Correlation of *CDA, ERCC1*, and *XPD* Polymorphisms with Response and Survival in Gemcitabine/Cisplatin–Treated Advanced Non–Small Cell Lung Cancer Patients

Carmelo Tibaldi,[1] Elisa Giovannetti,[2] Enrico Vasile,[1] Valentina Mey,[2] Adrie C. Laan,[4] Sara Nannizzi,[2] Roberta Di Marsico,[1] Andrea Antonuzzo,[1] Cinzia Orlandini,[3] Simona Ricciardi,[2] Mario Del Tacca,[2] Godefridus J. Peters,[4] Alfredo Falcone,[1,5] and Romano Danesi[2]

Abstract

Purpose: Selecting patients according to key genetic characteristics may help to tailor chemotherapy and optimize the treatment in non–small cell lung cancer (NSCLC). Polymorphisms at the *xeroderma pigmentosum group D* (*XPD*), *excision repair cross-complementing 1* (*ERCC1*), and *cytidine deaminase* (*CDA*) genes have been associated with alterations in enzymatic activity and may change sensitivity to the widely used cisplatin-gemcitabine regimen.

Experimental Design: Analyses of *CDA, XPD*, and *ERCC1* polymorphisms were done on blood samples of 65 chemotherapy-naïve, advanced NSCLC patients treated with cisplatin-gemcitabine. Furthermore, CDA enzymatic activity was evaluated by high-performance liquid chromatography analysis. Association between XPD Asp^{312}Asn and Lys^{751}Gln, ERCC1 *C*118*T*, and CDA Lys^{27}Gln polymorphisms and response, clinical benefit, toxicity, time to progression (TTP), and overall survival (OS) was estimated using Pearson's χ^2 tests, the Kaplan-Meier method, the log-rank test, and the Cox proportional hazards model.

Results: The CDA Lys^{27}Lys polymorphism significantly correlated with better clinical benefit ($P = 0.04$) and grade $\geq$3 neutropenia and thrombocytopenia, as well as with longer TTP and OS ($P = 0.006$ and $P = 0.002$, respectively), whereas no significant associations were found among ERCC1 and XPD polymorphisms and both response and clinical outcome. Finally, the enzymatic activity assay showed a significant lower mean in subjects harboring the CDA Lys^{27}Lys polymorphism.

Conclusions: Our data suggested the role of CDA Lys^{27}Lys polymorphism as a possible predictive marker of activity, toxicity, TTP, and OS in advanced NSCLC patients treated with cisplatin and gemcitabine. These results may be explained by the lower enzymatic activity associated with the Lys^{27}Lys CDA and offer a potential new tool for treatment optimization.

Lung cancer is the leading cause of cancer-related deaths in Western countries. Non–small cell lung cancer (NSCLC) accounts for >85% of primary lung cancers and approximately two thirds of NSCLC patients are diagnosed at an advanced stage (1).

Pooled data from older randomized trials of cisplatin-based chemotherapy versus best supportive care showed that cisplatin-based chemotherapy was associated with a modest improvement in overall survival (OS; ref. 2). In more recent randomized trials, new cytotoxic drugs such as paclitaxel, docetaxel, vinorelbine, or gemcitabine in combination with a platinum compound have shown an absolute 15% to 20% improvement of survival in favor of chemotherapy versus best supportive care. In particular, the 1-year survival rate for best supportive care was 11% to 17% versus 30% to 35% for chemotherapy, which prolonged median survival by 3 to 4 months (3). However, none of the last-generation doublets was shown to be superior to the others and they all seemed to have reached the therapeutic plateau, with objective response rates of 30% to 40%, median survival time of 8 to 10 months, and 1-year survival rate of 30% to 40% (4). Indeed, a four-arm randomized phase III trial showed no substantial differences in response rate, time to progression (TTP), and OS among paclitaxel (24-hour infusion)–cisplatin, docetaxel-cisplatin, paclitaxel-carboplatin, and gemcitabine-cisplatin combination (4). Therefore, the cisplatin-gemcitabine combination is one of the standard regimens for the treatment of advanced NSCLC (5).

In an attempt to improve activity and efficacy of current regimens, a pharmacogenetic approach has been advocated.

Authors' Affiliations: [1]Division of Oncology, Department of Oncology, Azienda USL-6 of Livorno, Livorno, Italy; [2]Division of Pharmacology and Chemotherapy, Department of Internal Medicine, University of Pisa, and [3]Division of Oncology, Department of Oncology, Pisa, Italy; [4]Department of Medical Oncology, VU University Medical Center, Amsterdam, the Netherlands; and [5]Istituto Tumori Toscano, Firenze, Italy

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Requests for reprints: Carmelo Tibaldi, U.O. Oncologia Medica, Presidio Ospedaliero, Viale Alfieri, 36, 57100 Livorno, Italy. Phone: 39-0586-223458; Fax: 39-0586-223457; E-mail: tiby@katamail.com.

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The main objective of pharmacogenetics is the identification of genotypes involved in clinically meaningful variations in drug responsiveness. Pharmacogenetics may reduce the variation in how individual patients respond to medicines by tailoring therapies to their genetic profile (6).

Cisplatin activity is mediated through the formation of cisplatin-DNA adducts. Removal of these adducts, which leads to chemoresistance, is mainly carried out by the nucleotide excision repair system, which consists of at least 30 identified polypeptides, including excision repair cross-complementing group 1 (ERCC1) and xeroderma pigmentosum group D (XPD) proteins (7). Single nucleotide polymorphisms (SNP) in any of these genes may modulate repair capacity and contribute to individual variations in chemotherapy response.

In vitro analysis showed that the *C/T* SNP at codon 118 of the *ERCC1* gene affected mRNA and protein levels, leading to differential cisplatin sensitivity (8). Furthermore, clinical data suggested a possible correlation of this SNP with clinical outcome and tumor response to platinum-based chemotherapy in advanced colorectal cancer as well as a significant correlation with survival in advanced NSCLC (9–12), whereas a low ERCC1 tumor expression was related to better outcome to adjuvant cisplatin-based chemotherapy in patients with completely resected NSCLC (13).

Two nonsynonymous polymorphisms occurring in XPD, the aspartic acid 312 asparagine ($Asp^{312}Asn$) resulting from a $G \rightarrow A$ substitution in exon 10, and the lysine 751 glutamine ($Lys^{751}Gln$), resulting from $A \rightarrow C$ in exon 23, have been associated with a differential DNA repair efficiency. Homozygous cells for the XPD 312 Asn mutated protein showed higher DNA repair capacity (14), whereas a reduction in DNA repair capacity was observed in NSCLC patients carrying the wild-type genotype for XPD 312 and 751 (15). A significant association was also observed between the wild-type genotype (*A/A*) for XPD 751 and longer OS in metastatic colorectal cancer patients treated with 5-fluorouracil and oxaliplatin (16), whereas significant differences in OS according to the XPD 312 polymorphism were reported in a retrospective study on locally advanced NSCLC patients treated with platinum-based chemotherapy (17).

Other studies in lung cancer patients yielded inconsistent results and showed no significant association between clinical outcome and the *C/C* genotype in codon 118 of ERCC1 (18, 19) and with polymorphisms at codons 312 and 751 in XPD (11, 12, 19, 20). Therefore, the use of genotypic analysis of nucleotide excision repair genes as a predictor of clinical outcome to platinum-based treatments is still controversial and further studies are warranted.

The metabolic inactivation of gemcitabine is catalyzed by cytidine deaminase (CDA), but, thus far, few studies evaluated the pharmacogenetics of this enzyme reporting controversial results. In particular, Gilbert et al. (21) have observed a significant decrease in deamination activity of gemcitabine in a CDA variant characterized by the nonsynonymous polymorphism of lysine 27 glutamine ($Lys^{27}Gln$), resulting from a $A \rightarrow C$ substitution in exon 79, whereas Kirch et al. (22) reported opposite results with cytarabine as the substrate. Moreover, sensitivity to cytarabine was not changed by introducing polymorphic 27Gln CDA into yeast CDA-null mutants (23). Other *in vitro* studies did not show any relationship between CDA activity and sensitivity to gemcitabine or cytarabine (24, 25); only cells with transfected CDA and hence with a very high CDA activity were less sensitive to gemcitabine (26). Hence, CDA might be a key enzyme in the mechanism of inactivation of gemcitabine, but the role of its polymorphism is still controversial and further investigations are warranted.

Taken together, these observations suggest that several SNPs may affect key genes involved in cisplatin and gemcitabine mechanism of action and may influence clinical outcome. Therefore, we retrospectively evaluated the correlations of XPD $Asp^{312}Asn$ and $Lys^{751}Gln$, ERCC1 *C118T* and CDA $Lys^{27}Gln$ polymorphisms on germ line DNA obtained from 65 NSCLC patients receiving gemcitabine-cisplatin with drug response and clinical benefit. Moreover, we studied the correlation between these polymorphisms and TTP, OS, and toxicity.

Patients and Methods

Patient selection criteria. Chemotherapy-naïve patients with histologically or cytologically proven NSCLC and measurable clinical stage IIIB or stage IV disease were eligible if they also met the following criteria: an Eastern Cooperative Oncology Group performance status ≤1, age >18 years, life expectancy >3 months, adequate bone marrow reserve (leukocyte count $>4.0 \times 10^9$/L, platelet count $\geq 100 \times 10^9$/L), adequate liver (bilirubin level ≤1.5 mg/dL, alanine aminotransferase/aspartate aminotransferase <3 times the upper limit of normal), and renal function (creatinine level ≤1.5 mg/dL).

The main exclusion criteria were active infections, concomitant malignancy, or a second primary malignancy, recent myocardial infarction, unstable angina, symptomatic brain metastases, or hypercalcemia. A written informed consent was obtained from each patient. The protocol was approved by the Pisa University Ethics Committee and the trial was conducted according to Good Clinical Practice guidelines and in accordance with the Helsinki declaration of the World Medical Association.

Evaluation criteria. Pretreatment evaluation included medical history, physical examination, assessment of performance status, complete blood cell count with differential routine chemistry, and computed tomography scan of the chest and abdomen.

Assessment of tumor response was carried out by computed tomography scan every three cycles. Responses were assessed using Response Evaluation Criteria in Solid Tumors (27). The best overall response for each patient was reported and all responses were reviewed by an independent radiologist and had to be confirmed 28 days or more after the initial documentation of response.

Hematologic and nonhematologic toxicities were recorded at day 1 and 8 of every treatment course. The worst toxicity grade for each patient in all chemotherapy cycles was reported. Toxicities were assessed using National Cancer Institute common terminology criteria (version 3.0).

The analysis of the samples was done in a blinded fashion relative to the clinical outcome.

Treatment. The chemotherapy regimen consisted of gemcitabine 1,200 mg/m^2 administered i.v. over 30 min on day 1 and 8, and cisplatin 80 mg/m^2 infused over 60 min given on day 1, every 3 wk for a maximum of six courses. Treatment was discontinued in case of disease progression, major toxicities, or according to the patient's or physician's decision.

Sample collection and DNA isolation. Genomic DNA was extracted from blood samples (5 mL) drawn from an antecubital vein before drug administration, using the QIAamp DNA mini Kit (Qiagen). DNA yields and integrity were checked by absorbance at 260 nm with an Uvikon-940 spectrophotometer (Kontron), whereas testing for contamination by proteins was done by measuring absorbance at 280 nm and calculating the 260/280 ratio.

SNP genotyping. The ERCC1 C118*T*, XPD Asp^{312}Asn, XPD Lys^{751}Gln, and CDA Lys^{27}Gln polymorphisms were studied with Taqman probe–based assays using the ABI PRISM 7900HT instrument equipped with the Sequence Detection System version 2.0 software (Applied Biosystems). Forward and reverse primers and probes (Applied Biosystems SNP Genotyping Assays products) were obtained using the File Builder version 1.0 software, on the basis of Genbank database, and sequences are available upon request. The PCR reactions were done using 20 ng of genomic DNA diluted in 11.875 μL DNase-RNase–free water, 12.5 μL of TaqMan Universal PCR Master Mix with AmpliTaq Gold, and 0.625 μL of the assay mix (forward and reverse specific primers and the specific probes), in a total volume of 25 μL. After thermal cycling, the 7900HT instrument determined the allelic content of each sample in the plate by reading the generated fluorescence.

Analysis of CDA enzymatic activity in blood samples. CDA activity was measured in blood samples from 60 of the 65 treated patients enrolled in the study, as described previously (24). RBC were lysed by mixing 10 μL RBC suspension with 100 μL double deionized water, left on ice for 20 min, and centrifuged for 10 min at 20,000 × *g*, 4°C. The supernatant was used as a crude cytoplasmic extract containing the CDA enzyme. For determination of the enzyme activity, 20 μL of this extract were mixed with 170 μL buffer [50 mmol/L β-mercaptoethanol in 0.1 mol/L Tris-HCl (pH 8.0)] and the substrate gemcitabine (final concentration, 250 μmol/L). The reaction mixture was incubated at 37°C for 15 and 30 min to ensure that enzyme activity was in a linear range; after incubation, the reaction was terminated with 50 μL of 40% trichloroacetic acid (final concentration, 8%), centrifuged, and the supernatant was neutralized with 400 μL trioctylamine/1,1,2-trichloro-trifluoroethane (1:4) and ready for high-performance liquid chromatography analysis. A previously described slightly modified reversed-phase ion pair high-performance liquid chromatography method using an Aqua C18 (Phenomenex) column with PIC B7 (Waters Chromatography) plus 3.5% acetonitrile (pH 2.8), flow 1 mL/min, was used for quantification of the product, dFdU. The system consisted of a Gynkotek pump (model 480, Dionex Gmbh), an automatic injection system Midas (Spark Holland), and a Gynkotek UVD 170U fixed wavelength detector (Dionex) set at 254 and 280 nm. Calibration lines of dFdU were prepared in water processed similarly as the samples in the range of 0.5 to 200 μm. Peak areas were quantified using the Chromeleon data acquisition software version 6.7 (Dionex). The high-performance liquid chromatography method was linear ($r^2 > 0.99$) over the analytic range and the limit of quantification (0.5 μmol/L) corresponded to the lower limit of the calibration curve. Retention times of gemcitabine and dFdU were 7.1 and 13.5 min, respectively. dFdU formation was normalized for protein concentration, measured with the Bradford assay (Sigma).

Statistical analysis. Demographic and clinical information were compared across genotype using Pearson's χ^2 tests (for categorical variables). To enhance the statistical power of analyses and in agreement with a previous study (28), we defined the patients achieving complete response or partial response as "responders," and the patients with stable disease or progressive disease as "nonresponders." Additional analyses were done by grouping patients with complete response, partial response, and stable disease (defined as "patients with clinical benefit") versus patients with progressive disease ("patients without clinical benefit").

TTP was calculated from the date of registration to the date of clinical and/or radiological evidence of progression or death, whichever occurred first, whereas OS was calculated from the day of treatment start to the end point (death or censoring). The Kaplan-Meier method was used to plot TTP and OS, and the log-rank test was used to compare curves in univariate analysis.

Factors included in univariate analysis were genotypes, sex (male versus female), performance status (0 versus 1), age (≤70 versus >70), clinical stage (IIIB versus IV), histology (adenocarcinoma including bronchioalveolar versus squamous and large cell and unspecified NSCLC), and smoking history (never versus former and current smokers). The prognostic variables of OS in univariate analysis were included in multivariate analysis, using the Cox proportional hazards model to identify factors of independent significance. In multivariate analysis, a step-down procedure was used based on the likelihood ratio test, and hazard ratios were calculated to estimate the magnitude and the direction of the effect.

Data were analyzed using SPSS/PC+11.5 statistical software (SPSS, Inc.). Statistical significance was set at $P < 0.05$.

Results

Patient characteristics and treatment. From November 2004 to February 2007, a total of 65 consecutive Caucasian patients, affected by advanced NSCLC, were enrolled. The majority of patients had stage IV disease (75.4%), whereas 16 (24.6%) had stage IIIB disease (Table 1).

Genotype information. For the ERCC1 codon 118 polymorphism, the frequencies of *T/T*, *C/T*, and *C/C* genotypes were 27.7%, 60.0%, and 12.3%, respectively. Although the *T/T* genotype has been termed the "variant" by convention, in several previous studies (8, 18) this genotype has had higher frequencies and has been used as reference group in all following analysis. The wild-type XPD 751 polymorphism (Lys/Lys) had a frequency of 30.8%, whereas the heterozygous Lys/Gln and homozygous Gln/Gln variants had a frequency of 47.7% and 21.5%, respectively. In terms of XPD 312

Table 1. Clinical characteristics of the NSCLC patients

	No. patients (%)
No. patients	65
Median age, y (range)	65 (44-77)
<70	57 (87.7)
≥70	8 (12.3)
Sex	
Male	51 (78.5)
Female	14 (21.5)
Smoking history	
Smokers	53 (81.5)
Never smokers	12 (18.5)
Clinical stage	
IIIB	16 (24.6)
IV	49 (75.4)
ECOG PS	
0	25 (38.5)
1	40 (61.5)
Histology	
Adenocarcinoma	24 (36.9)
Squamous	19 (29.2)
Large cells	4 (6.2)
Bronchoalveolar	1 (1.5)
Unspecified NSCLC	17 (26.2)
Metastatic sites (stage IV patients)	
Bone	20 (40.8)
Brain	10 (20.4)
Liver	6 (12.2)
Contralateral lung	21 (42.8)
Adrenal gland	5 (10.2)
Lymph nodes	5 (10.2)
Skin	2 (4.1)

Abbreviation: ECOG PS, Eastern Cooperative Oncology Group performance status.

Table 2. ERCC1, XPD, and CDA polymorphisms

Genotype	No. patients (%)	Allelic frequencies
ERCC1 C118T		C 0.42 T 0.58
C/C	8 (12.3)	
C/T	39 (60)	
T/T	18 (27.7)	
XPD Lys^{751}Gln		A(Lys) 0.55 C(Gln) 0.45
Lys/Lys	20 (30.8)	
Lys/Gln	31 (47.7)	
Gln/Gln	14 (21.5)	
XPD Asp312Asn		G(Asp) 0.57 A(Asn) 0.43
Asp/Asp	21 (32.3)	
Asp/Asn	32 (49.2)	
Asn/Asn	12 (18.5)	
CDA Lys^{27}Gln		A(Lys) 0.64 C(Gln) 0.36
Lys/Lys	27 (41.5)	
Lys/Gln	29 (44.6)	
Gln/Gln	9 (13.9)	

polymorphism, the wild-type (Asp/Asp) variant was found in 32.3% of cases, whereas the heterozygous Asp/Asn and Asn/Asn variant were observed in 49.2% and 18.5% of cases, respectively. The distribution of all these polymorphisms and the allelic frequencies are shown in Table 2, which also shows the frequencies of Lys/Lys, Lys/Gln, and Gln/Gln CDA genotypes (41.5%, 44.6%, and 13.9%, respectively).

All polymorphisms followed Hardy-Weinberg's equilibrium and genotype frequencies for ERCC1 118, XPD 751, and XPD 312 polymorphisms were comparable with those reported in previous studies in a Caucasian population of NSCLC patients (12, 18–20), whereas the frequencies of CDA 27 were similar to those observed in a control Caucasian population (29).

No significant correlations were detected between genotype and age, performance status, smoking status, gender, histology, or clinical stage.

Correlation between polymorphisms, response to chemotherapy, and clinical benefit. The overall response rate of the 65 patients enrolled in this study was 36.9% (Table 3). No significant correlations were observed between ERCC1 and XPD genotypes and objective response. A trend toward significant correlation was shown between response to cisplatin-gemcitabine chemotherapy and CDA genotype: 51.9% of the patients carrying the CDA Lys/Lys genotype experienced partial response, whereas only 31.0% of CDA Lys/Gln and 11.1% of CDA Gln/Gln patients responded to therapy ($P = 0.06$). By grouping patients with clinical benefit versus patients without clinical benefit, we observed a significant correlation between both ERCC1 and CDA genotype and clinical benefit. In particular, 92.6% of the patients carrying the CDA Lys/Lys genotype experienced a clinical benefit versus 75.9% of CDA Lys/Gln and 55.6% of CDA Gln/Gln patients ($P = 0.04$). Similarly, 94.4% of the patients carrying the ERCC1 *T/T* genotyping experienced a clinical benefit versus 79.5% of ERCC1 *C/T* and 50.0% of ERCC1 *C/C* patients ($P = 0.03$).

Correlation between polymorphisms and clinical outcome. Table 4 shows TTP and OS analysis data according to the examined polymorphisms. Overall median TTP was 6.1 months (95% confidence interval, 3.9-8.2 months), with a median TTP of 8.4 months for patients carrying the CDA 27 Lys/Lys polymorphism, compared with 5.0 and 3.3 months for those with Lys/Gln and Gln/Gln genotype (log-rank test, $P = 0.006$). The median OS was 13.0 months (95% confidence interval, 8.5-17.5 months); the difference in the median OS between patients carrying the CDA wild-type genotype (17.0 months) and those carrying the Lys/Gln (13.6 months) and Gln/Gln (4.1 months) variant was also statistically significant (log-rank test, $P = 0.002$).

The univariate analysis using Kaplan-Meier survival curves and the log-rank test also revealed that performance status and patient age were significantly associated with OS (Table 5), whereas clinical stage ($P = 0.18$), sex ($P = 0.83$), smoking history ($P = 0.58$), and histology ($P = 0.66$) were not significant prognostic factors. The Cox proportional hazards regression

Table 3. Response and clinical benefit according to ERCC1, XPD, and CDA genotype

Genotype	PR	SD+PD	*P*	PR+SD	PD	*P*
	No. patients (%)	No. patients (%)		No. patients (%)	No. patients (%)	
ERCC1 C118T						
C/C	2 (25.0)	6 (75.0)		4 (50.0)	4 (50.0)	
C/T	16 (41.0)	23 (59.0)	0.65	31 (79.5)	8 (20.5)	0.03
T/T	6 (33.3)	12 (66.7)		17 (94.4)	1 (5.6)	
XPD Lys^{751}Gln						
Lys/Lys	9 (45.0)	11 (55.0)		17 (85.0)	3 (15.0)	
Lys/Gln	13 (41.9)	18 (58.1)	0.30	26 (83.9)	5 (16.1)	0.25
Gln/Gln	2 (14.3)	12 (85.7)		9 (64.3)	5 (35.7)	
XPD Asp^{312}Asn						
Asp/Asp	9 (42.9)	12 (57.1)		18 (85.7)	3 (14.3)	
Asp/Asn	11 (34.4)	21 (65.6)	0.82	26 (81.2)	6 (18.8)	0.44
Asn/Asn	4 (33.3)	8 (66.7)		8 (66.7)	4 (33.3)	
CDA Lys^{27}Gln						
Lys/Lys	14 (51.9)	13 (48.1)		25 (92.6)	2 (7.4)	
Lys/Gln	9 (31.0)	20 (69.0)	0.06	22 (75.9)	7 (24.1)	0.04
Gln/Gln	1 (11.1)	8 (88.9)		5 (55.6)	4 (44.4)	

Abbreviations: PR, partial response; SD, stable disease; PD, progressive disease.

Table 4. TTP and OS according to ERCC1, XPD, and CDA genotypes

Genotype	Median TTP, mo (95% CI)	*P*	Median OS, mo (95% CI)	*P*
ERCC1 C118T				
C/C	3.3 (1.3-5.2)	0.09	8.8 (5.9-11.6)	0.37
C/T	7.5 (4.8-10.2)		16.7 (6.3-26.9)	
T/T	6.1 (3.7-8.4)		13.0 (6.9-19.1)	
XPD $Lys^{751}Gln$				
Lys/Lys	6.1 (2.0-10.1)	0.38	10.0 (8.1-11.9)	0.62
Lys/Gln	7.0 (4.0-9.9)		13.0 (5.8-20.1)	
Gln/Gln	5.0 (1.1-8.9)		13.6 (9.7-17.5)	
XPD $Asp^{312}Asn$				
Asp/Asp	6.1 (3.1-9.0)	0.72	10.0 (8.1-11.8)	0.78
Asp/Asn	7.0 (3.2-10.8)		12.6 (5.3-19.8)	
Asn/Asn	5.0 (2.5-5.0)		13.6 (0.0-29.0)	
CDA $Lys^{27}Gln$				
Lys/Lys	8.4 (6.7-10.2)	0.006	17.0 (5.9-28.1)	0.002
Lys/Gln	5.0 (3.2-6.8)		13.6 (5.0-22.1)	
Gln/Gln	3.3 (2.7-4.0)		4.1 (2.1-6.1)	

model used for the subsequent multivariate analysis confirmed the prognostic significance of CDA 27 genotype and age (Table 5).

No significant differences in TTP or OS were observed with respect to ERCC1 118, XPD 312, and XPD 751 genotypes.

Toxicity analysis. A significant difference in severe neutropenia was observed according to CDA genotype, with grade ≥3 neutropenia in 48.1% of homozygous patients for the wild-type CDA 27 polymorphism (Lys/Lys), in contrast to 10.3% of those who were Lys/Gln heterozygotes and 22.2% of those carrying the Gln/Gln variant ($P = 0.006$). Moreover, a higher percentage of thrombocytopenia grade ≥3 was reported for carriers of the CDA 27 Lys/Lys genotype (33.3%) with respect to carriers of Lys/Gln and Gln/Gln genotype (6.9% and 11.1%, respectively, $P = 0.03$).

No other significant differences in toxicity were found with respect to the other polymorphisms (Table 6).

Correlation between CDA genotype and enzymatic activity. The analysis of CDA activity was done in 60 of the 65 gemcitabine-cisplatin patients evaluable for response. In this population, the frequencies of CDA 27 Lys/Lys, Lys/Gln, and Gln/Gln genotypes were 38.3%, 46.7%, and 15.0%, respectively. The mean enzymatic activity in CDA $Lys^{27}Lys$ patients was 10,424.7 ± 1,957.4 pmol/h/mg proteins, whereas in patients carrying the $Lys^{27}Gln$ and the $Gln^{27}Gln$ genotype mean CDA activity was 17,745.5 ± 2,386.1 pmol/h/mg proteins. Statistical analysis with the unpaired Student's *t* test revealed a significant difference ($P = 0.018$) between patients harboring the wild-type and carriers of Lys/Gln and Gln/Gln genotypes. A significant difference ($P = 0.048$) was also detected between mean CDA activity in patients with clinical benefit (13,527.02 ± 1,938.9 pmol/h/mg) versus patients with progressive disease (20,588.1 ± 3,166.4 pmol/h/mg proteins).

Discussion

A pharmacogenetic approach to customize the chemotherapy treatment according to individual genetic characteristics or to genetic markers of cancer tissue represents a modern and intriguing challenge (6). Assessing germ line genetic polymorphisms as either predictive or prognostic markers is very appealing, especially in the advanced cancer setting. In this setting, diagnosis is usually done from small-needle biopsy samples, and tumors are either not resected or resected after neoadjuvant therapy, so that the handling of tumor material can be problematic. SNPs are inherited genetic variants harbored by all the cells of the body, and, although a genotype represents a static value unable to change in response to a different situation, such as exposure to chemotherapy, and it may not reflect changes in tumor DNA, such as loss of heterozygosity, previous studies showed no differences in SNPs analyzed in tumor and normal tissues (30). Moreover, their analysis can be easily done in blood tissue and is easier to adopt in the routine

Table 5. Factors associated with OS

	Median survival, mo (95% CI)	Univariate analysis		Multivariate analysis	
		Log-rank statistic	*P*	Hazard ratio (95% CI)	*P*
CDA $Lys^{27}Gln$					
Lys/Lys	17.0 (5.9-28.1)	12.7	0.002	1 (Reference)	0.03
Lys/Gln	13.6 (5.0-22.1)			1.3 (0.6-3.1)	
Gln/Gln	4.1 (2.1-6.1)			3.8 (1.4-10.7)	
Age, y (range)					
<70	13.6 (11.3-15.9)	6.9	0.009	1 (Reference)	0.03
≥70	7.0 (1.3-12.7)			3.5 (1.1-10.6)	
Performance status					
0	17.0 (12.3-21.7)	6.4	0.01	1 (Reference)	0.07
1	9.0 (6.6-11.4)			2.3 (0.9-5.6)	

Table 6. Grade 3 to 4 toxicity according to SNP genotype (percentage of patients)

Genotype	Neutropenia	*P*	Thrombocytopenia	*P*	Anemia	*P*	Nonhematologic	*P*
ERCC1 *C118T*								
C/C	50.0	0.31	25.0	0.72	0.0	0.35	12.5	0.35
C/T	25.6		15.4		7.7		5.1	
T/T	22.2		22.2		0.0		16.7	
XPD $Lys^{751}Gln$								
Lys/Lys	25	0.71	20.0	0.90	10.0	0.34	5.0	0.65
Lys/Gln	32.2		19.3		3.2		9.7	
Gln/Gln	21.4		14.3		0.0		14.3	
XPD $Asp^{312}Asn$								
Asp/Asp	23.8	0.17	14.3	0.35	4.7	0.66	4.7	0.60
Asp/Asn	22.6		16.1		6.4		12.9	
Asn/Asn	50.0		33.3		0.0		8.3	
CDA $Lys^{27}Gln$								
Lys/Lys	48.1	0.006	33.3	0.03	0.0	0.28	11.1	0.58
Lys/Gln	10.3		6.9		6.9		10.3	
Gln/Gln	22.2		11.1		11.1		0.0	

clinical setting than tumor gene expression arrays, which need core needle biopsies of patient's tumors with immediate freezing, laser microdissection, and subsequent sophisticated infrastructure (31).

Several SNPs of drug-metabolizing enzymes, such as thiopurine *S*-methyltransferase and UDP glucuronosyltransferase, have already been shown to play a critical role in predicting toxicity and clinical response of anticancer treatment (32, 33).

For the purposes of this study, we selected polymorphisms of key genes involved in the mechanism of action or metabolism of cisplatin and gemcitabine, respectively.

Our data show that, among NSCLC patients treated with cisplatin plus gemcitabine, CDA 27 Lys/Lys genotype predicted a better clinical benefit, a higher response rate, and longer TTP and OS than the other CDA genotypes. At multivariate Cox regression analysis, the polymorphisms of CDA 27 remained an independent predictive variable of treatment efficacy. Moreover, the trend toward correlation with response suggests that CDA 27 Lys/Lys may be a predictive factor of treatment activity and not only a prognostic factor of outcome. Toxicity data are in agreement with these findings and further confirm our hypothesis.

Our results seem to be in agreement with an *in vitro* study suggesting a correlation between very high levels of CDA and resistance to gemcitabine (26). Moreover, clinical studies showed a correlation between mRNA expression of CDA in peripheral blood mononuclear cells and clinical outcome in gemcitabine-treated patients (34) and *CDA* gene expression in bone marrow mononuclear cells and hematologic toxicity (35).

Nevertheless, other *in vitro* study showed a moderate decrease in CDA activity in the $Gln^{27}Gln$ variants with respect to the wild-type genotype (21), whereas the $Lys^{27}Lys$ haplotype did not show any significant effects on gemcitabine pharmacokinetics in a recent study carried out in 256 Japanese patients treated with gemcitabine (36).

This last study showed a clear relationship between pharmacokinetic variables and the CDA haplotype harboring the $Ala^{208}Thr$ polymorphic variant; however, this haplotype has a frequency of only 3.7% in the Japanese population, and has not yet been detected in Caucasians (29).

In our study, the activity and efficacy of treatment seem to decrease in a stepwise manner as the number of Gln copies increased, suggesting that CDA activity and gemcitabine metabolism is enhanced by polymorphic variants. Although in many cases genetic polymorphisms are associated with reduced activity of the encoded proteins, there are also allelic variants that encode proteins with enhanced activity (37).

To gain further insight into the relationship between genetic polymorphisms and the phenotype of CDA deamination, we evaluated enzymatic activity in a group of 60 NSCLC patients, showing a significantly lower activity in patients harboring the $Lys^{27}Lys$ genotype that was in agreement with clinical results. However, future functional studies to evaluate the possible influence of this SNP on gemcitabine-cisplatin pharmacologic interaction are warranted.

For the ERCC1 *C118T* polymorphism, we observed a significant correlation between the *T/T* genotype and clinical benefit. However, no such relationship was found with response, TTP, and survival. No other polymorphism in any of the genes included in the present study was significantly related to treatment activity and survival, and these data are in agreement with those of a recent study carried out by De las Peñas et al. (19) in advanced NSCLC patients treated with cisplatin plus gemcitabine. Interestingly, in this study, patients harboring XRCC3 $Met^{241}Met$ genotype lived longer than patients with other XRCC3 241 polymorphisms, even if no correlation with response has been reported.

Previous studies on ERCC1 and XPD SNPs and their predictive role of clinical outcome reported controversial results; furthermore, most of these trials were carried out in patients treated with different platinum-based regimens (11, 12, 16–18), not allowing definitive conclusions.

In conclusion, to our knowledge, this is the first *in vivo* study to analyze the predictive role of CDA $Lys^{27}Gln$ polymorphism. We reported a correlation of this polymorphism with enzyme activity, clinical benefit, TTP, and OS in advanced NSCLC patients treated with cisplatin plus gemcitabine. The results of our investigational study prompt us to perform a multicenter prospective clinical trial, to validate CDA $Lys^{27}Gln$ SNPs as possible predictive biomarker of gemcitabine-cisplatin activity and toxicity.

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Carmelo Tibaldi, Elisa Giovannetti, Enrico Vasile, et al.

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