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Nucleotide excision repair gene variants and association with survival in osteosarcoma patients treated with neoadjuvant chemotherapy

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Abstract

The aim of this study was to investigate the role of common polymorphisms in the NER pathway genes in the tumorigenesis of osteosarcoma and in the response to DNA damaging therapies, such as cisplatin-based neoadjuvant therapy. XPD (rs13181 and rs1799793), XPG (rs17655), and ERCC1 (rs3212986 and rs11615) polymorphisms were analysed in a group of 130 homogenously-treated patients with high-grade osteosarcoma for association with event free survival (EFS) using Kaplan-Meier plots and log-rank test. A positive association was observed between both XPD SNPs and an increased EFS (HR= 0.34, 95% CI 0.12-0.98 and HR= 0.19, 95% CI 0.05-0.77, respectively). We had also performed a case-control study for relative risk to develop osteosarcoma. Patients carrying at least one variant allele of XPD rs1799793 had a reduced risk of developing osteosarcoma compared to wild type patients (OR=0.55, 95% CI 0.36-0.84).This study suggests that XPD rs1799793 could be a marker of osteosarcoma associated with features conferring either a better prognosis or a better outcome after platinum therapy, or both.

Keywords

Nucleotide excision repair; osteosarcoma; DNA repair; polymorphism; event-free survival

INTRODUCTION

Osteosarcoma is the most common primary bone tumor occurring in children and adolescents. It most often develops in periods of rapid skeletal growth with more than 50% of tumors occurring in the long bones[1].

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Neoadjuvant chemotherapy followed by definitive resection with subsequent adjuvant chemotherapy is a well-established approach for the treatment of localized osteosarcoma since chemotherapy can eradicate the micrometastatic disease that is believed to be present in the majority of patients with clinically resectable cancer[2;3]. The neoadjuvant treatment of osteosarcoma includes the administration of cisplatin in combination with doxorubicin, methotrexate and ifosfamide[1;4]. This approach results in a 5-year survival rate of 60-70% for patients with non-metastatic osteosarcoma of the extremities[1;5].

The decision about the best treatment choice for these patients is still based upon the traditional assessment of the tumor characteristics, and there are no molecular markers that can guide therapy. The most consistent clinicopathologic prognostic markers are still clinical, including the presence of metastases at diagnosis, the histological response of the tumor to preoperative chemotherapy and tumor volume[1;6;7]. Among biological markers, previous studies have identified various factors which appeared to be associated with poor prognosis for patients with osteosarcoma, including overexpression of MDR1/P-glycoprotein[1;8], proliferation rate of tumor, P53 gene alteration and translocation[9;10]. However, amelioration of the prognosis of osteosarcoma should involve molecular approaches to offer patients additional, possibly tailored, therapies[1;11;12].

It has been suggested that deficiencies in DNA repair capacity could have a role in cancer onset or in its progression, as well as in affecting the response to DNA damaging agents[10;13;14]. DNA repair processes involve at least four different pathways acting on specific types of DNA damages. In particular, the nucleotide excision repair (NER) pathway repairs bulky lesions and has been associated with tumor progression and response to platinum-based chemotherapy[15;16]. Excision repair cross-complementing group 1 (ERCC1/XPA), group 2 (ERCC2/XPD), group 4 (ERCC4/XPF) and group 5 (ERCC5/XPG) are members of the NER pathway. It has been demonstrated that a deficiency of DNA repair capacity of NER genes is related to the presence of single nucleotide polymorphisms (SNPs) that result in altered mRNA expression or protein activity[13]. XPD 35931A>C (rs13181, Lys751Gln) and 23591G>A (rs1799793, Asp312Asn), XPG 3507G>C (rs17655, Asp1104His), ERCC1 8092C>A (rs3212986, Gln504Lys) and 19007C>T (rs11615, Asn118Asn) are common in Caucasians and lead to reduced DNA repair capacity[17-20]. The complex system of DNA repair plays a pivotal role in maintaining genomic integrity by counteracting endogenous or exogenous insults that can lead to an increase in cancer susceptibility. The main cytotoxic effect of cisplatin is due to the formation of mono or bi-functional DNA adducts causing inter- or intra-strand cross linking being removed by the NER pathway. A deficiency in the ability to repair DNA damages induced by platinum agents could therefore alter the response to treatment.

Several studies have evaluated the role of genetic variants in the NER pathway as either prognostic for survival or predictive for the efficacy of platinum-based chemotherapy[15;21-30]. However, from these findings, no single NER gene variant emerges as a validated marker with an effect that is consistently replicated across different studies.

The role of common variation in genes of the NER pathway has been also evaluated as risk factors for epithelial cancer, and the results are contradictory, with predisposing effect[29;31-39] or protective effect[40;41], whereas for mesenchimal tumors, like sarcomas, only few studies have been published so far[10;21;22].

In the present study, we have evaluated the effect of five NER gene SNPs for risk and survival in 130 patients with high-grade primary osteosarcoma homogeneously treated with neoadjuvant chemotherapy.

MATERIAL AND METHODS

Subjects

One hundred and thirty patients with high grade intramedullary osteosarcoma of the extremities, without prior treatment and with no evidence of metastasis at diagnosis, were accrued from the Istituto Ortopedico Rizzoli, Bologna. The characteristics of these patients are reported in Table 1. Patients were all treated with chemotherapy protocols including cisplatin together with doxorubicin, methotrexate, and ifosfamide. The treatment regimens were based on only two different protocols[4]: the IOR OS-N4 (cisplatin, doxorubicin, high dose methotrexate, ifosfamide) or the IOR OS-N5 (cisplatin, doxorubicin, high-doses methotrexate, high-doses ifosfamide).

Histological response to preoperative chemotherapy was defined on the basis of the evaluation of tumor necrosis. A good histological response was considered when the extent of tumor necrosis was 90% or greater[4].

After the end of treatment, patients were checked by imaging (radiographs or CT) every 2 months for two years, every 3 months in the third year and then every 6 months. After the fifth year, patients were checked annually with radiographs.

Median follow up was 38 months (range 1-278 months) from the time of diagnosis until death by any cause. Event-free survival (EFS) was calculated from the first day of preoperative chemotherapy to the most recent follow-up, local or systemic recurrence or death by any cause (median EFS was 28 months, range 1-278). Patients who were alive at the time of the last follow-up were censored at that time. Overall survival (OS) was calculated from the time of diagnosis until death by any cause or last follow-up.

Two hundred and fifty healthy controls (subjects without cancer at the time of enrolment) were recruited at the National Cancer Institute, Aviano, Italy, for the purpose of investigating the DNA repair variants as potential markers of cancer risk. Controls were matched for age and sex (median age 35 years, 156/101 males/females).

After signing an informed consent, blood (patients and controls) and non neoplastic (by histology) muscular tissue (patients only) samples were collected and frozen at –80°C until genetic analysis. Patients and controls are of Caucasian ethnicity. This study has been approved by the Institutional Review Boards of the accruing centers.

Genotyping assays

The genomic DNA was extracted with the High Pure PCR Template Preparation Kit (Roche Diagnostic GmbH, Mannheim, Germany). For XPD rs13181, XPD rs1799793 and XPG rs17655 the genotyping assays were performed using pyrosequencing (Biotage, Uppsala, Sweden) with specific 5’-biotinylated primers.

Amplification conditions were 35 cycles of 95°C for 30 seconds, each annealing temperature for 30 seconds, and 72°C for 30 seconds. A final 10 minutes extension at 72°C completed the amplification. Primer sequences and PCR conditions are described in the supplementary table. The sequencing primers used for the analysis were developed using SNP Primer Design software (version 1.0) from Biotage AB (Uppsala, Sweden).

ERCC1 rs3212986 and rs11615 were genotyped by TaqMan® pre-designed SNP genotyping assays by Applied Biosystem (https://products.appliedbiosystems.com/). The Applera TaqMan Genotyping Master Mix was employed together with the validated primers and probes mix at the usage concentration as provided by the manufacturer.

Statistical analysis

This study aims to identify the association between genetic variants and EFS. EFS was chosen as the primary endpoint because of the difficulty to take into account the effects of therapies administered after tumor recurrence. Additional analyses included the evaluation of the association between genetic variants and OS, relapse rate, as well as the risk of osteosarcoma using healthy controls in a case-control study.

Assuming alpha=0.05 and a statistical power of 0.8, the present sample size (N=130) allowed to estimate HRs equal 0.55 or lower. The association with EFS and OS was computed by Kaplan-Meier method[42], and log-rank test was used to test the differences between subgroups. Differences between subgroups were also tested in univariate analysis using the Cox proportional hazards model[43] to compute the hazard ratio (HR) and the corresponding 95% confidence interval (CI). Covariates that were significant in the univariate analysis were also tested in the multivariate model. As a final set, a Cox proportional hazards model was fitted with interaction terms between some covariates. Chi-squared analysis for trend was also evaluated.

Fisher's exact test[44] was used for a three genotype test to investigate the role of polymorphisms in histological response and in relative risk to develop osteosarcomas (allele frequencies between patients and controls). Odds ratios (ORs) and their 95% CIs were calculated.

The Hardy–Weinberg equilibrium was tested for each SNP, showing no significant deviation ($p>0.05$). For all the analysis conducted in this study, a $p<0.05$ was used as the cut-off for significance, not adjusted for multiple comparisons.

RESULTS

XPD rs13181 and rs1799793 are significantly associated with the EFS in univariate analysis: the variant allele confers increased EFS, with evidence for a trend for a gene-dosage effect (Table 2, Figures 1 and 2). Subjects carrying the homozygous mutated genotype showed a protective risk for relapse compared to wild type genotype (HR=0.34, 95%CI 0.12-0.98, p-trend=0.04 for XPD rs13181 variant allele and HR=0.19, 95%CI 0.05-0.77, p-trend=0.002 for XPD rs1799793 variant allele). Testing the impact of having at least one variant allele of these two SNPs, a correlation with EFS was confirmed for XPD rs1799793 (23591G>A; GA+AA vs GG, HR=0.40, 95%CI 0.22-0.74, p-trend=0.004) but not for XPD rs13181 (35931A>C; AC+CC vs AA, HR=0.65, 95%CI 0.38-1.10, p-trend=0.11) (Table 2). Patient characteristics (i.e. gender, age, histological type of tumor or necrosis at diagnosis) were not significant in the univariate analysis and were therefore not used in the multivariate analysis.

In a multivariate analysis, considering both XPD variants, only the association between the XPD rs1799793 and EFS remained significant (adjusted p-trend=0.01, Table 2). Patients carrying the XPD rs1799793 variant allele (GA or AA) had a higher probability to be event free compared to wild type patients (80% vs 40%, Table 2). In this patient population, most relapse events occurred within five years. No association could be observed between EFS and the other three SNPs of the NER genes ERCC1 and XPG (Table 2). Patient characteristics (i.e. gender, age, histological type of tumor or necrosis at diagnosis) were not significant in the univariate analysis of EFS and were not used in the multivariate analysis.

Combined analysis of both XPD polymorphisms on the influence of an increased variant allele number on EFS revealed that none of the cases carrying two variant alleles (in total, 4 variant alleles) relapsed (Table 3 and Figure 3). The probability for EFS at 5 years was

directly proportional to the number of variant alleles: patients with both homozygously mutated genotype had a better prognosis with a null risk of relapse (p-value for trend 0.03). The risk of relapse for patients with no variant allele was more than 3-fold higher than for patients with 3-4 variant alleles (HR=0.29, 95%CI 0.10-0.84, p-value=0.003). Inspite of the significant association between XPD rs1799793 and EFS, none of the five SNPs tested in this study was significantly associated with OS (Table 4). None of the polymorphisms analyzed was significantly associated with chemotherapy-induced necrosis as response to preoperative treatment (data not shown).

When the NER pathway gene SNPs were investigated for differences in allele frequencies between cases and controls, a significant association was observed for XPD rs1799793, indicating that patients carrying at least one variant allele (GA+AA) had a decreased risk to develop osteosarcoma compared to patients with no variant alleles (OR=0.55, 95% CI 0.36-0.84, Table 5). All the other SNPs did not show any association with the risk of osteosarcoma.

DISCUSSION

Osteosarcoma, despite being the eighth most common cancer of childhood, is classified as a “rare disease” representing 2.4% of all malignancies in pediatric patients and approximately 20% of all bone cancers[45]. Its incidence is about 400-500 cases per year in the United States, 800-1000 cases per year in Europe, and 100-120 cases per year in Italy[1]. Due to this rarity, it is very difficult to perform large studies, particularly in patients who are homogeneously treated in order to evaluate the possible clinical impact of the analyzed markers. In this study, we were able to collect a series of 130 osteosarcoma patients, with primary high-grade tumors located at the extremities, without metastasis at diagnosis, and all treated with neoadjuvant chemotherapy based on the administration of cisplatin in association with doxorubicin, high-dose methotrexate and ifosfamide.

The main finding of this study is that XPD rs13181 and rs1799793 are related to higher EFS in osteosarcoma patients treated with neoadjuvant chemotherapy. The presence of at least one of these alleles confers a protective role for relapse, with an HR of 0.34 and of 0.19 for XPD rs13181 and XPD rs1799793, respectively. Moreover, the effect appears to be even stronger after combining these two polymorphisms, as none of the seven homozygous variant patients for both SNPs relapsed compared to 24 (58.5%) patients without any variant allele after 60 months of follow up. However, considering that these two XPD SNPs are not in linkage disequilibrium, the multivariate analysis including both SNPs is suggestive of XPD rs1799793 being the main SNP driving these associations. The presence of a germline polymorphism translated to a variation in the aminoacid codified (Gln to Lys for XPD rs13181 and Asn to Asp for XPD rs1799793) reduces the repair capacity and can result into greater efficacy of platinum treatment, due to increased DNA damage and increased cytotoxic effect of platinum. In theory, patients with defective DNA repair should also be at increased risk of toxicities. However, since we did not collect the toxicity data from these patients this hypothesis cannot be verified in the present study.

In the current study, we found that the presence of XPD rs1799793 and XPD rs13181 could provide a therapeutic advantage from cisplatin chemotherapy, probably by reducing DNA repair activity. These results are in agreement with those reported for other cancers[22,26,28,30] where XPD variant alleles (rs13181 and rs1799793) were associated with a better clinical outcome after cisplatin therapy. Recently, Caronia et al. reported conflicting results on the role of XPD rs13181 in a small set of osteosarcoma patients[21]. It must be highlighted that the patients from Caronia study had different characteristics from our series. They considered also metastatic patients at diagnosis and neoadjuvant chemotherapy not included

cisplatin. However, this discrepancy requires further studies in a larger and homogeneous group of OS patients to better clarify the prognostic role of XPD rs13181.

Our data are suggestive of a predictive role of these two XPD polymorphisms, both alone or in combination, for patients who underwent platinum-based neoadjuvant treatment. However, due to the lack of a control group of patients who did not receive chemotherapy, our single-cohort study cannot ascertain their predictive role for platinum-mediated response. In addition, the significant improvement in EFS of XPD polymorphisms was not translated into longer OS, possibly because we were not able to take into account the effect of additional therapies given after recurrence or for a weak median follow-up time (3 years).

In this study, we also provide information of the effect of these SNPs as determinants of osteosarcoma development risk. Our results indicated that the XPD rs1799793 conferred a reduced risk of about 2-fold to develop osteosarcoma. Two other studies (one in soft tissue sarcomas (STS) and one in osteosarcoma) were not able to detect an effect of XPD rs1799793[10;46]. In particular, Nakayama et al.[46] did not find significant associations between 50 missense SNPs of DNA repair genes (including the XPD SNPs of our study) and the susceptibility to bone sarcomas and STS. The difference with our results could be explained by the absence of a histologic stratification of patients and ethnic differences between the two patient populations. In other tumor types, the results are discordant with XPD rs1799793 being either a protective (for cervical[47] and breast cancer[48]) or a predisposing SNP (for lung[49;50], gastric[51] and prostate cancers[52]). The recent experience with genome-wide association studies (GWAS) of cancer risk are suggestive of the involvement of multiple SNPs[53-55], each of them conferring a marginal effect on the risk. In light of the controversial results on XPD, genetic determinants of osteosarcoma risk remain to be discovered through GWAS.

For osteosarcoma patients, neoadjuvant treatment including cisplatin represents a strategy to lead to a reduction of limb amputation and to an improvement of survival. Unfortunately, despite the positive clinical results obtained with neoadjuvant treatments, there is still a significant proportion of patients who show a partial or very low response to conventional regimens [1]. The identification of genetic markers, which have prognostic value or are predictive of neoadjuvant chemotherapy response, would represent an important tool to reach informed decisions on how to select the subgroup of osteosarcoma patients who are likely to benefit from a more specific, tailored treatment[11]. Based on the results of our study, we propose that XPD rs1799793 is a germline variant to be subsequently tested prospectively in patients with high-grade osteosarcoma. This SNP might be a potential marker for a subset of osteosarcoma with hitherto unknown molecular features associated with a better prognosis after platinum-based therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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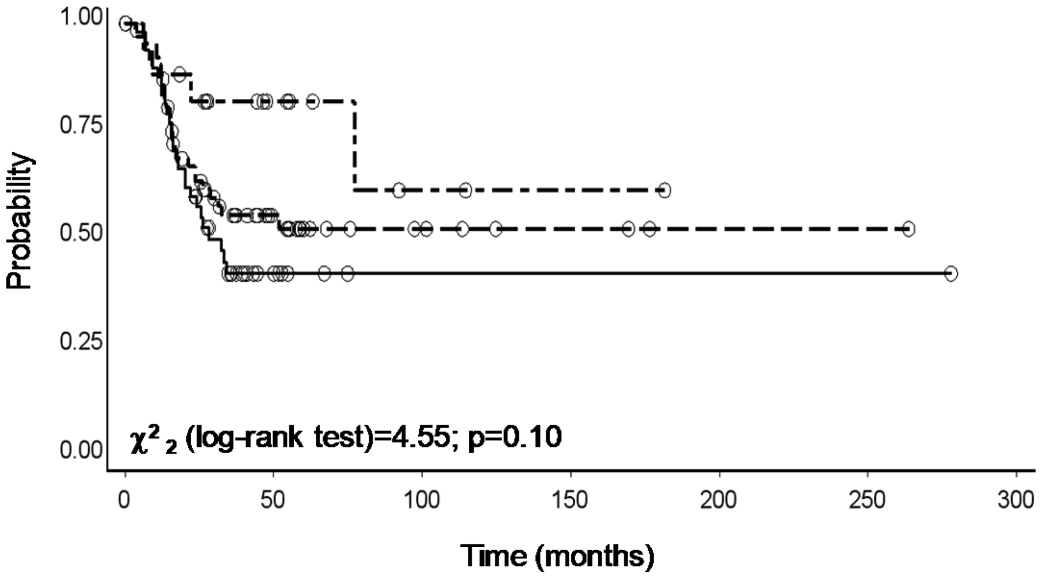

Figure 1.
XPD 35931A>C (rs13181) genotype curve's: ___AA vs. _ _AC vs. __ _ __CC

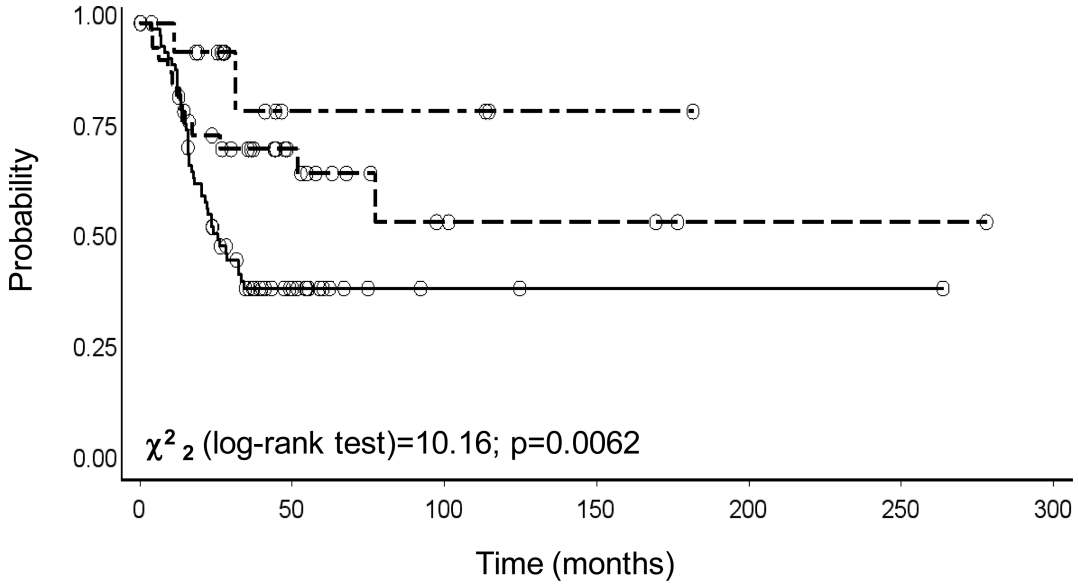

Figure 2.
XPD 23591G>A (rs1799793) genotype curve's: ___GG vs. _ _GA vs. __ _ __AA

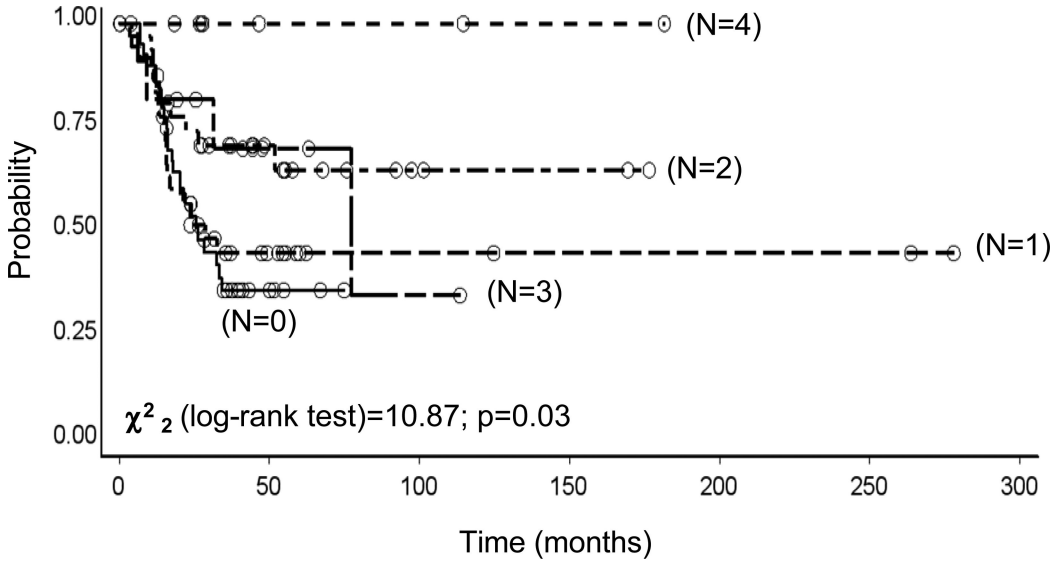

Figure 3.

The curves represent the increased number of variant alleles for combination of XPD rs13181 and XPD rs1799793: ____ N=O (none variant alleles), __ __ __ N=1 (one variant allele of XPD rs13181 or XPD rs1799793), __ _ __ N=2 (two variant alleles among XPD rs13181 and/or XPD rs1799793), ____ _ ____N=3 (three variant alleles among XPD rs13181 and/or XPD rs1799793), _ _ _ _N=4 (all four variant alleles).

Table 1

Clinicopathologic characteristics of 130 osteosarcoma patients treated with neoadjuvant chemotherapy.

		N (%)
Sex	Male	79 (60.8%)
	Female	51 (39.2%)
Age	Median	16
	Range	4-68
Stage	I-II	130 (100%)
	III-IV	0 (0%)
Site	Extremities	124 (95.4%)
	Other	6 (4.6%)
Histological Type	Osteoblastic	93 (71.6%)
	Non-osteoblastic	34 (26.1%)
	Not specified	3 (2.3%)
Surgical margins	Wide	121 (93.1%)
	Marginal	3 (2.3%)
	Radical	6 (4.6%)
Necrosis	≥ 90%	59 (45.4%)
	< 90%	71 (55.6%)
Neadjuvant treatment	IOR OS-N4*	116 (89.2%)
	IOR OS-N5**	10 (7.7%)
	Cisplatin, doxorubicin, high dose methotrexate, ifosfamide	10 (7.7%)
	Cisplatin, doxorubicin, high dose methotrexate	4 (3.1%)

N=number of patients

* IOR OS-N4 (Cisplatin, doxorubicin, high dose methotrexate, ifosfamide)

** IOR OS-N5 (Cisplatin, doxorubicin, high dose methotrexate, high dose ifosfamide)

Table 2

Distribution of relapses, probability for event free survival (EFS) at 5 years, hazard ratio (HR) for relapse and corresponding 95% confidence intervals (95% CI) according to DNA repair gene polymorphisms.

SNP	Relapse Rate		Probability to Event Free at 5 years	*p-value* **	Univariate analysis				Multivariate analysis	
	N. Relapse/N. Total (%)	*p-value* *			HR (95% CI)	*p-trend* ***	HR (95% CI)	*p-trend* ***	HR (95% CI)	*p-trend* ****
XPD rs13181 (35931 A>C)										
AA	26/49 (53.1%)		42%		1#		1#		1#	
AC	27/64 (42.2%)		53%		0.75 (0.44-1.29)		0.65 (0.38-1.10)		0.96 (0.54-1.69)	
CC	4/17 (23.5%)	0.10	82%	0.10	0.34 (0.12-0.98)	0.04		0.11	0.57 (0.19-1.73)	0.43
XPD rs1799793 (23591 G>A)										
GG	43/77 (55.8%)		40%		1#		1#		1#	
GA	12/38 (32.4%)		66%		0.50 (0.26-0.96)		0.40 (0.22-0.74)		0.53 (0.27-1.04)	
AA	2/15 (13.3%)	0.002	80%	0.0062	0.19 (0.05-0.77)	0.0023		0.0035	0.23 (0.05-0.99)	0.01
ERCC1 rs3212986 (8092 C>A)										
CC	32/72 (44.4%)		53%		1#		N.A.			
CA	17/44 (38.6%)		54%		0.86 (0.48-1.55)					
AA	5/8 (62.5%)	0.44	25%	0.35	1.78 (0.69-4.58)	0.67				
ERCC1 rs11615 (19007 T>C)										
TT	18/37 (48.6%)		48%		1#		N.A.			
TC	21/59 (35.6%)		63%		0.67 (0.36-1.26)					
CC	17/30 (56.7%)	0.14	37%	0.19	1.18 (0.61-2.29)	0.69				
XPG rs17655 (3507 G>C)										
GG	33/75 (44.0%)		51%		1#		N.A.			
GC	14/39 (35.9%)		60%		0.80 (0.43-1.49)					
CC	10/16 (62.5%)	0.20	27%	0.24	1.58 (0.78-3.22)	0.47				

N=number of patients; N.A., not available.

* Chi-square test

** Chi-square test Log-rank test for overall EFS

#Reference category

***Chi-square for trend

*****Chi-square for trend including all variables significant from the univariate analysis (XPD 35931A>C and XPD23591G>A)

Table 3

Distribution of relapses, probability for event free survival (EFS) at 5 years, hazard ratio (HR) for relapse and corresponding 95% confidence intervals (95% CI) according to combined XPD rs13181 and rs1799793.

N. of variant alleles	Relapse Rate		Probability to Event Free at 5 years	*p-value***	Univariate analysis	
	N. Relapse/N. Total (%)	*p-value**			HR (95% CI)	*p-trend****
0	24/41 (58.5%)		36%		1#	
1	19/38 (51.3%)		45%		0.87 (0.48-1.59)	
2	10/33 (30.3%)		65%		0.43 (0.20-0.91)	
3	4/11 (36.4%)		70%		0.29 (0.10-0.84)	
4	0/7 (0%)	0.0135	100%	0.03		0.003

N=number

*Chi-square test

**Chi-square Log-rank test for overall EFS

#Reference category

***Chi-square for trend

Table 4

Distribution of death, probability for overall survival (OS) at 5 years, hazard ratio (HR) for death and corresponding 95% confidence intervals (95% CI) according to DNA repair genes polymorphism.

SNP	Relapse Rate		Probability to be alive at 5 years	*p-value***	Univariate analysis	
	N. Death/N. Total (%)	*p-value**			HR (95% CI)	*p-trend****
XPD rs13181 (35931 A>C)						
AA	12/49 (24.5%)		67%		1#	
AC	13/64 (20.3%)		72%		0.73 (0.33-1.61)	
CC	2/17 (11.7%)	0.53	88%	0.43	0.41 (0.09-1.83)	0.20
XPD rs1799793 (23591 G>A)						
GG	20/77 (26.0%)		67%		1#	
GA	5/38 (13.5%)		85%		0.50 (0.19-1.34)	
AA	2/15 (13.3%)	0.23	69%	0.26	0.48 (0.11-2.05)	0.14
ERCC1 rs3212986 (8092 C>A)						
CC	12/72 (16.7%)		75%		1#	
CA	12/44 (27.3%)		67%		0.86 (0.48-1.55)	
AA	2/8 (25.0%)	0.38	69%	0.38	1.78 (0.69-4.58)	0.67
ERCC1 rs11615 (19007 T>C)						
TT	8/37 (21.6%)		76%		1#	
TC	9/59 (15.2%)		80%		0.71 (0.28-1.85)	
CC	9/30 (30.0%)	0.26	62%	0.43	1.31 (0.51-3.40)	0.58
XPG rs17655 (3507 G>C)						
GG	16/75 (21.3%)		72%		1#	
GC	6/39 (15.4%)		77%		0.72 (0.28-1.84)	
CC	5/16 (31.2%)	0.41	67%	0.42	1.58 (0.58-4.32)	0.66

N=number of patients

*Chi-square test

**Chi-square Log-rank Test for overall OS

#Reference Category

***Chi-square for trend

Table 5

Distribution of genetic frequencies of DNA repair SNPs in osteosarcoma cases and controls, Odd Ratio (OR) for cancer risk and corresponding 95% confidence intervals (95% CI).

SNP	Cases N. (%)	Controls N. (%)	*p-value**	OR (95% CI)
XPD rs13181 (35931 A>C)				
AA	49 (38%)	93 (38%)		1#
AC	64 (49%)	116 (46%)		1.05 (0.66-1.66)
CC	17 (13%)	41 (16%)	0.8	0.79 (0.40-1.52)
AC+CC	***81 (62%)***	***157 (63%)***	***1.00***	***0.98 (0.63-1.52)***
XPD rs1799793 (23591 G>A)				
GG	77 (59%)	112 (45%)		1#
GA	38 (28%)	100 (40%)		0.54 (0.33-0.87)
AA	15 (13%)	38 (15%)	0.11	0.57 (0.29-1.17)
GA+AA	***52 (41%)***	***138 (55%)***	***0.007***	***0.55 (0.36-0.84)***
ERCC1 rs3212986 (8092 C>A)				
CC	72 (58%)	129 (52%)		1#
CA	44 (35%)	98 (39%)		0.80 (0.51-1.27)
AA	8 (7%)	23 (9%)	0.31	0.62 (0.26-1.46)
CA+AA	***52 (42%)***	***121 (48%)***	***0.27***	***0.77 (0.50-1.19)***
ERCC1 rs11615 (19007 T>C)				
TT	37 (29%)	86 (34%)		1#
TC	59 (47%)	111 (45%)		1.23 (0.75-2.03)
CC	30 (24%)	53 (21%)	0.37	1.32 (0.73-2.38)
TC+CC	***89 (71%)***	***164 (66%)***	***0.35***	***1.261 (0.79-2.01)***
XPG rs17655 (3507 G>C)				
GG	75 (58%)	141 (56%)		1#
GC	39 (30%)	94 (38%)		0.78 (0.49-1.25)
CC	16 (12%)	15 (6%)	0.07	2.01 (0.94-4.28)
GC+CC	***55 (42%)***	***109 (44%)***	***0.8***	***0.95 (0.62-1.46)***

N=number of cases (patients or controls)

#reference category

*Fisher's Exact Test