IJC
International Journal of Cancer

Prognostic factors in gemcitabine–cisplatin polychemotherapy regimens in pancreatic cancer: *XPD-Lys751Gln* polymorphism strikes back

Amir Avan[1]*, Paola Pacetti[2]*, Michele Reni[3]*, Michele Milella[4], Enrico Vasile[5], Andrea Mambrini[2], Vanja Vaccaro[4], Sara Caponi[5], Stefano Cereda[3], Godefridus J. Peters[1], Maurizio Cantore[2] and Elisa Giovannetti[1]*

[1] Department of Medical Oncology, VU University Medical Center, Amsterdam, The Netherlands
[2] Department of Medical Oncology, Carrara Civic Hospital, Carrara, Italy
[3] Department of Medical Oncology, San Raffaele Scientific Institute, Milan, Italy
[4] Department of Medical Oncology A, Regina Elena National Cancer Institute, Rome, Italy
[5] Unit Medical Oncology-2, Azienda-Ospedaliera-Universitaria Pisa, Pisa, Italy

The use of platinated agents in combination chemotherapy regimens for advanced pancreatic cancer is controversial owing to the lack of an outstanding impact on the outcome and a substantial increase in hematologic and extra-hematologic toxicities. Pharmacogenetic studies to identify patients who could benefit most from such therapies are urgently needed. The *Xeroderma-Pigmentosum group-D* polymorphism at codon-751 (*XPD-Lys751Gln*) emerged as the most significant independent predictor for death- and progression-risk in our previous study on functional polymorphisms in 122 advanced pancreatic cancer patients treated with cisplatin–docetaxel–capecitabine–gemcitabine and cisplatin–epirubicin–capecitabine–gemcitabine (or EC-GemCap). To confirm the prognostic role of this variable, we further evaluated the correlation of *XPD-Lys751Gln* with outcome in another 125 patients treated with the same regimens, and 90 treated with gemcitabine monotherapy. Genotyping was successfully carried out in the vast majority of DNA samples. Genotype frequencies followed Hardy–Weinberg equilibrium, and *XPD-Lys751Gln* was associated with differential progression-free and overall-survival. Multivariate analysis confirmed its prognostic significance in platinum-based regimens. In particular, *XPD-Gln751Gln* was significantly associated with risk of death (hazard ratio, HR = 1.7, 95% confidence interval [CI], 1.1–2.6, $p = 0.011$) and risk of progression (HR = 1.7, 95% CI, 1.1–2.5, $p = 0.013$). No correlation was observed in gemcitabine monotherapy-treated patients. The analysis of DNA damage using extra-long-PCR in lymphocytes supported the association of *XPD-Gln751Gln* with greater resistance to cisplatin-induced damage. The increasing evidence of *XPD-Lys751Gln* impact on the outcome of gemcitabine–cisplatin-based polychemotherapy leads to plan prospective studies to validate the role of this polymorphism as a new tool for optimization of the currently available treatments in pancreatic cancer.

Key words: clinical outcome, pancreatic cancer, polychemotherapy, prognostic factor, *XPD-Lys751Gln* polymorphism

Abbreviations: HR: hazard ratio; NER: nucleotide excision repair; NSCLC: non small cell lung cancer; PDAC: pancreatic ductal adenocarcinoma; XL-PCR: extra-long PCR; XPD: Xeroderma-Pigmentosum group-D

Additional Supporting Information may be found in the online version of this article.

A.A., P.P. and M.R. contributed equally to this work

Grant sponsors: Netherlands Organization for Scientific Research, AIRC-Marie Curie, AIRC-fellowship, AIRC 5X1000 grant, CCA-Foundation

DOI: 10.1002/ijc.28078

History: Received 10 Aug 2012; Accepted 9 Jan 2013; Online 7 Feb 2013

Correspondence to: Dr. Elisa Giovannetti, Department of Medical Oncology, VU University Medical Center, Cancer Center Amsterdam, CCA 1.52, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands, Tel.: (31)-20-444267, Fax: +(31)-20-4443844, E-mail: e.giovannetti@vumc.nl

Short Report

Recently, the most relevant therapeutic progress in metastatic pancreatic ductal adenocarcinoma (PDAC) has come from the combination of several cytotoxic agents, such as the 5-fluorouracil, irinotecan and oxaliplatin regimen FOLFIRINOX.[1] Since 2001, we have reported the results of five studies,[2–6] including a phase III trial, showing superiority over single-agent gemcitabine,[3] of the four-drug regimens cisplatin–epirubicin–5-fluorouracil–gemcitabine, cisplatin–docetaxel–capecitabine–gemcitabine (PDXG) and cisplatin–epirubicin–capecitabine–gemcitabine (PEXG). Furthermore, two surveys mirroring the Italian clinical practice in the first-line therapeutic management of advanced PDAC suggested that four-drug combinations might yield a better outcome when compared to other regimens.[7,8]

However, the enthusiasm over the benefit of these regimens is tempered by their associated increased hematologic or extra-hematologic side effects. Analysis of accessible biomarkers, such as germ-line polymorphisms, may lend important insight into the selection of the most appropriate chemotherapeutic regimen to be used for any given patient.

The *Xeroderma-Pigmentosum group-D* (*XPD*) polymorphism at codon-751 (*XPD-Lys751Gln*) emerged as the most

What's new?

Recent advances in treatments of pancreatic cancer include a novel combination therapy of cisplatin-gemcitabine-based polychemotherapeutic regimens. However, this treatment shows increased hematologic or extra-hematologic side effects calling for the identification of biomarkers that predict treatment outcome. In this study, the XPD-Lys751Gln gene polymorphism was identified as the most significant independent predictor for death and progression-risk in pancreatic cancer patients who underwent such treatment. Determined by a simple blood test, the polymorphism offers an innovative tool for optimizing palliative chemotherapy in advanced pancreatic cancers. The authors call for prospective trials to validate their findings, which may ultimately lead to a more individualized treatment in selected pancreatic cancer patients.

significant independent predictor for death- and progression-risk in our previous pharmacogenetic study on 11 functional polymorphisms in 122 advanced PDAC patients treated with four-drug regimens.[9] Patients with the *XPD-Gln751Gln* genotype had a worse prognosis (median overall survival (OS) 10.3 months; 95% confidence interval [CI], 4.0–16.5) than patients with *XPD-Lys751Lys* or *XPD-Lys751Gln* (13.3 months, 95% CI, 10.9–15.7, $p = 0.003$). In the multivariate analysis, *XPD-Gln751Gln* remained a significant predictor for shorter survival, with an increased risk of death (hazard ratio, HR) of 1.9 (95% CI, 1.3–2.9; $p = 0.003$). Similarly, *XPD-Gln751Gln* was associated with a significantly increased risk of progression (HR, 2.1; 95% CI, 1.2–3.5; $p = 0.008$).

The XPD protein is a 5′-3′ superfamily-2 helicase involved in DNA unwinding during nucleotide excision repair (NER), which is the principal repair pathway for removing platinum-DNA adducts. Previous studies suggested that *XPD* polymorphisms might affect DNA repair capacity, and *XPD-Lys751Lys* genotype was associated with a reduced repair of X-ray-induced DNA damage, compared to genotypes having a *751Gln* allele. *XPD-Lys751Lys* was also associated with greater levels of chromatid aberrations and UVC-induced formation of strand breaks in lymphocytes.[10,11] Conversely, in a host-cell reactivation assay using BPDE-treated plasmids or UV-irradiated plasmids, DNA repair capacity was reduced in subjects carrying two *XPD* variant alleles (*Asn312Asn* and *Gln751Gln*) compared to those homozygous for the respective wild-type alleles.[12,13]

Therefore, our study was aimed at confirming the prognostic role of *XPD-Lys751Gln* in a new subset of 125 patients as well as in all the 247 patients treated with our four-drug regimens. Moreover, to test our hypothesis that *XPD-Gln751Gln* genotype was associated with a more efficient DNA repair after cisplatin exposure, we evaluated DNA damage using extra-long-PCR in lymphocytes harboring the different *XPD-Lys751Gln* genotypes.

Methods

Patients

In the current pharmacogenetic study, 337 patients (247 treated with gemcitabine–platinum regimens and 90 treated with gemcitabine) were selected based on the diagnosis of histologically confirmed locally advanced or metastatic PDAC. All the eligible patients were chemo-naive patients treated in four Italian oncology units: S. Raffaele Scientific Institute (Milano), Carrara Civic-Hospital (Carrara), Regina Elena National Cancer Institute (Roma) and Pisa University-Hospital (Pisa). Details on diagnosis and treatments are summarized in Supporting Information data and Supporting Information Table S1. The study was approved by local Hospital Ethic Committees.

Genotyping

Genomic DNA was extracted from blood samples at the Laboratory Medical Oncology (VUmc, Amsterdam, The Netherlands) using the QIAamp® DNA Mini-Kit according to the manufacturer's protocol (Qiagen, San Diego, CA). The concentration and purity of DNAs were determined with the NanoDrop®-1000-Detector (NanoDrop-Technologies, Wilmington, NC). Genotype analysis of *XPD-Lys751Gln* polymorphism was performed using Taqman®-probe-based assay; PCR reactions were carried out in 12.5 μL total volume, using 20 ng of DNA diluted in TaqMan® Universal Master Mix with specific primers and probes (c_3145033_10, Applied Biosystems, Foster City, CA). The ABIPRISM-7500 instrument equipped with the *SDS version-2.0* software was employed to evaluate the allelic content of the samples.

Isolation of lymphocytes from peripheral blood and extra-long PCR

The aim of our *in vitro* study was to evaluate whether the *XPD-Gln751Gln* genotype was associated with a more efficient DNA repair after cisplatin exposure. As other determinants of the NER system could affect cisplatin activity, we selected samples with the same genotypes for *ERCC1-C118T* and *XPD-Arg312Asn*.[9] Samples (10–14 mL) were taken from peripheral blood and mononuclear cells were isolated by Ficoll-Isopaque (Pharmacia, Uppsala, Sweden) density gradient centrifugation as described previously.[14] A total of 15 blood samples from chemo-naive healthy volunteers (five for each *XPD-Lys751Gln* genotype, as determined in preliminary genotyping analysis) were evaluated.

The cells were plated in four petri-dishes, and experiments were performed in triplicate, with inter- and intra-assay coefficients of variation below 12%. To estimate differential DNA repair ability in cells harboring the different *XPD-Lys751Gln* genotypes, these cells were exposed to cisplatin and gemcitabine. Then, a target sequence of β-globin was amplified by extra-long PCR (XL-PCR), followed by quantification with Taqman®-PCR.[15] XL-PCR was performed using the specific GeneAmp®

XL-PCR Kit (Applied Biosystems), starting from 100 ng of template DNA. This DNA was extracted from mononuclear cells untreated or exposed *in vitro* to 200 μM cisplatin, 1 μM gemcitabine or their combination for 24 hr as described previously.[16] Amplification of a 17.7-kb region of the β-globin gene was performed as described previously,[15] using specific primers (Supporting Information Table S2). These PCR reactions were performed in a GeneAmp®-PCR-9700 machine (Applied Biosystems), whereas the reaction products were quantified using TaqMan® assays in the ABIPRISM-7500 instrument, using specific primers and probes (Supporting Information Table S2). A standard curve of XL-PCR-amplified products for β-globin was generated by serial dilution of XL-PCR-amplified products from genomic DNA extracted from a mix of six untreated samples representative of the three *XPD-Lys751Gln* genotypes.

Statistics

Demographic and clinical information were compared across genotype using Pearson's χ^2-tests. In agreement with our previous study, the correlation with *XPD-Lys751Gln* genotypes was performed combining the homozygous *XPD-Lys751Lys* and heterozygous genotypes.[9] OS and PFS curves were analyzed from the day of treatment start to the end point (death or censoring) according to Kaplan–Meier method, and compared by log-rank and Wilcoxon tests. The significant prognostic variables in the univariate analysis were included in multivariate analyses, using Cox's proportional hazards model. This analysis included a step-down procedure based on the likelihood ratio test, where HRs were calculated to estimate the magnitude and the direction of the effect. Appropriate adjustment for false-positive report probability was performed according to the Wacholder method.[17] *In vitro* data were expressed as mean values ± S.E., analyzed by Student's *t*-test or ANOVA followed by Tukey's multiple comparison. Data were analyzed using SPSS-17 software (SPSS, Chicago, IL). All the analyses were two-sided and statistical significance was set at $p < 0.05$.

Results

Clinicopathological characteristics and outcome in patients treated with gemcitabine–cisplatin polychemotherapy regimens

To evaluate whether patient characteristics might influence clinical outcome, we analyzed data on OS or PFS according to the patients' clinicopathological features. Male gender, performance status (PS) ≤80, CA19.9 >median and stage-IV were associated with significantly shorter OS and PFS, whereas no differences were detected for age (Table 1).

XPD-Lys751Gln and clinical outcome in gemcitabine–cisplatin polychemotherapy regimens

To confirm the prognostic role of *XPD-Lys751Gln* with outcome, we performed genotyping analysis in a new subset of 125 patients as well as in all our 247 (*i.e.*, 122 + 125) patients treated with gemcitabine–cisplatin polychemotherapy. Genotyping was successfully carried out in 246 out of 247 samples. *XPD-Lys751Gln* genotype frequencies were followed Hardy–Weinberg equilibrium and were comparable with those reported in Caucasian populations in NCI-SNP500 databases. No associations were found between genotypes and age, gender, PS, CA19.9 or stage (data not shown).

As shown in the Kaplan–Meier curves in Figure 1, *XPD-Lys751Gln* polymorphism was associated with significantly differential OS. In the new subset, the patients with the *XPD-Lys751Lys* or *Lys751Gln* genotypes had a median OS of 12.0 months (95% CI, 10.1–13.9), whereas patients with the *XPD-Gln751Gln* genotype had a median OS of 7.0 months (95% CI, 4.3–9.7, log-rank $p = 0.01$, Wilcoxon $p = 0.01$). Moreover, we observed a trend toward a significant association for PFS, with median values of 7.0 (95% CI, 5.7–8.3) and 6.0 (95% CI, 2.5–9.5) months in the patients with the *XPD-Lys751Lys* or *Lys751Gln* and *XPD-Gln751Gln* genotype, respectively (log-rank $p = 0.05$, Wilcoxon $p = 0.09$).

The analysis of all the 246 patients showed that patients harboring the *XPD-Lys751Lys* or *Lys751Gln* genotypes had significantly longer median OS (log-rank $p < 0.01$, Wilcoxon $p < 0.01$). Similarly, the median PFS of patients carrying the *XPD-Gln751Gln* genotype was significantly shorter than the median PFS of *XPD-Lys751Lys* or *XPD-Lys751Gln* patients (log-rank $p < 0.01$, Wilcoxon $p = 0.01$, Table 1). None of the *XPD-Gln751Gln* patients survived more than 22 months. Conversely, a total of ten patients harboring the *XPD-Lys751Lys* or *XPD-Lys751Gln* genotypes survived more than 35 months after treatment started, including seven patients who did not progress at this time point. These data suggest that a subpopulation of PDAC cases with *XPD-Lys751Lys* or *XPD-Lys751Gln* genotypes clearly improves with cisplatin treatment. The 25, 50 and 75% OS and PFS values of the patients stratified by genotype are listed in Supporting Information Table 3.

In the multivariate analysis, male gender, PS ≤ 80 and stage-IV were significantly associated with increased risk of death and progression (Table 1). However, the Cox proportional hazards regression model also showed the prognostic significance of *XPD-Lys751Gln*. The *XPD-Gln751Gln* genotype was significantly associated with increased risk of death (HR = 2.1, $p < 0.01$) as well as with increased risk of progression (HR = 1.9, $p < 0.01$).

XPD-Lys751Gln and clinical outcome in gemcitabine monotherapy

A total of 90 patients treated with gemcitabine monotherapy were evaluated for an exploratory analysis to endorse the hypothesis of the specific value of *XPD-Lys751Gln* polymorphism in patients treated with platinum-based regimens. Although the purpose of our analysis was not to perform a case–control study, we compared the baseline demographic characteristics of these groups. No significant differences were measured across series (data not shown). *XPD-Lys751Gln* genotypes were successfully detectable in all these patients, and their allelic frequencies were similar to those in patients treated with gemcitabine–cisplatin polychemotherapy

Short Report

Table 1. Clinical outcome according to clinicopathological characteristics and *XPD-Lys751Gln* polymorphism of patients treated with the four-drug regimens PDXG, PEXG and ECGemCap

Characteristics	Patients, *n* (%)	OS median mo. (95% CI)	*p*-Value[1]	PFS median mo. (95% CI)	*p*-Value[1]
Univariate analysis					
No. patients	247	12.0 (11.0–13.0)		9.0 (7.9–10.1)	
Age (years)					
≤65	145 (58.7)	13.0 (10.5–15.5)	0.05	10.1 (8.7–11.5)	0.07
>65	102 (41.3)	11.0 (8.9–13.1)		7.0 (5.6–8.4)	
Sex					
Male	141 (57.1)	11.0 (9.7–12.3)	*0.01*	8.3 (7.0–9.6)	0.17
Female	106 (42.9)	16.0 (12.2–19.7)		10.0 (8.2–11.7)	
PS					
≤80	87 (35.2)	10.0 (7.4–12.6)	*<0.01*	6.0 (4.9–7.1)	*<0.01*
>80	160 (64.8)	13.0 (10.3–15.7)		10.1 (8.8–11.4)	
CA19.9					
≤Median	124 (50.2)	13.0 (10.8–15.2)	*0.04*	10.1 (8.4–11.8)	*0.04*
>Median	123 (49.2)	12.0 (10.3–13.7)		8.0 (6.6–9.4)	
Clinical stage					
III	81 (32.8)	18.0 (15.2–20.8)	*<0.01*	13.5 (10.5–16.4)	*<0.01*
IV	166 (67.2)	10.0 (8.7–11.2)		7.0 (5.7–8.2)	
XPD-Lys751Gln					
Lys751Lys + Lys751Gln	213 (86.2)	13.0 (11.4–14.6)	*<0.01*	9.3 (8.0–10.5)	*0.01*
Gln751Gln	33 (13.8)	7.0 (4.0–10.0)		6.0 (4.3–7.7)	
Multivariate analysis					
Covariates for OS	**Covariates**	**Hazard ratio**	**95%CI**	**df**	***p*-Value[2]**
Sex	Male	1.4	1.0–1.8	1	*0.04*
	Female	1 (ref)			
PS	≤80	1.6	1.1–2.2	1	*<0.01*
	>80	1 (ref)			
CA19.9	≤Median	1 (ref)		1	0.21
	>Median	1.2	0.9–1.6		
Clinical stage	III	1 (ref)		1	*<0.01*
	IV	1.8	1.3–2.6		
XPD-Lys751Gln	*Lys751Lys+Lys751Gln*	1 (ref)		1	*<0.01*
	Gln751Gln	2.1	1.4–3.3		
Covariates for PFS	**Covariates**	**Hazard ratio**	**95%CI**	**df**	***p*-Value[2]**
PS	≤80	1.5	1.1–2.0	1	*<0.01*
	>80	1 (ref)			
CA19.9	≤Median	1 (ref)		1	0.11
	>Median	1.2	0.9–1.6		
Clinical stage	III	1 (ref)		1	*<0.01*
	IV	1.8	1.3–2.4		
XPD-Lys751Gln	*Lys751Lys + Lys751Gln*	1 (ref)		1	*<0.01*
	Gln751Gln	1.9	1.3–2.9		

There were 189 deaths (even rate, 76.5%), whereas 33 patients were alive without progression at last contact (October 2011), with a median follow-up for living patients of 15.0 months (range, 4.4–63.5).
A total of 246 patients, including 122 patients from our previous study,[9] were assessable for the *XPD-Lys751Gln* polymorphism.
[1]Log-rank test *p*-values.
[2]Wald *p*-values.
Abbreviations: CA19.9, carbohydrate antigen 19.9; CI, confidence interval; df, degrees of freedom; mo., months; OS, overall survival; PFS, progression-free survival; PS, performance status and Ref, reference value.

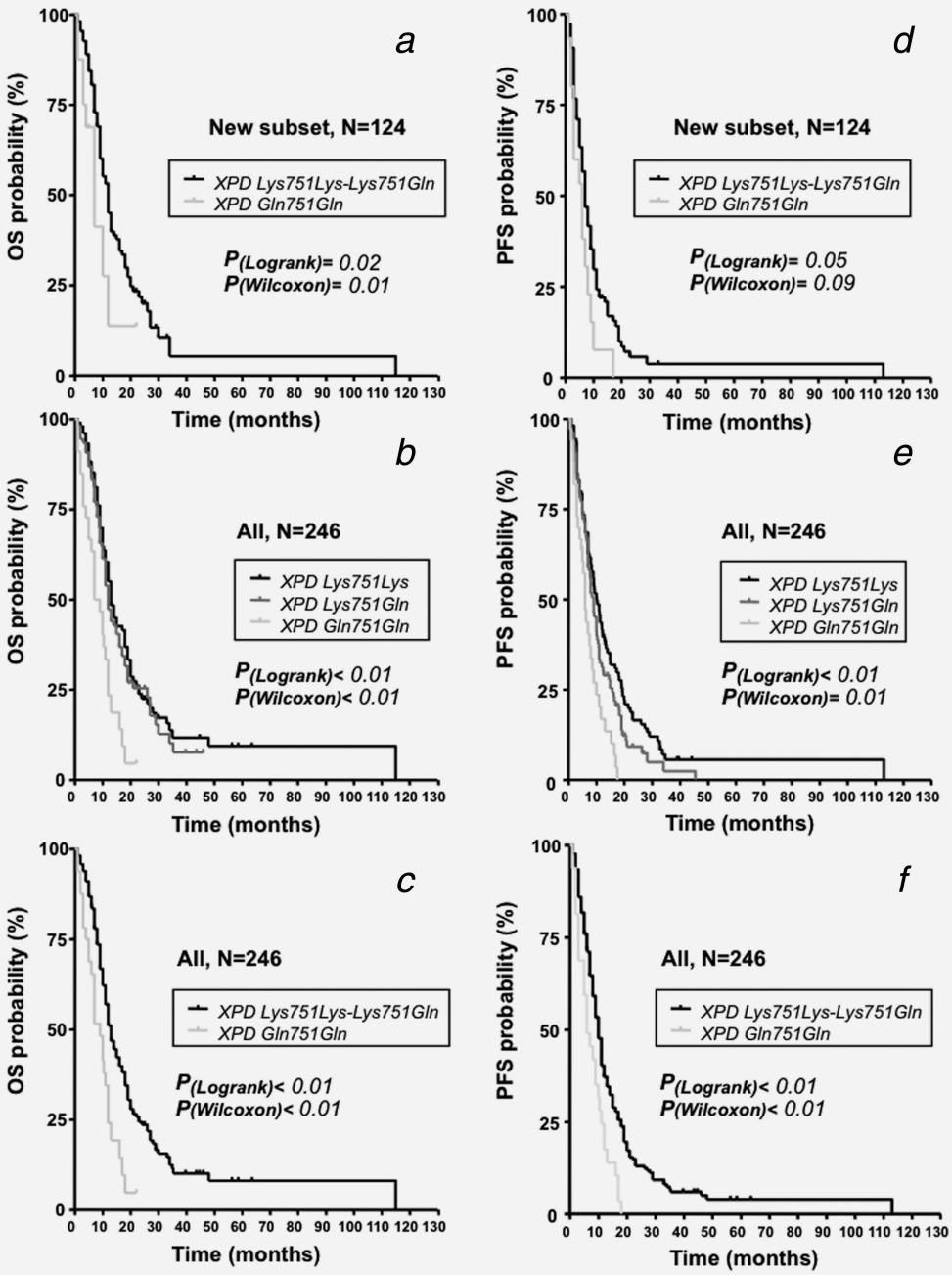

Figure 1. Kaplan–Meier survival curves. OS (*a–c*) and progression-free survival (PFS) (*d–f*) according to *XPD-Lys751Gln* genotypes in the new subset ($N = 124$) and in all of the PDAC patients treated upfront with gemcitabine–cisplatin-based four-drug polychemotherapy regimens. In the new subset of patients, a total of 108 and 16 patients had the *XPD-Lys751Lys* or *XPD-Lys751Gln*, and the *XPD-Gln751Gln* genotypes, respectively. Considering all the patients, a total of 213 and 33 patients had the *XPD-Lys751Lys* or *XPD-Lys751Gln*, and the *XPD-Gln751Gln* genotypes, respectively. *p*-Values were calculated with the log-rank and the Wilcoxon test.

regimens ($p > 0.05$ in the Pearson χ^2-test). No significant differences were observed in OS and PFS for *XPD-Lys751Gln* (Supporting Information Fig. 1).

XPD-Lys751Gln and XL-PCR results

The extent of DNA-repair ability was determined based on the reduction in PCR amplification of a target sequence in treated samples normalized to untreated controls. As shown in Figure 2, after cisplatin treatment the cells with the *XPD-Gln751Gln* genotypes had a significantly greater relative amplification in comparison to the cells with *XPD-Lys751Lys* ($p < 0.01$) or *XPD-Lys751Gln* genotypes ($P = 0.03$). These data suggest that the *XPD-Gln751Gln* genotype might confer an increased activity to XPD in its DNA-repair function, and

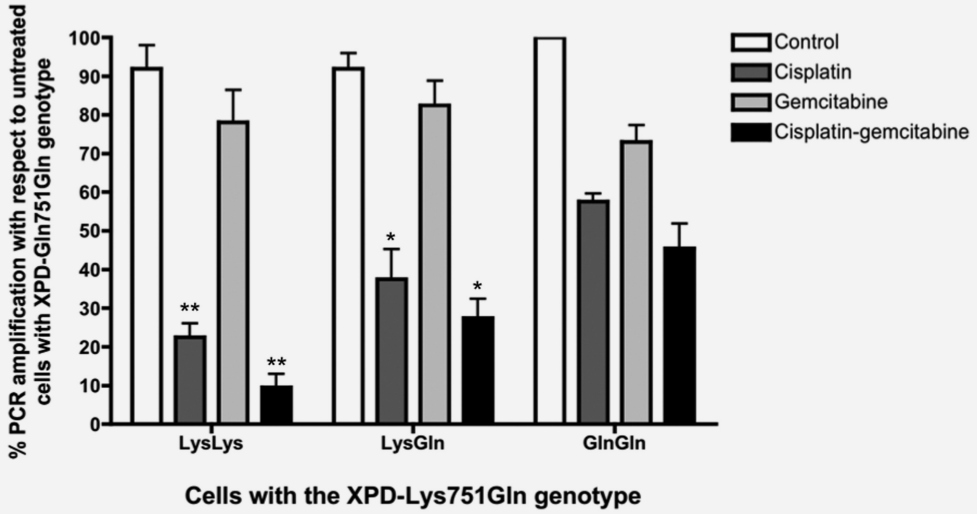

Figure 2. Evaluation of DNA repair after exposure to cisplatin and gemcitabine by XL-PCR. Lymphocytes from healthy volunteers characterized for their differential *XPD-Lys751Gln* genotypes ($N = 5$ in each group) were plated in Petri-dishes and treated as described in the **Methods** section. XL-PCR was performed using primers specific for a target sequence in the β-globin gene. Then, TaqMan quantification of XL-PCR amplicons was performed using specific primers and probe, and is presented as amplification relative to untreated control. *Columns*, mean values; *bars*, SD. *Significantly different from *XPD-Gln751Gln*. **Significantly different from *XPD-Gln751Gln* and *XPD-Lys751Gln* ($p < 0.05$).

thus reduce the anticancer activity of cisplatin. This effect was additionally increased by the cisplatin–gemcitabine combination ($p < 0.01$ for *XPD-Gln751Gln vs. XPD-Lys751Gln* and $p < 0.001$ for *XPD-Gln751Gln vs. XPD-Lys751Lys*).

Discussion

In our study, we demonstrated the importance of *XPD-Lys751Gln* polymorphism as a predictive marker for death- and progression-risk in a new panel of 125 as well as in a global analysis of 247 advanced PDAC patients treated with four-drug polychemotherapy regimens. These results are in agreement with our previous study,[9] and might be explained by the central role of XPD in DNA-repair and platinum activity. However, we performed further analyses in patients treated upfront with gemcitabine alone. In these patients, the lack of correlation between *XPD-Lys751Gln* polymorphism and outcome suggested that the NER system does not affect the repair of gemcitabine-induced DNA damage. These data are in accordance with the previous studies in PDAC patients treated with neoadjuvant gemcitabine concomitant to radiotherapy.[18]

The *XPD-Lys751Gln* polymorphism has been associated with survival after platinum-based chemotherapy in different tumor types, and we recently observed a significant association of *XPD-Gln751Gln* with shorter PFS ($p = 0.02$) and OS ($p = 0.04$) in 93 advanced non small cell lung cancer (NSCLC) patients treated with second-line carboplatin plus pemetrexed.[19] However, a recent meta-analysis of 12 studies reported inconclusive data in NSCLC.[20] These discrepancies suggest that pharmacogenetic associations are not always reproducible in small size studies, with different clinical settings, tumor types, stage and treatment. Larger multicentre studies are critical to explore the role of emerging biomarkers before planning of prospective trials.

Platinum compounds inhibit cell proliferation by damaging DNA through the formation of intrastrand crosslinks. Therefore, platinum resistance occurs mostly because of the removal of damaged DNA or efficient repair of DNA by the NER system. This system consists of at least 30 identified polypeptides, including XPD, which is a major player in the DNA repair.[21] Variations in the *XPD* sequence are found among the general population, and the codon-751 variant changes the electron configuration of the resulting peptide. This is a major change, located in the important domain of interaction between XPD and its helicase activator, p44 protein, inside the TFIIH complex.[22] In theory, the consequences of *Lys751Gln* polymorphism are the most important in terms of XPD activity and repair of cisplatin-induced DNA damage. Therefore, we performed functional studies to evaluate whether the *XPD-Gln751Gln* genotype might alter the cellular response to the DNA damage induced by cisplatin and gemcitabine. Using a validated XL-PCR technique, we found that *XPD-Gln751Gln* genotype was associated with more efficient DNA repair toward cisplatin-induced DNA damage. Our XL-PCR analyses, as well as other assays,[10–13] have several limitations in evaluating all relevant pathways affecting the repair of cisplatin damage, such as Fanconi pathways and recombinant and recombination or mismatch repair.[23] However, this is also the first XL-PCR study, suggesting a role of the *XPD-Lys751Gln* polymorphisms in the synergistic interaction of cisplatin and gemcitabine. Accordingly, previous *in vitro* studies showed that cisplatin–gemcitabine synergism was mainly dependent on an increase in platinum-adduct formation possibly related to changes caused by gemcitabine incorporation in DNA.[13,24]

A major strength of our study is that it was performed in a homogeneous setting of patients from a multicentric series. The results of multivariate analysis indicate the noteworthiness of the prognostic role of *XPD-Lys751Gln*. Moreover, the minor allele frequency of this polymorphism in a random Caucasian population is frequent (*i.e.*, 28% according to the SNP-NCBI cancer database). Thus, these findings might be relevant to a large number of patients.

However, a recent study showed that DNA repair capacity in peripheral lymphocytes was inversely correlated with survival in NSCLC patients treated with platinum-based chemotherapy. This suggests that a phenotypic DNA repair marker might be more accurate than genetic variations in the prediction of outcome.[25] Furthermore, the real predictive role of *XPD-Lys751Gln* for platinum-based chemotherapy activity should be established in prospective randomized studies with a control arm of patients treated with other regimens.

The increasing evidence that indicates that *XPD-Lys751Gln* has a direct impact on the outcome of PDAC patients treated with gemcitabine–cisplatin polychemotherapy leads to consider the analysis of this polymorphism as a relevant strategy to optimize treatment efficacy. Thus, prospective studies are ongoing, also in different clinical settings, such as in the perioperative setting. In particular, a pharmacogenetic study is currently underway within a three-arm phase II–III clinical trial (ClinicalTrials.gov-Identifier, NCT01150630) involving more than 20 Italian centers. Ultimately, the validation of the role of *XPD-Lys751Gln* might create new approaches in PDAC treatment.

Acknowledgements

This work was partially supported by grants from Netherlands Organization for Scientific Research (Elisa Giovannetti), AIRC-Marie Curie (Elisa Giovannetti), AIRC-fellowship (Vanja Vaccaro), AIRC 5X1000 grant (Michele Milella) and CCA-foundation (Amir Avan, Godefridus J. Peters and Elisa Giovannetti). The authors thank Sara Nannizzi, PhD, for her work on XL-PCR.

Competing interests

Michele Reni received consulting fees for Helsinn; a position on the advisory board of Abraxis, Merck, Clovis and Celgene; as well as lecture fees for Celgene.

References

1. Conroy T, Desseigne F, Ychou M, et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med* 2011;364:1817–25.
2. Reni M, Passoni P, Panucci MG, et al. Definitive results of phase II trial of cisplatin, epirubicin, continuous-infusion fluorouracil, and gemcitabine in stage IV pancreatic adenocarcinoma. *J Clin Oncol* 2001;19:2679–86.
3. Reni M, Cordio S, Milandri C, et al. Gemcitabine versus cisplatin, epirubicin, fluorouracil, and gemcitabine in advanced pancreatic cancer: a randomised controlled multicentre phase III trial. *Lancet Oncol* 2005;6:369–76.
4. Reni M, Cereda S, Bonetto E, et al. Dose-intense PEFG (cisplatin, epirubicin, 5-fluorouracil, gemcitabine) in advanced pancreatic adenocarcinoma: a dose-finding study. *Cancer Invest* 2007;25:594–8.
5. Reni M, Cereda S, Bonetto E, et al. Dose-intense PEFG (cisplatin, epirubicin, 5-fluorouracil, gemcitabine) in advanced pancreatic adenocarcinoma. *Cancer Chemother Pharmacol* 2007;59:361–7.
6. Reni M, Cereda S, Rognone A, et al. A randomized phase II trial of two different 4-drug combinations in advanced pancreatic adenocarcinoma: cisplatin, capecitabine, gemcitabine plus either epirubicin or docetaxel (PEXG or PDXG regimen). *Cancer Chemother Pharmacol* 2012;69:115–23.
7. Reni M, Sartori N, Mambrini A, et al. An Italian study on treatment trends and outcomes of patients with stage III pancreatic adenocarcinoma in the gemcitabine era: is it time to change? *Anticancer Drugs* 2010;21:459–64.
8. Reni M, Pasetto LM, Passardi A, et al. Treatment trends in metastatic pancreatic cancer patients: is it time to change? *Dig Liver Dis* 2011;43:225–30.
9. Giovannetti E, Pacetti P, Reni M, et al. Association between DNA-repair polymorphisms and survival in pancreatic cancer patients treated with combination chemotherapy. *Pharmacogenomics* 2011;12:1641–52.
10. Lunn RM, Helzlsouer KJ, Parshad R, et al. XPD polymorphisms: effects on DNA repair proficiency. *Carcinogenesis* 2000;21:551–5.
11. Moller P, Wallin H, Dybdahl M, et al. Psoriasis patients with basal cell carcinoma have more repair-mediated DNA strand-breaks after UVC damage in lymphocytes than psoriasis patients without basal cell carcinoma. *Cancer Lett* 2000, 151:187–92.
12. Spitz MR, Wu X, Wang Y, et al. Modulation of nucleotide excision repair capacity by XPD polymorphisms in lung cancer patients. *Cancer Res* 2001;61:1354–7.
13. Qiao Y, Spitz MR, Shen H, et al. Modulation of repair of ultraviolet damage in the host-cell reactivation assay by polymorphic XPC and XPD/ERCC2 genotypes. *Carcinogenesis* 2002;23:295–9.
14. Giovannetti E, Mey V, Loni L, et al. Cytotoxic activity of gemcitabine and correlation with expression profile of drug-related genes in human lymphoid cells. *Pharmacol Res* 2007;55:343–9.
15. Laws GM, Skopek TR, Reddy MV, et al. Detection of DNA adducts using a quantitative long PCR technique and the fluorogenic 5′ nuclease assay (TaqMan). *Mutat Res* 2001;484:3–18.
16. van Moorsel CJ, Pinedo HM, Veerman G, et al. Mechanisms of synergism between cisplatin and gemcitabine in ovarian and non-small-cell lung cancer cell lines. *Br J Cancer* 1999;80:981–90.
17. Wacholder S, Chanock S, Garcia-Closas M, et al. Assessing the probability that a positive report is false: an approach for molecular epidemiology studies. *J Natl Cancer Inst* 2004;96:434–42.
18. Li D, Frazier M, Evans DB, et al. Single nucleotide polymorphisms of RecQ1, RAD54L, and ATM genes are associated with reduced survival of pancreatic cancer. *J Clin Oncol* 2006;24:1720–8.
19. Tiseo M, Giovannetti E, Tibaldi C, et al. Pharmacogenetic study of patients with advanced non-small cell lung cancer (NSCLC) treated with second-line pemetrexed or pemetrexed-carboplatin. *Lung Cancer* 2012;78:92–9.
20. Wei SZ, Zhan P, Shi MQ, et al. Predictive value of ERCC1 and XPD polymorphism in patients with advanced non-small cell lung cancer receiving platinum-based chemotherapy: a systematic review and meta-analysis. *Med Oncol* 2011;28:315–21.
21. Sancar A. DNA repair in humans. *Annu Rev Genet* 1995;29:69–105.
22. Coin F, Marinoni JC, Rodolfo C, et al. Mutations in the XPD helicase gene result in XP and TTD phenotypes, preventing interaction between XPD and the p44 subunit of TFIIH. *Nat Genet* 1998;20:184–8.
23. Brabec V. DNA modifications by antitumor platinum and ruthenium compounds: their recognition and repair. *Prog Nucleic Acid Res Mol Biol* 2002;71:1–68.
24. Peters GJ, van der Wilt CL, van Moorsel CJ, et al. Basis for effective combination cancer chemotherapy with antimetabolites. *Pharmacol Ther* 2000;87:227–53.
25. Wang LE, Yin M, Dong Q, et al. DNA repair capacity in peripheral lymphocytes predicts survival of patients with non-small-cell lung cancer treated with first-line platinum-based chemotherapy. *J Clin Oncol* 2011;29:4121–8.

Short Report