Research Article

Cancer Epidemiology, Biomarkers & Prevention

Detection of Tumor Epidermal Growth Factor Receptor Pathway Dependence by Serum Mass Spectrometry in Cancer Patients

Christine H. Chung[1,2], Erin H. Seeley[3], Heinrich Roder[4], Julia Grigorieva[4], Maxim Tsypin[4], Joanna Roder[4], Barbara A. Burtness[5], Athanassios Argiris[6], Arlene A. Forastiere[7], Jill Gilbert[1], Barbara Murphy[1], Richard M. Caprioli[3], David P. Carbone[1,2], and Ezra E.W. Cohen[8]

Abstract

Background: We hypothesized that a serum proteomic profile predictive of survival benefit in non–small cell lung cancer patients treated with epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKI) reflects tumor EGFR dependency regardless of site of origin or class of therapeutic agent.

Methods: Pretreatment serum or plasma from 230 patients treated with cetuximab, EGFR-TKIs, or chemotherapy for recurrent/metastatic head and neck squamous cell carcinoma (HNSCC) or colorectal cancer (CRC) were analyzed by mass spectrometry. Each sample was classified into "good" or "poor" groups using VeriStrat, and survival analyses of each cohort were done based on this classification. For the CRC cohort, this classification was correlated with the tumor EGFR ligand levels and *KRAS* mutation status.

Results: In the EGFR inhibitor–treated cohorts, the classification predicted survival (HNSCC: gefitinib, $P = 0.007$ and erlotinib/bevacizumab, $P = 0.02$; CRC: cetuximab, $P = 0.0065$) whereas the chemotherapy cohort showed no survival difference. For CRC patients, tumor EGFR ligand RNA levels were significantly associated with the proteomic classification, and combined *KRAS* and proteomic classification provided improved survival classification.

Conclusions: Serum proteomic profiling can detect clinically significant tumor dependence on the EGFR pathway in non–small cell lung cancer, HNSCC, and CRC patients treated with either EGFR-TKIs or cetuximab. This classification is correlated with tumor EGFR ligand levels and provides a clinically practical way to identify patients with diverse cancer types most likely to benefit from EGFR inhibitors. Prospective studies are necessary to confirm these findings. *Cancer Epidemiol Biomarkers Prev; 19(2); 358–65. ©2010 AACR.*

Introduction

With the recent development of molecularly targeted agents, numerous epidermal growth factor receptor inhibitors (EGFRI) have been developed and some are approved for treatment of non–small cell lung cancer (NSCLC), head and neck squamous cell carcinoma (HNSCC), and colorectal cancer (CRC; refs. 1–5). There are two main classes of EGFRIs: (*a*) small-molecule tyrosine kinase inhibitors (TKI) such as gefitinib and erlotinib, and (*b*) monoclonal antibodies such as cetuximab and panitumumab. Although the response rates to these agents as monotherapy in unselected patients are modest, several studies have shown that survival is improved even in unselected patients (6–8). In NSCLC, patients with tyrosine kinase domain mutations have higher response rates after treatment with EGFR-TKIs, but the subset of patients with the mutation alone cannot explain the observed survival improvement in unselected patients (6, 9–12). The role of other genetic alterations, e.g., *KRAS* mutations and increased *EGFR* copy number, in NSCLC is also not very clear: the latest large randomized clinical trials [Gefitinib (Iressa) versus Taxotere as a second line therapy (INTEREST) and Gefitinib (Iressa) versus vinorelbine in chemonaive elderly patients (INVITE)] did not confirm their correlation with progression-free survival (PFS) or overall survival (OS; refs. 13, 14). Genetic markers associating benefits from cetuximab in NSCLC have not been defined to date. In CRC, *KRAS* mutation and low expression of tumor EGFR ligands [amphiregulin (AREG) and epiregulin (EREG)] have both been associated with lack of clinical benefit (5, 15–20). However, *EGFR* and *KRAS*

Authors' Affiliations: [1]Division of Hematology/Oncology, Department of Medicine, [2]Department of Cancer Biology, [3]Department of Biochemistry, Vanderbilt-Ingram Comprehensive Cancer Center, Vanderbilt University School of Medicine, Nashville, Tenessee; [4]Biodesix, Steamboat Springs, Colorado; [5]Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, Pennsylvania; [6]University of Pittsburgh, Pittsburgh, Pennsylvania; [7]Johns Hopkins University, Baltimore, Maryland; and [8]Section of Hematology/Oncology, Department of Medicine, University of Chicago, Chicago, Illinois

Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

Corresponding Author: Christine H. Chung, Division of Hematology/Oncology, Department of Medicine and Cancer Biology, Vanderbilt University School of Medicine, 2220 Pierce Avenue, 777 Preston Research Building, Nashville, TN 37232-6307. Phone: 615-322-4967; Fax: 615-343-7602. E-mail: Christine.Chung@Vanderbilt.edu

doi: 10.1158/1055-9965.EPI-09-0937

mutations are rare in HNSCC, and many NSCLC and CRC patients do not harbor these aberrations (21–23). There are thus no biomarkers available for reliably predicting survival benefit in the majority of patients currently being treated with EGFR inhibitors.

Recently, Taguchi et al. (24) have shown that classification of NSCLC patients based on the analyses of pretreatment sera or plasma using matrix-assisted laser desorption ionization mass spectrometry (MALDI MS) could predict OS benefit in those treated with erlotinib or gefitinib. This MALDI MS data analysis algorithm used a set of eight predefined mass-to-charge (*m/z*) features to classify the samples into "good" or "poor" groups in a training cohort, and the algorithm was validated in five independent cohorts as a treatment-specific indicator of survival benefit. The concordance of classification between spectra obtained from two independent institutions was shown to be 97%, confirming the reproducibility of the test, which is now commercially available. In addition, no difference in the classification label was observed between plasma and serum samples (24).

In the present study, we hypothesized that this established biomarker assay, based on the serum proteomic profile generated from NSCLC cohorts before treatment with EGFR-TKIs, is reflective of EGFR dependency of the tumor regardless of the site of origin or class of EGFR-targeted therapeutic agents; therefore, the profile may be predictive of survival in HNSCC and colon cancer patients treated with cetuximab or TKIs.

Materials and Methods

Patient and Sample Characteristics

Pretreatment serum or plasma samples from 230 patients were analyzed. The HNSCC and CRC samples were obtained from University of Chicago, Vanderbilt University Medical Center, Eastern Cooperative Oncology Group, and Bristol-Myers Squibb under Institutional Review Board–approved protocols. Three EGFRI-treated cohorts for recurrent and/or metastatic HNSCC were as follows: (*a*) 55 samples from patients treated with gefitinib 250 mg once daily (25); (*b*) 32 samples from patients treated with a combination therapy: erlotinib 150 mg once daily and bevacizumab (a monoclonal antibody against vascular endothelial growth factor) 15 mg/kg every 3 wk (26); and (*c*) 21 samples from patients treated with cetuximab 400 mg/m^2 loading dose and 250 mg/m^2 weekly. The CRC cohort consisted of 88 patients treated

Table 1. Patient characteristics

Cohorts	HNSCC				CRC
	Gefitinib (*n* = 55)	Erlotinib/ bevacizumab (*n* = 32)	Cetuximab (*n* = 21)	Control no EGFRI (*n* = 34)	Cetuximab (*n* = 88)
Age (median)	58	57	53	57	57
Sex					
Male	45	26	16	26	50
Female	10	6	5	8	38
Race					
White	49	27	20		75
Other	6	5	1	NA	13
PS					
0	13	17	NA	7	NA
1	35	14		25	
2	7	1		2	
Smoking					
Yes	31	24	12	27	NA
No	13	3	8	5	
unknown	11	5	1	2	
RECIST best response					
CR	0	1			1
PR	0	4	NA	NA	6
SD	20	19			21
PD	31	8			49
NE	4	0			11

Abbreviations: NA, data not available; PS, performance status; RECIST, Response Evaluation Criteria in Solid Tumors; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluable.

Table 2. OS (HNSCC) or PFS (CRC) outcomes in the patient cohorts based on the classification determined by MS profile in pretreatment plasma or sera

Cohorts	HNSCC				CRC
	Gefitinib (*n* = 55)	Erlotinib/bevacizumab (*n* = 32)	Cetuximab (*n* = 21)	Control no EGFRI (*n* = 34)	Cetuximab (*n* = 88)
MALDI MS algorithm					
Good (%)	31 (56)	24 (75)	16 (76)	22 (65)	49 (56)
Poor (%)	23 (42)	6 (19)	5 (24)	12 (35)	36 (41)
Undefined	1 (2)	1 (3)	0 (0)	0 (0)	3 (3)
Failed MS*	0 (0)	1 (3)	0 (0)	0 (0)	0 (0)
HR	0.41	0.2	0.26	0.88	0.51
(95% CI)	(0.22-0.79)	(0.05-0.78)	(0.06-1.06)	(0.4-1.94)	(0.31-0.83)
Log-rank *P*	0.007	0.02	0.06	0.76	0.007
Median time to death/progression (wk)					
Good	36.7	39.5	38.3	39.4	8.9
Poor	18.0	29.1	11.9	15.5	8.4

*Failed MS, proteomic profile by MS could not be obtained due to heavy RBC contamination.

with cetuximab monotherapy (16). There was a control HNSCC cohort of 34 samples collected through two phase II clinical trials with identical eligibility criteria, in which patients with previously nontreated recurrent and/or metastatic HNSCC were treated with docetaxel/bortezomib (a proteasome inhibitor of the chymotrypsin-like activity of the 26S proteasome) or docetaxel/irinotecan combinations.

MALDI MS Analysis

The samples were analyzed at the Vanderbilt University Mass Spectrometry Research Center using a Bruker Autoflex-II MALDI MS and/or at the University of Colorado Mass Spectrometry Shared Resource using a Voyager DE-PRO MALDI MS. Serum or plasma were thawed on ice, diluted with deionized water (1:20 dilution), spotted, and analyzed as previously described in detail (24). We have previously shown that classification results are identical when using serum or plasma (24).

Spectral Preprocessing and Classification Procedure

Raw spectra were analyzed as previously described (24). The predetermined classification algorithm, VeriStrat (Biodesix), was applied in a blinded fashion to each sample to classify into good or poor outcome groups as previously described (24). Briefly, the classification algorithm was initially established using a training set of spectra from two groups of NSCLC patients: those who benefited the most (defined as stable disease for >6 mo) and the least (defined as progressive disease in <1 mo) from EGFR TKIs. The spectral pattern that associates with the stable disease group was defined as good and the progressive disease group was defined as poor. The classification algorithm was further validated on independent cohorts of patients treated with EGFR TKIs, and it was confirmed that patients classified as poor are not likely to benefit from EGFR inhibitor therapy, whereas patients classified as good have statistically longer PFS and OS. Each sample was run in triplicate, and if there was discordance in replica classification, the sample was labeled as "undefined."

Statistical Analyses

Time from the date of enrollment in the clinical trials to death, or the date of the first treatment to death was used to compare OS between good and poor groups after treatment with EGFRIs or chemotherapy for the HNSCC cohorts. Time from the date of enrollment in the clinical trial to measured progression of disease was used to compare PFS in the CRC cohort because OS data were not available. The study groups (good versus poor) were compared for OS and/or PFS with Kaplan-Meier estimates and Log-rank tests. Multivariable Cox proportional hazard analysis was done to evaluate the relevance of various clinical features. All data analyses were done using the SAS/JMP software (27) or PRISM (28). All tests of significance were two sided, and differences were considered statistically significant for *P* values were <0.05. Hazard ratios (HR) were univariate and were calculated using the Mantel-Haenszel method unless otherwise specified.

Results

Acquisition of Spectra Using MALDI MS from Patient Plasma or Sera

Spectra were generated in a blinded fashion and in triplicate from 230 pretreatment plasma or serum samples from patients with HNSCC or CRC, and 224 samples

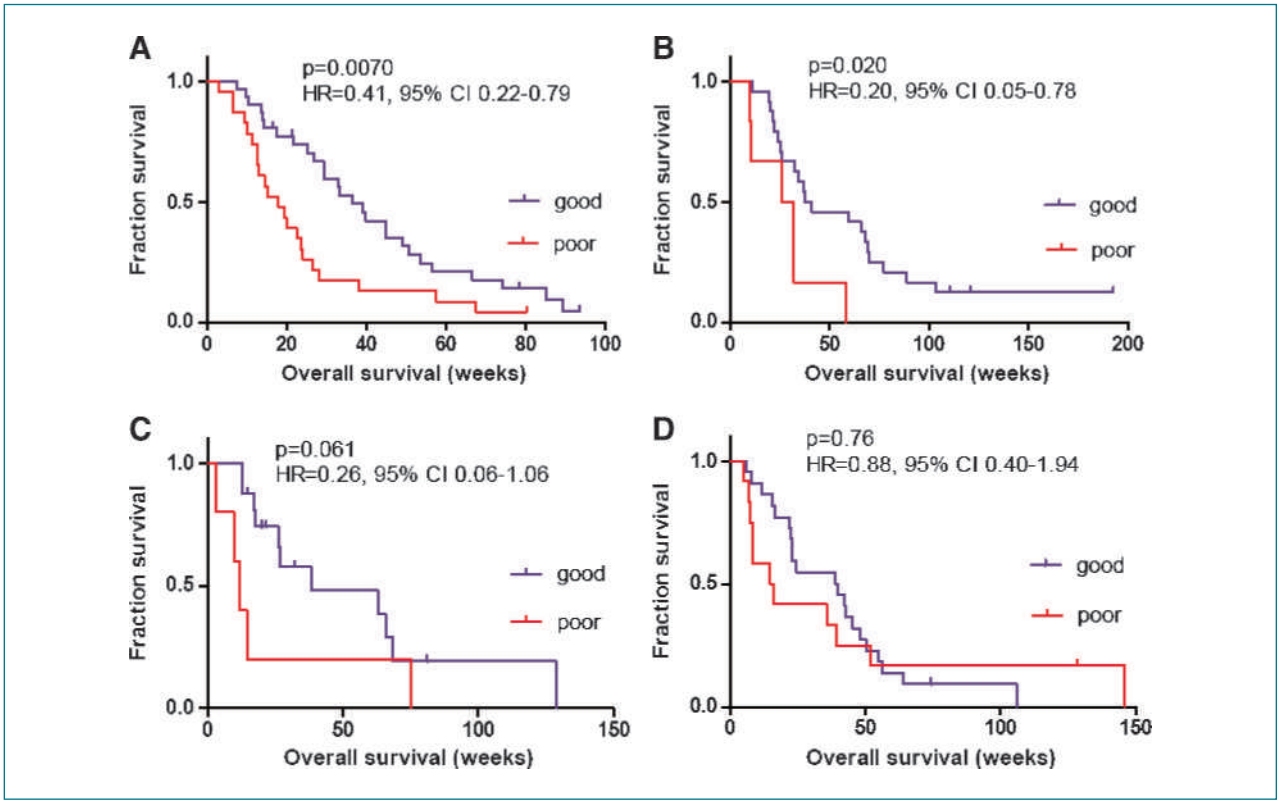

Figure 1. Kaplan-Meier plots for OS of patients with recurrent and/or metastatic HNSCC comparing the predictive groups of survival benefit, good and poor, determined by the MS profile, when treated with EGFRIs as previously described (24). A, a cohort of patients treated with gefitinib (n = 55); B, a cohort of patients treated with erlotinib and bevacizumab (n = 32); C, a cohort of patients treated with cetuximab (n = 21); and D, a cohort of patients treated with docetaxel-containing palliative chemotherapy as a control (n = 34).

(97%) yielded high-quality spectra for a definitive classification based on the previously published NSCLC predictive algorithm (24). The intrasample variability in these spectra was very much in line with what was reported previously for NSCLC samples, with an average feature intensity Coefficient of Variation (CVs) for the used peaks of <20%. Of the six samples that could not be classified, five were undefined due to discordance in the classification within the triplicate spectra, and one sample generated inadequate spectra due to hemoglobin contamination from RBC lysis during plasma separation. Detailed patient characteristics of each cohort are presented in Table 1.

Survival Analyses of Three HNSCC Cohorts Treated with EGFRIs

Among the 108 samples from three cohorts of recurrent and/or metastatic HNSCC patients treated with gefitinib, erlotinib/bevacizumab, or cetuximab, 71 (66%) were classified as good and 34 (32%) as poor outcome groups, whereas 2 (2%) were classified as undefined and one sample (1%) failed to generate usable spectra as described above. The three HNSCC cohorts treated with EGFRIs were analyzed separately based on this classification of each sample. The classification algorithm generated and validated in NSCLC predicted OS benefit in two HNSCC cohorts, with patients in the good group having significantly longer OS time compared with the patients in the poor group (Table 2; Fig. 1A-B): gefitinib-treated patients [log-rank P = 0.007; HR, 0.41, 95% confidence interval (95% CI), 0.22-0.79] and erlotinib/bevacizumab-treated patients (log-rank P = 0.02; HR, 0.20; 95% CI, 0.05-0.78). The OS of the cetuximab-treated cohort was close to statistical significance (log-rank P = 0.06; HR, 0.26; 95% CI, 0.06-1.06; Table 2; Fig. 1C), whereas PFS differed significantly between the two groups (log-rank P = 0.037; HR, 0.38; 95% CI, 0.05-0.91).

Cox univariate and multivariable analyses of OS were done on both the gefitinib and erlotinib/bevacizumab-treated cohorts using the classification and clinical features including patterns of disease failure (locoregional and distant metastases), presence of gastric tube, performance status, age, gender, race, and smoking history. Only age, performance status, and MALDI MS data analysis algorithm classification were independently associated with survival benefit in the gefitinib cohort (Supplementary Table S1). Performance status, gender, race, smoking history, and MALDI MS data analysis algorithm classification were independently associated with survival benefit in the erlotinib/bevacizumab cohort (Supplementary Table S2). A multivariable analysis was not done on the

cetuximab-treated cohort because the clinical data were not available.

Palliative Chemotherapy-Treated HNSCC Cohort as a Control

To determine whether the classification algorithm has a predictive association with therapeutic benefit specific to EGFR inhibition, 34 sera collected from two phase II clinical trials in patients with recurrent and/or metastatic HNSCC who did not receive EGFRI were analyzed. Among the 34 samples from patients treated with docetaxel-containing palliative chemotherapy, 22 samples were labeled as good and 12 samples as poor. There was no statistically significant difference in OS between the good and poor groups (log-rank $P = 0.76$; HR, 0.88; 95% CI, 0.40-1.94; Table 2; Fig. 1D). Although the sample size is too limited to exclude a potential separation with a small effect size, the result of this control set is consistent with multiple other chemotherapy alone or surgery alone control sets in our previous studies (24). The result of this set is also substantially different from the treatment samples to give a strong indication that the test performs differently for EGFRI-treated patients compared with non-EGFRI–treated patients.

Survival Analyses of the CRC Cohort

We further examined this proteomic classification in CRC patients, in which cetuximab is used frequently. Of 88 samples from recurrent and/or metastatic CRC patients treated with cetuximab monotherapy (16), 49 were characterized as good, 36 as poor, and 3 as undefined (Table 2). Because OS data were not available in this cohort, the classification was correlated with PFS, and the analysis showed statistically significant differences between the two groups (log-rank $P = 0.0065$; HR, 0.51; 95% CI, 0.31-0.83; Fig. 2A) with a wide separation of the two groups at longer progression-free intervals. These plots show that the PFS curves do not clearly separate until the later follow up time points. This is an artifact resulting from the fact that the planned tumor response assessments were at 9-week intervals. The dramatic drops in the PFS curve at 9 weeks are the result of early

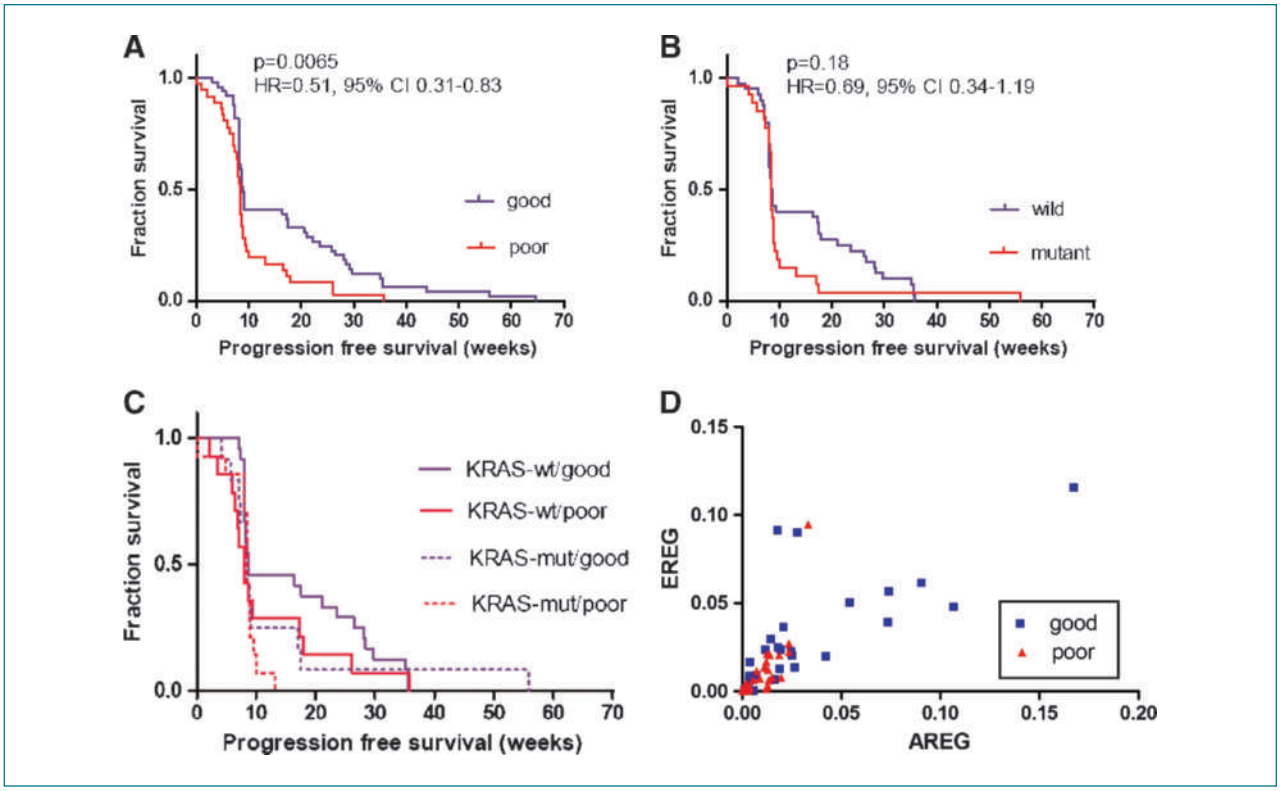

Figure 2. A, Kaplan-Meier plot for PFS of patients with recurrent and/or metastatic CRC comparing the predictive groups of survival benefit, good and poor, determined by the MS profile, when treated with cetuximab as previously described ($n = 88$; refs. 16, 24). B, Kaplan-Meier plot for PFS based on *KRAS* mutation status in the subset of CRC patients with both *KRAS* and MS profile classification data ($n = 64$; ref. 16). C, Kaplan-Meier plot for PFS of patients based on combined *KRAS* mutation status and the good/poor classification: *KRAS* mutant (*mut*)/poor versus *KRAS* wild-type (*wt*)/good groups (log-rank $P = 0.023$; HR, 0.37; 95% CI, 0.16-0.88); *KRAS*-wt/good versus *KRAS*-wt/poor (log-rank $P = 0.18$; HR, 0.59; 95% CI, 0.27-1.27); *KRAS*-mut/good versus *KRAS*-mut/poor (log-rank $P = 0.40$; HR, 0.69; 95% CI, 0.29-1.62); *KRAS*-mut/good versus *KRAS*-wt/poor (log-rank $P = 0.82$; HR, 1.10; 95% CI, 0.49-2.44); *KRAS*-wt/good ($n = 24$), *KRAS*-wt/poor ($n = 14$), *KRAS*-mut/good ($n = 12$), *KRAS*-mut/poor ($n = 14$); and D, correlation between the expression levels of AREG and EREG in tumors determined by qRT-PCR and the good/poor classification determined by the proteomic profile (n = 60; Mann-Whitney *U* test $P = 0.0053$ and 0.0015, respectively).

Table 3. Comparison of *KRAS* mutation status and MALDI classification for the 64 patients with available data

	Wild-type	Mutant
Good	24	12
Poor	14	14

progression in most of the patients, only documented at that time. This implies that the above effect size estimates (HRs and *P* values) are likely upper bounds (29, 30).

Comparison of the Classification Based on the Blood Proteomic Profile and Other Tumor Biomarkers

The *KRAS* mutation status and EGFR ligand RNA expression levels (AREG and EREG) in 80 tumor samples from this CRC cohort have been previously published, and showed that high expression levels of tumor AREG and EREG were associated with longer PFS whereas *KRAS* mutations were associated with poor response but not with PFS (16). The lack of association between the *KRAS* mutations and PFS in the original study was thought to be due to the small sample size. In our study, both *KRAS* data and the proteomic profile classification were available for 64 of the initial 88 tumor samples. *KRAS* mutation status did not associate with PFS in this subset as expected (Fig. 2B), and *KRAS* mutation status and the MALDI classification were not significantly correlated (Fisher's exact test, $P = 0.21$; Table 3). However, when the tumor *KRAS* mutation status and serum MS–based classification were analyzed together, the *KRAS* status enhanced the discrimination in PFS over MS-based classification alone between *KRAS* mutant/poor and *KRAS* wild-type/good groups (log-rank $P = 0.023$; HR, 0.37; 95% CI, 0.16-0.88, Fig. 2C).

In addition, 60 of the initial 88 tumor samples had the tumor EGFR ligand data and the serum MS–based classification. Consistent with the previous findings, tumors from the good outcome group of patients had higher expression levels of AREG and EREG in our study (Mann-Whitney *U* test: AREG versus MS-based classification, $P = 0.0053$; EREG versus MS-based classification, $P = 0.0015$), providing additional evidence that this group is most likely to benefit from the cetuximab therapy (Fig. 2D). Cox univariate analyses were done including tumor EGFR ligand data and other clinical features. The MS-based classification, tumor EGFR ligand levels, gender, and race were found to be associated with PFS benefit (Supplementary Table S3). As some of these features are correlated with the MS-based classification and to investigate multivariable dependencies, the cohort was stratified according to classification good and poor before a further Cox proportional hazards analysis. In the stratified groups, only gender and race showed any association with PFS benefit (Supplementary Table S4).

Discussion

One limitation of nearly all systemic cancer therapies is that most exhibits clinical activity in only a subset of patients. As the field of targeted therapy evolves, it is becoming apparent that predictive biomarkers are integral to the success of these therapies. The successful development of any drug should be linked to predictors of its efficacy, as these markers would considerably increase the likelihood that an individual patient will benefit. Given the morbidity and economic burden of treating cancer patients with expensive and ineffective agents, it is imperative that endeavors to identify biomarkers predictive of treatment benefit are undertaken.

Modest response and disease stabilization rates are observed when EGFRIs are administered as monotherapy in unselected HNSCC, NSCLC, and CRC patients, and a phase III trial comparing gefitinib to cytotoxic chemotherapy did not show a difference in OS (1, 25, 31). Nevertheless, there is compelling evidence that some patients significantly benefit from these agents, even when objective responses are not observed. This study found that a proteomic profile-based classification can be used to predict which patients, with a variety of malignancies, will benefit when treated with either EGFR-TKIs or cetuximab. By using survival as the end point and by testing the classification based on the proteomic profile in a control cohort of patients never treated with EGFRIs, it seems that this biomarker is associated with survival benefits from EGFRIs, which is of paramount importance to patients. Furthermore, the fact that the same signature was predictive in three different cancers and two different classes of EGFR pathway inhibitors suggests that this proteomic profile might detect tumor EGFR signal dependence and thus be useful in other populations in which EGFRI therapy might be appropriate, including almost all epithelial derived malignancies.

MALDI MS serum profiling has garnered criticism for being difficult to understand on a molecular basis. It seems that the information within the proteomic pattern is generated by specific, stable, and reproducible protein cleavages in the serum or plasma (32–35). This is underscored by the ability to analyze at multiple facilities either serum or plasma, from routinely processed, multisite collected samples with equivalent results. We are undertaking a study to identify the nature of peptides constituting the MS signature. Notwithstanding the precise mechanism underlying these observations, the aim of this study was to evaluate a standardized and commercialized MS profile classification (VeriStrat) in association with PFS and OS in HNSCC and CRC patients treated with EGFRIs. We specifically chose OS and/or PFS benefits, not response to treatment, as end points of our classifier and its application to patients in this study. There are numerous studies that show a correlation of a biomarker with response, but not with survival. However, in case of advanced diseases, survival prolongation is a more important indicator of a benefit for the patient, and is the

strength of this study. In our cohorts, the response rates were very low; however, we showed that good patients have a statistically significant lack of clinical progression and survival benefit over poor patients, even in the absence of clinical response. If we examine all of the patients that we have studied with NSCLC, we find that matched NSCLC patients classified as poor actually had a significantly worse survival when treated with EGFR TKIs than with chemotherapy.[9] Thus, potential negative effects of EGFRIs in a subset of patients make pretreatment patient selection for these therapies especially important.

There are several advantages to the serum MALDI MS profiling described here, especially compared with more labor intensive and technically challenging assays, such as immunohistochemistry, fluorescence *in situ* hybridization, and PCR, due to the unavailability of high-quality tumor specimens and intratumoral heterogeneity (9, 36). For recurrent/metastatic patients in community-based clinical settings, obtaining an adequate amount of tumor tissue for analysis can be challenging, especially because the tumor's characteristics may have changed after the initial diagnosis and staging and multiple lines of treatments. Getting a new biopsy immediately before a therapeutic decision point may be impractical and/or delay treatment. Recent data about the presence of *KRAS* mutations and treatment resistance to EGFRIs in NSCLC and CRC have been validated in several studies (15–17). However, the latest large randomized studies provided conflicting evidence on the role of these genetic biomarkers (13, 14). Besides, the frequency of mutations varies depending on the organ of origin: they are 3.5% in HNSCC and ~12% in NSCLC (15, 23, 37). Thus, there is no reliable way to select the majority of patients for therapy, and the burden and morbidity of inadequate treatment are very high. Our data suggest that MALDI MS profiling may provide an independent predictive marker that is reproducible, uses easily obtainable small volumes of pretreatment sera or plasma, does not require elaborate sample processing, and can provide results within days with an assay failure rate of only 2%. In the case when there are other biomarker measurements, proteomic tests can provide valuable additional information. Combining *KRAS* mutation status and this simple blood test may improve the stratification of patients if adequate tumor material is available for *KRAS* testing. The correlation found between the RNA expression levels of AREG and EREG in tumors and our serum-MS classification suggests that this proteomic profiling test may serve as a surrogate to identify high-AREG– and EREG-expressing CRC tumors.

Although most cancer therapies have been studied independently in each tumor type after phase I trials, highly specific targeted therapies should, in theory, work in any tumor that is driven by that pathway. There are few, if any, clinically relevant "pathway-specific" biomarkers available for the selection of therapy independent of tumor type. In this study, we propose that this biomarker, defined in lung cancer patients, seems to identify patients who will benefit from EGFRI therapy in two additional major tumor types treated with inhibitors of the EGFR pathway, regardless of the class of the inhibitor. Although the data generated in this study require further validation in prospective studies, we have shown that the classification of patients based on the standardized MALDI MS–based proteomic analysis VeriStrat predicts survival benefit in HNSCC, NSCLC, and CRC patients treated with both EGFR-TKIs or cetuximab, and the patients predicted to have poor outcome are unlikely to benefit from these treatments at the current doses and schedules administered. This predictive biomarker could be tremendously valuable given the financial and societal costs of treating unselected patients with EGFRIs.

Disclosure of Potential Conflicts of Interest

C.H. Chung received honoraria from Bristol-Myers Squibb (BMS) and Amgen for educational lectures and served on an advisory board, and received a research funding from Astra-Zeneca (AZ) and Lilly Oncology. H. Roder, J. Grigorieva, M. Tsypin, and J. Roder are full-time employees of Biodesix, Inc. B. Burtness received honoraria from BMS, Boehringer Ingelheim, Array Pharmaceuticals, and Pfizer for the participation in advisory boards. A. Argiris received research funding from BMS and AZ. E.W. Cohen serves in speakers' bureaus for BMS and Imclone.

Acknowledgments

We thank Lisa Lipp, Michelle Pham, Katherine Nichols, and Rosalyn Williams for the assistance in data management; Matthew Levine and Leslie Martin for sample handling; and the University of Chicago Head and Neck Cancer Tissue Core (Principal Investigator, Dr. Mark Lingen), the Vanderbilt University Head and Neck Cancer Tissue Bank (Principal Investigator-Dr. Wendell G. Yarbrough), and the Vanderbilt University Head and Neck Cancer Team for contributing to sample collection (Drs. James Netterville, Robert Sinard, Brian Burkey, Wendell G. Yarbrough, and Anthony Cmelak).

Grant Support

Damon Runyon Clinical Investigator Award (CL-28-05), the Robert J. and Helen C. Kleberg Foundation, and NIH grant R01-DE-017982 (C.H. Chung); the Barry Baker Laboratory for Head and Neck Oncology; and the Vanderbilt-Ingram Cancer Center Core Support Grant NIH/National Cancer Institute CA068485, and the Department of Defense grant W81XWH-05-1-0179 (R.M. Caprioli). This study in part was conducted by the Eastern Cooperative Oncology Group (Robert L. Comis, M.D., Chair) and supported in part by the Public Health Service grants CA23318, CA66636, CA21115, CA49957, CA39229, CA17145, CA27525, CA16116, and from the National Cancer Institute, NIH and the Department of Health and Human Services. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Cancer Institute. In addition, this study in part was funded by University of Chicago Cancer Research Center, University of Chicago Phase 2 Consortium (N01-CM-17102), Bristol-Myers Squibb, Astra-Zeneca, and American Society of Clinical Oncology (E.E. Cohen).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received 9/11/09; revised 11/18/09; accepted 12/8/09; published OnlineFirst 1/19/10.

[9] Gregorc, V, "Prospective studies with proteomics." Oral Presentation. 13th World Conference on Lung Cancer, San Francisco 2009.

References

1. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, et al. Erlotinib in previously treated non-small-cell lung cancer. N Engl J Med 2005;353:123–32.
2. Jonker DJ, O'Callaghan CJ, Karapetis CS, et al. Cetuximab for the treatment of colorectal cancer. N Engl J Med 2007;357:2040–8.
3. Cohen EE. Role of epidermal growth factor receptor pathway-targeted therapy in patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck. J Clin Oncol 2006;24:2659–65.
4. Chung KY, Shia J, Kemeny NE, et al. Cetuximab shows activity in colorectal cancer patients with tumors that do not express the epidermal growth factor receptor by immunohistochemistry. J Clin Oncol 2005;23:1803–10.
5. Van Cutsem E, Peeters M, Siena S, et al. Open-label phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy-refractory metastatic colorectal cancer. J Clin Oncol 2007;25:1658–64.
6. Tsao MS, Sakurada A, Cutz JC, et al. Erlotinib in lung cancer—molecular and clinical predictors of outcome. N Engl J Med 2005;353:133–44.
7. Vermorken JB, Mesia R, Rivera F, et al. Platinum-based chemotherapy plus cetuximab in head and neck cancer. N Engl J Med 2008;359:1116–27.
8. Cunningham D, Humblet Y, Siena S, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med 2004;351:337–45.
9. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004;304:1497–500.
10. Pao W, Miller V, Zakowski M, et al. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci U S A 2004;101:13306–11.
11. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004;350:2129–39.
12. Clark GM, Zborowski DM, Culbertson JL, et al. Clinical utility of epidermal growth factor receptor expression for selecting patients with advanced non-small cell lung cancer for treatment with erlotinib. J Thorac Oncol 2006;1:837–46.
13. Kim ES, Hirsh V, Mok T, et al. Gefitinib versus docetaxel in previously treated non-small-cell lung cancer (INTEREST): a randomised phase III trial. Lancet 2008;372:1809–18.
14. Crino L, Cappuzzo F, Zatloukal P, et al. Gefitinib versus vinorelbine in chemotherapy-naive elderly patients with advanced non-small-cell lung cancer (INVITE): a randomized, phase II study. J Clin Oncol 2008;26:4253–60.
15. Pao W, Wang TY, Riely GJ, et al. *KRAS* mutations and primary resistance of lung adenocarcinomas to gefitinib or erlotinib. PLoS Med 2005;2:e17.
16. Khambata-Ford S, Garrett CR, Meropol NJ, et al. Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab. J Clin Oncol 2007;25:3230–7.
17. Karapetis CS, Khambata-Ford S, Jonker DJ, et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N Engl J Med 2008;359:1757–65.
18. Amado RG, Wolf M, Peeters M, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol 2008;26:1626–34.
19. Sartore-Bianchi A, Moroni M, Veronese S, et al. Epidermal growth factor receptor gene copy number and clinical outcome of metastatic colorectal cancer treated with panitumumab. J Clin Oncol 2007;25:3238–45.
20. Van Cutsem E, Kohne CH, Hitre E, et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med 2009;360:1408–17.
21. Chung CH, Ely K, McGavran L, et al. Increased epidermal growth factor receptor gene copy number is associated with poor prognosis in head and neck squamous cell carcinomas. J Clin Oncol 2006;24:4170–6.
22. Cohen EE, Lingen MW, Martin LE, et al. Response of some head and neck cancers to epidermal growth factor receptor tyrosine kinase inhibitors may be linked to mutation of ERBB2 rather than EGFR. Clin Cancer Res 2005;11:8105–8.
23. Yarbrough WG, Shores C, Witsell DL, Weissler MC, Fidler ME, Gilmer TM. ras mutations and expression in head and neck squamous cell carcinomas. Laryngoscope 1994;104:1337–47.
24. Taguchi F, Solomon B, Gregorc V, et al. Mass spectrometry to classify non-small-cell lung cancer patients for clinical outcome after treatment with epidermal growth factor receptor tyrosine kinase inhibitors: a multicohort cross-institutional study. J Natl Cancer Inst 2007;99:838–46.
25. Cohen EE, Kane MA, List MA, et al. Phase II trial of gefitinib 250 mg daily in patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck. Clin Cancer Res 2005;11:8418–24.
26. Seiwert T, Davis D, Yan D, et al. pKDR/KDR ratio predicts response in a phase I/II pharmacodynamic study of erlotinib and bevacizumab for recurrent or metastatic head and neck cancer (HNC). Proc Am Soc Clin Oncol 2007;25:6021.
27. http://www.jmp.com.
28. http://www.graphpad.com.
29. Panageas KS, Ben-Porat L, Dickler MN, Chapman PB, Schrag D. When you look matters: the effect of assessment schedule on progression-free survival. J Natl Cancer Inst 2007;99:428–32.
30. Carroll KJ. Analysis of progression-free survival in oncology trials: some common statistical issues. Pharm Stat 2007;6:99–113.
31. Niho S, Ichinose Y, Tamura T, et al. Results of a randomized phase III study to compare the overall survival of gefitinib (IRESSA) versus docetaxel in Japanese patients with non-small-cell lung cancer who failed one or two chemotherapy regimens. Proc Am Soc Clin Oncol 2007;25:LBA7509.
32. Sidransky D, Irizarry R, Califano JA, et al. Serum protein MALDI profiling to distinguish upper aerodigestive tract cancer patients from control subjects. J Natl Cancer Inst 2003;95:1711–7.
33. Yanagisawa K, Shyr Y, Xu BJ, et al. Proteomic patterns of tumor subsets in non-small-cell lung cancer. Lancet 2003;362:433–9.
34. Rahman SM, Shyr Y, Yildiz PB, et al. Proteomic patterns of preinvasive bronchial lesions. Am J Respir Crit Care Med 2005;172:1556–62.
35. Yildiz PB, Shyr Y, Rahman JS, et al. Diagnostic accuracy of MALDI mass spectrometric analysis of unfractionated serum in lung cancer. J Thorac Oncol 2007;2:893–901.
36. Hirsch FR, Varella-Garcia M, Bunn PA, Jr., et al. Epidermal growth factor receptor in non-small-cell lung carcinomas: correlation between gene copy number and protein expression and impact on prognosis. J Clin Oncol 2003;21:3798–807.
37. Bissada E, Abou-Chacra Z, Weng X, et al. Prevalence of K-RAS codon 12 mutations in locally advanced head and neck squamous cell carcinoma and influence with regards to response to chemoradiation therapy. J Clin Oncol 2008;26:abstr 17005.