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ORIGINAL RESEARCH

# Single-arm, open label prospective trial to assess prediction of the role of ERCC1/XPF complex in the response of advanced NSCLC patients to platinum-based chemotherapy

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**Background:** Platinum-based therapy, combined or not with immune checkpoint inhibitors, represents a front-line choice for patients with non-small-cell lung cancer (NSCLC). Despite the improved outcomes in the last years for this malignancy, only a sub-group of patients have long-term benefit. Excision repair cross-complementation group 1 (ERCC1) has been considered a potential biomarker to predict the outcome of platinum-based chemotherapy in NSCLC. However, the *ERCC1* gene is transcribed in four splice variants where the isoform 202 was described as the only one active and able to complex Xeroderma pigmentosum group F-complementing protein (XPF). Here, we prospectively investigated if the active form of ERCC1, as assessed by the ERCC1/XPF complex (ERCC1/XPF), could predict the sensitivity to platinum compounds.

**Patients and methods:** Prospectively enrolled, patients with advanced NSCLC treated with a first-line regimen containing platinum were centrally evaluated for ERCC1/XPF by a proximity ligation assay. Overall survival (OS), progression-free survival (PFS) and objective response rate (ORR) were analyzed.

**Results:** The absence of the ERCC1/XPF in the tumor suggested a trend of worst outcomes in terms of both OS [hazard ratio (HR) 1.41, 95% confidence interval (CI) 0.67-2.94, $P = 0.373$] and PFS (HR 1.61, 95% CI 0.88-3.03, $P = 0.123$). ORR was marginally influenced in ERCC1/XPF-negative and -positive groups [odds ratio (stable disease + progressive disease versus complete response + partial response) 0.87, 95% CI 0.25-3.07, $P = 0.832$].

**Conclusion:** The lack of ERCC1/XPF complex in NSCLC tumor cells might delineate a group of patients with poor outcomes when treated with platinum compounds. ERCC1/XPF absence might well identify patients for whom a different therapeutic approach could be necessary.

**Key words:** ERCC1, NSCLC, platinum-based chemotherapy, proximity ligation assay, XPF

## INTRODUCTION

In a targeted and immunotherapy era, platinum compounds such as cisplatin and carboplatin are still a cornerstone for the first-line treatment of non-small-cell lung cancer (NSCLC) for a significant subgroup of patients. In fact, except for patients with tumors expressing programmed death-ligand 1 (PD-L1) >50% where single-agent immunotherapy is the best option,[1] platinum-based chemotherapy is the best additional component in first-line immunotherapy combinations.[2] Despite the significant beneficial impact of combination therapies, only a percentage of patients have long-term benefit. Therefore, even in the era of immune checkpoint inhibitors, there is an unmet need to discover biomarkers in order to explain the mechanisms that render the tumors insensitive to platinum compounds.

Platinum compounds are able to form DNA mono-adducts, DNA intra-strand and DNA inter-strand crosslinks.[3]

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The latter are particularly cytotoxic as they interfere with the transcription and the replication process inducing cell cycle arrest and apoptosis if not repaired.[4] Mammalian cells can activate different DNA repair mechanisms to repair the damage induced by platinum compounds.[5] The involvement of the nucleotide excision repair (NER) pathway in managing platinum compounds DNA lesions has been demonstrated by the high sensitivity to cisplatin of cells not expressing the excision repair cross-complementation group 1 (ERCC1) protein.[6] The ERCC1 protein interacts with Xeroderma pigmentosum group F-complementing protein (XPF) to form a complex able to cleave DNA near to the damaged DNA nucleotide.[7]

Given its role, ERCC1 expression has been considered for a long time as a potential biomarker to predict the outcome of platinum-based chemotherapy in tumors including NSCLC.[8-10] However, despite some existing evidence, this biomarker has not yet been implemented in everyday clinical practice in NSCLC. This is mainly because it has been studied in retrospective series and has been evaluated with different detection methods such as immunohistochemistry, reverse transcriptase PCR and analysis of single-nucleotide polymorphisms.[11] Moreover, the different performances of the antibodies against ERCC1 used in the different studies have been reported to be a further problem in defining the role of ERCC1 as a biomarker.[12]

Conflicting data about the inclusion of ERCC1 levels as a marker into clinical practice could be explained by a technical issue given that the *ERCC1* gene is transcribed in four splice variants (namely isoforms 201, 202, 203 and 204). Isoform 202 was described as the only one active and able to complex XPF, accounting for all ERCC1-mediated DNA-damage response.[12] The measure of the ERCC1/XPF complex (ERCC1/XPF) by proximity ligation assay (PLA) was reported to be a way to overcome the problem about the presence of different isoforms.[13]

In the present work, we prospectively investigated the potential of the ERCC1/XPF complex to identify NSCLC patients who could benefit from platinum-based therapy.

## METHODS

### *Study population and samples*

The Fondazione IRCCS Istituto Nazionale dei Tumori (Milan, Italy), Regina Elena National Cancer Institute (Rome, Italy), Hospital Papa Giovanni XXIII (Bergamo, Italy) Metropolitan Hospital and Attikon Hospital (Athens, Greece) were the centers involved. Consecutive patients with metastatic NSCLC who received platinum-based chemotherapy in combination with vinorelbine, gemcitabine or pemetrexed according to the physician's choice as first-line treatment between February 2014 and April 2017 were included in the BioRaRe prospective multicenter trial. Immunotherapy, if given, was administered as second-line or further treatment.

All patients had an Eastern Cooperative Oncology Group (ECOG) performance status (PS) between 0 and 2 and were at least 18 years of age. Exclusion criteria included any evidence of serious comorbidities that the investigator judged as a contraindication to the participation in the study, pregnancy and breast feeding.

Patients assessable for tumor response according to the RECIST 1.1 criteria were examined and their demographics, clinical and pathological characteristics were retrieved. Electronic case report forms and medical records were used to collect data.

The study was approved by the Fondazione IRCCS Istituto Nazionale dei Tumori Institutional Review Board (INT18/13) and conducted according to the Declaration of Helsinki ethical principles for medical research involving human subjects. All patients gave signed written informed consent.

### *PLA*

PLA was done centrally on single slides at the Istituto di Ricerche Farmacologiche Mario Negri IRCCS. Five µm thick slices put on to polylysine-coated glass slides were deparaffinized, quenched for the activity of endogenous peroxidase, blocked and incubated overnight with rabbit-ERCC1 (sc-10785, Santa Cruz Biotechnology, Santa Cruz, CA) 1:100 and mouse-XPF (MA56-12060, Thermo Scientific, Waltham, MA) 1 : 200. The slides were then incubated with Duolink® PLA probes (Minus and Plus, Sigma-Aldrich, St. Louis, MO) for the formation of oligonucleotides. The oligonucleotides were hybridized, ligated, amplified and detected using Duolink detection reagents for brightfield (Sigma-Aldrich). Slides were then counterstained with Nuclear Fast Red solution, dehydrated and mounted. Images were acquired with the VS120-Virtual Slide microscope (Olympus, Hamburg, Germany) at 40× magnification and processed with ImageJ software (Rasband, W.S., ImageJ, U.S. National Institutes of Health, Bethesda, MD). Each nuclear dot corresponded to one ERCC1/XPF complex. The numbers of dots were normalized by the numbers of nuclei in the area of interest. At least 150 cancer cells were analyzed in each sample and at least three different areas per core were examined.

### *Outcomes*

The primary outcome of the study was progression-free survival (PFS). Secondary outcomes were overall response rate and overall survival (OS).

PFS was defined as the time from the start of the platinum-based first-line therapy to the date of progression or death from any cause, whichever came first. OS was defined as the time from the platinum-based first-line therapy to the date of death from any cause. Patients who had not died or had no disease progression were censored at their last available information on status. Objective response rate (ORR) was defined as the proportion of patients with a complete or partial response to treatment.

### *Statistical methods*

Chi-square and Kruskall-Wallis tests were used to analyze the relations between ERCC1/XPF dots/cell and categorical

**Table 1. Patients' characteristics ($N = 95$)**

| | *n* | % |
|---|---|---|
| Age of diagnosis | | |
| Median (Q1-Q3) | 66.5 (60.2-70.4) | |
| Unknown | 3 | |
| Sex | | |
| Male | 59 | 64.1 |
| Female | 33 | 35.9 |
| Unknown | 3 | |
| ECOG-PS | | |
| 0 | 71 | 82.6 |
| 1 | 14 | 16.3 |
| 2 | 1 | 1.2 |
| Unknown | 9 | |
| Smoking | | |
| Never | 18 | 19.6 |
| Former smokers | 36 | 39.1 |
| Smokers | 38 | 41.3 |
| Unknown | 3 | |
| Stage at diagnosis | | |
| IIIB | 26 | 28.0 |
| IV | 67 | 72.0 |
| Unknown | 2[a] | |
| Histotype | | |
| Adenocarcinoma | 78 | 82.1 |
| Squamous | 15 | 15.8 |
| Other | 2 | 2.1 |
| Platinum-based therapy | | |
| Cisplatin | 29 | 34.1 |
| Carboplatin | 56 | 65.9 |
| Unknown | 1[b] | |
| Immunotherapy | | |
| No | 54 | 58.7 |
| Yes | 38 | 41.3 |
| Unknown | 2 | |
| ERCC1/XPF | | |
| ERCC1/XPF-negative | 23 | 24.2 |
| ERCC1/XPF-positive | 72 | 75.8 |
| ERCC1/XPF dots/cell | | |
| Median (Q1-Q3) | 0.7 (0.2-1.6) | |

ECOG, Eastern Cooperative Oncology Group; ERCC1, excision repair cross-complementation group 1; PS, performance status; Q1, first quartile; Q3, third quartile; XPF, xeroderma pigmentosum group F-complementing protein.
[a] The two patients with unknown stage were advanced NSCLC without further specification.
[b] The patient with unknown platinum-based therapy received platinum-based therapy without further specification.

clinical variables. The Spearman correlation coefficient was used for measuring the correlation between ERCC1/XPF and continuous clinical variables. ERCC1/XPF was analyzed as a continuous and dichotomous variable (ERCC1/XPF score = 0 as negative and ERCC1/XPF score > 0 as positive).

Survival curves were calculated with the Kaplan–Meier method and tested by the log-rank test. Cox proportional hazard models were used to analyze the impact of ERCC1/XPF on PFS and OS, adjusting for clinical and pathological characteristics ECOG-PS, age, histology, smoking habit, therapy and, only for OS, immunotherapy. Patients were considered former smokers if they smoked more than 100 cigarettes in their life and smoker if they smoke any tobacco product at least once a day. Results were expressed as hazard ratios (HRs) with their 95% confidence intervals (95% CIs).

The impact of ERCC1/XPF on ORR was analyzed with logistic regression models and expressed as odds ratios (OR) with their 95% CI.

**Table 2. Association between ERCC1/XPF continuous or ERCC1/XPF pos versus neg and patient/tumor characteristics**

| | *P* ERCC1/XPF continuous | *P* ERCC1/XPF-positive versus -negative |
|---|---|---|
| Age of diagnosis | 0.916[a] | 0.756[b] |
| Sex | 0.305[b] | 1.000[c] |
| ECOG-PS | 0.090[b] | 0.034[c] |
| Smoking | 0.030[b] | 0.606[c] |
| Stage at diagnosis | 0.502[b] | 0.598[c] |
| Histotype | 0.483[b] | 0.530[c] |
| Platinum-based therapy | 0.012[b] | 0.034[c] |
| Immunotherapy | 0.684[b] | 1.000[c] |

ECOG, Eastern Cooperative Oncology Group; ERCC1, excision repair cross-complementation group 1; PS, performance status; XPF, xeroderma pigmentosum group F-complementing protein.
[a] Spearman correlation.
[b] Kruskall-Wallis test.
[c] Fisher exact test.

All statistical tests were two-sided and $P < 0.05$ was considered statistically significant. Statistical analyses were done using SAS version 9.4 (SAS Institute, Cary, NC).

## RESULTS

The demographic characteristics of the analyzed population ($N = 95$) are reported in Table 1. The majority of patients were males (64.1%), had an ECOG-PS of 0-1 (98.9%), had tumors of adenocarcinoma histology (82.1%), were smokers (41.3%) or ex-smokers (39.1%) and were not treated with immunotherapy (58.7%). All patients were diagnosed with advanced (stage IIIb-IV) disease and received platinum-based chemotherapy as their first-line treatment of advanced disease. When considered as a continuous variable, ERCC1/XPF complex was associated with smoking (ERCC1/XPF median for ex-smokers was 1.1, for current smokers was 0.55 and for never smokers was 0.58 dots/cell, $P = 0.030$) and the type of platinum-based therapy ($P = 0.012$), whereas when considered as a dichotomous variable (negative versus positive), ERCC1/XPF complex was associated with ECOG-PS (87% of ERCC1/XPF positive had PS equal to 0, $P = 0.034$) and the type of platinum-based therapy (89% of patients treated with cisplatin had ERCC1/XPF positive complex in comparison with 68% of patients treated with carboplatin, $P = 0.034$) (Table 2). The distribution of ERCC1/XPF complex in the population is shown in Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2020.100034.

At a median follow-up of 17.5 months [first quartile (Q1)-third quartile (Q3): 8.3-48.9] 77 progressions, 56 deaths and 87 deaths or progressions were observed. The multivariable analysis of the role of ERCC1/XPF complex, considered as a continuous variable, showed a non-significant HR for PFS of 0.95 (95% CI 0.69-1.30, $P = 0.748$) and 0.84 for OS (95% CI 0.59-1.21, $P = 0.355$). Detailed results on multivariable analyses for OS and PFS are reported in Table 3.

We then investigated if the absence of the ERCC1/XPF complex influences outcomes. We considered the ERCC1/XPF complex as a dichotomous variable (negative versus positive). Median PFS were 4.3 (Q1-Q3: 2.4-8.4) and 7.0

**Table 3. PFS and OS by ERCC1/XPF continuous score**

| | PFS | | OS | |
|---|---|---|---|---|
| | HR (95% CI) | *P* | HR (95% CI) | *P* |
| Univariable | | | | |
| ERCC1/XPF[a] | 0.99 (0.76-1.28) | 0.949 | 1.00 (0.74-1.35) | 0.984 |
| Multivariable | | | | |
| ERCC1/XPF[a] | 0.95 (0.69-1.30) | 0.739 | 0.84 (0.59-1.20) | 0.340 |
| Age at diagnosis[b] | 0.97 (0.94-1.00) | 0.026 | 0.98 (0.95-1.01) | 0.222 |
| Histology | | | | |
| Adenocarcinoma | Reference | 0.885 | Reference | 0.160 |
| Squamous | 1.00 (0.52-1.95) | | 0.79 (0.35-1.78) | |
| Nos or other | 1.69 (0.21-13.7) | | 7.52 (0.80-70.8) | |
| Smoking | | | | |
| Never | Reference | 0.876 | Reference | 0.069 |
| Previous | 1.17 (0.63-2.16) | | 2.85 (1.17-6.93) | |
| Current | 1.26 (0.61-2.62) | | 2.22 (0.79-6.22) | |
| ECOG-PS | | | | |
| 0 | Reference | 0.982 | Reference | 0.567 |
| 1 or 2 | 0.99 (0.45-2.18) | | 0.73 (0.25-2.15) | |
| Immunotherapy | | | | |
| No | – | – | Reference | 0.037 |
| Yes | – | | 0.53 (0.29-0.96) | |

CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; ERCC1, excision repair cross-complementation group 1; HR, hazard ratio; Nos, not otherwise specified; OS, overall survival; PFS, progression-free survival; PS, performance status; XPF, xeroderma pigmentosum group F-complementing protein.

[a] One-point score increment.

[b] One-year increment.

(Q1-Q3: 3.6-12) months, for negative and positive ERCC1/XPF groups, respectively. PFS in ERCC1/XPF-negative patients was worse than that in ERCC1/XPF-positive patients, although the difference was not statistically significant (HR 1.61 95% CI 0.88-3.03, $P = 0.123$) (Figure 1A). When OS was considered, ERCC1/XPF-negative patients showed a median of 16.5 (Q1-Q3: 6.3-not reached) compared with 20.5 (Q1-Q3: 8.5-48.9) months reached in the ERCC1/XPF-positive group (HR 1.41, 95% CI 0.67-2.94, $P = 0.373$) (Figure 1B). Detailed results on univariable and multivariable analyses for OS and PFS are reported in Table 4.

There was no difference between the ERCC1/XPF-negative and -positive groups [OR (stable disease + progressive disease versus complete response + partial response) 0.87, 95% CI 0.25-3.07, $P = 0.832$] or for ERCC1/XPF as a continuous variable in the ORR to platinum-based first-line treatment (OR 1.03, 95% CI 0.60-1.76, $P = 0.916$) (Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2020.100034).

## DISCUSSION

Since the 1970s, platinum compounds have constituted the cornerstone of the treatment of early and advanced NSCLC yielding responses in about 25% of patients.[14] Despite our ability to control side-effects it represents one of the worst tolerated chemotherapy agents.[14,15] For this reason, the possibility to select patients for this treatment remains a major goal, to protect those from potentially deleterious effects who would be unlikely to derive benefit.

While research into biomarkers for selection of patients for several targeted therapies has been fruitful, the search for biomarkers able to stratify patients for chemotherapy has been difficult, often generating controversial results. Most of the evidence has been obtained retrospectively, rendering the interpretation of results difficult, which have often not been reproducible in prospective studies.[16]

The cytotoxic activity of platinum compounds is driven by the ability of these molecules to form DNA adducts.[17] The presence of platinum adducts induces DNA double helix distortion and this status activates cellular mechanisms able to remove the DNA lesions and restore the DNA integrity. The ability of cells to repair the lesions is generally related to the efficacy of alkylating agents such as platinum compounds. DNA repair status has been considered a potential biomarker to select patients based on the hypothesis that tumors which harbor a defective DNA repair system might benefit more than those without.[11,18,19]

As the activity of ERCC1 is the limiting step in the NER pathway and the NER pathway is deeply involved in the repair of the platinum compounds adducts, the researcher investigated the role of this protein as a biomarker for the selection of patients that potentially could benefit or not the treatment. Many papers describe the role of ERCC1 as mediators of platinum response, but results are contradictory.[11,20-22] Several studies suggest that patients with ERCC1-negative tumors appear to benefit more from platinum-based chemotherapy than patients with ERCC1-positive tumors.[23-25] However, the activity of ERCC1 has been evaluated by the analysis of surrogate markers with indirect endpoints such as the study of the protein level (IHC or western blot), RNA expression levels with different techniques and single nucleotide polymorphisms.[26,27] In addition, ERCC1 has different isoforms and isoform 202 was claimed to be the only one active, but no specific antibodies are available against this particular isoform.[12,13]

In our study we employed the PLA between ERCC1 and XPF to measure the active complex processing the platinum adduct, to overcome the issues about the different isoforms. Our results suggest that different amounts of ERCC1/XPF complex do not necessarily impact on outcomes. Only when we separated patients into overall negative or positive for the presence of ERCC1/XPF complex was it possible to delineate a potential role for this marker, although statistical significance was not reached, possibly due to the small number of patients. The study highlighted the possibility that patients negative for the presence of the complex in tumor cells would present worse survival in terms of both PFS and OS. These results are unexpected, given that the absence of ERCC1 was associated with higher sensitivity of the cells to exposure to cis-platinum *in vitro*.[6] In addition, as previously mentioned, many studies suggest that patients with ERCC1-negative tumors seem to benefit more from platinum-based therapy than patients with ERCC1-positive

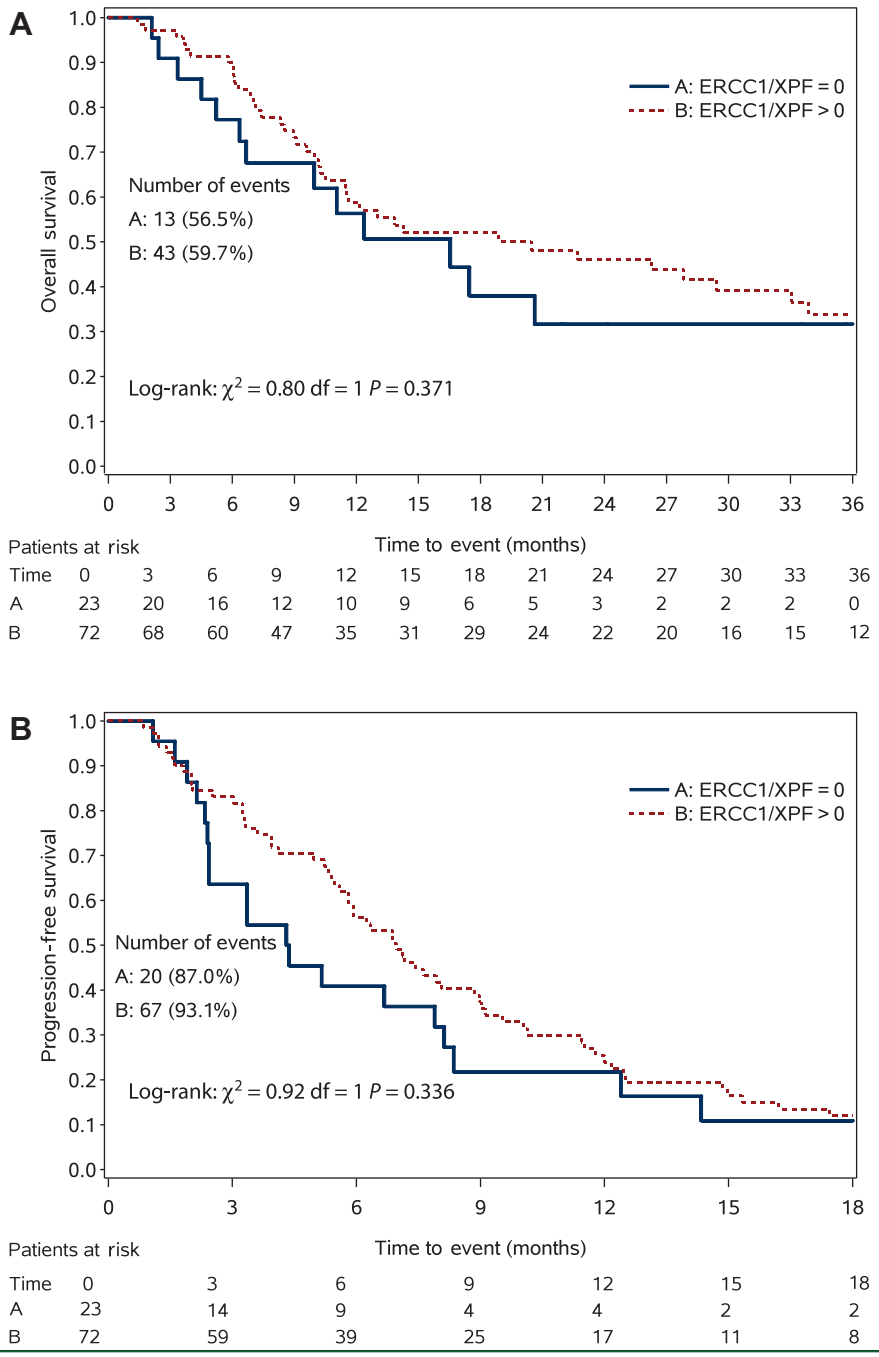


**Figure 1. (A) Kaplan–Meier curves for progression-free survival according to the value of ERCC1/XPF complex positive or negative. (B) Kaplan–Meier curves for overall survival according to the value of ERCC1/XPF complex positive or negative.**
ERCC1, excision repair cross-complementation group 1; XPF, xeroderma pigmentosum group F-complementing protein.

tumors.[23-25] We have to consider that all these studies were carried out without discriminating the active form of ERCC1 and data on the expression of the different isoforms of this gene are not available. A manuscript that discriminates the active form of ERCC1, by PLA, was recently published. The authors investigated the role of ERCC1 as a predictor of platinum response in a panel of ovarian cancer xenografts. In this report, no role was detected for ERCC1 in ovarian cancer.[28]

To our knowledge, this is the first study that has investigated the role and the value of the ERCC1/XPF complex as a platinum-based therapy response biomarker in NSCLC.

NSCLC tumors that do not express ERCC1/XPF complex may unexpectedly delineate a group of patients with poor outcomes compared with patients positive for the complex. This biomarker could therefore identify a subgroup of patients for which alternatives to platinum-based chemotherapy should be used.

**Table 4. PFS and OS by ERCC1/XPF-positive versus -negative**

| | PFS | | OS | |
|---|---|---|---|---|
| | HR (95% CI) | *P* | HR (95% CI) | *P* |
| Univariable | | | | |
| ERCC1/XPF | | | | |
| Positive | Reference | 0.338 | Reference | 0.372 |
| Negative | 1.28 (0.77-2.13) | | 1.33 (0.71-2.50) | |
| Multivariable | | | | |
| ERCC1/XPF | | | | |
| Positive | Reference | 0.123 | Reference | 0.373 |
| Negative | 1.61 (0.88-3.03) | | 1.41 (0.67-2.94) | |
| Age at diagnosis[a] | 0.96 (0.93-0.98) | 0.003 | 0.97 (0.94-1.00) | 0.077 |
| Histology | | | | |
| Adenocarcinoma | Reference | | Reference | 0.071 |
| Squamous | 1.04 (0.54-2.00) | 0.815 | 0.88 (0.39-1.95) | |
| Nos or other | 2.04 (0.23-18.2) | | 14.7 (1.36-159) | |
| Smoking | | | | |
| Never | Reference | 0.744 | Reference | 0.042 |
| Previous | 1.28 (0.68-2.44) | | 3.16 (1.28-7.81) | |
| Current | 1.19 (0.58-2.47) | | 2.05 (0.73-5.76) | |
| ECOG-PS | | | | |
| 0 | Reference | 0.650 | Reference | 0.458 |
| 1 or 2 | 0.83 (0.37-1.86) | | 0.66 (0.21-2.00) | |
| Therapy | | | | |
| Cisplatin | Reference | 0.111 | Reference | 0.031 |
| Carboplatin | 1.58 (0.90-2.78) | | 2.16 (1.07-4.32) | |
| Immunotherapy | | | | |
| No | — | — | Reference | 0.054 |
| Yes | — | — | 0.55 (0.30-1.01) | |

CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; ERCC1, excision repair cross-complementation group 1; HR, hazard ratio; OS, overall survival; PFS, progression-free survival; PS, performance status; XPF, xeroderma pigmentosum group F-complementing protein.
[a] One-year increment.

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## DISCLOSURE

MCG reported personal fees from Merck, Bristol-Myers Squibb, AstraZeneca, Roche, Takeda, Celgene, Pfizer, and GlaxoSmithKline. No other disclosures were reported.

## DATA SHARING

All datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by Fondazione IRCCS Istituto Nazionale dei Tumori Institutional Review Board (INT18/13) and conducted according to the ethical principles for medical research involving human subjects adopted in the Declaration of Helsinki. All patients signed a written informed consent.

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