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ORIGINAL REPORT

Feasibility and Efficacy of Molecular Analysis-Directed Individualized Therapy in Advanced Non–Small-Cell Lung Cancer

George Simon, Anupama Sharma, Xueli Li, Todd Hazelton, Frank Walsh, Charles Williams, Alberto Chiappori, Eric Haura, Tawee Tanvetyanon, Scott Antonia, Alan Cantor, and Gerold Bepler

From the Program and Division of Thoracic Oncology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL.

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Terms in blue are defined in the glossary, found at the end of this article and online at www.jco.org.

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Address reprint requests to Gerold Bepler, MD, PhD, H. Lee Moffitt Cancer Center and Research Institute, MRC-4W, Room 4046, 12902 Magnolia Dr, Tampa, FL 33612-9497; e-mail: beplerg@moffitt.usf.edu.

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ABSTRACT

Purpose

The treatment of patients with advanced non–small-cell lung cancer (NSCLC) is based on clinical trials experience. Molecular characteristics that impact metabolism and efficacy of chemotherapeutic agents are not used for decision making. Ribonucleotide reductase subunit 1 (RRM1) is crucial for nucleotide metabolism, and it is the dominant molecular determinant of gemcitabine efficacy. Excision repair cross-complementing group 1 gene *(ERCC1)*, a component of the nucleotide excision repair complex, is important for platinum-induced DNA adduct repair. We hypothesized that selection of double-agent chemotherapy based on tumoral *RRM1* and *ERCC1* expression would be feasible and beneficial for patients with advanced NSCLC.

Patients and Methods

We conducted a prospective phase II clinical trial in patients with advanced NSCLC. Patients were required to have a dedicated tumor biopsy for determination of *RRM1* and *ERCC1* gene expression by real-time quantitative reverse transcriptase polymerase chain reaction. Double-agent chemotherapy consisting of carboplatin, gemcitabine, docetaxel, and vinorelbine was selected based on gene expression. Disease response and patient survival were monitored.

Results

Eighty-five patients were registered, 75 had the required biopsy without significant complications, 60 fulfilled all eligibility criteria, and gene expression analysis was not feasible in five patients. *RRM1* expression ranged from 0 to 1,637, *ERCC1* expression ranged from 1 to 8,103, and their expression was correlated (Spearman's *rho* = 0.46; $P < .01$). Disease response was 44%. Overall survival was 59% and progression-free survival was 14% at 12 months, with a median of 13.3 and 6.6 months, respectively.

Conclusion

Therapeutic decision making based on *RRM1* and *ERCC1* gene expression for patients with advanced NSCLC is feasible and promising for improvement in patient outcome.

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INTRODUCTION

Double-agent chemotherapy is the standard of care for first-line treatment of patients with advanced non–small-cell lung cancer (NSCLC). It produces response rates of approximately 20%, a median overall survival (OS) of 8 months, and a 1-year survival rate of 33%.[1] No single platinum-based doublet regimen has emerged as the best choice in terms of efficacy.[1-5] More recent trials have demonstrated that nonplatinum-containing doublet regimens result in survival rates equal to those of platinum-containing regimens.[6-10] As a result, therapeutic decisions on chemotherapy for this group of patients are based on the oncologist's personal preference and familiarity, convenience of delivery, and regimen-specific toxicity.

Recent pharmacogenomic research has produced promising results in linking tumor-specific molecular characteristics with response to the epidermal growth factor receptor inhibitors gefitinib and erlotinib.[11-13] Likewise, we and others have described a strong association between the genes ribonucleotide reductase subunit 1 (*RRM1*) and excision repair cross-complementing (*ERCC1*) and therapeutic benefit from gemcitabine and platinum.[14-18] Both genes are critical components of the DNA synthesis and DNA damage repair pathways.[19,20] This article provides results from a prospective, single-institution phase II clinical trial

that utilized tumoral expression of the genes *RRM1* and *ERCC1* for selection of double-agent chemotherapy.

PATIENTS AND METHODS

Clinical Trial Design

A single-institution phase II trial was designed to prospectively assess the feasibility and efficacy of selecting double-agent chemotherapy based on tumoral *RRM1* and *ERCC1* expression in previously untreated patients with advanced NSCLC (ClinicalTrials.gov identifier: NCT00215930). Double-agent chemotherapy was chosen because single-agent therapy was deemed inadequate for previously untreated patients with advanced-stage NSCLC and a good performance status (National Comprehensive Cancer Network guidelines for NSCLC treatment, June 2006; www.nccn.org). Trial participation required a dedicated biopsy of the tumor specifically for gene expression analysis, which was performed by real-time quantitative reverse transcriptase polymerase chain reaction. Predetermined values for *RRM1* and *ERCC1* were used for decisions regarding use of the drugs gemcitabine and carboplatin. If *RRM1* was equal to or less than the value of 16.5, gemcitabine was used in the treatment doublet. If *ERCC1* was equal to or less than the value of 8.7, carboplatin was used in the treatment doublet (Fig 1). These levels were selected based on our experience and published reports with expression analysis in patients with NSCLC.[14,15,21,22] This strategy resulted in four possible gene expression strata with the following doublet therapies: The low *RRM1* and low *ERCC1* group (gemcitabine and carboplatin [GC] group) was treated with gemcitabine (1,250 mg/m^2 on days 1 and 8) and carboplatin (area under the concentration-time curve [AUC] of 5 on day 1) every 21 days. The low *RRM1* and high *ERCC1* group (gemcitabine and docetaxel [GD] group) was treated with gemcitabine (1,250 mg/m^2 on days 1 and 8) and docetaxel (40 mg/m^2 on days 1 and 8) every 21 days. The high *RRM1* and low *ERCC1* group (docetaxel and carboplatin [DC] group) was treated with docetaxel (75 mg/m^2 on day 1) and carboplatin (AUC 5 on day 1) every 21 days. The high *RRM1* and high *ERCC1* group (docetaxel and vinorelbine [DV] group) was treated with vinorelbine (45 mg/m^2 on days 1 and 15) and docetaxel (60 mg/m^2 on days 1 and 15) every 28 days. Disease response was sequentially assessed after every two cycles by computed tomography (CT) of the chest, upper abdomen, and other areas as indicated. Other imaging modalities used for disease response assessment included magnetic resonance imaging of the brain and soft tissues. Patients without disease progression were continued on therapy for at least four cycles. Subsequent clinical management was at the discretion of the treating physician. The primary end point was best disease response after a maximum of six cycles. Secondary end points were OS and progression-free survival (PFS). An interim analysis after the first 25 patients was planned with

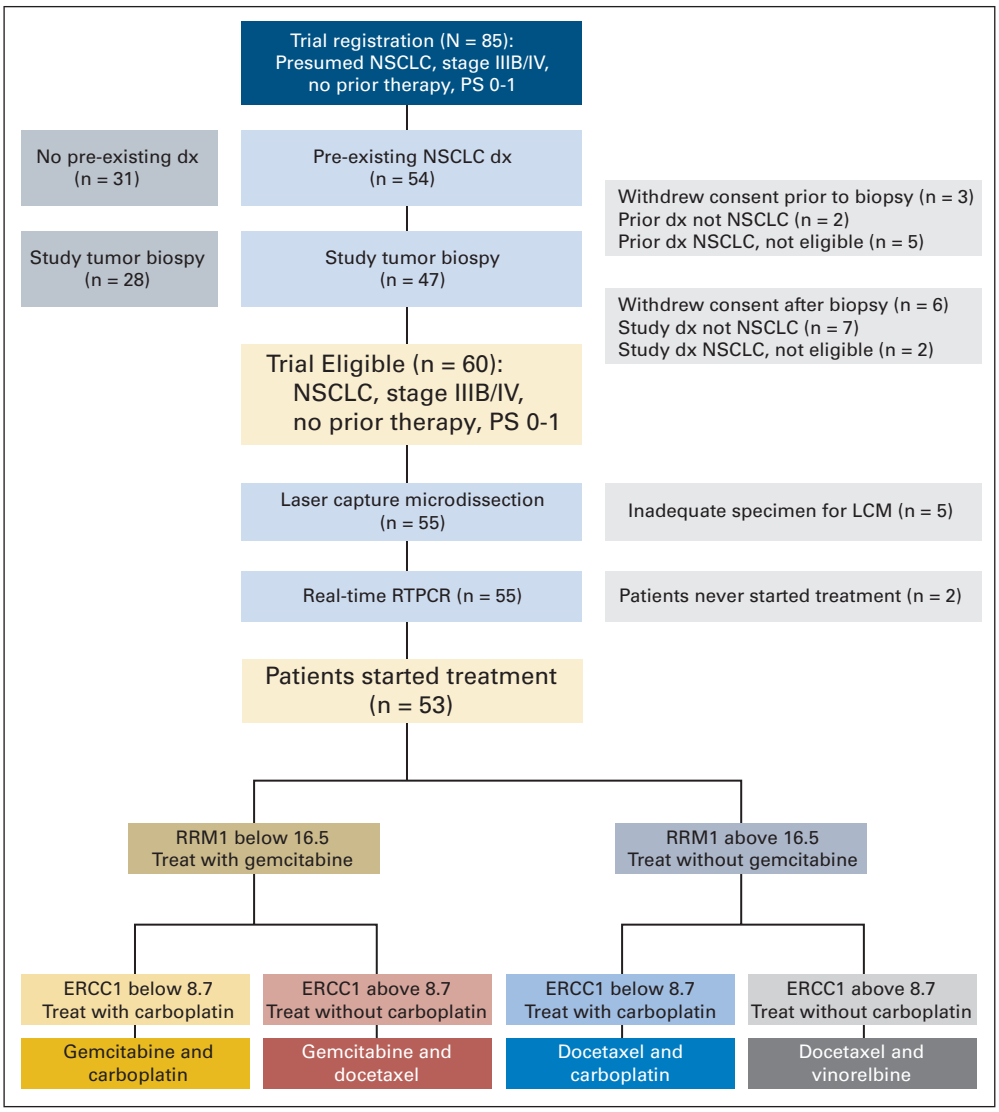

Fig 1. Flow chart and treatment algorithm used for selection of double-agent chemotherapy based on tumoral ribonucleotide reductase subunit 1 (*RRM1*) and excision repair cross-complementing group 1 gene (*ERCC1*) expression. NSCLC, non–small-cell lung cancer; PS, performance status; dx, diagonsis; LCM, laser capture microdissection; RTPCR, reverse transcription polymerase chain reaction.

the goal to terminate the study if no more than eight patients had an objective response to therapy. After completion of the study-selected chemotherapy, patients were observed at least every 3 months with CT and magnetic resonance imaging scans, if indicated, for determination of disease status. The trial was approved by the institutional review board, and all subjects provided written informed consent.

Eligibility

Trial eligibility required pathologically confirmed NSCLC; stage IV or wet IIIB disease (patients with cytologically positive pleural effusion); measurable or assessable disease by Response Evaluation Criteria in Solid Tumors Group (RECIST)[23]; no prior systemic therapy with cytotoxic, molecularly targeted, or immunologic agents; performance status 0 or 1 by Eastern Cooperative Oncology Group criteria; age 18 years or older; and adequate bone marrow, liver, and kidney function. Patients with prior surgery or radiation for lung cancer were eligible provided they had at least one measurable target lesion outside of the field of prior therapy. Patients with CNS metastases were eligible if no immediate intervention was required or if they had completed radiation more than 28 days before the planned chemotherapy. Patients with prior malignancies were eligible if there was no evidence for recurrence for at least 3 years. Because the trial required a dedicated tumor biopsy for gene expression analysis, we allowed registration (ie, informed consenting) on the trial before full eligibility was established to avoid a potential second biopsy in patients that did not yet have a confirmed diagnosis.

Disease Response, Survival, and Toxicity Assessment

For disease response determination, at least one and up to eight separate cancer lesions were measured in greatest diameter using images obtained with intravenous contrast on a multichannel helical CT scanner. Measurements were performed on a picture archive communication system workstation (Siemens MagicView 1000; Malvern, PA), and they were repeated at 6 to 8 weekly intervals. The percentage of change of the sum of tumor diameters comparing the post-treatment with the pretreatment measurements was calculated using the formula 1−(SumCTpost/SumCTpre). A positive value indicated tumor shrinkage, and a negative value indicated tumor growth. The appearance of a new and previously unobserved tumor lesion on imaging studies or physical exam was coded as disease progression. Disease response was categorized as progressive disease (PD), stable disease (SD), partial remission (PR), and complete remission according to RECIST. OS was recorded as the time elapsed from the date of first treatment to the date of death. PFS was recorded as the time elapsed from the date of first treatment to the date of first evidence for disease progression or death. Patients without an event were censored at the last date of follow-up (December 27, 2006). Toxicity was recorded according to the Common Terminology Criteria for Adverse Events version 3 (http://ctep.cancer.gov).

Specimen Collection, Processing, and Gene Expression Analysis

The study required collection of fresh-frozen tumor specimens before therapy. This was performed by core needle biopsy that produced a tissue specimen of 0.8 mm in diameter (20-gauge needle). The biopsy was performed percutaneously or endoscopically under radiographic or sonographic guidance. Specimens were immediately frozen in liquid nitrogen. They were embedded in optimal cutting temperature medium (OCT) and cut in 5 to 7 μm sections. Tumor cells were collected by laser capture microdissection using the Arcturus (Mountain View, CA) system (60 mW, 1.5 milliseconds, intensity 100, spot size approximately 20 μm), and total RNA was extracted using a commercially available method (PicoPure RNA Isolation Kit KIT0204; Arcturus). Complementary DNA was generated with Superscript II (InVitrogen, Carlsbad, CA). Real-time quantitative polymerase chain reaction gene analysis was performed in triplicate per sample and gene in 96-well plates (ABI prism 7700; Perkin-Elmer, Foster City, CA). Each plate contained a serial dilution of reference cDNA for standard curve determination and negative controls without template. We had designed and validated the primers and probes used for *RRM1* and *ERCC1* expression analysis as previously reported.[21,22] Commercially available primers and probes were used for expression analysis of the housekeeping gene 18S*rRNA* (Perkin-Elmer; #4310893E-0203015), which was used as internal reference standard. The relative amount of target RNA in a sample was determined by comparing the threshold cycle with the standard curve, and the standardized amount was then determined by dividing the target amount by the 18S*rRNA* amount.

Statistical Methods

Correlation coefficients between gene expression and the continuous variables tumor response and patient's age were calculated according to Spearman. Cox proportional hazards analysis was used to assess the impact of gene expression on survival. The *t* test was used to test for significance between gene expression and sex or other dichotomous patient variables. The one-way analysis of variance test was used to test for significance between gene expression and patients' smoking status or other noncontinuous patient variables with more than 2 values. OS and PFS probabilities were estimated using the Kaplan-Meier method. For statistical purposes, it is important to note that this trial was not designed to compare outcomes among patients assigned to the different chemotherapies, but rather that molecular analysis directed individualized chemotherapy assignment is feasible and yields promising results in outcomes.

RESULTS

Feasibility

From February 2004 to December 2005, 85 patients were registered to the trial. Sixteen were ineligible after completion of the required tests, and nine withdrew consent because they desired treatment closer to home (Fig 1).

Seventy-five patients underwent the required biopsy—48 had CT-guided lung biopsies, seven had bronchoscopy-guided lung biopsies, and 20 had biopsies from organs other than lung. A complication was noted in one instance of a CT-guided lung biopsy, which resulted in a small pneumothorax that spontaneously resolved.

A gene expression analysis could not be performed in five of the 60 eligible patients because the samples consisted of necrosis and inflammatory cells. Thus, gene expression analysis was successful in 92% (55 of 60) of patients. The time elapsed from tumor biopsy to gene analysis was 14 or fewer days, except for two patients, who required extensive work-up and palliative intervention before chemotherapy. Two patients never received the assigned treatment because of natural disasters in Florida during the summer 2004 (Fig 1 and Table 1).

Efficacy

In the 53 patients that were treated with the assigned therapy, *RRM1* expression ranged from 0.0 to 1,637.3 (median, 12.1; mean, 71.6), and *ERCC1* expression ranged from 0.9 to 8,102.8 (median, 12.4; mean 186.3). The expression of both genes was significantly correlated (Spearman's *rho* = 0.458; $P < .01$). Twelve patients received GC, 20 GD, seven DC, and 14 DV as therapeutic regimens. At a planned interim analysis, 11 (44%) of the first 25 patients had achieved a PR, and the study was continued.

The total number of treatment cycles was one in two patients, two in eight patients, three in four patients, four in 21 patients, five in two patients, and six in 16 patients. Treatment response was not assessed in one of the two patients that received only one cycle.

The best treatment response was PR in 23 patients (44%; 95% CI, 31% to 59%), SD in 23 patients (44%; 95% CI ,31% to 59%) and progressive disease in six patients (12%; 95% CI, 4% to 23%; four developed new metastases, and two had > 20% increase in tumor diameters). Thus, the disease control rate (PR/SD) was 88.5% (95% CI, 76.6% to 95.6%). In the 23 patients with PR, the tumor reduction ranged from 30% to 77%, and the best response was observed after

Table 1. *RRM1* and *ERCC1* Expression and Patients' Characteristics

Characteristic	No.	%	95% CI
Patients registered	85		
Patients eligible	60		
Patients with successful gene analysis	55		
Patients treated	53		
Tumor cells used for gene analysis	180-3,000		
RRM1/18SrRNA expression			
Median	12.1		
Range	0.0-1,637.3		
ERCC1/18SrRNA			
Median	12.4		
Range	0.9-8102.8		
Disease response			
CR	0	0	
PR	23	44	
SD	23	44	
PD	6	12	
Not assessable	1		
Overall survival, months			
6		83	70 to 91
12		59	42 to 72
18		42	25 to 57
24		37	21 to 54
Median	13.3		11.5 to ∞ < 24
Progression-free survival, months			
6		56	42 to 68
12		14	5 to 27
18		14	5 to 27
24		7	1 to 19
Median	6.6		4.7 to 8.8
Tumor histology			
Adenocarcinoma	33	62	
Squamous cell carcinoma	2	4	
Large-cell or unspecified NSCLC	18	34	
Tumor stage			
IIIB, malignant pleural effusion	1	2	
IV	52	98	
Sex			
Male	31		
Female	22		
Age, years			
Median	63		
Range	38-78		
Smoking status			
Lifetime never-smoker	6	11	
Quit > 1 year	35	66	
Active	12	23	
ECOG performance status			
0	25	47	
1	28	53	
Weight loss ≥ 5% in 3 months			
Absent	49	92	
Present	4	8	

Abbreviations: RRM1, ribonucleotide reductase subunit 1; ERCC1, excision repair cross-complementing group 1 gene; CR, complete remission; PR, partial remission; SD, stable disease; PD, progressive disease; NSCLC, non–small-cell lung cancer; ECOG, Eastern Cooperative Oncology Group.

two cycles in four patients, after four cycles in nine patients, and after six cycles in 10 patients.

The 12-month OS rate was 59%, and the 12-month PFS rate was 14% (Fig 2 and Table 1). The median OS was 13.3 months (95% CI, 11.5 months to < 24 and the median PFS was 6.6 months (95% CI, 4.7 to 8.8 months). A total of 31 patients have died—25 from progressive disease and six from other causes. Of the 22 patients alive, six have not progressed (8.2 to 29.8 months).

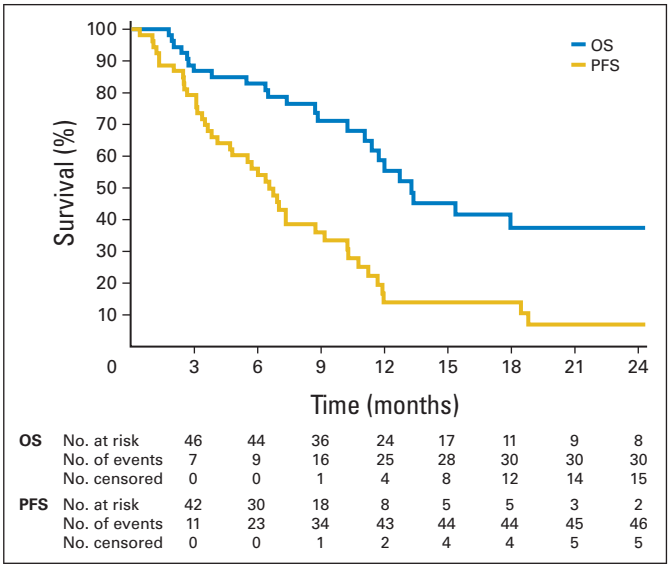

Fig 2. Overall survival (OS) and progression-free survival (PFS) of 53 patients with advanced non–small-cell lung cancer treated with chemotherapy based on expression of the genes ribonucleotide reductase subunit 1 (*RRM1*) and excision repair cross-complementing group 1 gene (*ERCC1*).

As expected from the study design, there was no significant correlation between gene expression and response to therapy—*RRM1*: Spearman's *rho* = 0.15, *P* = .28; and *ERCC1*: Spearman's *rho* = −0.14, *P* = .33. In addition, there was no significant correlation between gene expression and survival—*RRM1* and OS: HR = 0.996, *P* = .24; *RRM1* and PFS: HR = 0.998, *P* = .36; *ERCC1* and OS: HR = 1.00, *P* = .19; and *ERCC1* and PFS: HR = 1.00, *P* = .23. Patients assigned to the four different therapeutic regimens appeared to have indistinguishable outcomes—OS: *P* = .98; PFS: *P* = .58; Figure 3.

Toxicity

No symptomatic toxicities or complications requiring intervention were observed as a result of the required tumor biopsy. One patient died of intracranial hemorrhage from treatment-related thrombocytopenia, and one patient died from possibly treatment-related gastrointestinal toxicity. No symptomatic grade 4 toxicities

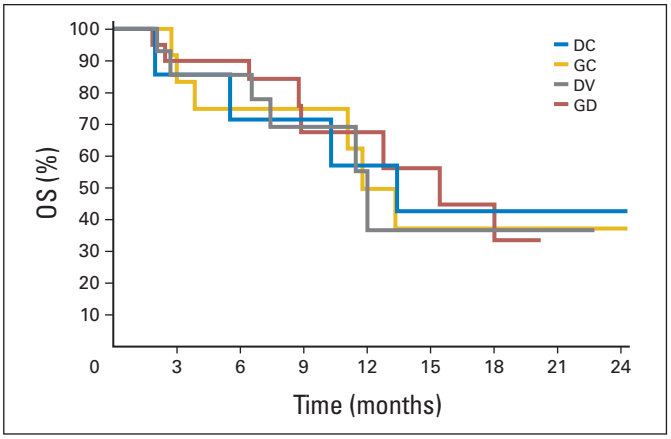

Fig 3. Overall survival (OS) by assigned chemotherapy. DC, docetaxel and carboplatin; GC, gemcitabine and carboplatin; DV, docetaxel and vinorelbine; GD, gemcitabine and docetaxel.

were noted. Symptomatic toxicities of grade 3 severity included fatigue (four patients), pain (one patient), nausea and vomiting (one patient), hand-foot skin reaction (two patients), nail changes (one patient), tearing (one patient), motor and sensory neuropathy (one patient), hypersensitivity reaction (one patient), vasovagal episode (one patient), deep vein thrombosis (one patient), and lower extremity edema (one patient).

DISCUSSION

To test whether selection of chemotherapy based on gene expression is feasible and would improve patient survival, we conducted a phase II single-institution treatment trial in patients with advanced and incurable NSCLC. In this study, the decision on a double-agent chemotherapy regimen was based on the expression of the genes *RRM1* and *ERCC1*. A core needle biopsy was required for study participation. Specimens were immediately frozen, sectioned, and subjected to laser capture microdissection for tumor cell collection and mRNA expression analysis of *RRM1* and *ERCC1* (Fig 1).

RRM1 is the only known mammalian gene that regulates substrate specificity and activity of ribonucleotide reductase subunit 1, which catalyzes deoxynucleotide production.[19,24] It is the major cellular determinant of gemcitabine (2′,2′-difluorodeoxycytidine) efficacy.[16-18] ERCC1 is a component of the nucleotide excision repair pathway, which is responsible for repair of platinum-induced DNA adducts.[25,26] High levels of tumoral expression of these genes had been associated with poor survival in NSCLC patients treated with gemcitabine/platinum-based chemotherapy in retrospective analyses.[14,15] We had conducted a prospective analysis and found that the level of *RRM1* expression and, to a lesser degree, the level of *ERCC1* expression were inversely correlated with tumor response to gemcitabine and carboplatin in patients with NSCLC.[18] In the prospective trial reported here, we used the tumoral expression of these genes to choose double-agent chemotherapy regimens that did or did not contain these agents for first-line therapy of patients with advanced NSCLC.

We used this knowledge for selection of chemotherapy. Our data suggest that treatment of patients with advanced NSCLC based on the intratumoral expression of *RRM1* and *ERCC1* results in promising patient outcome with a response rate of 44%, a 1-year survival of 59%, and a median OS time of 13.3 months. These results compare favorably with our own prior experience with phase II trials in similar patient populations.[27]

Participants in the study were required to undergo collection of a histologic tumor specimen under controlled conditions for determination of gene expression. Although the trial was not designed to assess patients' acceptance of this additional invasive procedure, we did not observe reluctance to participate. In fact, 54 of the 85 patients registered on the trial had a pre-existing pathological diagnosis of lung cancer. The additional biopsy did not result in symptomatic toxicity, gene expression analysis led to successful treatment assignment in more than 90% of patients, and it may be responsible for a 1-week delay in systemic treatment initiation. The high rate (29%) of patients that were study ineligible (n = 16) or withdrew their consent (n = 9) before treatment initiation was unexpected. It is unlikely that the required tumor biopsy was a significant factor for this attrition rate, since only three patients withdrew their consent before the biopsy (two of these did not have a diagnosis), and six patients withdrew consent after the biopsy (one of these did have a prior diagnosis). However, we did allow trial registration without a definitive or confirmed diagnosis of NSCLC to avoid a second biopsy if possible, and NSCLC was not the final diagnosis in 11 (13%) of the 85 patients (Fig 1). Seven (8%) patients with NSCLC withdrew consent because they desired therapy closer to home, and seven (8%) were ineligible for various reasons (prior therapy in three, laboratory values outside of the eligibility range in two, psychosocial reasons in one, and tumor lesion inaccessible without a major surgical procedure in one). We thus conclude that a dedicated tumor biopsy with gene expression analysis for treatment assignment is technically feasible and acceptable to patients.

Several issues should be addressed before a general recommendation for implementation of our gene expression–based therapeutic approach can be given. First, it is important to verify our results in a large multi-institutional trial. Second, the general feasibility of performing core needle biopsies of patients' tumors with immediate freezing, laser capture microdissection, and subsequent sophisticated gene expression analysis appears limited given the required infrastructure. Thus, the development of a more generally applicable methodology based on technology familiar to clinical laboratories and pathologists, such as immunochemistry, is desirable.[28-30] Third, it is likely that other molecular characteristics of tumor cells and the host significantly impact the RRM1- and ERCC1-affected treatment response and patient outcome. Therefore, it is important to further elucidate specific molecular features and pathways that modulate therapeutic efficacy to these agents used for lung cancer therapy.

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: George Simon, Gerold Bepler
Financial support: George Simon
Administrative support: Gerold Bepler
Provision of study materials or patients: George Simon, Anupama Sharma, Xueli Li, Todd Hazelton, Frank Walsh, Charles Williams, Alberto Chiappori, Eric Haura, Tawee Tanvetyanon, Scott Antonia, Gerold Bepler
Collection and assembly of data: George Simon, Gerold Bepler
Data analysis and interpretation: George Simon, Alan Cantor, Gerold Bepler
Manuscript writing: George Simon, Gerold Bepler
Final approval of manuscript: George Simon, Anupama Sharma, Xueli Li, Todd Hazelton, Frank Walsh, Charles Williams, Alberto Chiappori, Eric Haura, Tawee Tanvetyanon, Scott Antonia, Alan Cantor, Gerold Bepler

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GLOSSARY

ERCC1 (excision repair cross-complementing group 1 gene): Encodes a nucleotide excision repair protein that repairs a range of lesions, including UV-induced thymine dimers and other photoproducts, and also lesions caused by a variety of chemical agents.

RECIST (Response Evaluation Criteria in Solid Tumors): The Response Evaluation Criteria Group proposed a model by which a combined assessment of all existing lesions, characterized by target lesions (to be measured) and nontarget lesions, is used to extrapolate an overall response to treatment.

RRM1 (ribonucleotide reductase subunit 1): A gene that encodes the regulatory subunit of ribonucleotide reductase subunit 1 and is a molecular target of gemcitabine.