Prospective Assessment of XPD Lys751Gln and XRCC1 Arg399Gln Single Nucleotide Polymorphisms in Lung Cancer

Daniela F. Giachino,1 Paolo Ghio,2 Silvia Regazzoni,1 Giorgia Mandri,1 Silvia Novello,2 Giovanni Selvaggi,2 Dario Gregori,3 Mario DeMarchi,1 and Giorgio V. Scagliotti2

Abstract  Purpose: XRCC1 and XPD play key roles in the repair of DNA lesions and adducts. Contrasting findings have been reported on the effect of polymorphisms of these genes on the response to platinum-based chemotherapy in advanced non–small-cell lung cancer (NSCLC). This study aimed to investigate the relationship between the XPD Lys751Gln and XRCC1 Arg399Gln genotypes and outcome in lung cancer patients.

Experimental Design: We genotyped 203 NSCLC and 45 small-cell lung carcinoma (SCLC) patients for the two polymorphisms. Most of the patients (81%) received a platinum-based chemotherapy.

Results: The patients’ genotype frequencies did not significantly differ from controls and both groups were in Hardy-Weinberg equilibrium for the two polymorphisms. The XRCC1 Gln/Gln variant genotype was associated with a higher median survival time (80 weeks versus 54.6 weeks for the Arg/Gln heterozygous and 55.6 weeks for the wild-type Arg/Arg genotype; P = 0.09). At the multivariable analysis adjusted for histology, stage of the disease, performance status, age, and gender, the Gln/Gln genotype was associated with a better survival of borderline significance in the subgroup of patients treated with cisplatin (hazard ratio, 0.55; 95% CI, 0.30-1.00); this association became significant for those with grade 3-4 clinical toxicity (hazard ratio, 0.46; 95% CI, 0.22-0.98). No association between XPD Lys751Gln genotype and clinical outcome was found.

Conclusion: This prospective investigation provides suggestive evidence of a favorable effect of the XRCC1 Gln/Gln genotype on survival in platinum-treated NSCLC and, for the first time, in SCLC patients also. This contrasts with other authors who did not include non–platinum-treated patients, but it does fit the expectation for a suboptimal ability to remove DNA adducts.

Most patients with a diagnosis of lung cancer are treated with cytotoxic chemotherapy, and the response is related to tumor and clinical characteristics, such as stage and performance status, as well as to drug sensitivity of the host and tumor tissues (1). In recent years, pharmacogenetic studies have tried to identify candidate genes that may account for the cancer- and/or the patient-related components of the outcome variability.

Because both carcinogens and platinum compounds produce adducts and breaks in the DNA double helix, individual variability of DNA repair not only can affect the susceptibility to environmental causes of cancer (ref. 2; e.g., tobacco smoking) but can also be relevant in modulating the efficacy of cytotoxic agents. Therefore, gene polymorphisms of DNA repair factors are obvious candidates as determinants of the efficacy of chemotherapy, and this variation may be regarded as a double-edged sword: suboptimal repair capacity in tissues favors the carcinogenetic process but ensures tumor sensitivity to the drug, whereas repair proficiency protects tissues from cancer but may make the tumor resistant to chemotherapy, and, conversely, an impaired DNA damage response can result in resistance of tumor cells to chemotherapy. Indeed, high tumor mRNA levels of the nucleotide excision repair factor ERCC1 have been related to chemoresistance in ovarian cancer and chronic lymphocytic leukemia (3) and to poor survival in gemcitabine/cisplatin-treated NSCLC (4, 5). This approach is restricted by tumor tissue availability whereas the determination of the germ-line genotypes of candidate genes offers an alternative to overcome this limitation. Several studies have focused on XPD, a subunit of the nucleotide excision repair pathway factor ERCC1 and on XRCC1, a scaffold factor involved in base excision repair, for which both functional polymorphisms have been identified (7).

In a retrospective study in 103 patients with stage III and IV NSCLC, single nucleotide polymorphisms (SNP) of XRCC1 and XPD were found to be a prognostic factor mainly in stage III patients (8). However, in this same study, several selection
biases, acknowledged by the authors themselves, may have been responsible for a spurious association with survival. More recently, a significant association of XRCC1 and XPD polymorphisms with survival, in addition to a stronger effect of XRCC3, has been reported in 145 NSCLC patients treated with cisplatin-gemcitabine (9); however, discrepancies in the pattern of genotype associations between these two studies prompted us to reassess the issue in an independent group of patients.

The aim of our work was to prospectively assess the relationship of the two SNPs, XPD Lys751Gln (rs17955147:A>C) and XRCC1 Arg99Gln (rs17435395:A>G), with response, toxicity, and survival in a series of consecutive patients with newly diagnosed lung cancer and treated with chemotherapy.

Materials and Methods

Patient recruitment and follow-up. This study included 248 consecutive patients with advanced NSCLC (stages IIIA-IV) or small-cell lung carcinoma (SCLC; limited or extensive disease) who were diagnosed from July 1, 2002 to October 31, 2004 and referred for cytotoxic chemotherapy at the Thoracic Oncology Unit, Department of Clinical and Biological Sciences, University of Torino, Italy. Written informed consent for genotypic analyses was specifically obtained from each patient. The study was conducted under the approval of the appropriate ethical review boards and the guidelines for good clinical practice. The recommendations of the Declaration of Helsinki for biomedical research involving human subjects were also followed.

Clinical data were systematically recorded at entry (age at diagnosis, gender, smoking habit, clinical stage, tumor histology) and along follow-up (type of treatment, objective response, relapses, adverse effects of the therapy, death).

Before starting any treatment, patients underwent a medical history and physical examination, tumor measurement of palpable or visible lesions, chest X-ray, full blood count, blood chemistries, urinalysis, electrocardiogram, vital signs, and calculated creatinine clearance. Prestudy assessments also included computed tomography scan or magnetic resonance imaging when needed for tumor measurement or when clinical or laboratory data suggested disease in a site not otherwise valuable. The same assessment method used to determine disease status at baseline was consistently used for efficacy evaluation throughout the patient’s follow-up.

Chemotherapy regimens. Patients with stage IIIA for pleural effusion or positive supraclavicular nodes or stage IV NSCLC with good (0-1 Eastern Cooperative Oncology Group) performance status or extensive SCLC were exclusively treated with chemotherapy (for NSCLC: gemcitabine 1,250 mg/m², days 1 and 8 every 3 weeks in combination with cisplatin 75 mg/m² or carboplatin AUC 6, both administered on day 1, every 3 weeks; for SCLC and five patients with NSCLC: etoposide 100 mg/m², days 1-3 every 3 weeks in combination with cisplatin 75 mg/m² or carboplatin AUC 6, both administered on day 1, every 3 weeks). Few patients (n = 12; 5%) with stage IV NSCLC were treated with a non–platinum-based doublet. Patients with performance status 2 or elderly (>70 years old; n = 34 [14%]) received single-agent gemcitabine (1,250 mg/m², days 1 and 8 every 3 weeks). Patients with locally advanced NSCLC (stage IIIA and IIIB) received a multimodality treatment including surgery (for stage IIIA) or sequential radiotherapy (for stage IIIB; total dose, 59.4 Gy). Limited disease SCLC was sequentially treated with thoracic radiotherapy to a total dose of 59.4 Gy.

Response and survival assessments. Tumor response was evaluated according to Response Evaluation Criteria in Solid Tumors, the revised version of the International Union Against Cancer/WHO criteria (10). Overall survival and time to progression were assessed from the first day of chemotherapy administration to the date of death (any cause) and the date of objective disease progression, respectively (death was considered a progression event in patients who died before disease progression). Patients without documented death or objective progression at the time of the final analysis were censored at the date last known to be alive or their last objective tumor assessment. For the purpose of this report, the survival data were censored at December 31, 2005.

Genotype analyses. Blood samples were collected for genotyping at the time of study entry. Genomic DNA was isolated from blood by Wizard Genomic kit (Promega Corp.). Allele and genotype frequencies were also assessed in a control group of 253 healthy medical students. SNPs were characterized through PCR-RFLP by digesting the amplified XPD exon 23 with PstI (Promega) and the XRCC1 exon 10 with MspI (Fermentas International, Inc.). Briefly, DNA (100 ng) was amplified in a mixture containing 0.2 μmol/L × 4 deoxynucleotide triphosphates, 0.4 μmol/L of each primer [XPD Lys751Gln forward and reverse primer (11), XRCC1 Arg99Gln forward primer (12), and reverse primer (13)], 1 unit of AmpliTaq polymerase (Applied Biosystems) in a final volume of 25 μL. Initial 5′ denaturation was followed by 35 cycles of 30 s at 95°C, 45 s annealing at 59°C for XPD and 60°C for XRCC1, 45 s at 72°C, and final extension at 72°C for 7 min. The PCR products were digested overnight with 1 unit of the restriction enzyme at 37°C, following the manufacturer’s recommendation, run in 2% agarose gel with ethidium bromide, and visualized under UV light. The PstI-restricted product of XPD had sizes of 218 + 104 bp for the Lys751 allele and 155 + 104 + 63 bp for Gln751. The MspI-restricted product of XRCC1 had sizes of 162 + 78 bp for the Arg99 allele and 240 bp for Gln99.

Statistical analyses. Time to death was modeled using a Cox proportional hazard model. Proportional hazard assumption was checked using the Grambsch and Therneau test and diagnostic plots based on Shoenfeld residual (14). Response, as an ordinal variable from progression up to complete response, was modeled using a proportional-odds logit regression (15). Main effects for the multivariable analysis of polymorphisms and their interactions were tested using the Akaike Information Criterion using a backward strategy (16).

Kaplan-Meier survival curves were estimated. The statistical significance was settled at P < 0.05. S-plus (release 2000) statistical package and the Harrell’s Design and Hmisc libraries were used for analysis.

Results

Descriptive statistics and univariate analysis. Main clinical features are summarized in Table 1. Two hundred (81%) patients were males; median age at diagnosis was 62 years (range, 41-79 years); and 82% had a cytologic or histologic diagnosis of NSCLC and the remaining of SCLC. Metastatic disease at diagnosis was reported in 62% of NSCLC and in 47% of SCLC. Smoking habit data indicate that only 58% were current smokers.

Among NSCLC patients, 152 received cisplatin-gemcitabine, 12 received a non-platinum doublet (mainly docetaxel-gemcitabine), and 34 received gemcitabine alone. All SCLC patients were treated with cisplatin-etoposide (Table 2).

Overall objective response rate to chemotherapy was 46% (82% in SCLC; in NSCLC: 38% treated with cisplatin-gemcitabine, 33% with the non-platinum doublets, and 23% with gemcitabine alone). Progressive disease rate was 18% in NSCLC and 4% in SCLC. Fifty-nine percent of the patients with gemcitabine alone). Progressive disease rate was 18% in NSCLC and 4% in SCLC. Fifty-nine percent of the patients

At univariate analysis, performance status (P < 0.00001), response to therapy (P < 0.0001), and extent of disease (only for NSCLC, P < 0.002) were significantly associated with survival. Although the median survival time for extensive SCLC was clearly lower than for limited SCLC (69 versus 43 weeks), the difference was not statistically significant, probably due to the limited number of patients. After adjustment for stage of disease and performance status, the median survival time for

### Table 1. Patient and tumor characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. patients (N = 248)</th>
<th>Status</th>
<th>ORR (n = 108, 44%)</th>
<th>HR for death (95% CI)</th>
<th>OR for response (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dead (n = 187)</td>
<td>Alive (n = 61)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Male, n (%)</td>
<td>200 (81)</td>
<td>152 (81)</td>
<td>48 (79)</td>
<td>87 (81)</td>
<td>1.13 (0.78-1.63)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>56/62/68</td>
<td>56/62/68</td>
<td>56/63/67</td>
<td>55/61/65</td>
<td>1.18 (0.95-1.46)</td>
</tr>
<tr>
<td>Clinical stage, n (%)</td>
<td></td>
<td></td>
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<tr>
<td>NSCLC (n = 203)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Locally advanced</td>
<td>79 (32)</td>
<td>49 (26)</td>
<td>30 (49)</td>
<td>31 (29)</td>
<td>1</td>
</tr>
<tr>
<td>Metastatic</td>
<td>124 (50)</td>
<td>106 (57)</td>
<td>18 (30)</td>
<td>39 (36)</td>
<td>1.72 (1.23-2.42)</td>
</tr>
<tr>
<td>SCLC (n = 45)</td>
<td></td>
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<tr>
<td>Limited</td>
<td>24 (10)</td>
<td>16 (8.5)</td>
<td>8 (13)</td>
<td>21 (19)</td>
<td>1</td>
</tr>
<tr>
<td>Metastatic</td>
<td>21 (8)</td>
<td>16 (8.5)</td>
<td>5 (8)</td>
<td>17 (16)</td>
<td>1.80 (0.88-3.65)</td>
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<tr>
<td>Histology, n (%)</td>
<td></td>
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<tr>
<td>Adenocarcinoma</td>
<td>94 (38)</td>
<td>79 (25)</td>
<td>15 (42)</td>
<td>36 (33)</td>
<td>1</td>
</tr>
<tr>
<td>Large-cell carcinoma</td>
<td>42 (17)</td>
<td>10 (16)</td>
<td>32 (17)</td>
<td>13 (10)</td>
<td>0.71 (0.47-1.08)</td>
</tr>
<tr>
<td>Squamous-cell carcinoma</td>
<td>67 (27)</td>
<td>22 (36)</td>
<td>45 (24)</td>
<td>22 (20)</td>
<td>0.87 (0.60-1.25)</td>
</tr>
<tr>
<td>Small-cell carcinoma</td>
<td>45 (18)</td>
<td>14 (23)</td>
<td>31 (17)</td>
<td>37 (34)</td>
<td>0.81 (0.53-1.23)</td>
</tr>
<tr>
<td>Performance status (ECOG), n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>119 (48)</td>
<td>76 (41)</td>
<td>43 (70)</td>
<td>55 (51)</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>99 (40)</td>
<td>82 (44)</td>
<td>17 (28)</td>
<td>42 (39)</td>
<td>1.84 (1.34-2.52)</td>
</tr>
<tr>
<td>2</td>
<td>30 (12)</td>
<td>29 (16)</td>
<td>1 (2)</td>
<td>11 (10)</td>
<td>3.88 (2.51-6.00)</td>
</tr>
<tr>
<td>Smoking habit, n (%)</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Never</td>
<td>28 (11)</td>
<td>25 (13)</td>
<td>3 (5)</td>
<td>9 (8)</td>
<td>1.14 (0.73-1.76)</td>
</tr>
<tr>
<td>Current</td>
<td>144 (58)</td>
<td>102 (55)</td>
<td>42 (69)</td>
<td>71 (66)</td>
<td>1</td>
</tr>
<tr>
<td>Former</td>
<td>76 (31)</td>
<td>60 (32)</td>
<td>16 (26)</td>
<td>28 (26)</td>
<td>1.26 (0.92-1.74)</td>
</tr>
<tr>
<td>XPD genotype, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys/Lys</td>
<td>107 (43)</td>
<td>77 (41)</td>
<td>30 (49)</td>
<td>50 (46)</td>
<td>1</td>
</tr>
<tr>
<td>Lys/Gln</td>
<td>109 (44)</td>
<td>86 (46)</td>
<td>23 (38)</td>
<td>45 (42)</td>
<td>1.11 (0.82-1.51)</td>
</tr>
<tr>
<td>Gln/Gln</td>
<td>32 (13)</td>
<td>24 (13)</td>
<td>8 (13)</td>
<td>13 (12)</td>
<td>1.10 (0.70-1.75)</td>
</tr>
<tr>
<td>XRCC1 genotype, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gln/Gln</td>
<td>29 (12)</td>
<td>17 (9)</td>
<td>12 (20)</td>
<td>10 (9)</td>
<td>0.61 (0.36-1.03)</td>
</tr>
<tr>
<td>Gln/Arg</td>
<td>100 (40)</td>
<td>82 (44)</td>
<td>18 (30)</td>
<td>45 (42)</td>
<td>1.09 (0.80-1.47)</td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>119 (48)</td>
<td>88 (47)</td>
<td>31 (51)</td>
<td>53 (49)</td>
<td>1</td>
</tr>
</tbody>
</table>

NOTE: Distribution of observed variables for the overall sample (Combined), according to event (Status) and to response (Response). Data are percentages (absolute numbers in parentheses) or median (1 quartile/median/III quartile) for continuous variables. Unadjusted HRs and unadjusted odds ratio are presented along with their 95% CIs. Both HR and odds ratio for continuous variables are computed with reference to their interquartile difference.

### Table 2. Treatment characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. patients (N = 248)</th>
<th>Status</th>
<th>ORR (n = 108, 44%)</th>
<th>HR for death (95% CI)</th>
<th>OR for response (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dead (n = 187)</td>
<td>Alive (n = 61)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment, n (%)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Platinum-gemcitabine</td>
<td>152 (61)</td>
<td>108 (58)</td>
<td>44 (72)</td>
<td>58 (54)</td>
<td>1</td>
</tr>
<tr>
<td>Gemcitabine alone</td>
<td>34 (14)</td>
<td>33 (18)</td>
<td>1 (2)</td>
<td>8 (7)</td>
<td>2.05 (1.38-3.03)</td>
</tr>
<tr>
<td>Platinum-etoposide</td>
<td>50 (20)</td>
<td>34 (18)</td>
<td>16 (26)</td>
<td>38 (35)</td>
<td>1.00 (0.68-1.47)</td>
</tr>
<tr>
<td>Non-platinum doublets</td>
<td>12 (5)</td>
<td>12 (6)</td>
<td>0 (0)</td>
<td>4 (4)</td>
<td>1.40 (0.77-2.55)</td>
</tr>
<tr>
<td>Side effects, n (%)</td>
<td></td>
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</tr>
<tr>
<td>Grade 0-2</td>
<td>105 (42)</td>
<td>80 (43)</td>
<td>25 (41)</td>
<td>41 (38)</td>
<td>1</td>
</tr>
<tr>
<td>Grade 3-4</td>
<td>143 (58)</td>
<td>107 (57)</td>
<td>36 (59)</td>
<td>67 (62)</td>
<td>0.92 (0.69-1.23)</td>
</tr>
</tbody>
</table>

NOTE: Distribution of observed variables for the overall sample (Combined), according to event (Status) and to response (Response). Data are percentages (absolute numbers in parentheses) or median (1 quartile/median/III quartile) for continuous variables. Unadjusted HRs and unadjusted odds ratio are presented along with their 95% CIs. Both HR and odds ratio, for continuous variables, are computed with reference to their interquartile difference.
patients treated with doublet chemotherapies was significantly lower than the single-agent chemotherapy ($P < 0.02$).

**Genotype associations and clinical correlations.** Allele and genotype frequencies did not significantly differ between patients and controls (XPD751 Lys/Lys 40%, Lys/Gln 43%, Gln/Gln 17%, patients versus controls $\chi^2 = 1.9$, $P = 0.38$; XRCC1 399 Gln/Gln 12%, Gln/Arg 44%, Arg/Arg 44%, patients versus controls $\chi^2 = 0.64$, $P = 0.72$). Both groups were in Hardy-Weinberg equilibrium for the two polymorphisms (not shown).

None of the evaluated genotypes were associated with patient and tumor characteristics such as age, smoke, gender, performance status, histology, and disease stage.

The XPD and XRCC1 genotypes were not associated with each other (not shown; Spearman $\chi^2$, $P = 0.37$).

**XPD Lys751Gln.** In our study, the variant Gln allele of the XPD gene was not associated with decreased overall survival: patients with the wild-type Lys/Lys genotype had a median survival time of 61 weeks (95% CI, 47.4-75 weeks), just slightly superior to that of the variant Lys/Gln heterozygotes (56.3 weeks; 95% CI, 47.6-61.4 weeks) and Gln/Gln homozygotes (56.9 weeks; 95% CI, 44.3-67 weeks; log-rank test, $P = 0.77$; Fig. 1). Similarly, no association was found between the XPD genotype and the objective response rate reported.

In the Cox multivariable model, after adjusting for stage, histology, performance status, age, smoke, and considering genotype as an indicator variable, the hazard ratios (HR) for the Lys/Gln heterozygotes (HRs, 1.00 for the heterozygous and 1.06 for the homozygous group and 55.6 weeks (95% CI, 45.4-62.1 weeks) in the heterozygous variant Gln/Gln genotype with a better survival (HR, 1.00; 95% CI, 0.60-1.61) in the homoyzogous Gln homozygotes (HR, 0.88; 95% CI, 0.55-1.42) showed no significance (Table 3).

In the Cox multivariable model, after adjusting for stage, histology, performance status, age, smoke, and gender, performance status, and considering genotype as an indicator variable, the hazard ratios (HR) for the Arg/Gln heterozygotes (HRs, 0.89 for the heterozygous and 1.00 for the homozygous group and 55.6 weeks (95% CI, 45.4-62.1 weeks) in the homozygous wild-type genotype (log-rank test, $P = 0.092$; Fig. 2). No association was found between the XRCC1 genotype and the objective response rate reported.

In the Cox multivariable model, after adjusting for stage, histology, performance status, age, smoke, and considering genotype as an indicator variable, the HRs for the Arg/Gln heterozygotes (HR, 1.17; 95% CI, 0.85-1.59) and Gln/Gln homozygotes (HR, 0.60; 95% CI, 0.35-1.03) did not differ significantly from the wild-type genotype.

Preplanned subgroup analysis of platinum-treated patients resulted in a borderline significant association of the homozygous variant Gln/Gln genotype with a better survival (HR, 0.55; 95% CI, 0.30-1.00), which was more significant in the subgroup of platinum-treated patients who experienced relevant clinical toxicity (grade 3-4; HR, 0.46; 95% CI, 0.22-0.98). In contrast, among non-platinum-treated patients, we observed no effect at all of the XRCC1 genotype on survival (HR, 1.00 for the heterozygous and 1.06 for the homozygous variant genotype).

All the interactions between factors in the multivariable model were not significant.

**Discussion**

In lung cancer, the choice of the cytotoxic chemotherapy is currently based on tumor (histology and disease extent) and patient features (age and performance status). To customize
chemotherapy, better prognostic scoring of the tumor and predictions on the efficacy of therapy are needed. In early-stage patients who undergo surgery, this is possible through an extensive molecular characterization of the resected neoplastic tissue. Assessing germ-line genetic polymorphisms as either prognostic or predictive markers has much appeal especially in the setting of advanced NSCLC where tumor tissues are rarely available.

A number of promising pharmacogenetic candidates have been identified for prediction of chemotherapy efficacy and toxicity, as well as survival, especially in patients with gastrointestinal malignancies (17). Data in NSCLC are much more conflicting. Moreover, few attempts have been made to address these questions in the context of prospective studies. To conduct such evaluations in a clinical context where therapy is not assigned at random, it seems mandatory to discriminate the influence of genetic factors from those of many potential confounding factors.

In patients undergoing cytotoxic chemotherapy with DNA-damaging agents, a highly efficient repair mechanism may result in a decreased number of adducts and, consequently, in a defective clinical response. A seminal study by Bosken et al. (18) investigated the relationship between DNA repair capacity and survival in NSCLC patients treated with cisplatin-based chemotherapy. Patients in the top quartile had a risk of death twice that of patients in the lower quartile.

Nucleotide excision repair is a repair system that removes a wide variety of DNA lesions, including UV-induced lesions, bulky chemical adducts such as those generated by cytotoxic drugs, and some forms of oxidative damage. It involves the action of at least 30 proteins in a “cut-and-paste”–like mechanism (19). Among these, XPD is a DNA helicase involved in transcription and nucleotide excision repair. Several polymorphisms in the XPD gene have been identified (e.g., at codons 199, 312, and 751).

Other repair mechanisms may also play a role in the response to cisplatin therapy. The XRCC1 protein physically interacts with ligase III and poly(ADP-ribose) polymerase. It is thought to act as a scaffold in the removal of adducts through both single-strand break repair and base excision repair (20) and in the repair of other types of cisplatin-induced damage, including double-strand breaks, through a nonhomologous end-joining pathway, which is alternative to the predominant ATM-XRCC4-DNA ligase IV pathway (21). More than 60 SNPs in XRCC1 gene have been validated and those more extensively studied are located at codons 194, 280, and 399. The latter is located in the critical COOH-terminal poly(ADP-ribose) polymerase–binding BRCT-I domain and, in an epidemiologic study, was the most common and showed no major variations by ethnicity (7).

There is little evidence on the functional effects of these polymorphisms. In a study of lung cancer patients, the XPD Lys751Gln and Asp312Asn SNPs were consistently associated with lower DNA repair capacity as assessed by a cell reactivation assay (22). In addition, cytogenetic challenge assays indicate defective base excision repair function in X-irradiated cells homozygous for the XRCC1 399Gln variant and defective nucleotide excision repair function in UV-irradiated cells homozygous for the XPD 751Gln variant (23).

At the epidemiologic level, several studies have investigated the association of the XRCC1 399Gln variant with various cancers (24) and its interactions with smoking habit, without conclusive results (7, 25). Interestingly, it may be associated with the spectrum of p53 mutations in the tumor (26).

In two small retrospective studies in patients with advanced NSCLC, a modest association of survival with polymorphic variants of ERCC1, another nucleotide excision repair component, was observed (27, 28), whereas in two other retrospective investigations, XRCC1 and XPD genetic polymorphisms were found to be prognostic in stage III and, to a lesser extent, stage IV NSCLC (8, 9). In two different studies in Chinese patients, polymorphisms in XRCC1 (29) but not in XPD (30) have been recently associated with response to cisplatin-based chemotherapy.

All these studies were done retrospectively, with some case selection, and exclusively included patients with NSCLC. In contrast, our study aimed at investigating prospectively the value of the constitutional patient’s genotype which, unless altered by somatic mutations, is also shared by the tumor tissue in every consecutive patient admitted to our thoracic oncology clinic for cytotoxic chemotherapy, including both NSCLC and SCLC and any type of chemotherapy. Basically, this “broad approach” is crucial in discriminating whether any role of a polymorphism should be ascribed to an influence on tumor features or response to therapy.

When the XPD and XRCC1 genotypes were considered as possible additional explanatory variables, neither was a truly independent factor for survival and response, but in patients treated with cisplatin and with clinically significant toxicities related to chemotherapy, the association between the XRCC1 genotype and survival reached statistical significance.

Our findings support the hypothesis that DNA repair gene polymorphisms may modulate the response to cytotoxic chemotherapy and, for the first time, show this not only in NSCLC but in SCLC patients as well. It is surprising that in our study, the XRCC1 399 Gln/Gln genotype is associated with a better survival following platinum-based chemotherapy [i.e., opposite to the findings of other authors (8, 9)].

A similar favorable influence of the XRCC1 399 Gln allele on platinum response has been recently reported in patients with
head and neck carcinoma (31) and in women with bulky cervical carcinoma (32). Because in other cancers the XRCCI 399 Gln/Gln genotype has been associated with susceptibility to adverse effects of radiotherapy (33), the poor prognosis reported by these authors (8, 9) might be tentatively ascribed to different patient characteristics or treatments. Indeed, the defective function ascribed to the XRCCI 399 Gln/Gln homozygotes could fit with both increased radiosensitivity, which might have been a confounder responsible for poor clinical outcome as acknowledged by Gurubhagavatula et al. (8), and on the other side with a more effectice action of cisplatin chemotherapy, which, in turn, could translate in an increased survival. Interestingly, the effect was even more evident when platinum-treated patients with clinical relevant toxicities were only considered.

In conclusion, this preliminary work indicates for the XRCCI Arg399Gln polymorphism a moderate association with survival especially in patients receiving cisplatin-gemcitabine, but no contribution of the XPD Lys751Gln polymorphism in predicting response to chemotherapy and in assisting the choice of treatment. This study has a number of limitations. First, the effects of the two SNPs on the enzymatic or nonenzymatic functions of the XPD and XRCCI proteins are not exactly known. Nevertheless, our study adds weight to the existing notion that rare and probably hypomorphic variants of DNA repair genes, as shown here for XRCCI Arg399Gln, may improve the response to adduct-forming chemotherapy in a similar way as the sensitivity to xenobiotic mutagens. Second, these findings should be validated in a prospective study with a larger group of patients and a more comprehensive series of polymorphisms.

Third, it is quite unlikely that the effect of a single common sequence variant on outcomes will be easily detectable in a population-based study as the present one, whereas a combination of multiple sequence variants functioning in the same biochemical pathway might be more important in the identification of different risks for survival and other clinical relevant efficacy outcomes (34).

References

Prospective Assessment of XPD Lys751Gln and XRCC1 Arg399Gln Single Nucleotide Polymorphisms in Lung Cancer

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