Ribonucleotide Reductase Messenger RNA Expression and Survival in Gemcitabine/Cisplatin-Treated Advanced Non-Small Cell Lung Cancer Patients

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ABSTRACT

Purpose: **No chemotherapy regimen, including the widely used combination of gemcitabine/cisplatin, confers significantly improved survival over any other in metastatic non-small cell lung cancer (NSCLC); however, the selection of patients according to key genetic characteristics can help to tailor chemotherapy. Ribonucleotide reductase subunit M1 (RRM1) is involved in DNA synthesis and repair and in gemcitabine metabolism, and the excision repair cross-complementing group 1 (*ERCC1*) gene has been related to cisplatin activity.**

Experimental Design: **Patients were part of a large randomized trial carried out from September 1998 to July 2000, comparing gemcitabine/cisplatin *versus* gemcitabine/cisplatin/vinorelbine *versus* gemcitabine/vinorelbine followed by vinorelbine/ifosfamide. We analyzed RRM1 and ERCC1 mRNA expression in paraffin-embedded samples obtained from bronchoscopy by real-time quantitative reverse transcription-PCR. Results were correlated with survival using the Kaplan-Meier method.**

Results: **A total of 100 patients were assessed. There was a strong correlation between RRM1 and ERCC1 mRNA expression levels (Spearman $r = 0.410$; $P < 0.001$). In the gemcitabine/cisplatin arm, patients with low RRM1 mRNA expression levels had significantly longer median survival than those with high levels [13.7 *versus* 3.6 months; 95% confidence interval (CI), 9.6–17.8 months; $P = 0.009$]. Median survival was also significantly longer among patients with low mRNA expression levels of both *RRM1* and *ERCC1* (not reached), than among those with high levels of both genes (6.8 months; 95% CI, 2.6–11.1 months; $P = 0.016$).**

Conclusions: RRM1 **mRNA expression is a crucial predictive marker of survival in gemcitabine/cisplatin-treated patients. Genetic testing of *RRM1* mRNA expression levels can and should be used to personalize chemotherapy.**

INTRODUCTION

The prognosis of advanced non-small cell lung cancer (NSCLC) is dismal. A recent Eastern Cooperative Oncology Group trial of 1155 patients showed no differences among cisplatin/paclitaxel, cisplatin/gemcitabine, cisplatin/docetaxel, and carboplatin/paclitaxel. Overall median time to progression was 3.6 months, and median survival was 7.9 months (1). Although clear pharmacogenetic guidelines for personalized chemotherapy have not yet been exactly defined, the assessment of abnormalities in DNA repair pathways and enzymes involved in gemcitabine metabolism can help to distinguish between patients who are sensitive or resistant to gemcitabine or cisplatin.

Ribonucleotide reduction is the reaction whereby ribonucleotides, the precursors of RNA synthesis, are reduced to form deoxyribonucleotides, the precursors of DNA synthesis (2). Ribonucleotide reductase converts ribonucleotide 5′-diphosphate to deoxyribonucleotide 5′-diphosphate, which is essential for DNA synthesis (Fig. 1). Gemcitabine, an analogue of deoxycytidine (2′,2′-difluorodeoxycytidine), is phosphorylated to the 5′-monophosphate form by deoxycytidine kinase. Subsequent phosphorylation by uridylate-cytidylate monophosphate kinase generates difluorodeoxycytidine 5′-diphosphate, which interferes with the function of ribonucleotide reductase and reduces the pool of deoxyribonucleotide 5′-diphosphate available for DNA synthesis (Ref. 3; fig. 1). Although *RRM1* is a tumor suppressor gene (4), up-regulation of human *RRM1* has been observed during DNA repair after chemotherapy damage (5). Overexpression of ribonucleotide reductase was observed in a gemcitabine-resistant human oropharyngeal carcinoma KB clone (6) and in a gemcitabine-resistant human leukemic cell line K562 (7).

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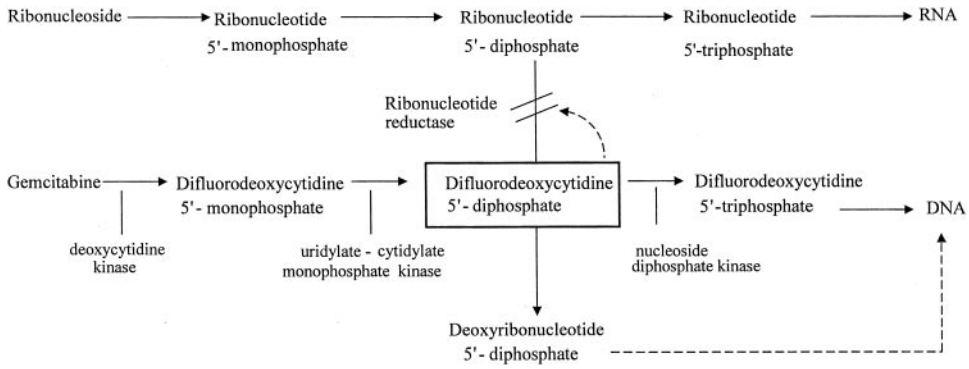

Fig. 1 Gemcitabine and cisplatin DNA damage. Gemcitabine metabolism competes with ribonucleotide 5′-diphosphate for incorporation into DNA. Ribonucleotide reductase converts ribonucleotide 5′-diphosphate to deoxyribonucleotide 5′-diphosphate, which is essential for DNA synthesis. Gemcitabine, an analogue of deoxycytidine (2′,2′-difluorodeoxycytidine), is phosphorylated to the 5′-monophosphate form by deoxycytidine kinase. Subsequent phosphorylation by uridylate-cytidylate monophosphate kinase generates difluorideoxycytidine 5′-diphosphate, which interferes with the function of ribonucleotide reductase and reduces the pool of deoxyribonucleotide diphosphate available for DNA synthesis. Overexpression of ribonucleotide reductase abrogates gemcitabine depletion of deoxyribonucleotide diphosphate, leading to efficient DNA synthesis and repair.

Table 1 Clinical and genetic characteristics[a]

Characteristic	All patients	Gemcitabine plus cisplatin	Gemcitabine plus cisplatin plus vinorelbine	Gemcitabine plus vinorelbine–vinorelbine plus ifosfamide
No. of patients	81	21 (25.9)	31 (38.3)	29 (35.8)
Age, yr (range)	59 (37–75)	61 (45–75)	57.4 (37–75)	60.3 (48–73)
Sex				
Male	77 (95.1)	21 (100)	30 (96.8)	26 (89.7)
Female	4 (4.9)		1 (3.2)	3 (10.3)
Performance status				
0–1	63 (77.8)	16 (76.2)	24 (77.4)	23 (79.3)
2	18 (22.2)	5 (23.8)	7 (22.6)	6 (20.7)
Weight loss >5%	34 (42)	8 (38.1)	15 (48.1)	11 (37.9)
Histology				
Adenocarcinoma	39 (48.1)	10 (47.6)	13 (41.9)	16 (55.2)
Squamous cell carcinoma	39 (48.1)	10 (47.6)	16 (51.6)	13 (44.8)
Large cell carcinoma	3 (3.7)	1 (4.8)	2 (6.5)	—
Stage				
IIIB	12 (14.8)	5 (23.8)	5 (16.1)	2 (6.9)
IV	69 (85.2)	16 (76.2)	26 (83.9)	27 (93.1)
Metastatic site				
Liver	15 (18.5)	5 (23.8)	5 (16.1)	5 (17.2)
Lung	35 (43.2)	9 (42.9)	14 (45.2)	12 (41.4)
Bone	22 (27.2)	3 (14.3)	8 (25.8)	11 (37.9)
Brain	4 (4.9)			4 (13.8)
Adrenal	20 (24.7)	3 (14.3)	9 (45)	8 (27.6)
Skin	6 (7.4)	3 (14.3)	2 (6.5)	1 (3.4)
Lymph node	9 (11.1)	3 (14.3)	3 (9.7)	3 (10.3)
Others	8 (9.9)	3 (14.3)	3 (9.7)	2 (6.9)
ERCC1[b] mRNA, median (range)	1.6 (0.6–10.7)	1.5 (0.6–3.9)	1.7 (0.6–10.7)	1.6 (0.7–4.3)
RRM1 mRNA, median (range)	1.1 (0.3–6.9)	1.3 (0.3–3.5)	1.1 (0.3–6.9)	0.9 (0.3–4.9)
ERCC1 mRNA[b]				
≤1.7	45 (55.6)	13 (61.9)	15 (48.4)	17 (58.6)
>1.7	36 (44.4)	8 (38.1)	16 (51.6)	12 (41.4)
RRM1 mRNA[c]				
≤1.4	46 (63)	12 (60)	17 (63)	17 (65.4)
>1.4	27 (37)	8 (40)	10 (37)	9 (34.6)
No. of chemotherapy cycles, median (range)	5 (1–7)	5 (1–6)	4 (1–7)	5 (1–7)

[a] Numbers in parentheses indicate percentages except when specified as a range.
[b] ERCC1, excision repair cross-complementing group 1; RRM1, ribonucleotide reductase subunit M1.
[c] Possible discrepancies between numbers of patients may occur because PCR was not amplified in some instances.

The nucleotide excision repair (NER) pathway is involved in eliminating both cisplatin DNA adducts and nucleotides damaged by UV-irradiation, and the resulting gaps are filled with precursors for DNA synthesis provided by RRM1 (8). ERCC1 is a NER component that participates in DNA damage recognition and DNA strand incision. The inhibition of ERCC1 expression by antisense approaches has been reported to reduce cisplatin-DNA adduct repair (9). In human cell lines and in mouse organs, ERCC1 has been reported to be transcribed at low constitutive levels (10), and in human ovarian and gastric cancer, elevated *ERCC1* mRNA expression in tumor tissue has been related to cisplatin resistance (11, 12). Overexpression of ERCC1 has also been associated with resistance to cisplatin in tumor cell lines (13) and to nitrogen mustards in chronic lymphocytic leukemia (14). We analyzed the relative mRNA expression levels of ERCC1/β-actin by real-time quantitative reverse-transcription-PCR in 56 advanced NSCLC patients treated with gemcitabine/cisplatin, and we found significantly longer median survival in patients with low *ERCC1* mRNA expression levels (15 months) than in those with higher levels (5 months) (15).

To clarify the influence of RRM1 and ERCC1 on survival in patients treated with gemcitabine/cisplatin, we analyzed *RRM1* and *ERCC1* mRNA expression in tumors from 100 advanced NSCLC patients who were part of a larger randomized trial (16), and we compared gemcitabine/cisplatin *versus* gemcitabine/cisplatin/vinorelbine *versus* sequential doublets of gemcitabine/vinorelbine followed by vinorelbine/ifosfamide.

PATIENTS AND METHODS

Patients. A total of 100 tumor samples were analyzed to determine *RRM1* and *ERCC1* mRNA expression levels. They were obtained from 100 NSCLC patients derived from a group of 557 patients who were treated as part of a multicenter, randomized study (16) between September 1998 and July 2000. This study was carried out in accordance with the institutional review board at each participating institution, and written informed consent was obtained from all of the patients. The genetic analysis was carried out between July and September 2002, at which time 493 of 557 patients had died. For this reason, institutional review boards were informed that the genetic analysis was considered to be minimal-risk research, which would not affect the treatment administered and did not require a renewal of patient consent. Eligibility criteria for the randomized study included a histologically or cytologically confirmed diagnosis of advanced NSCLC with malignant pleural effusion or metastatic disease and the absence of prior chemotherapy. Patients were randomized to receive a maximum of six cycles of one of the following: cisplatin 100 mg/m^2 on day 1 plus gemcitabine 1250 mg/m^2 on days 1 and 8, every 21 days; triplets of cisplatin 100 mg/m^2 on day 1 plus gemcitabine 1000 mg/m^2 and vinorelbine 25 mg/m^2 on days 1 and 8, every 21 days; or sequential doublets of gemcitabine 1000 mg/m^2 and vinorelbine 30 mg/m^2 on days 1 and 8 for three cycles followed by vinorelbine 30 mg/m^2 on days 1 and 8 plus ifosfamide 3 g/m^2 on day 1 for three cycles.

Laboratory Methods. Malignant cells were selectively procured from serial microdissected tissue sections of 10 μm stained with nuclear Fast Red. RNA isolation from paraffin-embedded specimens was performed according to a proprietary procedure (United States patent number 6,248,535). After RNA isolation, cDNA was prepared from each sample as described previously (17). Relative cDNA quantitation for *RRM1*, *ERCC1*, and an internal reference gene (β-*actin*) was done using a fluorescence-based, real-time detection method (ABI PRISM 7700 Sequence Detection System; TaqMan; Applied Biosystems, Foster City, CA), as described previously (17). Primers and probe sequences and PCR conditions used for gene expression analysis have previously been described in detail (15, 17). The GenBank accession numbers are L10342 for *RRM1* human genomic DNA and NT_028310 for a genomic clone (contig) on chromosome 11. The GenBank accession numbers are M13194 for *ERCC1* human genomic DNA and NT_011109 for a genomic clone (contig) on chromosome 19. The threshold cycle (C_T) was the fractional cycle number at which the fluorescence generated by cleavage of the probe exceeded a fixed level above baseline. The relative amount of tissue target mRNA standard-

Table 2 Survival and time to progression according to biological markers

	Median survival			Median time to progression		
	Mo	95% CI[a]	*P*	Mo	95% CI	*P*
Gemcitabine plus cisplatin						
RRM1						
≤1.4	13.7	9.6–17.8	0.009	8.4	5.4–11.3	0.020
>1.4	3.6	0–8.1		2.7	2.5–2.9	
ERCC1						
≤1.7	13.7	0.3–27	0.190	8.4	4.5–12.2	0.070
>1.7	9.5	6.3–12.8		5.1	1–9.3	
RRM1 and *ERCC1*						
≤1.4 and ≤1.7	NR		0.020	11	4.2–17.8	0.030
>1.4 and >1.7	6.8	2.6–11.1		2.7	2.6–2.9	
Others	8.3	0–21.7		5.1	3.3–6.9	
Gemcitabine plus cisplatin plus vinorelbine						
RRM1						
≤1.4	11.1	4.9–17.3	0.820	7.1	4.3–9.9	0.890
>1.4	11	0–24.7		7.1	0–16.2	
ERCC1						
≤1.7	10.6	3–18.2	0.870	7.1	3.1–11.1	0.830
>1.7	11	6.7–15.2		7.1	0.9–13.4	
RRM1 and *ERCC1*						
≤1.4 and ≤1.7	11.1	3.4–18.7	0.290	7.1	3.4–10.8	0.630
>1.4 and >1.7	18.9	3.3–34.5		10.8	1.4–20.1	
Others	3.2	0–12.8		2.2	0–8.8	
Gemcitabine plus vinorelbine followed by vinorelbine plus ifosfamide						
RRM1						
≤1.4	8.8	4.9–12.7	0.780	6	4.3–7.7	0.760
>1.4	6.9	3.9–9.9		5.1	3.8–6.3	
ERCC1						
≤1.7	6.9	2.5–11.3	0.530	5.5	3.9–7	0.840
>1.7	7.5	0–15.9		5.1	2.8–7.4	
RRM1 and *ERCC1*						
≤1.4 and ≤1.7	8.8	4.3–13.3	0.740	5.5	3.6–7.4	0.090
>1.4 and >1.7	5.9	0.9–14		4.6	0–9	
Others	7.5	5–6.7		9.9	0–20.4	

[a] CI, confidence interval; NR, not reached; RRM1, ribonucleotide reductase subunit M1; ERCC1, excision repair cross-complementary group 1.

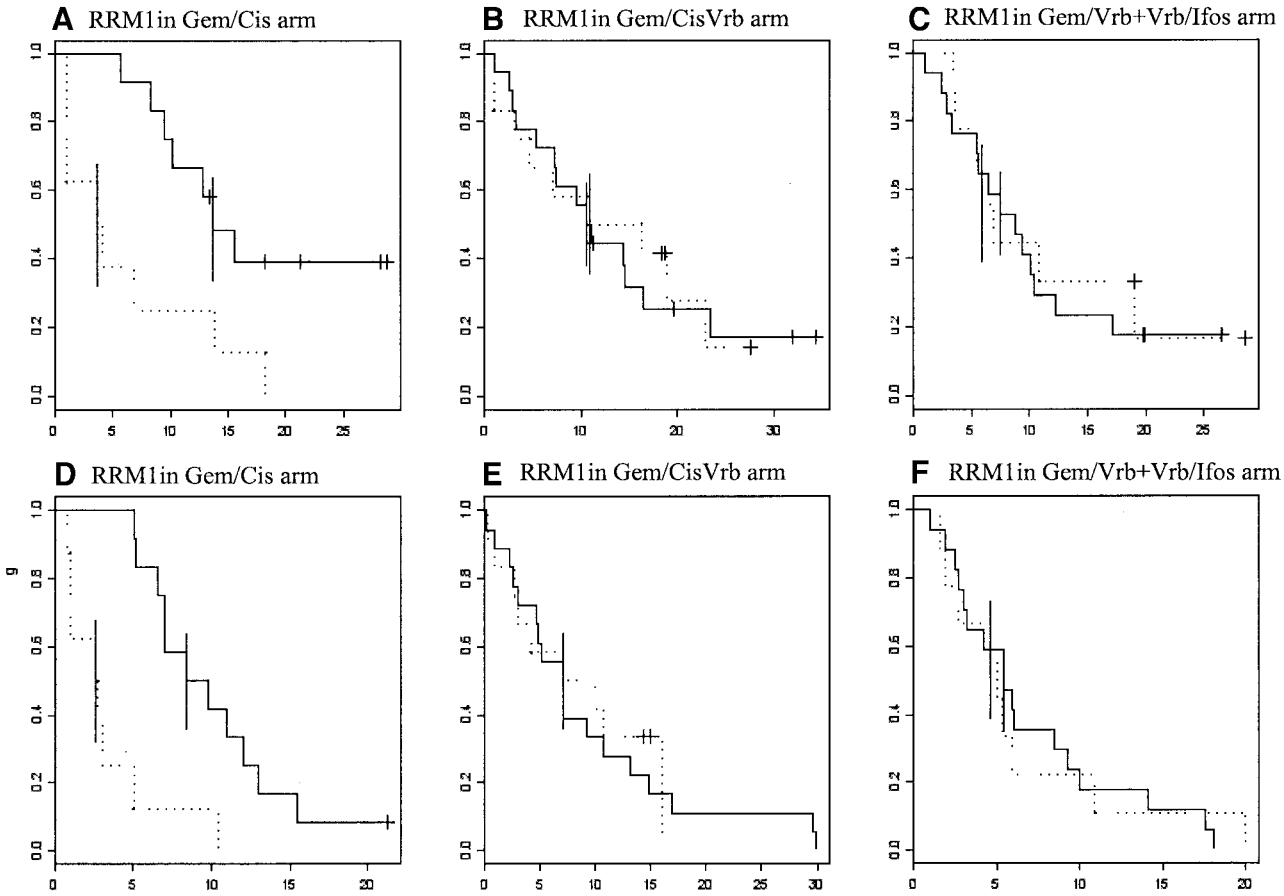

Fig. 2 Kaplan-Meier estimates of survival and time to progression according to ribonucleotide reductase subunit M1 (*RRM1*) mRNA expression, as measured by quantitative PCR. *A*, survival for patients treated with gemcitabine plus cisplatin. *B*, survival for patients treated with gemcitabine plus cisplatin plus vinorelbine. *C*, survival for patients treated with gemcitabine plus vinorelbine followed by vinorelbine plus ifosfamide. *D*, time to progression for patients treated with gemcitabine plus cisplatin. *E*, time to progression for patients treated with gemcitabine plus cisplatin plus vinorelbine. *F*, time to progression for patients treated with gemcitabine plus vinorelbine followed by vinorelbine plus ifosfamide. *Vertical lines*, the SE of survival probability.

ized against the amount of β-*actin* mRNA was expressed as $-\Delta C_T = -(C_{T(\text{target gene-1})} - C_{T(\beta\text{-}actin)})$. The ratio of the number of target mRNA copies to the number of β-*actin* mRNA copies was then calculated as $2^{-\Delta CT} \times K$, where K is a constant. Significant contamination with genomic DNA was excluded by amplifying non-reverse-transcribed RNA.

Statistical Analysis. Quantitative PCR analyses yield values that are expressed as ratios between two absolute measurements (gene of interest:internal reference gene). The maximal χ^2 method of Halpern was adapted to determine which cutoff value best dichotomized patients into low-expression and high-expression *RRM1* and *ERCC1* subgroups. Proportions were compared by use of Fisher's exact test. The Kaplan-Meier method was used to calculate survival and time to progression, and the Brookmeyer-Crowley method was used for the 95% CI. Vertical lines on survival curves indicate the SE of survival probability. Univariate analyses of survival and time to progression according to levels of RRM1 and ERCC1 and other factors (age, disease stage, performance status, and chemotherapy) were performed using a two-sided log-rank test. Because of the small sample size, bootstrap-like simulations were used to confirm results obtained from the log-rank test; *P*s were determined for the statistical differences in median survival values when comparing *RRM1* and *ERCC1* mRNA expression levels. Multiple logistic-regression analysis was used to determine risk factors for time to progression. Spearman correlation coefficients were calculated to assess associations between the mRNA expression levels of *RRM1* and *ERCC1*. Differences of $P \leq 0.05$ were considered statistically significant. Calculations were performed with the SPSS software package, version 10.0.5 (SPSS Inc, Chicago, IL). Bootstrap analyses and graphics were performed with the S Plus 6 statistical package.

RESULTS

Patient Characteristics. A total of 81 of 100 patients were assessable for *RRM1* and *ERCC1* mRNA expression levels. The remaining patients were not quantifiable because of fibrotic or necrotic tumor tissue. The clinical characteristics of patients in the three arms were similar (Table 1). The median

age was 59 years; 77 were male; 77% of patients had a performance status of 1. Forty-two % of patients had a weight loss of more than 5% in the previous 6 months. Eighty-five % had stage IV disease. Forty-three % had lung, 27% bone, 24% adrenal, and 18% liver metastases; other metastatic sites were observed at lower frequencies. Median survival for all 81 patients was 10.2 months; survival rate at 1 year was 41.3% and at 2 years was 18%. Median survival was 12.8 months in the gemcitabine/cisplatin arm, 11.1 months in the gemcitabine/cisplatin/vinorelbine arm, and 7.5 months in the sequential doublet arm ($P = 0.600$). Median time to progression was 7 months for the gemcitabine/cisplatin arm, 9 months for the gemcitabine/cisplatin/vinorelbine arm, and 5 months for the sequential doublet arm ($P = 0.030$).

Biological markers. The median *RRM1* mRNA expression relative to the housekeeping gene β-*actin* was 1.1×10^{-3} (minimum expression, 0.3×10^{-3}; maximum expression, 6.9×10^{-3}). (From here to the end of the article, values will be given without "$\times 10^{-3}$"). The median *ERCC1* mRNA expression was 1.6 (minimum, 0.6; maximum, 10.7). There were no differences in median gene expression among the treatment arms (Table 1). There was a significant association between *RRM1* and *ERCC1* mRNA expression (Spearman *r*, 0.410; $P < 0.001$). With a cutoff of 1.4, 46 patients (63%) had low *RRM1* levels, and 27 patients (37%) had high *RRM1* levels. With a cutoff of 1.7, 45 patients (55.6%) had low *ERCC1* levels, and 36 patients (44.4%) had high *ERCC1* levels. Similar levels of gene expression were observed across the three treatment arms.

Clinical Outcome According to Biological Markers. Table 2 shows median survival and time to progression in the three treatment arms according to biological markers. Median survival was significantly longer in the gemcitabine/cisplatin arm among patients with low *RRM1* levels. Median survival was 13.7 months (95% CI, 9.6–17.8 months) for patients with low levels and 3.6 months (95% CI, 0–8.1 months) for those with high levels ($P = 0.009$; Fig. 2*A*). There was a tendency towards longer median survival and time to progression in the gemcitabine/cisplatin arm among patients with low *ERCC1* levels, although the difference was not statistically significant. Median survival was 13.7 months (95% CI, 0.3–27 months) for patients with low levels and 9.5 months (95% CI, 6.3–12.8 months) for those with high levels ($P = 0.190$; Fig. 3*A*). Figure 4 shows

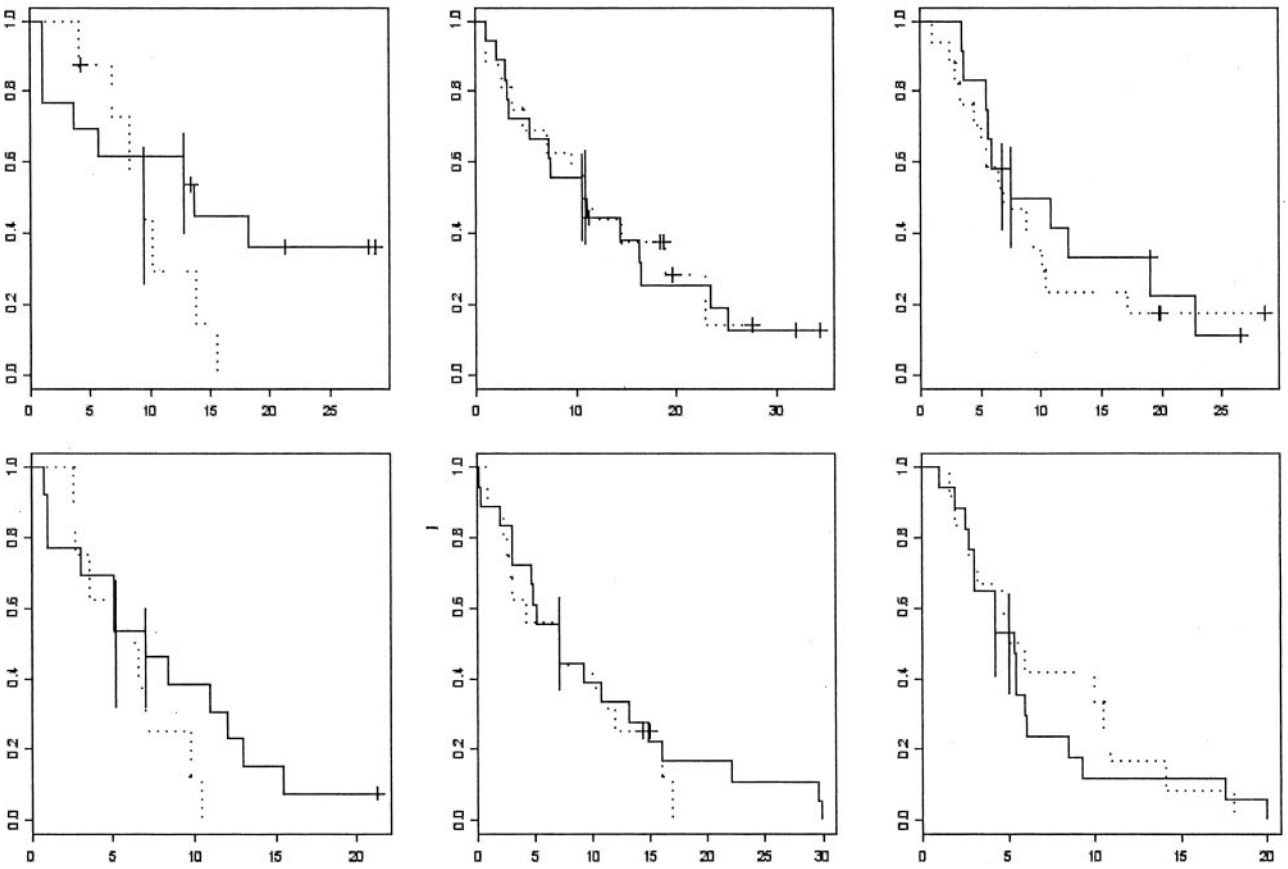

Fig. 3 Kaplan-Meier estimates of survival and time to progression according to excision repair cross-complementing 1 (*ERCC1*) mRNA expression, as measured by quantitative PCR. *A*, survival for patients treated with gemcitabine plus cisplatin. *B*, survival for patients treated with gemcitabine plus cisplatin plus vinorelbine. *C*, survival for patients treated with gemcitabine plus vinorelbine followed by vinorelbine plus ifosfamide. *D*, time to progression for patients treated with gemcitabine plus cisplatin. *E*, time to progression for patients treated with gemcitabine plus cisplatin plus vinorelbine. *F*, time to progression for patients treated with gemcitabine plus vinorelbine followed by vinorelbine plus ifosfamide. *Vertical lines*, the SE of survival probability.

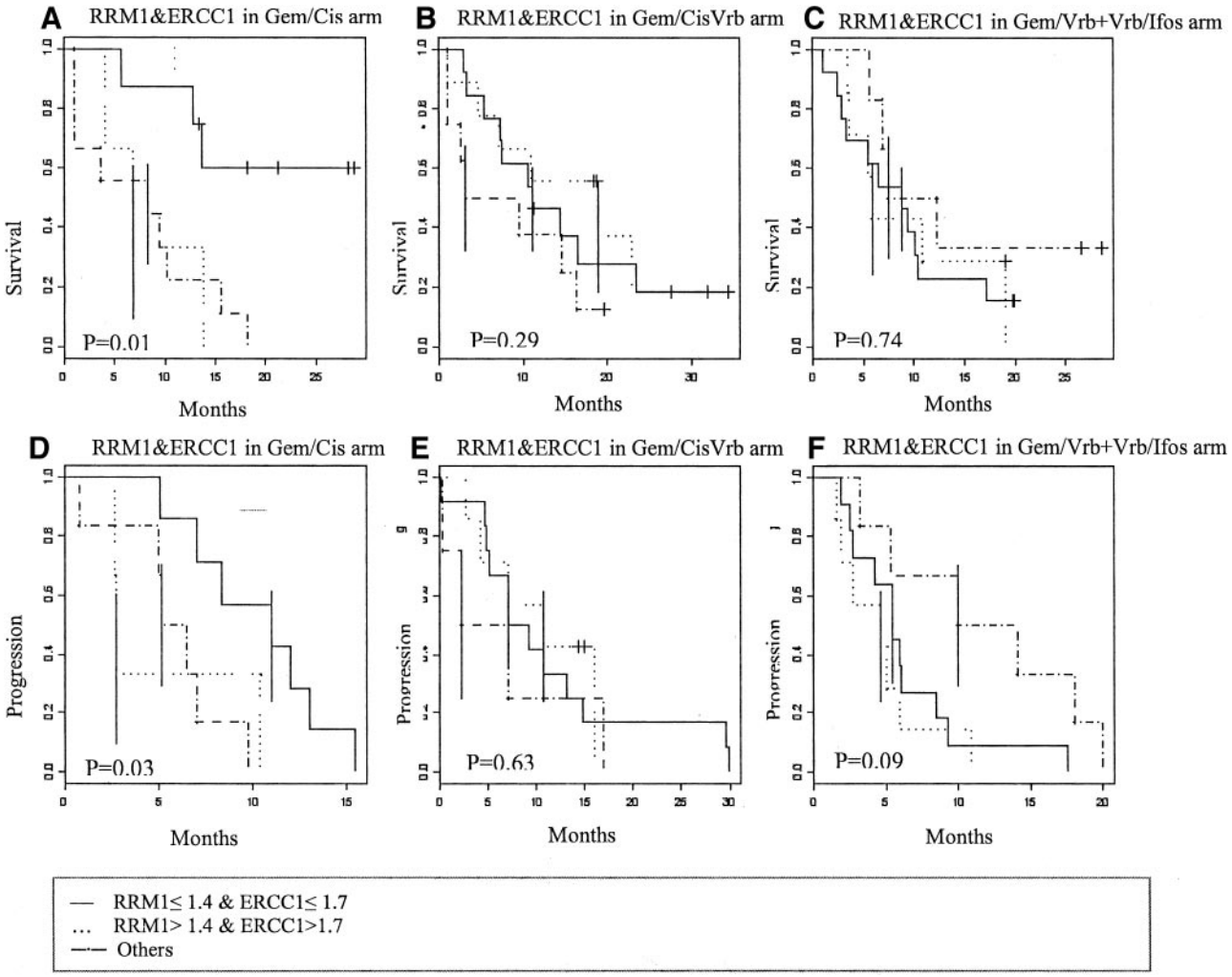

Fig. 4 Kaplan-Meier estimates of survival and time to progression according to ribonucleotide reductase subunit M1 (*RRM1*) and excision repair cross-complementing 1 (*ERCC1*) mRNA expression, as measured by quantitative PCR. *A*, survival for patients treated with gemcitabine plus cisplatin. *B*, survival for patients treated with gemcitabine plus cisplatin plus vinorelbine. *C*, survival for patients treated with gemcitabine plus vinorelbine followed by vinorelbine plus ifosfamide. *D*, time to progression for patients treated with gemcitabine plus cisplatin. *E*, time to progression for patients treated with gemcitabine plus cisplatin plus vinorelbine. *F*, time to progression for patients treated with gemcitabine plus vinorelbine followed by vinorelbine plus ifosfamide. *Vertical lines*, the SE of survival probability.

median survival and time to progression for each of the treatment arms according to levels of both *RRM1* and *ERCC1*. Median survival was significantly longer in the gemcitabine/cisplatin arm among patients with low levels of both *RRM1* and *ERCC1*. Median survival has not been reached for these patients, whereas patients with high levels of both genes had a median survival of 6.8 months (95% CI, 2.6–11.1 months). Patients with low levels of one gene and high levels of the other had a median survival of 8.3 months (95% CI, 0–21.7 months; $P = 0.020$; Table 2; Fig. 4*A*). No other significant differences in median survival were observed according to gene expression levels (Table 2; Figs. 2–4).

Bootstrap-like simulations showed that low levels of either *RRM1* or *ERCC1* improved survival in patients treated with gemcitabine/cisplatin (*RRM1*, $P = 0.004$; *ERCC1*, $P = 0.001$). However, in patients treated with gemcitabine/cisplatin/vinorelbine, there was no difference in survival according to either *RRM1* or *ERCC1* levels (RRM1, $P = 0.835$; *ERCC1*, $P = 0.321$).

Significant differences in time to progression were observed only in the gemcitabine/cisplatin arm. Median time to progression was significantly longer in the gemcitabine/cisplatin arm among patients with low *RRM1* levels. Median time to progression was 8.4 months (95% CI, 5.3–11.3 months) for patients with low levels and 2.7 months (95% CI, 2.5–2.9 months) for those with high levels ($P = 0.020$; Fig. 2*D*). No significant differences in time to progression were observed in the other two treatment arms according to *RRM1* levels (Table 2; Fig. 2, *E* and *F*). Time to progression in the gemcitabine/cisplatin arm was 8.4 months (95% CI, 4.5–12.2 months) for patients with low *ERCC1* levels and 5.1 months (95% CI, 1–9.3 months) for those with high levels ($P =$

0.070; Fig. 3*D*). No significant differences were observed according to *ERCC1* levels in the other treatment arms (Table 2; Fig. 3, *B*, *C*, *E*, and *F*). Time to progression was significantly longer in the gemcitabine/cisplatin arm among patients with low levels of both *RRM1* and *ERCC1*. Time to progression for these patients was 11 months (95% CI, 4.2–17.8 months), whereas patients with high levels of both genes had a time to progression of 2.7 months (95% CI, 2.6–2.9 months). Patients with low levels of one gene and high levels of the other had a time to progression of 5.1 months (95% CI, 3.3–6.9 months) ($P = 0.030$; Table 2; Fig. 4*D*). No other significant differences were observed according to combined *RRM1* and ERCC1 *levels* (Table 2; Fig. 4, *B*, *C*, *E*, and *F*).

The logistic regression analysis of factors predictive of disease progression indicated that high *RRM1* levels increased the risk of disease progression 4-fold for patients treated with gemcitabine/cisplatin (odds ratio, 4.3; 95% CI, 1.4–13.1; $P = 0.009$). However, the addition of vinorelbine to this combination significantly decreased the risk of disease progression for patients with high *RRM1* levels (odds ratio, 0.25; 95% CI, 0.09–0.73; $P = 0.01$).

DISCUSSION

Pooled data on older randomized trials of cisplatin-based chemotherapy *versus* supportive care showed a hazard ratio of 0.73 in favor of chemotherapy, but with only a slight absolute improvement in 1-year survival of 10% (from 5% to 15%) and an increase in median survival of 1.5 months (18). However, in more recent randomized trials, paclitaxel, gemcitabine, docetaxel, or vinorelbine in combination with a platinum compound have shown an absolute improvement of 15–20% for chemotherapy over best supportive care. One-year survival for best supportive care was 11–17% *versus* 30–35% for chemotherapy, and median survival improved by 3–4 months (19, 20). Although no single chemotherapy combination has demonstrated a clear overall superiority to any other in survival benefit (1, 21) or in quality of life (21) in metastatic NSCLC, Fossella *et al.* (22) have recently shown an increase in median survival with docetaxel/cisplatin over vinorelbine/cisplatin.

We have previously observed that overexpression of *ERCC1* (15) or *RRM1* (23) is each separately correlated with strikingly shorter survival in gemcitabine/cisplatin (but not in vinorelbine/cisplatin)-treated stage IV NSCLC patients (23). The present study is the first to report both a high correlation between *ERCC1* and *RRM1* mRNA levels and a specific effect of *RRM1* expression on survival in gemcitabine/cisplatin-treated stage IV NSCLC.

The first suggestions of a relation between dysregulation of gene expression and differential cisplatin sensitivity are now emerging. Up-regulation of *BRCA1*, which is involved in transcription-coupled NER (24), confers cisplatin resistance (25). An association between the degree of *BRCA1* expression and taxane sensitivity in an inducible breast cancer model cell line has also been reported (26), and decreased *BRCA1* mRNA expression enhanced cisplatin sensitivity and resistance to antimicrotubule agents (27). Collectively, these preclinical and clinical findings indicate that antimicrotubule/cisplatin combinations could be active in NER-efficient tumors (those with high *RRM1* levels). This hypothesis should be tested in tailored chemotherapy trials, in both early and advanced NSCLC, in which *RRM1* could be used as a surrogate of NER functional status. Patients with low *RRM1* levels could receive gemcitabine/cisplatin, whereas those with higher *RRM1* levels could receive an antimicrotubule/cisplatin combination.

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