

Randomized Phase III Trial of Gemcitabine-Based Chemotherapy With In Situ RRM1 and ERCC1 Protein Levels for Response Prediction in Non–Small-Cell Lung Cancer

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ABSTRACT

Purpose

We evaluated the efficacy of gemcitabine versus gemcitabine and carboplatin in patients with advanced non–small-cell lung cancer (NSCLC) and a performance status (PS) of 2 and assessed if tumoral RRM1 and ERCC1 protein levels are predictive of response to therapy.

Patients and Methods

A randomized phase III trial was conducted in community-based oncology practices. Tumor specimens were collected a priori and shipped to a single laboratory for blinded determination of in situ RRM1 and ERCC1 protein expression levels by an automated quantitative immunofluorescent-based technology.

Results

One hundred seventy patients were randomly assigned. Overall median survival was 5.1 months for gemcitabine and 6.7 months for gemcitabine and carboplatin ($P = .24$). RRM1 (range, 5.3 to 105.6; median, 34.1) and ERCC1 (range, 5.2 to 131.3; median, 34.7) values were significantly and inversely correlated with disease response ($r = -0.41$; $P = .001$ for RRM1; $r = -0.39$; $P = .003$ for ERCC1; ie, response was better for patients with low levels of expression). A model for response prediction that included RRM1, ERCC1, and treatment arm, was highly predictive of the treatment response observed ($P = .0005$). We did not find statistically significant associations between survival and RRM1 or ERCC1 levels.

Conclusion

Single-agent chemotherapy remains the standard of care for patients with advanced NSCLC and poor PS. Quantitative analysis of RRM1 and ERCC1 protein expression in routinely collected tumor specimens in community oncology practices is predictive of response to gemcitabine and gemcitabine and carboplatin therapy. Oncologists should consider including in situ expression analysis for these proteins into their therapeutic decisions.

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INTRODUCTION

The combination of two cytotoxic drugs, a platinum and a nonplatinum agent, is the standard of care for first-line treatment of patients with advanced non–small-cell lung cancer (NSCLC) and good performance status (PS, 0 to 1).¹ This treatment yields response rates of approximately 25% to 30% and a median overall survival (OS) of 9 to 11 months in otherwise unselected patients.²⁻⁴ Because a high rate of toxicity has been observed with dual-agent therapy in patients with a poor PS (PS, 2), single-agent therapy has become an accepted standard. However, subgroup analyses of cooperative group trials have suggested improved survival with dual-agent single-agent therapy for this group of patients.^{5,6}

Promising results on the utility of molecular parameters in predicting efficacy of systemic cytotoxic therapy in NSCLC have been reported. Two molecules involved in DNA synthesis and damage repair, ERCC1 and RRM1, have been associated with efficacy of platinum agents and gemcitabine.⁷⁻¹¹ ERCC1 is a component of the nucleotide excision repair complex, and it is responsible for the 5' incision required for the removal of DNA adducts that are the basis for platinum cytotoxicity.¹² RRM1 is the molecular target of gemcitabine and a component of ribonucleotide reductase, which is required for deoxynucleotide production.¹³ However, with one exception,¹¹ these studies have evaluated gene expression at the RNA level; and the relationship

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Clinical Trials repository link available on [JCO.org](http://www.jco.org).

Clinical Trial Identifier: NCT00190710 (<http://clinicaltrials.gov>).

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

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between cellular levels of RNA and protein, the molecule responsible for function, is tenuous.^{14,15} In addition, most of these studies have demonstrated differential survival by gene expression and not differences in response rates.^{7,8,10,11}

We studied if significant survival differences exist between NSCLC patients with PS 2 treated with gemcitabine alone or gemcitabine and carboplatin, and the predictive utility of RRM1 and ERCC1 protein expression on therapeutic efficacy in tumor specimens collected under standard conditions in community oncology practices.

PATIENTS AND METHODS

Clinical Trial

A randomized phase III clinical trial (USO-03012) was designed and conducted in community oncology practices in patients with previously untreated stage IIIB/IV NSCLC,¹⁶ a PS of 2,¹⁷ and measurable disease by Response Evaluation Criteria in Solid Tumors (RECIST),¹⁸ (registration #: NCT00190710). Up to 6 cycles of therapy was given. Gemcitabine and carboplatin was given every 3 weeks at doses of 1,000 mg/m² of gemcitabine on days 1 and 8 and of carboplatin at an area under the curve of 5 on day 1. Gemcitabine was given every 3 weeks at a dose of 1,250 mg/m² on days 1 and 8 (Appendix, online only).

Therapeutic Efficacy Assessment

Survival analyses were performed on an intent-to-treat basis. OS was the time interval between the dates of first treatment and death; progression-free survival (PFS) was the time interval between the dates of first treatment and disease progression or death, whichever occurred first. For patients who did not start treatment, the date of registration was used for survival calculations. The data lock occurred on February 28, 2008.

The sum of maximal diameters of all measurable tumor lesions was recorded at baseline and after treatment cycles 2, 4, and 6. These measurements were used to calculate the largest percentage of shrinkage or smallest percentage of growth using the baseline assessment as reference. The best response to therapy was categorized according to RECIST. Adverse events were assessed using National Cancer Institute's Common Terminology Criteria version 3.0.

Specimen Collection and Protein Expression Analysis

Tumor specimens from patients whose diagnosis was performed on histologic or equivalent cytological samples were shipped to a single central laboratory for biomarker analysis. Full specimen sections of 4- μ m thickness were cut from paraffin blocks and mounted on adhesive coated or charged glass slides. A fluorescent-based immunohistochemical (IHC) method combined with automated quantitative analysis (AQUA) was used to determine in situ expression levels for RRM1 and ERCC1 as previously described.¹⁹ For ERCC1 detection, mouse clone 8F1 (1:300 dilution, lot #9475) produced by Exalpha Biologicals (Maynard, MA) and distributed by Sigma-Aldrich (St Louis, MO) was used. For RRM1 detection, a custom-made rabbit antiserum (RIAS-6b, 1:300 dilution) was used.¹⁵

For each patient, two full specimen sections were analyzed, one slide for RRM1 and one slide for ERCC1. For each slide, random spots (spot diameter 0.6 mm) ranging in number from 5 to 66 (median, 21) for ERCC1 and from 3 to 69 (median, 19) for RRM1 were scanned with SpotGrabber (HistoRx, New Haven, CT), and image data were analyzed with AQUA (PM-2000, version 1.2, score range 0 to 255, HistoRx).

Determination of Cross-Analysis-Adjusted RRM1 and ERCC1 Protein Expression Values

In order to adjust for variances in AQUA scores among tumor specimens analyzed in different runs, we incorporated a uniform calibration tissue microarray in each run. The array included three specimen replicates of 10 permanent human cell lines that displayed a range of expression for the target molecules. Representative RRM1 and ERCC1 scores from each experimental tumor specimen and the calibration specimens were obtained using quarter-root power transformations. Adjusted means were

obtained for each experimental specimen by averaging their standardized quarter-rooted transformed raw scores. These adjusted means were used for all statistical analyses (Appendix).

Statistical Analyses

OS was the primary end point of the clinical trial. It was estimated that 220 eligible patients were required to detect an improvement from 2.4 months for gemcitabine to 3.8 months for gemcitabine and carboplatin (ie, a 36.8% reduction in the hazard rate of death, with a power of 0.90 and a type 1 error of 0.05). The OS targets were derived from the subset analysis of PS 2 patients in trial Cancer and Leukemia Group B 9730.⁵ The anticipated patient accrual time was 22 months. OS and PFS were estimated using the Kaplan-Meier method. Tumor response rates were calculated as the sum of the number of observed CRs and PRs divided by the number of patients qualifying for the efficacy analysis (ie, patients who met enrollment criteria and received at least one dose of study medication). Two-sided 95% CI were calculated based on the exact binomial probability.

The primary goal of the biomarker analysis was to determine if RRM1 and ERCC1 protein levels were predictive of best tumor response. First, we determined that no transformation was needed for values of best tumor response. Separate weighted linear regression models for ERCC1 and RRM1 were performed using best measured tumor response as the dependent variable and the adjusted biomarker means as the predictor variables. The number of ERCC1 scores (range, 5 to 66) and RRM1 scores (range, 3 to 69) per patient was used as the weight. Follow-up analyses included a weighted regression for ERCC1 and RRM1, including the study arm and a nonweighted regression including ERCC1, RRM1, and study arm.

In order to compare demographic and clinical features of the treatment arms within the clinical and biomarker groups, and to compare patients with and without biomarker data, the Wilcoxon rank sum test was used for continuous variables while the Fisher's exact test was used for categorical variables, both based on exact distribution theory. We used the maximal χ^2 method and log-rank testing for optimal cut point determination with appropriate adjustments of the *P* values for multiple looks to evaluate OS and PFS differences.²⁰ Only cutpoints within the central 80% of ordered RRM1 and ERCC1 levels were considered.

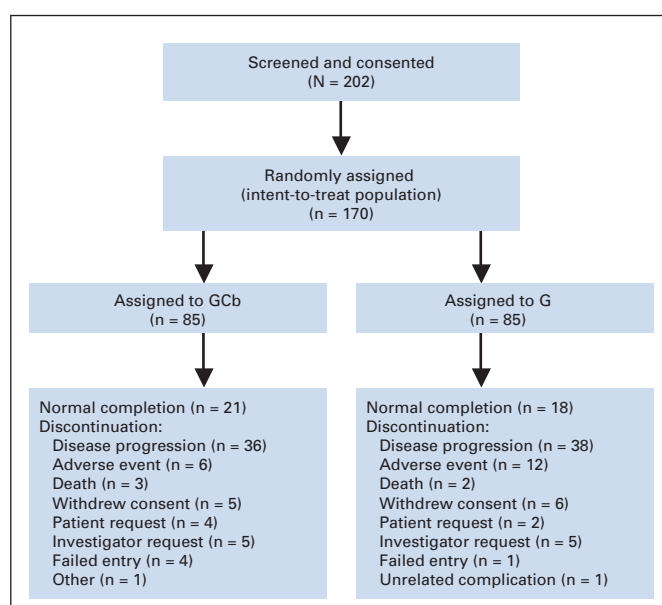


Fig 1. CONSORT diagram. G, gemcitabine; G/Cb, gemcitabine and carboplatin.

RESULTS

Clinical Trial

A total of 202 patients from 84 community-based oncology practices were consented between March 2004 and December 2006 (Fig 1). Patient accrual was 50% of the expected rate, and the trial was terminated. Thirty-two patients withdrew consent before randomization. Both arms, with 85 patients each, were demographically well balanced (Table 1). At least one dose of chemotherapy was given to 160 patients, 81 in the gemcitabine and 79 in the gemcitabine and carboplatin arm.

The median number of cycles was 4 in the gemcitabine and carboplatin arm, 38% received 6 cycles, and it was 3 in the gemcit-

abine arm, 13% received 6 cycles. More patients received dose reductions in the gemcitabine and carboplatin arm (55.7% had ≥ 1 dose reduction of gemcitabine and 34.2% had ≥ 1 reduction of carboplatin in the gemcitabine and carboplatin arm versus 13.6% with ≥ 1 reduction in the gemcitabine arm). The median relative dose intensity of gemcitabine was 93.8% in the gemcitabine and carboplatin arm and 97.1% in the gemcitabine arm; and it was 96.5% for carboplatin in the gemcitabine and carboplatin arm.

There was a statistically significantly higher confirmed response rate in the group of patients treated with gemcitabine and carboplatin (21.1%) compared with the group treated with gemcitabine (6.3%; $P = .01$). The best measurable response rates by CT

Table 1. Patients' Characteristics by Treatment Arm and Biomarker Analysis

Characteristic	Gemcitabine and Carboplatin (n = 85)		Gemcitabine (n = 85)		P	Nonbiomarker Group (n = 101)		Biomarker Group (n = 69)		P
	No.	%	No.	%		No.	%	No.	%	
Age, years					.820*					.718*
Median		72.9		75.0			73.8		75.0	
Range		46.3-88.4		45.2-89.7			45.2-89.7		49.4-85.5	
Sex					1.000†					.275†
Male	47		48			60		35		
Female	38		37			41		34		
Histology					.311†					.609†
Adenocarcinoma	53		45			55		43		
Squamous carcinoma	14		22			23		13		
Other	18		18			23		13		
Stage					.035†					.180†
IV	70		80			86		64		
IIIB	13		5			14		4		
Unknown	2		0			1		1		
Best measurable response by CT§					.003†					.977†
CR	0	0	0	0		0	0	0	0	
PR	29	43.9	10	16.4		21	31.3	18	30.0	
SD	23	34.8	35	57.4		30	44.8	28	46.7	
PD	14	21.2	16	26.2		16	23.9	14	23.3	
Best measurable response by CT					< .001†					1.000†
CR and PR	29	43.9	10	16.4		21	31.3	18	30.0	
95% CI		24.2 to 54.2		8.2 to 28.1			20.6 to 43.8		18.9 to 43.2	
Best confirmed response category					.050†					.668†
CR	0	0	0	0		0	0	0	0	
PR	16	21.1	5	6.3		10	11.5	11	15.9	
SD	34	44.7	38	47.5		39	44.8	33	47.8	
PD	20	26.3	25	31.3		25	28.7	20	29.1	
Unknown	6	7.9	12	15.0		13	14.9	5	7.2	
Best confirmed response					.010†					.240†
CR and PR	16	21.1	5	6.3		10	12.7	11	17.2	
95% CI		12.5 to 31.9		2.1 to 14.0			4.8 to 17.5		8.2 to 26.7	
OS, months					.242‡					.312‡
Median		6.7		5.1			4.9		6.0	
95% CI		4.9 to 10.0		3.9 to 6.3			3.9 to 6.7		5.1 to 8.4	
PFS, months					.143‡					.698‡
Median		3.8		2.7			2.6		3.1	
95% CI		2.6 to 4.6		1.9 to 3.6			2.0 to 4.1		2.8 to 4.3	

Abbreviations: CT, computed tomography; CR, complete response; PR, progressive disease; SD, stable disease; PD, progressive disease; OS, overall survival; PFS, progression-free survival.

*Wilcoxon rank-sum test.

†Fisher's exact test.

‡Log-rank test.

§Best measurable response by CT was available in 66 patients treated with gemcitabine and carboplatin and 61 patients treated with gemcitabine.

||Best confirmed response was available in 70 patients treated with gemcitabine and carboplatin and 68 patients treated with gemcitabine.

were 43.9% and 16.4%, respectively ($P < .001$). However, the survival differences between the treatment arms were not statistically significant (median OS, 6.7; 95% CI, 4.9 to 10.0 v 5.1; 95% CI, 3.9 to 6.3 months; $P = .24$; median PFS, 3.8; 95% CI, 2.6 to 4.6; v 2.7; 95% CI, 1.9 to 3.6 months; $P = .14$). At 1 year, the OS rate was 31.3% for gemcitabine and carboplatin and 21.2% for gemcitabine (Fig 2).

Common toxicities reported in the patients who received at least one dose of study treatment are summarized in Table 2. The rates of grade 3 or 4 cytopenias were notably higher in the gemcitabine and carboplatin arm compared with the gemcitabine arm. Grade 3 or 4 febrile neutropenia, however, did not occur in either treatment arm. Nonhematologic toxicities were not substantially different between treatment arms. The most common grade 2 or higher nonhematologic toxicities included fatigue, dyspnea, anorexia, and nausea, all of which occurred in more than 10% of patients.

Tumor Specimens and Patient Characteristics

Specimens from 87 patients and 42 different study sites were received by the biomarker laboratory. Eight specimens had been

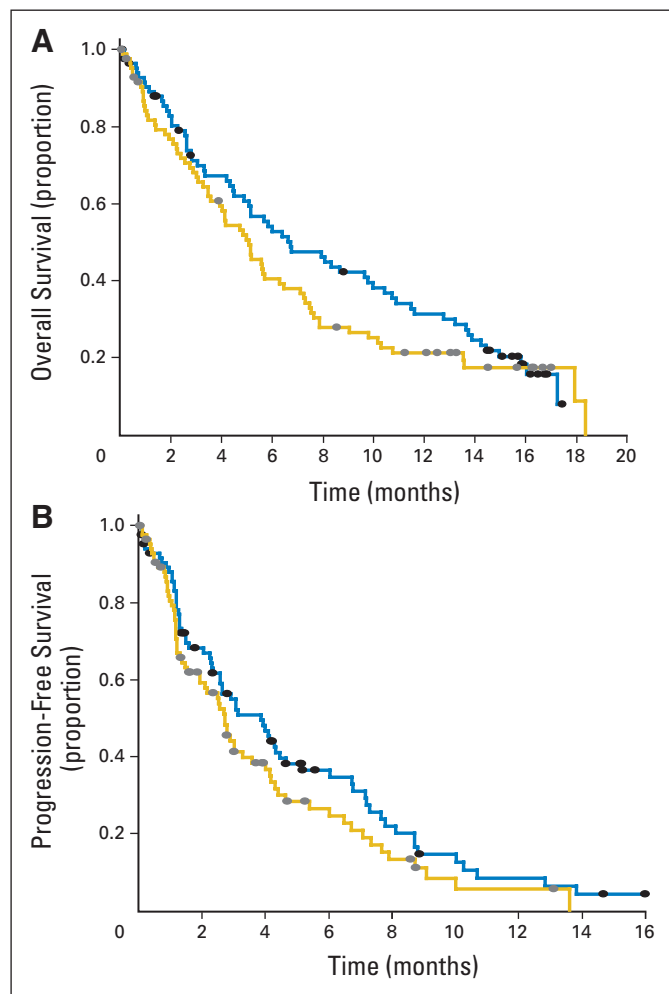


Fig 2. (A) Overall survival (OS) and (B) progression-free survival (PFS) estimates by treatment arm. (gold line) treatment with gemcitabine; (blue line) treatment with gemcitabine and carboplatin; (solid oval) censored patients. The differences in median OS and PFS were not statistically significant (log-rank test $P = .14$).

stained, which resulted in 79 specimens useful for analysis. Specimens from 10 patients were inadequate to produce results because the specimen had washed off during the procedure or because of absence of tumor cells. RRM1 data were obtained on 69 and ERCC1 data on 65 patients (four specimens were exhausted after RRM1 analysis).

Of the 69 patients with protein expression data, 34 had been treated with gemcitabine and carboplatin and 35 with gemcitabine. There were no significant differences between the groups of patients with and without biomarker data (Table 1).

Predictive Impact of RRM1 and ERCC1 Levels on Measurable Tumor Response

Since full section specimens from different patients were processed at separate time points, we standardized the biomarker values by incorporating calibration specimens in each analysis to adjust for inter-analysis variability. The adjusted values for RRM1 ranged from 5.3 to 105.6 (median, 34.1), and those for ERCC1 ranged from 5.2 to 131.3 (median, 34.7). They were not statistically significantly associated with patients' age, sex, tumor histology, and stage; and they were not significantly different between both treatment arms (Table 3). For comparison, the raw values ranged from 8.2 to 157.3 (median, 46.7) for RRM1 and from 8.2 to 115.2 (median, 40.2) for ERCC1. Best measurable responses ranged from a 100% reduction (-100%) to a 133% increase in tumor sizes. In situ RRM1 levels were significantly and inversely correlated with disease response ($r = -0.41$; $P = .001$; Fig 3A) in the 58 patients with both parameters available. Likewise, ERCC1 levels were significantly and inversely correlated with disease response ($r = -0.39$; $P = .003$) (Fig 3B) in the 55 patients with both parameters available. RRM1 and ERCC1 expression levels were significantly and positively correlated ($r = 0.36$; $P = .003$; $N = 65$).

We evaluated if an interaction between RRM1 or ERCC1 levels and treatment arm existed (ie, if RRM1 or ERCC1 had a differential predictive impact in the gemcitabine or gemcitabine and carboplatin treatment arms). We did not find a statistically significant interaction ($P = .64$ for RRM1; $P = .79$ for ERCC1). However, the slope of the best-fit line between RRM1 values and disease response was steeper (0.47) than the ERCC1 response slope (0.34) in the gemcitabine arm (Fig 3A). This difference was less notable in the gemcitabine and carboplatin arm (0.35 for RRM1; 0.29 for ERCC1; Fig 3B).

We generated a model for response prediction that included RRM1, ERCC1, and treatment arm. The predicted response based on the model was positively correlated ($r = 0.54$; $P = .0005$) with the observed response (Fig 3C). Of the 42 patients predicted to have a reduction in tumor size as best response, 38 did have an observed reduction (positive predictive value = 0.90); whereas 6 of 13 tumors predicted to increase in size actually grew (negative predictive value = 0.46).

Impact of RRM1 and ERCC1 Levels on Survival

We analyzed if RRM1 or ERCC1 protein levels would be associated with patients' survival using an optimal cut point method. The median OS for the 30 patients with RRM1 levels below 32.1 was 8.2 months (95% CI, 5.1 to 13.6), and it was 5.6 months (95% CI, 4.1 to 7.9) for the 39 patients with RRM1 levels above 32.1 (Fig 4A). The median OS for the 46 patients with ERCC1 levels below 65.0 was 6.8 months (95% CI, 5.2 to 10.8), and it was 3.4 months (95% CI, 2.6 to 8.1) for the 19 patients with ERCC1 levels above this value (Fig 4B).

Table 2. Toxicity Summary

Toxicity	Occurrences by Grade											
	Gemcitabine and Carboplatin (n = 79)						Gemcitabine (n = 81)					
	2		3		4		2		3		4	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Hematologic												
Neutropenia	9	11.4	29	36.7	17	21.5	6	7.4	7	8.6	2	2.5
Anemia	19	24.1	12	15.2	0	0.0	19	23.5	5	6.2	1	1.2
Thrombocytopenia	5	6.3	14	17.7	21	26.6	3	3.7	3	3.7	0	0.0
Febrile neutropenia	—	—	0	0.0	0	0.0	—	—	0	0.0	0	0.0
Nonhematologic												
Fatigue	19	24.1	3	3.8	0	0.0	15	18.5	13	16.0	2	2.5
Dyspnea	9	11.4	12	15.2	1	1.3	9	11.1	15	18.5	1	1.2
Anorexia	11	13.9	1	1.3	0	0.0	14	17.3	2	2.5	0	0.0
Nausea	9	11.4	0	0.0	0	0.0	8	9.9	2	2.5	0	0.0
Pneumonia	0	0.0	5	6.3	0	0.0	2	2.5	6	7.4	1	1.2
Dehydration	4	5.1	1	1.3	0	0.0	4	4.9	4	4.9	0	0.0
Diarrhea	3	3.8	0	0.0	0	0.0	4	4.9	3	3.7	0	0.0
Vomiting	4	5.1	0	0.0	0	0.0	3	3.7	2	2.5	0	0.0
Pleural effusion	1	1.3	4	5.1	0	0.0	1	1.2	2	2.5	0	0.0
Dizziness	4	5.1	1	1.3	0	0.0	2	2.5	0	0.0	0	0.0
Rash	3	3.8	0	0.0	0	0.0	1	1.2	0	0.0	0	0.0
Chest pain	0	0.0	2	2.5	0	0.0	2	2.5	1	1.2	1	1.2
Alopecia	2	2.5	—	—	—	—	0	0.0	—	—	—	—

These differences were not statistically significant ($P = .16$ for RRM1; $P = .19$ for ERCC1; P values adjusted for multiple looks). We did also not find a statistically significant difference in PFS for RRM1 or ERCC1 between patients with high and low expression levels (median

PFS, 4.0 and 3.0 months for low and high RRM1, $P = .31$; median PFS, 3.6 and 2.9 months for low and high ERCC1, $P = .53$). A Cox regression analysis also produced statistically nonsignificant associations between survival and RRM1 or ERCC1.

Table 3. RRM1 and ERCC1 Protein Levels by Clinical Characteristics

Characteristic	Protein Level					
	RRM1 (n = 69)			ERCC1 (n = 65)		
	Median	Range	P	Median	Range	P
Age, years			.552			.650
≥ 75.0	33.4	8.5-105.6		34.7	5.2-131.3	
< 75.0	34.2	5.3-90.1		35.4	10.1-102.3	
Sex			.722			.401
Male	36.1	5.3-105.6		34.4	5.2-127.6	
Female	33.2	8.6-93.5		47.3	11.5-131.3	
Histology			.570			.893
Adeno	36.9	5.3-105.6		39.9	5.2-131.3	
Squamous	23.7	9.9-93.5		34.1	6.0-104.9	
Large cell and NOS	34.1	14.7-90.1		27.9	11.5-127.6	
Stage			.312			.029
IV	35.1	5.3-105.6		34.4	5.2-127.6	
IIIB	27.2	14.8-36.9		69.7	51.3-131.3	
Treatment			.196			.610
Gemcitabine and carboplatin	32.9	6.4-105.6		36.7	12.8-131.3	
Gemcitabine	39.1	5.3-90.1		33.4	5.2-127.6	
Best measurable response category by CT			.023			.402
PR	22.9	5.3-81.4		34.6	10.1-84.7	
SD	39.0	8.5-105.6		34.1	5.2-131.3	
PD	42.2	15.2-89.4		51.8	18.7-102.3	

NOTE. Wilcoxon rank sum test (with Van der Waerden quantile normal scores) for two groups and Kruskal-Wallis test for three groups. The χ^2 rank test P value for associations of best response with RRM1 was $< .01$, and it was $> .10$ for best response with ERCC1.²⁷

Abbreviations: NOS, not otherwise specified; CT, computed tomography; PR, partial response; SD, stable disease; PD, progressive disease.

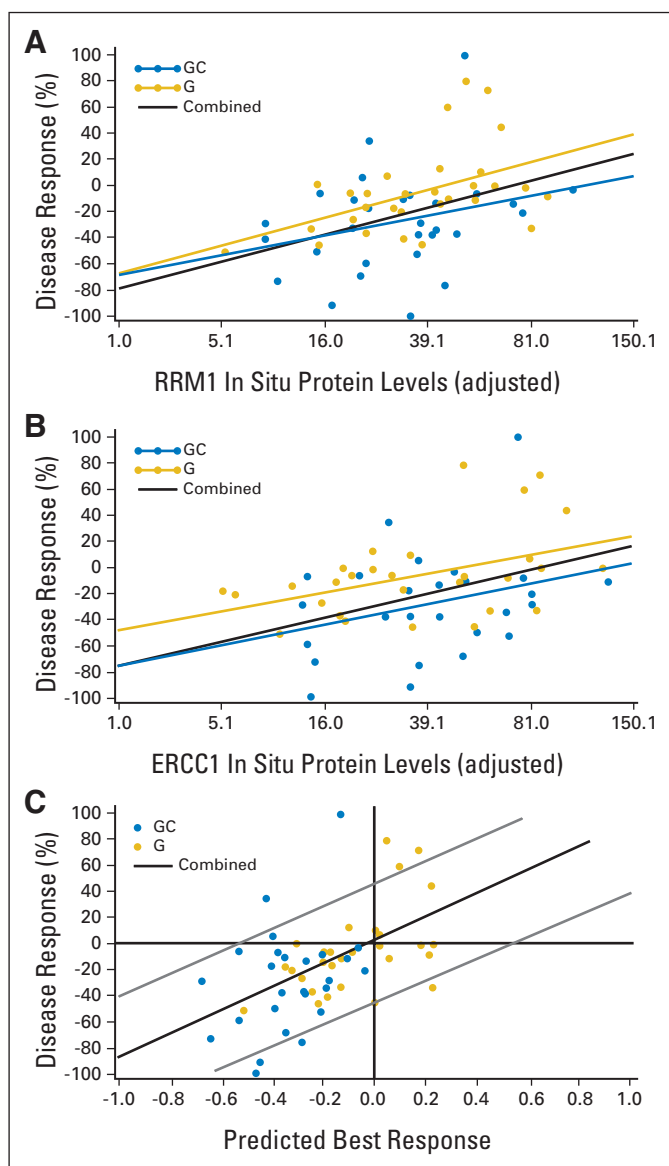


Fig 3. (A) RRM1 in situ protein levels, (B) ERCC1 in situ protein levels, and (C) predicted tumor response by combining both markers and treatment arm into a model displayed on the x-axis in relation to tumor response displayed on the y-axis (negative values denote tumor shrinkage and positive values tumor growth; [gold circle] treatment with gemcitabine [G]; [blue circle] G and carboplatin [GC]; [black line] both combined: [A] $\rho = -0.41$, $P = .001$; [B] $\rho = -0.39$, $P = .003$; [C] $\rho = 0.54$, $P = .0005$).

DISCUSSION

The primary objective of the clinical trial was to demonstrate superior OS for gemcitabine and carboplatin over gemcitabine in patients with advanced NSCLC and PS 2. Although there was a trend toward longer survival in the group of patients treated with gemcitabine and carboplatin compared with gemcitabine, this difference was not statistically significant. We thus conclude that single-agent chemotherapy remains the standard of care for this group of patients. However, the possibility exists that this result may be spurious. Reasons may be early closure of the trial and a higher than anticipated survival in the single-agent treatment arm.

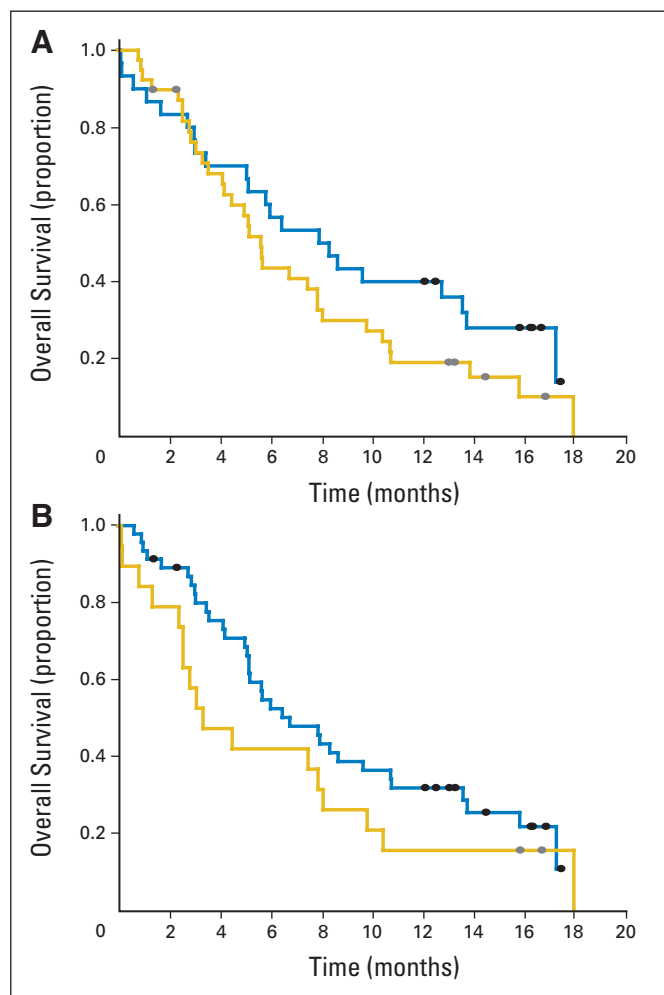


Fig 4. (A) Differential overall survival for patients dichotomized into high and low groups using the optimal RRM1 level of 32.1 as cut point ($P = .16$; log-rank test with Miller-Siegmund adjustment).²⁰ (B) Differential survival for patients dichotomized into high and low groups using the optimal ERCC1 level of 65.0 as cut point ($P = .19$; log-rank test with Miller-Siegmund adjustment).²⁰ (gold line) high protein levels; (blue line) low protein levels.

We based our a priori survival estimates on a published subgroup analysis of a randomized phase III trial that reported a median OS of 2.4 months (Cancer and Leukemia Group B 9730) in 50 patients with PS 2 treated with single-agent paclitaxel.⁵ This estimate was clearly too low as evidenced by our result (5.1 months), a prior single-arm trial of gemcitabine in a similar group of patients (4.8 months),²¹ a PS 2 subgroup analysis of a randomized phase III Italian trial (3.5 to 4.2 months in 86 patients treated with single-agent vinorelbine or gemcitabine),²² and data from a randomized phase II trial in PS 2 patients showing a median OS of 4.8 months for single-agent gemcitabine.²³ It is conceivable that the survival of patients enrolled in trials specifically designed for those with PS 2 may be better than the survival observed in PS 2 subgroup patients from a trial open to patients with PS 0 to 2 as a result of a biased interpretation of the PS classification by enrolling physicians.

It is noteworthy that the survival of patients with PS 2 receiving dual-agent therapy was approximately 2 months longer than the survival of patients receiving single-agent therapy in the three referenced

studies^{5,22,23} and our study, which suggests that a lack of statistical significance in the OS differences may have been caused by the small sample sizes.

Another objective of the trial was to evaluate the predictive utility of RRM1 and ERCC1 protein levels on tumor response to gemcitabine and gemcitabine and carboplatin. Both molecules are promising predictive markers because of their demonstrated impact on gemcitabine and platinum efficacy in experimental models.^{9,24} Two earlier clinical investigations reported a significant association between *RRM1* mRNA levels and disease response.^{9,24} Both studies had small patient numbers, 35 and 18 respectively, and both utilized specimen procurement conditions that required a sophisticated infrastructure. Two additional studies had reported a significantly better survival for patients with low *RRM1* mRNA levels compared with patients with high levels.^{8,10} Similarly, low *ERCC1* mRNA levels were associated with disease response to platinum-based therapy in lung, ovarian, and colorectal cancer.^{7,9,25,26} Finally, high tumoral *ERCC1* levels were associated with lack of benefit from adjuvant cisplatin-based therapy in patients with resected NSCLC.¹¹

Our data demonstrate that in situ RRM1 and ERCC1 protein levels in tumor specimens collected under routine conditions in community oncology practices are significantly predictive of disease response in patients with advanced NSCLC. The result that ERCC1 protein levels are predictive of response to gemcitabine alone can be explained by the highly significant association between ERCC1 and RRM1 levels.^{8-10,15} In addition, the mechanism of gemcitabine cytotoxicity is not only mediated through targeting RRM1, but also involves direct interference with DNA synthesis through incorporation of gemcitabine, a cytosine analog.²⁷ It is thus possible that ERCC1, through its role in nucleotide excision repair, may impact on gemcitabine efficacy. In addition, we found that the integration of both markers into a model for response prediction was significantly correlated with the observed disease response, having a positive predictive value of 0.90.

The lack of association of RRM1 levels with patients' age, sex, and histology is consistent with prior reports.^{9,10,15} Likewise, most studies found no significant associations between ERCC1 levels and these parameters.^{7,9,10,15} However, Olaussen et al¹¹ reported that ERCC1 levels were significantly higher in older patients, men, and squamous cell carcinomas. This discrepancy is possibly explained by the relatively low number of patients included in our study (65 patients with ERCC1 levels) and the other reported studies (between 35 and 184 patients) compared with the study by Olaussen et al (761 patients).

We conclude that single-agent chemotherapy remains the standard of care in patients with poor PS NSCLC. We further conclude that RRM1 and ERCC1 are predictive markers of gemcitabine and gemcitabine and carboplatin efficacy, and that a determination of protein expression levels of both markers in routinely collected spec-

imens from patients with advanced NSCLC in community oncology practices is feasible in approximately half of all patients without requiring a change practice patterns. Future trials that use gemcitabine or a platinum-agent should incorporate RRM1 and ERCC1 expression levels into the analysis plan. In addition, large prospective trials that further validate and refine the predictive utility of both markers are desirable.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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