Original Article

A Phase 2 Cooperative Group Adjuvant Trial Using a Biomarker-Based Decision Algorithm in Patients With Stage I Non-Small Cell Lung Cancer (SWOG-0720, NCT00792701)

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BACKGROUND: This cooperative group adjuvant phase 2 trial in patients with completely resected stage I non-small cell lung cancer with tumor diameters measuring $\geq$2 cm was designed to assess the feasibility and preliminary efficacy of assigning patients to therapy or observation using a molecularly based decision algorithm. **METHODS:** At least a lobectomy and sampling of recommended mediastinal lymph node stations, good Zubrod performance status, adequate organ function, and a formalin-fixed and paraffin-embedded tumor specimen were required. Excision repair cross-complementing group 1 (ERCC1) and ribonucleotide reductase M1 (RRM1) were analyzed using immunofluorescence-based in situ automated quantitative image analysis and categorized as high or low using prespecified cutoff values. Patients with high ERCC1 and RRM1 were assigned to observation and all others to 4 cycles of cisplatin and gemcitabine. Feasibility was defined as treatment assignment within 84 days from surgery in >85% of patients. Secondary objectives were to estimate the 2-year survival. **RESULTS:** Treatment assignment met the feasibility criteria in 88% of eligible patients (71 of 81 patients). The collective 2-year disease-free and overall survival rates were 80% and 96%, respectively. Protein levels for RRM1 fell within the previously established range, ERCC1 levels were slightly lower than expected, and they were significantly correlated (correlation coefficient, 0.4). The rates of assignment of patients to observation (22%) and chemotherapy (78%) were as expected. **CONCLUSIONS:** Gene expression analysis for treatment assignment is feasible. Survival results are encouraging and require future validation. Real-time performance of quantitative in situ ERCC1 and RRM1 analysis requires further development. ***Cancer* 2014;120:2343–51**.

KEYWORDS: lung cancer, adjuvant therapy, personalized medicine, ERCC1 (excision repair cross-complementing group 1), RRM1 (ribonucleotide reductase M1).

INTRODUCTION

After publication of the International Adjuvant Lung Cancer Trial in 2004, adjuvant chemotherapy containing a platinum agent has become the standard of care for patients with a complete surgical resection of American Joint Committee on Cancer stage II to III (version 6) non-small cell lung cancer (NSCLC).[1] The trial included patients with stage I to III disease and demonstrated an absolute 4.1% improvement in overall survival (OS), and a subgroup analysis indicated that the OS benefit increased with stage: the hazards ratio (HR) for death among patients receiving adjuvant chemotherapy compared with controls was approximately 0.98 for patients with stage I disease, 0.88 for patients with stage II disease, and 0.79 for patients with stage III disease.[1] The data were confirmed by the National Cancer Institute of Canada Clinical Trials Group JBR.10 trial in 2005, which included patients with stage IB and stage II disease.[2] A third trial, Cancer and Leukemia Group B (CALGB) 9633, which included only patients with stage IB disease, was terminated early and also reported a therapeutic benefit for adjuvant chemotherapy.[3] However, a final analysis of mature data revealed no statistically significant OS benefit (HR, 0.83), but demonstrated a benefit for patients with tumor diameters of $\geq$ 4 cm (HR, 0.69).[4]

During the same time period, an increasing number of correlative biomarker analyses demonstrated that the efficacy of platinum agents was associated with intratumoral levels of the excision repair cross-complementing group 1 (ERCC1) gene, with high levels indicating resistance.[5-9] Similarly, high intratumoral levels of the regulatory subunit of

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ribonucleotide reductase M1 (RRM1) were reported to be predictive of resistance to gemcitabine.[9-13] Finally, both biomarkers had also been reported to be prognostic of survival in patients who had not received chemotherapy or radiation, with high levels indicating longer survival.[8,14-16]

Based on these data, we designed an adjuvant trial in 2007. The underlying hypothesis was that patients with high intratumoral levels of ERCC1 and RRM1 would not benefit from chemotherapy and would have a good prognosis because of a less aggressive tumor phenotype. In contrast, patients with low levels of ERCC1 and RRM1 would have tumors that were sensitive to chemotherapy but with a more aggressive phenotype. Because a biomarker-driven adjuvant chemotherapy selection trial had not been performed in patients with NSCLC, we focused on demonstrating the feasibility of such an approach before launching a phase 3 trial. In addition, because adjuvant chemotherapy had quickly become the standard of care for patients with stage II/IIIA disease, we focused our efforts on patients with stage I disease. After discussions within the SWOG (formerly the Southwest Oncology Group) lung cancer working group and the National Cancer Institute (NCI)'s Cancer Therapy Evaluation Program, and after peer review by a National Institutes of Health study section, the consensus was to focus this feasibility trial on patients with stage I disease and tumor diameters of $\geq$2 cm.

MATERIALS AND METHODS

Trial Design and Treatment Plan

The trial (NCT00792701, SWOG-0720) complied with the Declaration of Helsinki and was approved by the Institutional Review Boards of the study institutions. Eligibility criteria included a diagnosis of NSCLC; stage I disease (according to version 6 of the American Joint Committee on Cancer staging manual) with a tumor diameter $\geq$2 cm; a complete surgical resection by lobectomy, bilobectomy, or pneumonectomy; surgical staging of the mediastinum through sampling of at least 2 lymph node stations; a positron emission tomography scan; a computed tomographic scan of the chest and abdomen; adequate bone marrow, liver, and renal function; a Zubrod performance status of 0 or 1; and willingness to provide a smoking history. Patients with a prior malignancy, prior radiation to the chest, or other significant illnesses according to good medical practice were excluded.

Patients had to be registered on the trial within 35 days of surgery. Tumor specimens were then retrieved and shipped to a central laboratory. They were analyzed for in situ tumor levels of ERCC1 and RRM1 using an immunofluorescence-based automated quantitative analysis method.[17] Prespecified cutoff levels that had been determined in 187 patients with stage I disease ($\geq$65 for ERCC1 and $\geq$40 for RRM1) were used to categorize specimens as high or low expressors for each marker (Fig. 1).[16] The appropriate therapeutic assignment was then passed on to the statistical center and the participating therapeutic center; however, specific protein levels were not communicated to the treatment center. Therapeutic assignment was based solely on biomarker categories, and no other stratification parameters were used.

Patients with high levels of both biomarkers received active surveillance and patients with low levels of one or both biomarkers received 4 cycles of cisplatin (at a dose of 80 mg/m^2 on day 1) and gemcitabine (at a dose of 1 g/m^2 on days 1 and 8) every 21 days. The protocol included provisions for dose reductions or treatment delays. The addition of other targeted or cytotoxic agents during therapy or as maintenance was not permitted.

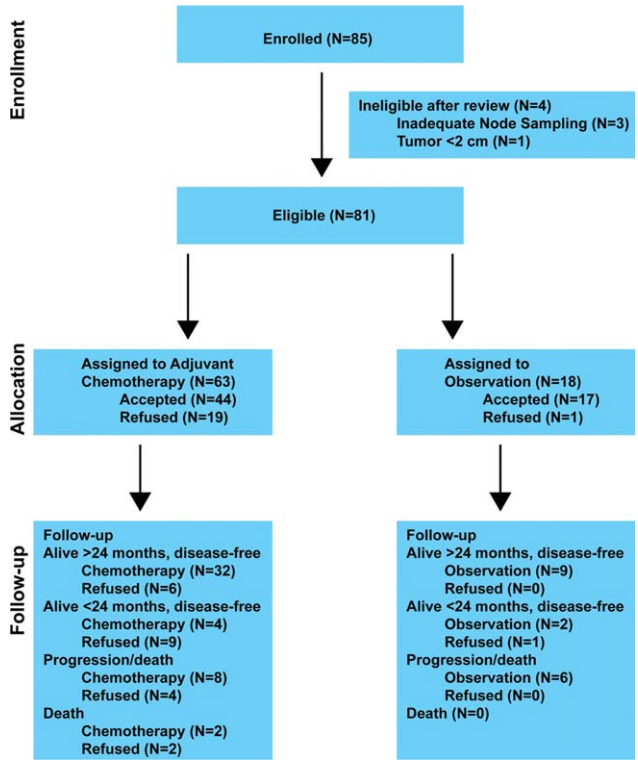

Figure 1. CONSORT (Consolidated Standards Of Reporting Trials) diagram of the trial is shown.

Specimen Collection, Processing, and Gene Expression Analysis

The study required the collection and shipment of formalin-fixed and paraffin-embedded tumor blocks before therapy. However, if local policies did not permit submission of a tissue block, 10 serial unstained sections could be submitted. Processing was done in a reference laboratory by 1 of 2 investigators (V.O. and Z.Z.). Sections measuring 5 μm in thickness were placed on frosted glass slides, and in situ quantification was performed by the automated quantitative analysis method (PM-2000 [version 1]; HistoRx Inc, New Haven, CT) as previously described.[9,16,18]

The primary antibody for the detection of ERCC1 was clone 8F1 (product code NB500-704, lots G412 and H347 from Novus Biologicals [Littleton, Colo]), and the antiserum for RRM1 was R1AS-6 (generated in a rabbit in 2003 against a keyhole limpet hemocyanin [KLH]-conjugated 21-aminoacid peptide specific to the N-terminal of RRM1, column purification lot 09-2008). Slides were scanned with SpotGrabber (HistoRx, New Haven, Conn.), and image data were captured with a digital camera and fluorescence microscope and analyzed. Scores were adjusted to range from 1 to 255. Because full sections were evaluated for each specimen, multiple spots with diameters of 0.6 mm were analyzed to obtain a representative level of protein expression. The number of spots was dependent on suitable areas with tumor cells, and it ranged from 5 to 25 spots (median, 10 spots) for both targets. Runs included a tissue microarray of 15 control specimens in triplicate for control purposes.

Statistical Analysis

The primary objective of the current study was the feasibility of a biomarker-based treatment assignment in the cooperative group setting. If the true success rate were $\leq 75\%$, then a biomarker-based treatment assignment would not be considered feasible, but if the true success rate were $\geq 90\%$ it would be feasible. If ≥ 47 of 55 eligible patients (85%) were successfully assigned to treatment or active monitoring within 84 days from surgery, this would be considered evidence of feasibility. The design had 91% power using an exact binomial test with a 1-sided type I error of 5%.

Secondary objectives included estimating the collective 2-year disease-free survival (DFS) for patients who accepted their treatment assignment and in the subset of patients who received adjuvant chemotherapy. However, there would be no comparison made between treatment arms. To assess DFS, the disease status was monitored every 2 months for the first 6 months and subsequently every 3 months by computed tomography after enrollment and according to good medical practice. Toxicities related to the administration of chemotherapy were assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 3.0; ctep.cancer.gov).

DFS was defined as the time from the date of enrollment to disease recurrence or death due to any cause and estimated according to the Kaplan-Meier method. A Cox regression model was fit with the time from surgery to enrollment as a covariate to evaluate its effect on DFS. A natural log transformation was applied to the raw protein measurement data, and the Pearson correlation coefficient was used to test associations. Bivariate comparison of baseline characteristics between the assigned treatment groups was performed using the Fisher exact test for categorical variables or the Student *t* test or Wilcoxon rank sum test for continuous variables. A multivariable logistic model to evaluate baseline factors and treatment assignment was fit using backwards selection. Median ERCC1 and RRM1 expression levels were compared with historical medians using the 1-sample Wilcoxon signed rank test. The percentage of patients with both ERCC1 ≥ 65 and RRM1 ≥ 40 was compared with the historical rate using a chi-square test. All statistical analyses and graphics were performed using SAS statistical software (version 9.2; SAS Institute Inc, Cary, NC). A significance level of 5% was used for all analyses.

RESULTS

Patient and Trial Characteristics

To ensure an adequate sample size of eligible patients and biomarker-specific subgroups, a total of 85 patients was registered between April 2, 2009 and April 1, 2011 from 27 participating sites. Four patients were ineligible; 3 had inadequate lymph node sampling and 1 did not have a tumor measuring ≥ 2 cm. Table 1 provides the characteristics of the 81 eligible patients.

The distribution of assignment to chemotherapy and observation was 63 patients (78%) and 18 patients (22%), respectively, which was not significantly different ($P = .20$, Fisher exact test) from the expected rates of 70% (129 patients) and 30% (55 patients), respectively.[16] Based on protein levels in these 81 patients, the number of those with low ERCC1 and low RRM1 was 31 patients (38%), 22 patients had low ERCC1 and high RRM1 (27%), 10 patients had high ERCC1 and low RRM1 (12%), and 18 patients had high ERCC1 and

TABLE 1. Patient Demographics and Disease Characteristics

Variables[a]	All Patients N = 81	Assigned to Chemotherapy N = 63	Assigned to Observation N = 18	*P*	Refused Assignment N = 20	Accepted Assignment N = 61	*P*
Age, y				.37			.39
Median	64	63.3	68.8		67.2	63.3	
Mean	63.5	62.9	65.5		65.2	62.9	
Range	41.6–84.2	41.6–84.2	41.6–81.7		44.2–82.9	41.6–84.2	
Sex				.18			.61
Female	44 (54%)	37 (59%)	7 (39%)		12 (60%)	32 (52%)	
Male	37 (46%)	26 (41%)	11 (61%)		8 (40%)	29 (48%)	
Ethnicity				.65			.18
Unknown	7 (8%)	5 (8%)	2 (11%)		0 (0%)	7 (11%)	
Non-Hispanic	74 (91%)	58 (92%)	16 (89%)		20 (100%)	54 (89%)	
Race				.73[b]			.75[b]
African American	8 (10%)	8 (13%)	0 (0%)		2 (10%)	6 (10%)	
Asian	3 (4%)	2 (3%)	1 (6%)		0 (0%)	3 (5%)	
Pacific Islander	2 (2%)	1 (2%)	1 (6%)		0 (0%)	2 (3%)	
White	66 (81%)	52 (83%)	14 (78%)		17 (85%)	49 (80%)	
Unspecified	2 (2%)	0 (0%)	2 (11%)		1 (5%)	1 (2%)	
Histology				.06[c]			.60[c]
Adeno	52 (64%)	44 (70%)	8 (44%)		14 (70%)	38 (62%)	
Squamous	25 (31%)	17 (27%)	8 (44%)		6 (30%)	19 (31%)	
Large	1 (1%)	1 (2%)	0 (0%)		0 (0%)	1 (2%)	
Bronchioloalveolar	1 (1%)	0 (0%)	1 (6%)		0 (0%)	1 (2%)	
Other	2 (2%)	1 (2%)	1 (6%)		0 (0%)	2 (3%)	
Stage of disease				.16			.27
IA (<3 cm)	25 (31%)	22 (35%)	3 (17%)		4 (20%)	21 (34%)	
IB (≥3 cm)	56 (69%)	41 (65%)	15 (83%)		16 (80%)	40 (66%)	
Zubrod performance status				.11			1.00
0	44 (54%)	31 (49%)	13 (72%)		11 (55%)	33 (54%)	
1	37 (46%)	32 (51%)	5 (28%)		9 (45%)	28 (46%)	
Weight loss (6 mo)				1.00[d]			.31[d]
<5%	64 (79%)	49 (78%)	15 (83%)		14 (70%)	50 (82%)	
5-<10%	9 (11%)	7 (11%)	2 (11%)		3 (15%)	6 (10%)	
10–20%	4 (5%)	3 (5%)	1 (6%)		2 (10%)	2 (3%)	
>20%	1 (1%)	1 (2%)	0 (0%)		0 (0%)	1 (2%)	
Unknown	3 (4%)	3 (5%)	0 (0%)		1 (5%)	2 (3%)	
Smoking status							
Current	33 (41%)	26 (41%)	7 (39%)		8 (40%)	25 (41%)	
Former (quit ≥1 y)	39 (48%)	30 (48%)	9 (50%)		10 (50%)	29 (48%)	
Never	9 (11%)	7 (11%)	2 (11%)	1.00[e]	2 (10%)	7 (11%)	1.00[e]

Abbreviation: Adeno, adenocarcinoma.
[a] All *P* values shown are 2-sided.
[b] White versus all other races.
[c] Adenocarcinoma versus all other histologies.
[d] Weight loss <5% versus ≥5%.
[e] Derived using the Freeman-Halton exact test.

RRM1 (22%), which is not significantly different from prior results ($P = .14$, Fisher exact test; 54 of 184, 29%; 38 of 184, 21%; 37 of 184, 20%; and 55 of 184, 30%, respectively).

We investigated whether treatment arm assignment varied by patients' smoking status, histology, age, and sex. In bivariate comparisons, no statistically significant associations were found. However, the multivariable logistic model found that patients with adenocarcinoma ($P = .03$) and potentially stage IA disease ($P = .06$) were more likely to be assigned to adjuvant chemotherapy (ie, they were more likely to have low levels of ERCC1, RRM1, or both).

One of the 18 patients assigned to observation and 19 of the 63 patients assigned to chemotherapy rejected this choice and withdrew consent. There was no statistically significant difference in patient characteristics between those who accepted and those who refused their treatment assignment (Table 1).

Feasibility

The trial achieved its primary feasibility objective with a treatment assignment within the prespecified timeframe in 71 of 81 patients (88%). We successfully determined protein levels in all 85 patients. Ten of the 81 eligible patients did not achieve assignment to treatment

versus observation within the 84-day time interval from surgical resection. The time interval from surgery to assignment ranged from 86 days to 105 days in these 10 patients. For 3 patients, the specimens were received after the 84-day limit had passed. For the other 7 patients, the time interval from receipt to reporting ranged from 7 days to 25 days (median, 18 days). For the 71 patients with a successful assignment within the 84-day time interval from surgical resection, the time from receipt to reporting ranged from 3 days to 26 days (median, 8 days). The reasons for reporting results in excess of 14 days were equipment failure and inadequate expression values in control specimens, which required equipment recalibration and a repeat processing of the specimens. Overall, the time from receipt of specimens to reporting ranged from 1 day to 27 days (median, 11 days; mean, 12 days), which is similar to that reported for patients with advanced NSCLC (range, 1 day-47 days; median, 11 days; mean, 12 days).[18]

Survival and Toxicity

Survival analyses were performed on the 61 patients who accepted assignment to treatment (44 patients) or surveillance (17 patients). Patients who rejected their treatment assignment withdrew consent and thus could not be followed for survival. Fourteen patients had DFS events; 2 had died (1 from disease recurrence and the other from cardiac disease without recurrence). The median follow-up among those patients still alive at the time of last follow-up was 27 months (range, 3 months-44 months). Six patients had < 24 months of follow-up.

The collective 2-year DFS and OS rates were 80% (95% confidence interval [95% CI], 67%-88%) (Fig. 2A) and 96% (95% CI, 87%-99%) from the date of registration. The 2-year DFS rate was 83% (95% CI, 68%-92%) for patients who received chemotherapy (Fig. 2B), and it was 71% (95% CI, 43%-87%) for those observed (Fig. 2C). Table 2 includes 2-year DFS estimates within each of the 3 gene expression categories in the chemotherapy arm. The median time from surgery to enrollment was 41 days (range, 11 days-79 days). The time from surgery was added as a covariate to a Cox regression model and was not found to be significantly related to DFS ($P = .22$) or OS ($P = .36$).

A total of 22 patients discontinued chemotherapy because of treatment-related toxicity (50%). None of the patients died because of treatment-related toxicity. Details are provided in Table 3.

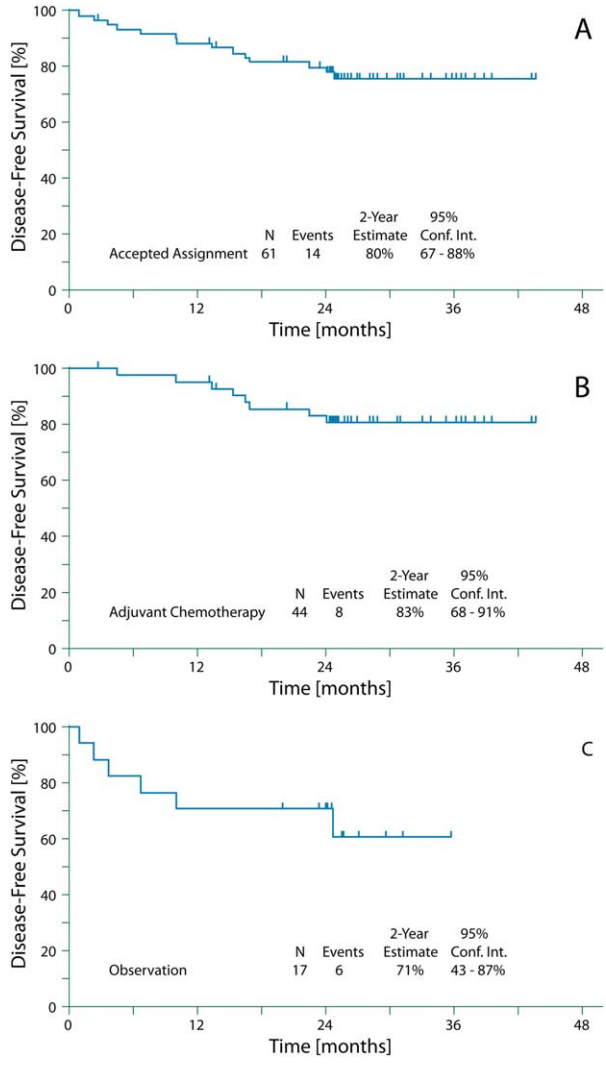

Figure 2. Kaplan-Meier survival estimates are shown. (A) Collective disease-free survival is shown for patients who accepted adjuvant chemotherapy or observation based on gene expression analysis. (B) Disease-free survival is shown for patients who received adjuvant chemotherapy. (C) Disease-free survival is shown for patients in the observation group. Conf Int indicates confidence interval.

In Situ ERCC1 and RRM1 Protein Levels

RRM1 levels ranged from 2.4 to 234.3 (median, 39.7; mean, 48.1), which were not significantly different from the expected values (median, 40.5; range, 8.3-96.2) ($P = .87$).[16] ERCC1 protein levels ranged from 4.3 to 211.2 (median, 41.9; mean, 58.8), and these values were significantly different from the expected values (median, 65.9; range, 1.9-178.7) ($P = 0.02$). There was a significant correlation noted between ERCC1 and RRM1 levels (correlation coefficient, 0.39; $P = .0003$) (Fig. 3), as previously reported.[9,16,18]

TABLE 2. Disease-Free Survival Rates

Patient Group	No.	DFS (95% CI)	
		1-Year	2-Year
Accepted assigned treatment	61	88% (77%-94%)	80% (67%-88%)
Received chemotherapy	44	95% (83%-99%)	83% (68%-92%)
By protein level category (for those that received chemotherapy)			
Low ERCC1/low RRM1	20	95% (69%-99%)	84% (59%-95%)
Low ERCC1/high RRM1	18	94% (65%-99%)	82% (55%-94%)
High ERCC1/low RRM1	6	100% (100%-100%)	100% (100%-100%)

Abbreviations: 95% CI, 95% confidence interval; DFS, disease-free survival; ERCC1, excision repair cross-complementing group 1; RRM1, ribonucleotide reductase M1.

TABLE 3. Number of Patients With Grade 3 and Grade 4 Adverse Events Among the 44 Patients Who Received Chemotherapy[a]

Adverse Event	Level of Severity	
	Grade 3	Grade 4
No. of patients with events	13	14
Type of events		
Neutropenia	11	6
Thrombocytopenia	4	4
Nausea	4	0
Vomiting	4	0
Anemia	2	0
Anorexia	2	0
Fatigue	2	0
Febrile neutropenia	1	1
Thromboembolism	1	1
Dehydration	1	0
Hearing impairment	1	0
Mucositis	1	0
Pleural effusion	1	0
Renal failure	1	0
Bradycardia (sinus)	1	0
Syncope	1	0
ALT elevation	1	0
Hypokalemia	1	0
Hyponatremia	0	2

Abbreviation: ALT, alanine aminotransferase.
[a] Adverse events were assessed according to the Common Terminology Criteria for Adverse Events (version 3.0).

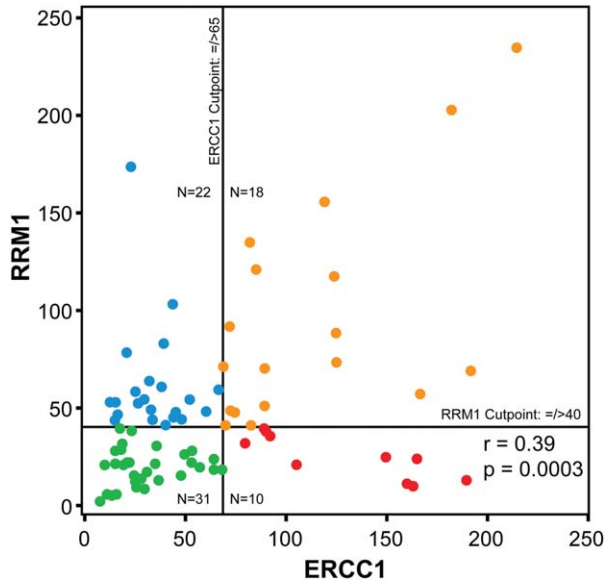

Figure 3. Distribution of excision repair cross-complementing group 1 (ERCC1) and ribonucleotide reductase M1 (RRM1) levels in eligible patients is shown.

The median protein levels of ERCC1 in adenocarcinomas, squamous cell carcinomas, and the other histologies were 34.2, 57.1, and 121.5, respectively. The corresponding median levels of RRM1 were 38.1, 42.6, and 48.9, respectively. Although the levels were higher in squamous cell carcinomas compared with adenocarcinomas, the medians were not statistically significant (ERCC1: $P = .16$; RRM1: $P = .72$).

DISCUSSION

Disease stage is a predictor of benefit from adjuvant chemotherapy in patients with NSCLC. Patients with stage III disease derive the most benefit and those with stage I are reported to derive the least.[1,2,4,19-23] Although not statistically significant, for patients with stage I disease and a tumor diameter > 3 cm, a numerical risk reduction of 7% has been reported and for those with tumors measuring ≤ 3 cm a numerical risk increase of 40% has been reported.[23] A significant treatment-related toxicity is febrile neutropenia, which has been reported in 7% to 24% of patients.[2,4,20,22] Treatment-related deaths occur in 0.5% to 2% of patients.[1,2,20,22] The inclusion of molecular markers predictive of therapeutic efficacy into adjuvant decision algorithms would greatly improve the clinical benefit and reduce toxicity for patients with NSCLC. This approach is particularly attractive for patients with stage I disease, in whom the parameters for weighing risks

and benefits are to our knowledge the least well defined. Recent advances in molecular diagnostics have resulted in improved outcomes for patients whose tumors harbor mutations in oncogenic signal transduction molecules that can be inactivated by therapeutic agents. Similarly, platinum agents target DNA, and gemcitabine targets ribonucleotide reductase; both are unequivocally required not only for cellular proliferation but also for other essential cellular functions. Although to our knowledge specific oncogenic mutations have not been identified to date, ERCC1 and RRM1 have emerged as promising predictors of efficacy for cisplatin and gemcitabine, respectively. We conducted a phase 2 trial of treatment selection based on the levels of protein expression of ERCC1 and RRM1 for patients with completely resected stage I NSCLC and tumor diameters ≥ 2 cm primarily to establish feasibility but also to evaluate preliminary efficacy as assessed by 2-year survival rates.

We achieved our primary goal by demonstrating within a cooperative group environment that treatment assignment can be achieved for $> 85\%$ of patients within 84 days (12 weeks), the established timeframe for the initiation of adjuvant therapy from surgery in patients with NSCLC.[1,2,4,20-22] At first glance, our demonstration of feasibility should not be surprising. However, it is important to note that surgical practice has not usually engaged a medical oncologist at the time of initial therapeutic planning but rather after complete recovery, which substantially reduces the time available for molecular testing before the initiation of adjuvant treatment. We found no difference ($P = .20$) between academic and community sites in the time elapsed from surgery to the receipt of specimens in the reference laboratory (community sites: 57 patients; median, 48 days [range, 18 days-90 days]; academic sites: 24 patients; median, 53 days [range, 20 days-90 days]). The time elapsed from specimen receipt to reporting (median, 12 days; range, 1 day-27 days) was similar to our previous experience in an international trial of patients with advanced NSCLC (median, 11 days; range, 1 day-47 days).[18] Based on these observations, we conclude that the current process for routine specimen procurement, handling, and shipping to a reference laboratory requires substantial improvements to facilitate implementation of molecularly based therapeutic decision-making. For example, a developing National Cancer Institute-sponsored project, Adjuvant Lung Cancer Enrichment Marker Identification and Sequencing Trial (ALCHEMIST) which will randomize patients with epidermal growth factor receptor-mutated or anaplastic lymphoma kinase (ALK)-rearranged NSCLC to targeted therapy or not, will need to carefully consider these logistical issues.

Prior results from adjuvant trials and a retrospective staging project in patients with stage I disease after complete surgical resection have reported 2-year DFS rates of 72% to 74%[20] and rates of 68% to 75% for patients with stage IB disease.[4] The corresponding 2-year OS rates were 80% to 88% for patients with stage I disease[20,24] 65% to 90% for patients with stage IB disease,[2,4,22,25] and 85% for those with stage IA disease.[25] Thus, our results of a 2-year DFS rate of 80% and OS rate of 96% appear favorable by comparison. However, it is prudent to be cautious because we lost 20 of 81 patients from the survival analysis because of consent withdrawal, and a direct comparison of outcomes data among trials cannot account for differences in study populations, eligibility and staging criteria, and provisions for data collection and analysis.

The spectrum of protein levels for ERCC1 and RRM1, significant correlation of levels between both molecules, and distribution of patients into the 4 gene expression categories in the current study is consistent with previous experience.[9,12,13,16,18,26] However, the current analysis method for biomarker evaluation (ie, antibody-based assessment of in situ protein levels) is not suitable for general clinical implementation for several reasons. First, ERCC1 has multiple isoforms that cannot be specifically distinguished by the available reagents, and only 1 isoform appears to be involved in platinum-induced DNA damage repair.[27] Second, the monoclonal antibody 8F1, which is consistently used for ERCC1 protein expression analysis, detects a second and unrelated protein that shares a common epitope with ERCC1.[28-30] This observation may account for the highly batch-dependent performance of this antibody,[18,27] which may explain the significantly lower ERCC1 values in the current study compared with prior results.[16] Third, protein levels for RRM1 in particular, and to a lesser degree for ERCC1, appear to be influenced by the specimen processing and handling procedures used at collection sites.[26] Finally, although the method for immunofluorescence-based quantitative detection of both molecules performs well if all specimens to be analyzed are processed simultaneously, there is considerable interassay variability if specimens need to be processed individually over an extended period of time as required for real-time patient decision-making.[18] However, it is important to note that the biochemical, biophysical, and cell biological evidence for ERCC1 and RRM1 as predictive molecules for platinum and gemcitabine efficacy remains undisputed.[5,10-12,27,31,32]

A small number of recent clinical trials have used ERCC1 prospectively for therapeutic decision-making. These include 2 randomized phase 3 trials in patients with advanced-stage NSCLC (1 published [NCT00499109][18] and the other terminated and unpublished [NCT00801736]) and 2 adjuvant trials, 1 of which was a terminated and not yet published phase 2 trial [TAilored Post-Surgical Therapy in Early Stage NSCLC (TASTE), NCT00775385] and the other an ongoing phase 3 trial [International TAilored Chemotherapy Adjuvant trial (ITACA); EudraCT 2008-001764-36]. Results from the first trial (NCT00499109) demonstrated no improvement in patient survival; however, the authors raised the possibility of a false-negative result because of an inexplicably divergent survival in an internal control group.[18] The second trial (NCT00801736) and third trial (NCT00775385) were terminated early after the discovery of ERCC1 isoforms[27] and specificity problems with the 8F1 antibody.[28-30] The fourth trial is using ERCC1 and tumor thymidylate synthase mRNA expression levels for treatment assignment compared with a cisplatin-based control treatment with OS as the primary endpoint and a planned accrual of 700 patients. Results from these trials will help to further delineate the feasibility and technical issues mentioned above.

The results of the current study demonstrated the feasibility of our biomarker-based decision algorithm in a multiinstitutional cooperative group environment for patients with surgically resected NSCLC. We identified that the current practice of evaluation and treatment for these patients may present an obstacle to rapid molecular-based decision-making. Although encouraging efficacy data emerged from this trial, bioassays that specifically measure platinum-induced DNA damage repair must be developed before further clinical trials are launched that seek to tailor the use of these agents.

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CONFLICT OF INTEREST DISCLOSURES

Dr. Bepler has a patent pending for the use of RRM1 and ERCC1 as biomarkers of treatment benefit for therapeutic decision-making in patients with cancer.

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