Low ERCC1 Expression Correlates with Prolonged Survival after Cisplatin plus Gemcitabine Chemotherapy in Non-Small Cell Lung Cancer

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ABSTRACT

Purpose: Overexpression of the excision repair cross-complementing 1 (ERCC1) gene, which is crucial in the repair of cisplatin (CDDP)-DNA adducts, is reported to negatively influence the effectiveness of CDDP-based therapy for gastric and ovarian cancers. Recent evidence indicates that Gemcitabine (Gem) may modulate ERCC1 nucleotide excision repair activity, and down-regulation of DNA repair activity by ERCC1 antisense RNA reportedly inhibits synergism of CDDP/Gem. We investigated whether ERCC1 mRNA expression levels were associated with clinical outcomes after treatment with a combination Gem/CDDP regimen for patients with advanced stage non-small cell lung cancer (NSCLC).

Experimental Design: Response and survival were correlated with the level of ERCC1 expression in 56 patients with advanced (stage IIIb or IV) NSCLC treated as part of a multicenter randomized trial with Gem 1250 mg/m² days 1 and 8 plus CDDP 100 mg/m² on day 1 every 3 weeks. mRNA was isolated from paraffin-embedded pretreatment primary tumor specimens, and relative expression levels of ERCC1/β-actin were measured using a quantitative reverse transcription-PCR (Taqman) system.

Results: ERCC1 expression was detectable in all tumors. There were no significant differences in ERCC1 levels by gender, age, performance status, weight loss, or tumor stage. The overall response rate was 44.7%. There were no significant associations between ERCC1 expression and response. Median overall survival was significantly longer in patients with low ERCC1 expression tumors (61.6 weeks; 95% confidence interval, 42.4–80.7 weeks) compared to patients with high expression tumors (20.4 weeks, 95% confidence interval, 6.9–33.9 weeks). ERCC1 expression, Eastern Cooperative Oncology Group performance status, and presence of weight loss were significant prognostic factors for survival in a Cox proportional hazards multivariable analysis.

Conclusions: These data suggest that ERCC1 expression is a predictive factor for survival after CDDP/Gem therapy in advanced NSCLC. Although there was a trend toward decreased response with high ERCC1 mRNA levels, this difference failed to reach statistical significance. This result may reflect the impact of Gem and the requirement for ERCC1 expression for CDDP/Gem synergism or may be attributable to the relatively small patient sample size in this study. Prospective studies of ERCC1 as a predictive marker for activity of CDDP-based regimens in NSCLC are warranted.

INTRODUCTION

Lung cancer is the leading cause of cancer death in both men and women in many countries, including Spain and the United States. More than 75% of lung cancers are NSCLC. Except for some patients with surgically resectable disease, the prognosis for patients with NSCLC is poor. Platinum-based chemotherapy has been shown to provide survival and quality of life benefits for patients with advanced stage, unresectable NSCLC, but overall 2-year survival rates for this group remain <15% (1, 2).

The abbreviations used are: NSCLC, non-small cell lung cancer; CDDP, cisplatin, cis-diaminedichloroplatinum; Gem, Gemcitabine, 2’,2’-difluorodeoxycytidine; ERCC1, excision repair cross-complementing gene 1; XPA, xeroderma pigmentosum group A protein; EOCG, Eastern Cooperative Oncology Group; SLCG, Spanish Lung Cancer Group; CI, confidence interval.
Pharmacogenetics, the study of genes that influence drug activity and toxicity, offers the possibility of tailoring therapy to the specific genetic profile of individual patients and tumors. A pharmacogenetic approach can thus potentially increase response rates and survival outcomes while decreasing toxicity and overall treatment costs. The cytotoxic effect of the anticancer drug CDDP is principally attributable to the formation of bulky intrastrand platinum-DNA adducts. Removal of these adducts from genomic DNA is mediated by the nucleotide excision repair pathway. ERCC1, a critical element of which for DNA repair can be attenuated by blocking the interaction between ERCC1 protein and the XPA (7), and high ERCC1 expression is associated with resistance to platinum-containing therapy in human ovarian and gastric tumor specimens (8, 9). Cytotoxic synergism has been demonstrated between Gem and CDDP, (10–13), and a higher response rate was found for the combination of Gem plus CDDP compared with a standard CDDP plus etoposide regimen in patients with advanced NSCLC (14). Importantly, this Gem/CDDP synergism has been shown to involve ERCC1 and the nucleotide excision repair pathway; expression of ERCC1 antisense RNA abrogates gemcitabine-mediated cytotoxic synergism with CDDP in vitro in human colon cancer cells defective in mismatch repair but proficient in nucleotide excision repair (15). In this study, we investigated whether these in vitro findings apply in vivo by measuring ERCC1 mRNA levels in primary NSCLC tissues and correlating these with the clinical outcomes for patients treated as part of a prospective randomized trial with a combined Gem/CDDP regimen.

MATERIALS AND METHODS

Patients and Samples. Clinical data were retrieved from the medical and trial database records of patients with advanced NSCLC who were treated with a standardized Gem/CDDP regimen at various hospitals of the SLCG. All patients were enrolled in the Gem/CDDP arm of a prospective multicenter three-arm randomized trial (GECP/98/02), the SLCG Phase III trial of Gem/CDDP versus Gem/CDDP/vinorelbine versus sequential doublets of Gem/vinorelbine followed by ifosfamide/vinorelbine in advanced NSCLC (16). The patients were treated between October 1998 and September 2000. All patients received Gem 1250 mg/m² on days 1 and 8 plus CDDP 100 mg/m² on day 1 every 3 weeks. Eligibility criteria for GECP/98-02 were measurable stage IV (with brain metastases eligible if asymptomatic) or stage IIIB (malignant pleural and/or pericardial effusion and/or supraclavicular adenopathy) NSCLC and ECOG performance score 0–2.

All patients had chest X-ray and a computed tomography scan of the chest and upper abdomen before entry into the study and underwent repeat evaluations at least every 6 weeks. Tumor response was assessed according to WHO criteria as complete response, partial response, stable disease, and progressive disease. Tumors were reassessed during treatment with the same imaging methods used to establish the baseline tumor measurement. All patients gave signed informed consent, and the study was approved by the institutional ethics review boards. Archival primary tumor specimens from each patient were retrieved from the participating SLCG centers after review of the H&E-stained slides.

RNA Isolation and cDNA Synthesis. RNA isolation from paraffin-embedded specimens was done according to a proprietary procedure (US patent number 6,248,535). After RNA isolation, cDNA was prepared from each sample as described previously (17).

Reverse Transcription-PCR Quantification of mRNA Expression. Relative cDNA quantitation for 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 (17–19).

The primers and probe sequences used are given below. In each case, the first primer is the forward PCR primer, the second is the reverse PCR primer, and the third is the Taqman probe: ERCC1, GGGAATTTGGCGACGTATTC, GCGGAGGTGAGGAAACAG, and 6FM (carboxyfluorescein) 5′-CACAGTGCTCTGGCCACACATA-3′ TAMRA (N,N,N′,N′-tetramethyl-6-carboxyxyrhomadine); β-actin, TGAGCGGCTACAGCTT, TCCCTTAATGTCACGAGATTT, and 6FAM5′-ACCACACCGCAGCGC-3′ TAMRA.

The PCR reaction mixture consisted of 600 nM of each primer, 200 nM probe, 2.5 units of AmpliTaq Gold polymerase, 200 μM each dATP, dCTP, dGTP, 400 μM dUTP, 5.5 mM MgCl₂, and 1× Taqman Buffer A containing a reference dye, to a final volume of 25 μl (all reagents were from Applied Biosystems, Foster City, CA). Cycling conditions were 50°C for 10 s, 95°C for 10 min, followed by 46 cycles at 95°C for 15 s and 60°C for 1 min. Colon, liver, and lung RNAs (all from Stratagene, La Jolla, CA) were used as control calibrators on each plate. All gene expression analyses were performed in a blinded fashion with the laboratory investigators unaware of the clinical data.

Statistical Analysis. TaqMan analyses yield values that are expressed as ratios between two absolute measurements (gene of interest/internal reference gene). The Mann-Whitney t test was used to test for significant associations between the continuous test variable ERCC1 expression and dichotomous variables (patient sex, age above and below the median age, presence of weight loss, presence of pleural effusion, and tumor stage). The Kruskal-Wallis test was used to test for significant differences in ERCC1 expressions within multiple groups (ECOG performance status and histology). Fisher’s exact test was used for the analysis of categorical clinicopathological values including response and dichotomized ERCC1 values.

All patients were followed from first study treatment until death or until the data were censored, with the patient considered to be alive as of April 2001. Kaplan-Meier survival curves and the log-rank test were used to analyze univariate distributions for survival and disease-free survival. The maximal χ² method of Miller and Siegmund (20) and Halpern (21) was adapted to determine which expression value best segregated patients into poor and good prognosis subgroups (in terms of likelihood of surviving), with the log-rank test as the statistic used to measure the strength of the grouping. To determine a P that would be interpreted as a measure of the strength of the association based on the maximal χ² analysis, 1000 boot-strap-like simulations were used to estimate the distribution of the
ERCC1 mRNA > median value (6.7)

ERCC1 mRNA < median value (6.7)

Fig. 1 Kaplan-Meier survival curve for patients with intratumoral ERCC1 levels above and below the median ERCC1 level.

levels were significantly higher in squamous cell carcinomas (median, 8.6) compared with adenocarcinomas (median, 5.2; \( P = 0.015 \), Mann-Whitney test).

**Response to Chemotherapy.** The tumor response frequencies for the 47 patients who were evaluable for response are shown in Table 1. The overall response rate was 44.7%. The ERCC1 expression levels in the complete response and partial response, i.e., “responding” tumors (median, 4.3; range, 1.2–24.6) were not significantly different from the levels in the stable disease and progressive disease, i.e., “non-responding” tumors (median, 7.85; range, 0.8–24.3; \( P = 0.31 \), Mann-Whitney test). There were also no significant differences between the proportion of responding and non-responding tumors with ERCC1 values greater and less than any ERCC1 level (all Fisher’s exact test). The response rate in tumors with ERCC1 expression below the median value (“low” expression, 52% responders) was higher than for tumors with ERCC1 expression above the median value (“high” expression, 36.4% responders; Fisher’s exact test, \( P = 0.38 \)).

**Association between Patient Overall Survival and ERCC1 Levels.** The median overall survival time was 36.6 weeks (range, 0–113.4 weeks), and the median time to progression was 24.4 weeks (range, 0–102.9 weeks). Use of the log-rank test and the maximal \( \chi^2 \) statistic to identify ERCC1 levels that segregated patients into poor and good prognosis subgroups showed that the range of discriminatory values included the median value, which was therefore used as the cutoff value for the survival analysis. Fig. 1 shows the Kaplan-Meier survival curve for patients with intratumoral ERCC1 levels above and below the median ERCC1 level. As shown in Table 2, patients with ERCC1 levels below the median had a significantly longer median survival of 61.6 weeks (95% CI, 42.4–80.7 weeks) compared with 20.4 weeks (95% CI, 6.9–33.9 weeks) for patients with ERCC1 levels above the median. Adjusted for tumor stage, the log-rank statistic for the association between low or high ERCC1 expression and overall survival was 3.97, and the \( P \) was 0.046. The unadjusted log-rank results are shown in Table 2.

### RESULTS

**Patient and Tumor Characteristics.** Demographic details on the 56 patients included in the study and tumor stage and cell type details are shown in Table 1. The median number of treatment cycles received was three (range, one to six). Three of the 56 patients had received radiotherapy, and 5 patients had undergone surgical resection of the primary tumor.

**ERCC1 Expression Levels.** ERCC1 mRNA expression was detectable in all 56 samples analyzed. The median ERCC1 expression, relative to the expression of the internal control housekeeping gene \( \beta\)-actin, was \( 6.7 \times 10^{-3} \) (range, \( 0.8 \times 10^{-3} \) to \( 24.6 \times 10^{-3} \); values shown hereafter without \( \times 10^{-3} \), e.g., median, 6.7). Twenty-eight (50%) patients had an ERCC1 level greater than the median, and 50% had a level <6.7. There were no significant associations between ERCC1 levels and any of the factors age (\( P = 0.66 \)), sex (\( P = 0.18 \)), presence of weight loss in the 6 months before randomization (\( P = 0.74 \)), tumor stage (IIIB versus IV; \( P = 0.39 \)), or presence of pleural effusion (\( P = 0.25 \), all Mann-Whitney \( t \) test). There were also no significant differences between the ERCC1 levels among patients with different performance status grades (\( P = 0.48 \), Kruskal-Wallis test) or different tumor cell types (all four tumor types, \( P = 0.10 \), Kruskal-Wallis test), but ERCC1 expression

<table>
<thead>
<tr>
<th>Table 1 Patient characteristics</th>
<th>( n ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>56 (100)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>48 (85.7)</td>
</tr>
<tr>
<td>Female</td>
<td>8 (14.3)</td>
</tr>
<tr>
<td>Age, yr</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>60.5</td>
</tr>
<tr>
<td>Range</td>
<td>32–75</td>
</tr>
<tr>
<td>ECOG performance status</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>13 (23.2)</td>
</tr>
<tr>
<td>1</td>
<td>35 (62.5)</td>
</tr>
<tr>
<td>2</td>
<td>8 (14.3)</td>
</tr>
<tr>
<td>Weight loss</td>
<td>21 (37.5)</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
</tr>
<tr>
<td>IIIB</td>
<td>16 (28.6)</td>
</tr>
<tr>
<td>IV</td>
<td>40 (71.4)</td>
</tr>
<tr>
<td>Pleural effusion</td>
<td>11 (19.6)</td>
</tr>
<tr>
<td>Histopathology</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>30 (53.6)</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>20 (35.7)</td>
</tr>
<tr>
<td>Large cell carcinoma</td>
<td>4 (7.1)</td>
</tr>
<tr>
<td>Unspecified</td>
<td>2 (3.6)</td>
</tr>
<tr>
<td>Response</td>
<td></td>
</tr>
<tr>
<td>Complete response</td>
<td>3 (5.4)</td>
</tr>
<tr>
<td>Partial response</td>
<td>18 (32.1)</td>
</tr>
<tr>
<td>Stable disease</td>
<td>8 (14.3)</td>
</tr>
<tr>
<td>Progressive disease</td>
<td>18 (32.1)</td>
</tr>
<tr>
<td>Not evaluable (^a)</td>
<td>9 (16.1)</td>
</tr>
</tbody>
</table>

\(^a\) Due to early death or malignant pleural effusion.
An ERCC1 cutoff value of 5.8 was tested because this value was shown in a previous study to be associated with overall survival for patients with gastric cancer (9). Overall survival was significantly better for the group of NSCLC patients in this study with ERCC1 levels <5.8 (median, 74.71 weeks; 95% CI, 71.77–77.66 weeks) compared with those with ERCC1 levels ≤5.8 (median, 61.0 weeks; 95% CI, 45.61–76.39 weeks; unadjusted log-rank statistic, 6.37; P = 0.011).

Other factors that were significantly associated with overall survival on univariable analysis using Kaplan Meier survival curves and the log-rank test were the presence of pretreatment weight loss and the ECOG performance status (Table 2). Patient age (P = 0.18), sex (P = 0.87), tumor stage (P = 0.99), tumor cell type (SCC versus adenocarcinoma P = 0.63), and presence of pleural effusion (P = 0.71) were not significant prognostic factors for overall survival. ERCC1 level, ECOG performance status, and weight loss remained significant prognostic factors for survival in the Cox proportional hazards regression model multivariable analysis (Table 2). Ps for a Cox regression model stratified on tumor stage were 0.038 for ERCC1, 0.017 for weight loss and 0.02 for ECOG performance status (performance status 0 versus 1 or 2).

### DISCUSSION

This study found an association between lower ERCC1 mRNA expression levels and improved survival after treatment with a combination Gem/CDDP regimen for patients with advanced stage NSCLC. Experimental studies have shown that high ERCC1 levels are associated with increased removal of CDDP-induced DNA adducts and relative CDDP resistance (5), and ERCC1-defective cells or knockout mice are highly sensitive to DNA cross-linking agents (22, 23). Lee et al. (24) showed that transfecing ERCC1 into a UV repair-deficient [ERCC1(−)] Chinese hamster ovary cell line conferred DNA adduct repair capability and CDDP resistance. These findings suggest that the likely explanation for our results is that intratumoral ERCC1 levels are associated with the effectiveness of CDDP therapy because ERCC1 expression influences ERCC1-mediated DNA adduct repair activity (25).

Confidence in our results is derived from the fact that, although the genetic analysis was performed retrospectively after the trial was closed, the clinical data were collected prospectively under the conditions of a multicenter randomized trial, and the laboratory work was performed in a blinded fashion. Furthermore, other studies have also found an association between lower intratumoral ERCC1 expressions and improved clinical outcomes for patients treated with platinum-containing regimens. Metzger et al. (9) found a significant association between ERCC1 levels and survival after CDDP/5-fluorouracil therapy for patients with gastric cancer. Metzger et al. (9) used a cutoff ERCC1 mRNA expression value of 5.8, which also divided patients in a statistically significant way into good and poor survival arms in our study, although a higher ERCC1 level was a more powerful discriminator. Dabholkar et al. (26) reported that patients with ovarian cancer who were clinically resistant to platinum-based therapy had a statistically significant 2.6-fold higher expression level of ERCC1 in their tumor tissue than patients who responded to that therapy. A further study showed that both ERCC1 and XPAC (the human excision repair gene that corrects the defect in xeroderma pigmentosum group A cells) were important for response to platinum-based chemotherapy in ovarian cancer tissues (8). Other studies have also reported associations between higher ERCC1 expressions and worse clinical outcomes for CDDP-based therapy for esophageal cancer (27, 28) and for oxaliplatin/5-fluorouracil treatment for colorectal cancer (29).

In addition to associations with survival outcomes, several of these studies found associations between ERCC1 expression and chemoresponse (8, 9, 26, 28, 29). These studies included patients treated with CDDP but not with Gem. A significant association with response was not found in our study, but this finding was not unexpected because of the requirement for ERCC1 for CDDP/Gem synergism (15). Despite this requirement, ERCC1 levels remain important, as indicated by the association with survival and the trend toward a lower response rate in patients with high ERCC1 mRNA tumor levels. It is also noteworthy that the 52% response rate in the low ERCC1 group is considerably higher than the 21–40% response rates reported in other CDDP/Gem NSCLC randomized trials (14, 30–32).

Cisplatin- or carboplatin-containing regimens have been considered a standard of care in the therapy of advanced stage

<table>
<thead>
<tr>
<th>Table 2 Factors associated with overall survival</th>
<th>Univariable analysis</th>
<th>Multivariable analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Medians (wk)</strong></td>
<td><strong>Log-rank statistic</strong></td>
<td><strong>P</strong></td>
</tr>
<tr>
<td><strong>ERCC1 expression</strong></td>
<td><strong>Low</strong></td>
<td>62</td>
</tr>
<tr>
<td><strong>High</strong></td>
<td>20</td>
<td></td>
</tr>
<tr>
<td><strong>Weight loss</strong></td>
<td><strong>Absent</strong></td>
<td>46</td>
</tr>
<tr>
<td><strong>Present</strong></td>
<td>14</td>
<td></td>
</tr>
<tr>
<td><strong>ECOG performance status</strong></td>
<td><strong>0</strong></td>
<td>61</td>
</tr>
<tr>
<td><strong>1</strong></td>
<td>31</td>
<td></td>
</tr>
<tr>
<td><strong>2</strong></td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

*ERCC1 expression values are categorized according to whether there was less than the ERCC1 median value of 6.7 ("low expression") or greater than the median ("high expression").
NSCLCs for >15 years (1). Recently, randomized studies have sought to determine whether nonplatinum combinations of newer agents were either more efficacious or less toxic. Results have been inconclusive, suggesting that a therapeutic plateau for currently available chemotherapy has been reached (33–38). Instead, novel therapeutic approaches will likely be required to optimize chemotherapy effectiveness in individual patients, and the use of potential molecular predictors of response and survival in individual NSCLC patients may well become important criteria for chemotherapy selection (39, 40). Recent studies of NSCLC cells or tissues have identified several potentially valuable chemosensitivity markers in addition to ERCC1 (8, 39, 41–43), but validation of these markers is still required. Another potential clinical consequence of our findings is that, as suggested by Li et al. (5), pharmacological approaches that inhibit ERCC1 expression may increase cellular sensitivity to CDDP. UCN-01 (7-hydroxystaurosporine) is a cell cycle checkpoint abrogator that has been shown to inhibit nucleotide excision repair, attenuate the interaction of ERCC1 with XPA (7), and potentiate CDDP cytotoxicity (44). In the present study, the multivariate analyses confirmed the strength of the ERCC1 mRNA levels, which was even more significant than that of performance status. Further validation of these findings could lead to a dramatic change in clinical practice, avoiding unnecessary CDDP chemotherapy in almost half of NSCLC patients. The Preliminary Genotypic International Lung Trial, a prospective randomized trial testing the importance of ERCC1 expression and other factors for CDDP effectiveness in NSCLC patients, is under way.

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