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ORIGINAL REPORT

Predictive and Pharmacodynamic Biomarker Studies in Tumor and Skin Tissue Samples of Patients With Recurrent or Metastatic Squamous Cell Carcinoma of the Head and Neck Treated With Erlotinib

Mark Agulnik, Gilda da Cunha Santos, David Hedley, Trudey Nicklee, Patricia Pintor dos Reis, James Ho, Gregory R. Pond, Heidi Chen, Shuo Chen, Yu Shyr, Eric Winquist, Denis Soulieres, Eric X. Chen, Jeremy A. Squire, Paula Marrano, Suzanne Kamel-Reid, Janet Dancey, Lillian L. Siu, and Ming S. Tsao

From the Princess Margaret Hospital Phase II Consortium; Ontario Cancer Institute, Toronto; National Cancer Institute of Canada Clinical Trials Group, Kingston, Ontario, Canada; Vanderbilt University Medical Center, Nashville, TN; and the National Cancer Institute, Bethesda, MD.

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M.A. and G.d.C.S. contributed equally to this work.

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Address reprint requests to Lillian L. Siu, MD, FRCPC, Department of Medical Oncology and Hematology, Princess Margaret Hospital, University Health Network, 610 University Ave, Suite 5-718, Toronto, Ontario, M5G 2M9, Canada; e-mail: lillian.siu@uhn.on.ca.

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ABSTRACT

Purpose
Pharmacodynamic tissue studies were conducted on a phase I/II trial of erlotinib and cisplatin in patients with recurrent or metastatic head and neck squamous cell carcinoma (HNSCC). Levels of epidermal growth factor receptor (EGFR), downstream signaling components, and markers of angiogenesis and apoptosis were evaluated to determine the relationship between correlative end points and clinical outcomes.

Patients and Methods
Pretreatment and during-treatment tumor and skin biopsies, and archival tumor specimens were evaluated for EGFR, phosphorylated (p) -EGFR, extracellular signal-regulated kinase (ERK), p-ERK, Akt, p-Akt, Ki67, p27, p-nuclear factor kappa B (NFκB), p-signal transducer and activator of transcription 3 (STAT3), and *EGFR* gene copy number.

Results
On 37 archival samples, response to therapy was evident in two of four (50%) patients with high *EGFR* gene copy number tumors and in four of 27 (15%) patients with low gene copy number tumors. On nine paired tumor biopsies, elevated pretreatment levels of p27 and p-STAT3 predicted for prolonged time to progression (TTP) and overall survival (OS; $P \leq .03$). With treatment, a decrease in p-EGFR, p-NFκB, and p27 correlated with increased TTP, OS, or both TTP and OS, respectively ($P \leq .04$). Multidimensional scaling (MDS) models revealed clustering profiles of tumor markers by immunofluorescence could predict response. On 32 paired skin biopsies, suppression of p-EGFR with therapy correlated with increased OS ($P = .045$).

Conclusion
High *EGFR* gene copy in tumor specimens may predict which patients have an increased likelihood of response to erlotinib, and decreased p-EGFR level in skin biopsies during therapy may represent a potential surrogate marker for improved clinical outcome. MDS represents a novel way to evaluate the relationships between molecular markers and clinical outcome. Additional biomarker studies with larger sample sizes are required to elucidate HNSCC patients who may benefit from this targeted therapy.

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INTRODUCTION

Epidermal growth factor receptor (EGFR) and its ligands are fundamental for cell proliferation, motility, adhesion, invasion, and angiogenesis.[1] Dysregulation of the EGFR signal transduction pathway plays a critical role in the process of tumor formation, growth, and metastasis.[2-4] Biochemical and immunohistochemical studies have demonstrated overexpression of EGFR in a number of solid tumors, including 80% to 90% of head and neck squamous cell carcinomas (HNSCC),[5-7] and elevated levels of EGFR protein portend for a decreased disease-free survival.[6,8]

EGFR inhibition is an important strategy in the treatment of HNSCC. Erlotinib is a small-molecule quinazoline derivative that inhibits autophosphorylation of EGFR tyrosine kinase (TK) and exerts antiproliferative effects resulting in cell cycle arrest and induction of apoptosis.[9,10] Preliminary results suggest that erlotinib inhibits EGFR signaling at the tumor level, and that p27

upregulation may represent a marker of effective downstream inhibition in skin tissues.[11,12]

To maximize the clinical potential of erlotinib in HNSCC and expand on the single-agent activity,[13] its use in combination with cisplatin was evaluated in a phase I/II trial. Pre- and during treatment levels of EGFR, its downstream signaling components, and markers of angiogenesis and apoptosis in tumor and skin biopsies were examined to determine the relationship between these correlative end points and clinical outcome.

PATIENTS AND METHODS

Clinical Study

Tumor and skin samples were procured from participants in a phase I/II trial of erlotinib and cisplatin in recurrent or metastatic HNSCC. Eligible patients were treated either in the phase I portion of the trial or at the recommended phase II dose of erlotinib 100 mg orally daily on a continuous schedule and cisplatin 75 mg/m^2 intravenously on day 1 every 21 days. For the first cycle, erlotinib was taken alone for 7 days (days −6 to day 0) as a run-in period, to enable a steady-state concentration before initiating cisplatin. The primary end point was objective response rate assessed using Response Evaluation Criteria in Solid Tumors.[14] Secondary end points included clinical parameters of efficacy, safety assessments, and molecular correlations using tumor and skin biopsies. Separate written consent was obtained for optional tissue banking and correlative studies. Institutional review board approval was obtained at all participating centers.

Tissue Acquisition

All patients enrolled onto the study had representative archival paraffin block or unstained sections from their primary tumor diagnostic specimen sent for evaluation. In those patients with accessible tumor tissues and written consent, fresh tumor biopsies were taken up to 7 days before and after 1 week of erlotinib treatment. Biopsies obtained during erlotinib treatment were taken between 4 and 8 hours after the drug dose and before cisplatin administration. In consenting patients, paired skin biopsies were performed at the same time points. For tumor tissue biopsies, a 14-gauge tru-cut biopsy was performed using standard surgical technique. For skin biopsies, a 3- to 4-mm punch specimen was procured pretreatment, and repeated during treatment at or near the original site. All samples obtained were placed immediately in 10% neutral buffered formalin, fixed overnight, and transferred to 70% ethyl alcohol for storage before paraffin embedding.

Immunohistochemistry and Immunofluorescence

Biomarkers related to EGFR and its associated pathways were analyzed in tumor and surrogate tissues using either colorimetric immunohistochemistry (IHC) or immunofluorescence (IF) techniques. Phosphorylation of EGFR activates downstream signaling pathways, including Ras-Raf-MEK–extracellular signal-regulated kinase (ERK), phosphatidylinositol 3′kinase/Akt, and the signal transducer and activator of transcription (STAT) pathways.[15,16] Ki67 is an index of cellular proliferation; p27 is a cell cycle inhibitor; and nuclear factor kappa B (NFκB) is a marker of cell survival. Phosphorylation denotes the activation state of the cellular proteins under evaluation. Archival specimens were assessed for EGFR expression using IHC. Paired tumor specimens were assessed for EGFR, phosphorylated (p) -EGFR, ERK, p-ERK, Akt, p-Akt, p27, Ki67, p-NFκB, p-STAT3 using both IHC and IF. Paired skin biopsies were assessed for EGFR, p-EGFR, ERK, p-ERK, Akt, p-Akt, p27, and Ki67 using IHC.

For IHC and IF, sections were pretreated with 0.4% pepsin, pH 2.0, or subjected to antigen retrieval in a Milestone T/T Mega microwave oven (Milestone srl, Sorisole [BG], Italy) for 10 minutes at 120°C in 10 mmol/L citrate buffer, pH 6.0. After cooling, sections were covered with normal serum and incubated in primary antibodies. IF tumor biopsies were dual stained with antibodies against the marker protein and cytokeratin (cocktail of Dako monoclonal antibodies to AE1/AE3, HMWK, CK7, CK20 or Dako rabbit polyclonal antikeratin wide spectrum screening; Dako Cytomation, Carpinteria, CA) to identify epithelial tumor cells. After primary antibody incubation, slides were rinsed in phosphate-buffered saline and secondary antibodies (Cy5 conjugate, specific to the cell signaling markers, and Cy3 conjugate, specific to the cytokeratin) were applied for 30 minutes at room temperature in darkness. Sections were counterstained with the DNA-specific dye 4,6-diamidino-2-phenylindole (DAPI; Roche Applied Science, Laval, Quebec, Canada) at 1 μg/mL for 5 minutes at 4°C, then rinsed twice for 5 minutes with distilled water and air dried.

Image Analysis

Biopsies stained by IF were imaged using a wide-field laser-scanning microscope (TISSUEscope; Biomedical Photometrics Inc, Waterloo, Ontario, Canada) using dual 532- and 639-nm excitation. The entire tissue area was scanned to give a pixel resolution of approximately 2 μm. Using the cytokeratin-stained image, the tumoral area was outlined as described previously.[17] This outline creates masks of tumor areas, which were superimposed on the specific cell-signaling marker tagged with Cy5 fluorochrome image. The change in staining brightness was measured in gray levels for the pretreatment and during-treatment biopsies. Results were expressed as the percent of positive stained areas in square micrometers within the tumor regions, and the staining intensity reported as mean integrated optical density in gray levels, except for p27, Ki67, p-STAT3, and NFκB, for which the estimated number of positive nuclei per tumor area with mean nuclear size of 75 μm^2 was determined.

IHC-stained biopsies were acquired using Zeiss transmitted-light microscope (Carl Zeiss, Oberkochen, West Germany), 20× objective, motorized stage, and a Sony DXC970 color camera (Toyko, Japan) controlled by MCID Elite software (St Catherine's, Ontario, Canada). Manually selecting the epidermis and setting a threshold on positive NovaRed stained areas, the software computed the proportional positive stained area for all markers except p27 and Ki67, for which the estimated number of positive nuclei per tumor area with mean nuclear size of 75 μm^2 was determined.

IHC Scoring

The IHC for EGFR was evaluated for the extent of tumor cell membranous staining and staining intensity, and was scored from 0 to 5 (0%, < 1%, 1% to 10%, 11% to 33%, 34% to 67%, > 67%) and 0 to 3 (absent, weak, medium, strong), respectively.

Fluorescent In Situ Hybridization and Analysis

Archival tumor specimens were analyzed for *EGFR* gene amplification status using fluorescent in situ hybridization (FISH), as described previously.[18]

Analyses were done with a fluorescence microscope by two independent observers (G.C.S. and P.M.). The observers were blinded to the patient's clinical response and each other's assessments. One hundred nonoverlapping interphase nuclei were scored for EGFR and CEP7 copy number and classified into six categories, as reported previously.[18,19]

Statistical Analysis

Expression levels were averaged across slides from the same tumor biopsy. Changes due to treatment for paired biopsies were evaluated using Wilcoxon rank sum tests on the difference in expression (during treatment minus pretreatment). Predictions of clinical outcomes were evaluated using χ^2 tests when the outcome was response, and analysis of variance or Spearman correlation coefficients for survival outcomes (all patients had experienced disease progression and/or died as of the final analysis). Generalized estimating equations were used to verify and explore results further. Grouping of variables based on response, FISH status, and for IHC analyses based on staining extent and intensity was done to increase statistical power. All tests were two sided and a P value of .05 or less was considered statistically significant. No multiple-testing P value adjustment was performed.

To provide an analysis of multiple markers at the same time, multidimensional scaling (MDS) was used.[20] MDS can be considered to be an alternative to factor analysis. MDS is a data analysis technique that displays the structure of distance-like data as a geometric picture. MDS illustrates the structure of a set of study samples from data that approximate the distances between pairs of the study samples. Two similar study samples are represented by two points that are close together, and two dissimilar study samples are

Table 1. Response Rates, TTP, and OS for Patients With Assessable Archival Specimens, Paired Skin Biopsies, and Paired Tumor Biopsies

Outcome	Archival Specimens No.	Archival Specimens %	Skin Biopsies No.	Skin Biopsies %	Tumor Biopsies No.	Tumor Biopsies %
No. of patients	37		32		9	
Response						
CR	1	3	1	3	0	0
PR	6	16	5	16	1	11
SD	18*	49	16	50	7*	78
PD	11	30	10	31	1	11
NA	1	3	0	0	0	0
TTP, months						
Median	3.1		3.2		3.3	
Range	1.2-10.1		1.2-10.1		0.9-8.9	
OS, months						
Median	6.1		8.2		8.3	
Range	1.2-28.8		1.2-28.8		0.9-28.8	

Abbreviations: TTP, time to progression; OS, overall survival; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NA, not assessable.
*One patient had unconfirmed PR.

represented by two points that are far apart. The result of MDS is usually a three-dimensional Euclidean space.

RESULTS

Samples Available for Analysis and Clinical Outcomes

Of the 51 patients accrued for enrollment onto the phase I/II trial, 42 archival tumor samples from 37 different patients, 32 paired skin and nine paired tumor biopsy samples were available for analysis. Response rates, time to progression (TTP) and overall survival (OS) for these patients are outlined in Table 1. Details of the clinical results of this study are provided in a companion article.[21]

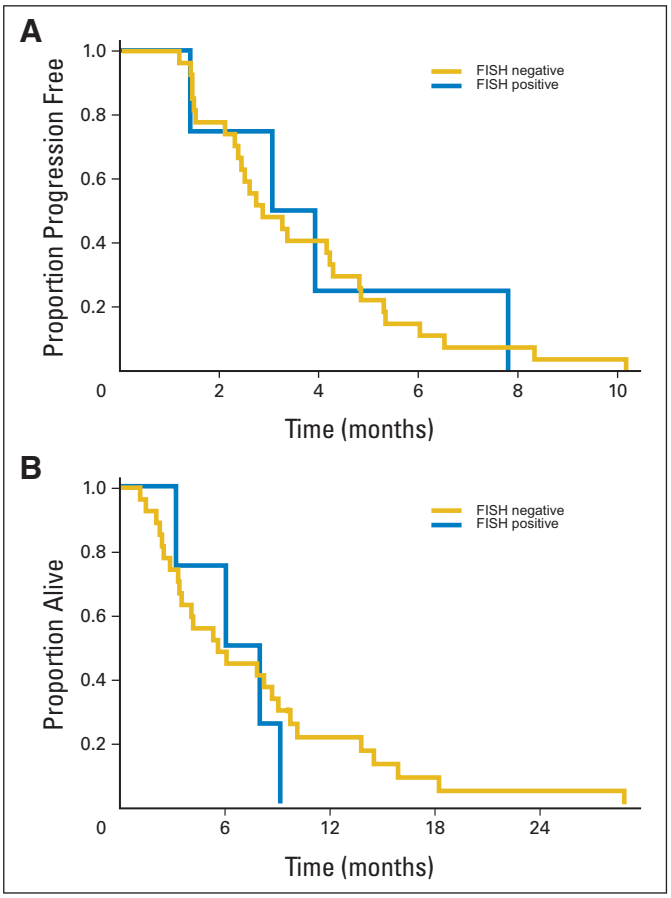

Fig 1. (A) Kaplan-Meier curves of (A) time to progression and (B) overall survival for fluorescent in situ hybridization (FISH) -positive versus FISH-negative patients analyzed for epidermal growth factor receptor (*EGFR*) gene amplification status.

Table 2. Objective Tumor Response, TTP, and OS, As Analyzed by *EGFR* Gene Copy and Protein Expression Assays on Archival Specimens

EGFR Assay	No. (n = 35)	Response Rate (%)	TTP (months)	OS (months)
Gene copy number by FISH				
Low	27	15	2.8	5.6
High (HP + AMP)	4 (2 + 2)	50	3.5	7.1
P		.33	.79	.88
Protein by IHC				
Staining extent, %				
0-10	1	0	2.6	7.8
11-33	0	NA	NA	NA
34-66	10	10	3.0	4.1
67-100	24	25	3.2	6.1
P		.39	.82	.55
Staining intensity				
Absent or weak	4	0	3.7	8.4
Medium	17	12	3.2	5.4
Strong	14	36	2.4	7.7
P		.090	.46	.89
Staining extent and intensity				
67%-100% and strong	12	33	2.4	7.7
< 67% and < strong	23	13	3.1	5.6
P		.33	.35	.96

Abbreviations: TTP, time to progression; OS, overall survival; EGFR, epidermal growth factor receptor; FISH, fluorescent in situ hybridization; HP, high polysomy; AMP, amplification; IHC, immunohistochemistry.

Archival Tumor Samples

FISH was used to assess the *EGFR* gene copy number on all archival tumor samples and complete analysis was achieved on 32 (86%) samples (Table 2). Patients with high *EGFR* gene copy numbers (amplification and high polysomy) are designated as FISH positive and patients in all other categories are designated FISH negative. FISH-positive patients had a high response rate to therapy (two of four; 50%), longer TTP (3.5 months), and a median survival of 7.1 months. Among FISH-negative patients, response to therapy was observed in four of 27 patients (15%), and TTP and median OS were 2.8 and 5.6 months, respectively (Fig 1).

Among the 37 patients whose archival tumor samples were subjected to IHC analysis, 36 samples (97%) were EGFR positive. The relationships among EGFR status, clinical parameters of tumor response, TTP, and OS are summarized in Table 2. Of those patients with ≥ 67% of cells staining for EGFR, six of 24 (25%) responded to therapy, whereas only one of 11 (9%) patients with less than 67% of cells staining for EGFR achieved an objective response. With respect to EGFR staining intensity, objective responses were seen in five (36%) of

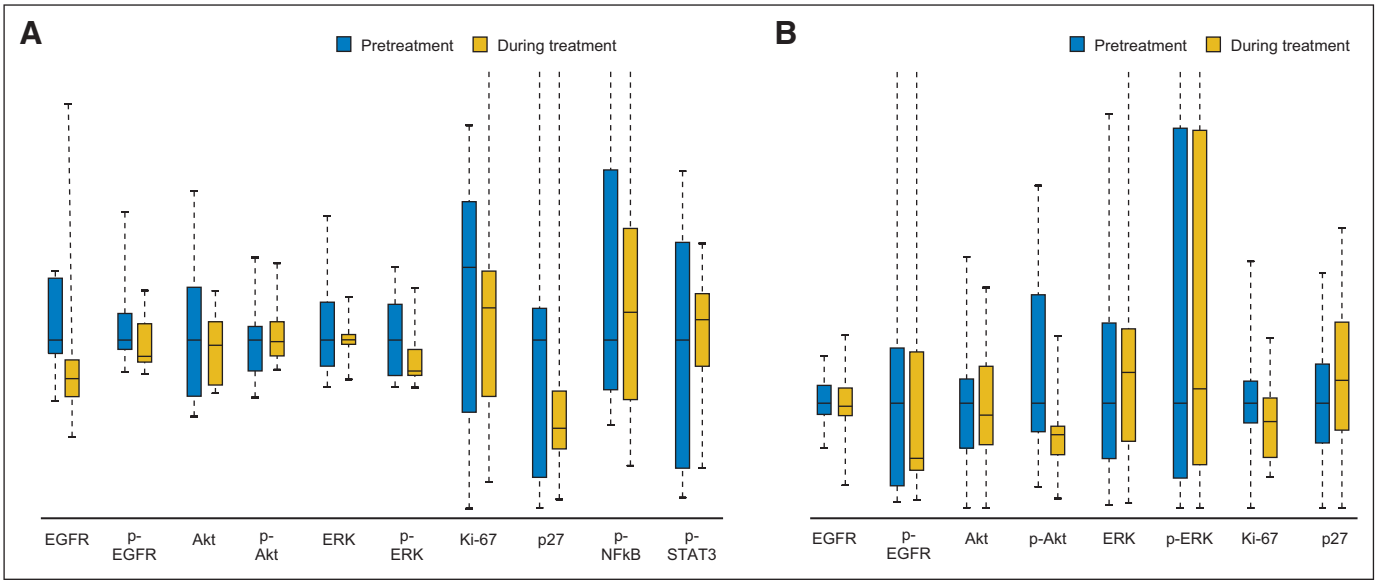

Fig 2. Whisker plot of change in markers pretreatment and during treatment. Plots were scaled by dividing by the median pretreatment value, for visual purposes only. (A) Whisker plot of immunofluorescence markers on paired tumor biopsies. (B) Whisker plot of immunohistochemistry markers on paired skin biopsies. No statistically significant changes are observed. EGFR, epidermal growth factor receptor; p, phosphorylated; ERK, extracellular signal-regulated kinase; NFκB, nuclear factor kappa B; STAT3, signal transducer and activator of transcription 3.

14 patients with strong staining intensity, compared with two (10%) of 21 patients who had weaker or no staining intensity. No statistically significant association between FISH and IHC evaluation of EGFR status was established.

Paired Tumor Biopsies

Pretreatment expression levels of EGFR, p-EGFR, ERK, p-ERK, Akt, p-Akt, Ki67, p27, p-NFκB, and p-STAT3 were analyzed in the nine paired tumor biopsies. Levels were compared with expression levels after 7 days of treatment with erlotinib (Fig 2A). IF analysis suggests that several markers may predict or correlate with TTP and OS. An elevated baseline p27 and p-STAT3 predicted for prolonged TTP and OS ($P \leq .03$). Decreases in p-EGFR and p-NFκB with treatment were associated with increased TTP ($P = .04$) and OS ($P = .03$), respectively, and decrease in p27 with treatment was associated with both increased TTP and OS ($P \leq .04$). All calculated expression levels of the markers are a reflection of the area of the tumor that has stained for the specific cell-signaling marker. Intratumor heterogeneity of molecular markers has been observed in biopsy specimens and may reflect an underestimate of the true expression levels of the markers (Fig 3).

MDS was used to explore the impact of the simultaneous grouping of expression levels of multiple markers on response rates, TTP, or OS. These models illustrated that expression levels of tumor markers by IF can predict for response (Appendix Fig A1A, online only). No clustering patterns were visualized when IHC evaluation of tumor markers was used. MDS graphs did not illustrate clustering patterns as predictors of TTP or OS (Appendix Fig A1B, online only).

Paired Skin Biopsies

Pretreatment expression levels of EGFR, p-EGFR, ERK, p-ERK, Akt, p-Akt, Ki67, and p27 were analyzed in the 32 paired skin biopsies. Levels were compared with expression levels after 7 days of treatment with erlotinib (Fig 2B). When comparing levels of expression as a result of therapy, increased OS was observed in patients exhibiting a suppression of p-EGFR with therapy (median survival of 10.1 months [range, 6.1 to 15.9 months] *v* 4.8 months [range, 2.5 to 8.7 months]). Expression of the cell cycle inhibitor p27 was found to increase with therapy, but neither predicted nor correlated with clinical outcome. No other marker showed a significant association with response, survival, or TTP.

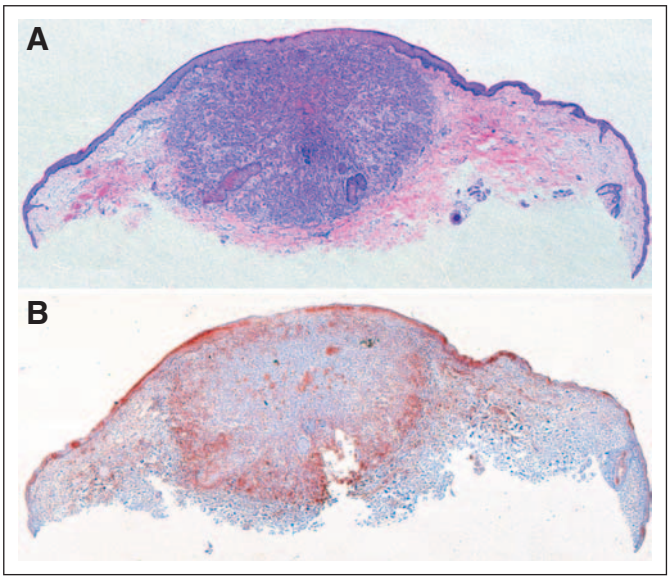

Fig 3. Representative example of staining heterogenicity. (A) Histologic appearance of the tumor prestaining; (B) immunohistochemical result showing positive staining of tumor cells for phosphorylated extracellular signal-regulated kinase, with concentration of staining around the periphery of the tumor and absence of staining in the center.

MDS graphs were established from the paired skin samples in an identical way as that for the paired tumor biopsy samples. No distinct clustering patterns in the three-dimensional models were visualized when associating grouped skin markers with responses, TTP, or OS (Appendix Fig A1C, online only).

DISCUSSION

Erlotinib as a single agent has been shown to produce prolonged disease stabilization in patients with recurrent or metastatic HNSCC.[13] Identification of a subset of patients who may benefit the most from erlotinib therapy has not been well established in this disease. Our study sought to establish if baseline levels of *EGFR* expression and receptor phosphorylation status in tumor and skin biopsy samples could predict outcome to therapy, if the effect of erlotinib on downstream signaling components can be measured in tumor and skin tissues, and if skin biopsies can be used as a surrogate to predict or follow clinical outcome.

To the best of our knowledge, this study is the first to suggest that HNSCC patients with high gene copy number may have higher response rates to erlotinib therapy than patients with low *EGFR* gene copy number. Three recent reports, in patients with non–small-cell lung cancer (NSCLC), confirmed an association between high *EGFR* gene copy number with either survival or response to EGFR TK inhibitor therapy. Cappuzzo et al[19] reported on NSCLC patients treated with gefitinib, and showed a statistically significant survival advantage for patients with EGFR FISH-positive tumors. Similar results were seen in a subset of patients with lung adenocarcinoma with bronchoalveolar growth pattern.[22] Tsao et al[18] have recently confirmed, by univariate analysis, a prolonged survival in EGFR FISH-positive NSCLC patients treated with erlotinib; however, by multivariate analysis, this advantage was not conferred.

Most previous studies have found the expression of EGFR protein in archival specimens by IHC an unreliable predictor of responsiveness to EGFR inhibitor therapies.[23-25] Consistent with the phenotype of squamous cell carcinoma, we found that nearly all of our patients expressed the EGFR protein, thus rendering the differentiation of clinical outcome between EGFR-positive and -negative patients to be statistically impossible. We then attempted to analyze our results by stratification based on percentage of positively stained cells and intensity of staining. Response rates tended to favor those patients with increased percentage of cells staining for EGFR and those with increased staining intensity. Other studies have not demonstrated this association[26] and most recently a study of the anti-EGFR monoclonal antibody cetuximab in HNSCC has shown the opposite finding.[27] It should be reminded that IHC is a subjective assay lacking the dynamic range for quantitative evaluation of protein expression level. With most HNSCC expressing high EGFR protein, the lack of correlation between EGFR gene dosage and the IHC protein levels is not surprising, as demonstrated by our data and that of Mrhalova et al.[28]

With EGFR TK inhibition by erlotinib, downstream effector signals are expected to decrease and theoretically, p27 should be increased.[9,10] We observed a decrease in p27 in patients receiving erlotinib therapy that was associated with improved survival outcomes. A concern has arisen about the potential antagonistic effect of EGFR inhibitors and chemotherapeutic agents.[29,30] Although cytotoxic chemotherapeutic agents act on dividing cells, EGFR inhibitors cause G_1 cell cycle arrest. By causing this cell cycle arrest, erlotinib may antagonize the antitumor effects of cisplatin. It is therefore a possibility that patients whose tumor cells continued to proliferate and did not undergo cell cycle arrest, despite erlotinib, were more likely to experience benefits from cisplatin and thus had an improved survival outcome with an observed decrease in p27.

As a prognostic marker, overexpression of p-NFκB has been found to relate to poor survival in a series of tonsillar HNSCC.[31] Our study did not indicate any significant association between the pretreatment p-NFκB level and clinical outcome. As a pharmacodynamic marker, EGFR inhibitors have been shown to inhibit NFκB phosphorylation in vitro.[32] Although significant change in p-NFκB levels after 1 week of erlotinib was not observed, among patients whose p-NFκB did decrease with erlotinib, there was an increase in OS ($P = .03$) in a univariate analysis. This result is logical, given that NFκB is a survival factor for tumor cells, hence a decrease in the activation of this factor would confer a better outcome. However, given the small sample size and the fact that this is only a univariate analysis, this finding should be interpreted as exploratory.

Given the small number of samples obtained, and by comparison, the large number of markers evaluated, individual examinations of the effects of each marker on objective response, TTP, and OS by univariate analyses are plagued by multiple significance testing and are statistically flawed. From our nine paired tumor biopsy samples and 32 paired skin biopsy samples, the only uniform finding was that a decrease in p-EGFR level after 1 week of erlotinib in both tissue types was associated with increased TTP and OS, respectively. Therefore, p-EGFR may represent a useful surrogate pharmacodynamic marker of erlotinib effect in recurrent or metastatic HNSCC. Our group has shown similar results of a decreased p-EGFR level in tumor tissue during treatment with erlotinib for patients with metastatic colorectal cancer.[17]

Recent publications have explored the role of MDS as a means to visualize multiple results without the use of a *P* value.[33-35] Our MDS models illustrated that clustering patterns of molecular markers in tumor biopsy samples may help distinguish responders from nonresponders, but no significant patterns were identified using the skin samples from our patients. Although MDS models of baseline and during treatment expressions of tumor markers seemed to predict for objective responses with erlotinib, no associations with TTP or OS were found.

This study illustrates that when serial tumor biopsies are optional rather than mandatory, a paucity of research samples are obtained. Given the number of assessable samples, the inclusion of multiple markers for analysis, the issue of tumor heterogeneity, the variability of reagents and antibodies used, and the differences in sample handling and storage procedures, it becomes difficult to draw definitive conclusions with sufficient power. Although our ability to formulate any association between tumor or surrogate tissue samples with clinical outcome is limited, additional evaluation of the association

between *EGFR* gene copy and response to EGFR inhibition in HNSCC needs to be explored.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following authors or their immediate family members indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. 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.

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AUTHOR CONTRIBUTIONS

Conception and design: David Hedley, Janet Dancey, Lillian L. Siu, Ming S. Tsao

Administrative support: Janet Dancey

Provision of study materials or patients: Eric Winquist, Denis Soulieres, Eric X. Chen, Lillian L. Siu

Collection and assembly of data: Gilda da Cunha Santos, David Hedley, Trudey Nicklee, Patricia Pintor dos Reis, James Ho, Gregory R. Pond, Jeremy A. Squire, Paula Marrano, Suzanne Kamel-Reid, Ming S. Tsao

Data analysis and interpretation: Mark Agulnik, Gilda da Cunha Santos, David Hedley, Trudey Nicklee, Patricia Pintor dos Reis, James Ho, Gregory R. Pond, Heidi Chen, Shuo Chen, Yu Shyr, Jeremy A. Squire, Paula Marrano, Suzanne Kamel-Reid, Lillian L. Siu, Ming S. Tsao

Manuscript writing: Mark Agulnik

Final approval of manuscript: Mark Agulnik, Gilda da Cunha Santos, David Hedley, Trudey Nicklee, Patricia Pintor dos Reis, James Ho, Gregory R. Pond, Heidi Chen, Shuo Chen, Yu Shyr, Eric Winquist, Denis Soulieres, Eric X. Chen, Jeremy A. Squire, Paula Marrano, Suzanne Kamel-Reid, Janet Dancey, Lillian L. Siu, Ming S. Tsao

REFERENCES

1. Woodburn JR: The epidermal growth factor receptor and its inhibition in cancer therapy. Pharmacol Ther 82:241-250, 1999

2. Yarden Y: The EGFR family and its ligands in human cancer: Signalling mechanisms and therapeutic opportunities. Eur J Cancer 37:S3-S8, 2001 (suppl 4)

3. Schlessinger J: Cell signaling by receptor tyrosine kinases. Cell 103:211-225, 2000

4. Mendelsohn J, Baselga J: The EGF receptor family as targets for cancer therapy. Oncogene 19:6550-6565, 2000

5. Rubin Grandis J, Melhem MF, Barnes EL, et al: Quantitative immunohistochemical analysis of transforming growth factor-alpha and epidermal growth factor receptor in patients with squamous cell carcinoma of the head and neck. Cancer 78: 1284-1292, 1996

6. Rubin Grandis J, Melhem MF, Gooding WE, et al: Levels of TGF-alpha and EGFR protein in head and neck squamous cell carcinoma and patient survival. J Natl Cancer Inst 90:824-832, 1998

7. Santini J, Formento JL, Francoual M, et al: Characterization, quantification, and potential clinical value of the epidermal growth factor receptor in head and neck squamous cell carcinomas. Head Neck 13:132-139, 1991

8. Shin DM, Ro JY, Hong WK, et al: Dysregulation of epidermal growth factor receptor expression in premalignant lesions during head and neck tumorigenesis. Cancer Res 54:3153-3159, 1994

9. Moyer JD, Barbacci EG, Iwata KK, et al: Induction of apoptosis and cell cycle arrest by CP-358,774, an inhibitor of epidermal growth factor receptor tyrosine kinase. Cancer Res 57:4838-4848, 1997

10. Pollack VA, Savage DM, Baker DA, et al: Inhibition of epidermal growth factor receptor-associated tyrosine phosphorylation in human carcinomas with CP-358,774: Dynamics of receptor inhibition in situ and antitumor effects in athymic mice. J Pharmacol Exp Ther 291:739-748, 1999

11. Malik SN, Siu LL, Rowinsky EK, et al: Pharmacodynamic evaluation of the epidermal growth factor receptor inhibitor OSI-774 in human epidermis of cancer patients. Clin Cancer Res 9:2478-2486, 2003

12. Hidalgo M, Shazly M, Rowinsky EK, et al: Inhibition of the epidermal growth factor receptor (EGFR) by OSI-774, a specific EGFR inhibitor in malignant and normal tissue of cancer patients. Proc Am Soc Clin Oncol 20:71a, 2001 (abstr 281)

13. Soulieres D, Senzer NN, Vokes EE, et al: Multicenter phase II study of erlotinib, an oral epidermal growth factor receptor tyrosine kinase inhibitor, in patients with recurrent or metastatic squamous cell cancer of the head and neck. J Clin Oncol 22:77-85, 2004

14. Therasse P, Arbuck SG, Eisenhauer EA, et al: New guidelines to evaluate the response to treatment in solid tumors: European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 92:205-216, 2000

15. Bunn PA Jr, Franklin W: Epidermal growth factor receptor expression, signal pathway, and inhibitors in non-small cell lung cancer. Semin Oncol 29:38-44, 2002

16. Haura EB, Zheng Z, Song L, et al: Activated epidermal growth factor receptor-Stat-3 signaling promotes tumor survival in vivo in non-small cell lung cancer. Clin Cancer Res 11:8288-8294, 2005

17. Townsley CA, Major P, Siu LL, et al: Phase II study of erlotinib (OSI-774) in patients with metastatic colorectal cancer. Br J Cancer 94:1136-1143, 2006

18. Tsao MS, Sakurada A, Cutz JC, et al: Erlotinib in lung cancer: Molecular and clinical predictors of outcome. N Engl J Med 353:133-144, 2005

19. Cappuzzo F, Hirsch FR, Rossi E, et al: Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. J Natl Cancer Inst 97:643-655, 2005

20. Steyvers M: Multidimensional scaling, in Encyclopedia of Cognitive Science. London, United Kingdom, Nature Publishing Group, 2002

21. Siu LL, Soulieres D, Chen EX, et al: Phase I-II trial of erlotinib and cisplatin in patients with recurrent or metastatic squamous cell carcinoma of the head and neck: A Princess Margaret Hospital Phase II Consortium and National Cancer Institute of Canada Clinical Trials Group Study. J Clin Oncol 2510.1200/JCO.2006.07.6547

22. Hirsch FR, Varella-Garcia M, McCoy J, et al: Increased epidermal growth factor receptor gene copy number detected by fluorescence in situ hybridization associates with increased sensitivity to gefitinib in patients with bronchioloalveolar carcinoma subtypes: A Southwest Oncology Group Study. J Clin Oncol 23:6838-6845, 2005

23. Dancey JE: Predictive factors for epidermal growth factor receptor inhibitors: The bull's-eye hits the arrow. Cancer Cell 5:411-415, 2004

24. Parra HS, Cavina R, Latteri F, et al: Analysis of epidermal growth factor receptor expression as a predictive factor for response to gefitinib ('Iressa', ZD1839) in non-small-cell lung cancer. Br J Cancer 91:208-212, 2004

25. Perez-Soler R, Chachoua A, Hammond LA, et al: Determinants of tumor response and survival with erlotinib in patients with non–small-cell lung cancer. J Clin Oncol 22:3238-3247, 2004

26. Baselga J, Trigo JM, Bourhis J, et al: Phase II multicenter study of the antiepidermal growth factor receptor monoclonal antibody cetuximab in combination with platinum-based chemotherapy in patients with platinum-refractory metastatic and/or recurrent squamous cell carcinoma of the head and neck. J Clin Oncol 23:5568-5577, 2005

27. Burtness B, Goldwasser MA, Flood W, et al: Phase III randomized trial of cisplatin plus placebo compared with cisplatin plus cetuximab in metastatic/recurrent head and neck cancer: An Eastern Cooperative Oncology Group study. J Clin Oncol 23: 8646-8654, 2005

28. Mrhalova M, Plzak J, Betka J, et al: Epidermal growth factor receptor: Its expression and copy numbers of EGFR gene in patients with head and neck squamous cell carcinomas. Neoplasma 52:338-343, 2005

29. Piperdi B, Ling Y, Kroog G, et al: Schedule-dependent interaction between epidermal growth factor inhibitors (EGFRI) and G2/M blocking chemotherapeutic agents (G2/MB) on human NSCLC cell lines in vitro. J Clin Oncol 22:623s, 2004 (abstr 7028)

30. Baselga J: Combining the anti-EGFR agent gefitinib with chemotherapy in non-small-cell lung cancer: How do we go from INTACT to impact? J Clin Oncol 22:759-761, 2004

31. Zhang PL, Pellitteri PK, Law A, et al: Overexpression of phosphorylated nuclear factor-kappa B in tonsillar squamous cell carcinoma and high-grade dysplasia is associated with poor prognosis. Mod Pathol 18:924-932, 2005

32. Bancroft CC, Chen Z, Yeh J, et al: Effects of pharmacologic antagonists of epidermal growth factor receptor, PI3K and MEK signal kinases on NF-kappaB and AP-1 activation and IL-8 and VEGF expression in human head and neck squamous cell carcinoma lines. Int J Cancer 99:538-548, 2002

33. Gruvberger SK, Ringner M, Eden P, et al: Expression profiling to predict outcome in breast cancer: The influence of sample selection. Breast Cancer Res 5:23-26, 2003

34. Ross ME, Mahfouz R, Onciu M, et al: Gene expression profiling of pediatric acute myelogenous leukemia. Blood 104:3679-3687, 2004

35. Rouzier R, Perou CM, Symmans WF, et al: Breast cancer molecular subtypes respond differently to preoperative chemotherapy. Clin Cancer Res 11:5678-5685, 2005

Appendix

The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).