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TP53 and *P21* polymorphisms: Response to cisplatinum/paclitaxel-based chemotherapy in ovarian cancer

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Abstract

Ovarian cancer (OC) is the most lethal gynaecologic cancer and its standard treatment consists of platinum-based chemotherapy after cytoreductive surgery. The p53 protein plays a critical role on different cellular processes in response to DNA damage and it is responsible for transcriptional induction of the *P21* gene. We have analysed 114 blood samples in order to investigate the effect of the *TP53* codon 72 and the *P21* 3′UTR polymorphisms in response to cisplatinum/paclitaxel chemotherapy for OC treatment. The genotypes of the *TP53* codon 72 and *P21* 3′UTR polymorphism were identified using AS-PCR and PCR-RFLP, respectively. Our results indicate that the *TP53* P allele is associated with a worse prognosis ($P = 0.011$) while *P21* polymorphism genotypes did not reveal any statistically significant result ($P > 0.05$). Furthermore, simultaneous carriers of the *TP53* AA genotype and the *P21* CC genotype demonstrate a longer progression-free interval ($P = 0.020$). This study suggests that the characterisation of a genetic profile can contribute to the definition of a better chemotherapy treatment.

Keywords: Chemotherapy; Cisplatin; Paclitaxel; Pharmacogenomics; Ovarian cancer; *P21* 3′UTR polymorphism; *TP53* codon 72 polymorphism

Ovarian cancer (OC) is one of the most common gynaecologic malignancies and the seventh most frequent cause of death by cancer in women. In 2002, the estimated incidence of OC was 204,000 new cases worldwide and the mortality rate was 125,000 deaths [1]. In Portugal, there are approximately 600 new cases of OC each year, with an incidence rate of 9.9/100,000 and a mortality rate of 5.4/100,000 [2].

Ovarian cancer is often asymptomatic and approximately 70% of all cases, when diagnosed, present an advanced stage of the disease [3,4]. Nevertheless, early stages of OC are curable in a high percentage of patients and the standard treatment for advanced ovarian carcinoma women is surgery, followed by the combination of platinum–taxanos chemotherapy [5]. However, the efficiency of a chemotherapy treatment is limited by inter-individual variations in responses and toxicities, and genetic factors are believed to play an important role in drug response [6–9]. In fact, genetic polymorphisms have been described to have an effect on numerous cancer susceptibilities [10–17].

The *TP53* tumour suppressor gene, located at chromosome 17p13, is described as mutated in 50–55% of cancer cases [18] and in 40–80% of epithelial ovarian cancers [9]. *TP53* encodes the p53 protein that plays a critical role in cell cycle arrest, gene transcription, DNA repair, and apoptosis. Codon 72 polymorphism of the *TP53* gene encodes either arginine (CGC) or proline (CCC) and these two allelic variants are structurally and functionally different, conferring different susceptibilities to cancer development [10,19–26]. Lavarino et al. [8] described that patients with

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mutant p53 ovarian tumours were more responsive to paclitaxel-based chemotherapy. Nevertheless, Sullivan et al. [27] demonstrated that anticancer drugs respond differently according to the single nucleotide polymorphism (SNP) on codon 72 of the *TP53* that confers differential cellular resistance.

P53 is also responsible for the transcriptional induction of the *P21* gene, located at chromosome 6p21.2, that encodes the p21 protein. This protein, also known as WAF1/CIP1, is a cyclin kinase inhibitor that binds to and inhibits all cyclin-dependent kinases complexes, causing cell cycle arrest in the G1 phase of the cell cycle.

Mutations in either *TP53* or *P21* gene may affect the regulation of cellular proliferation, increasing the susceptibility to cancer. This is the reason why several studies have evaluated the effects of *TP53* and *P21* polymorphisms on gastric [28], breast [29,30], endometrial [31], prostate [32], cervical [33], hepatocellular [34], and ovarian carcinomas [3,17].

The aim of this study was to evaluate the influence of *TP53* codon 72 polymorphism and the *P21* nt590 polymorphism in ovarian cancer women treated with combined platinum and taxanos chemotherapy in order to understand their role as a predictive factors. To the best of our knowledge, this is the first study that correlates these two SNP and the chemotherapy treatment for OC patients.

Materials and methods

Population. This study was conducted on blood samples of 114 women with histological confirmed ovarian cancer, according to previous studies from our group [6,7]. Between 1996 and 2002, these patients were admitted and treated at the Portuguese Institute of Oncology, Oporto, Portugal, and underwent a platinum-based chemotherapy after cytoreductive surgery. Assessment of the tumour response to chemotherapy was based on World Health Organization (WHO). The chemotherapy protocol consisted of paclitaxel (175 mg/m^2) and cisplatin (75 mg/m^2). Resistance to treatment was deemed present if the disease persisted or recurred within 6 months. If this occurred, patients underwent another course of the same combined chemotherapy. Patients were treated by the same medical oncologist and evaluated according to the FIGO staging system. All blood samples were collected after informed consent, according to the Declaration of Helsinki.

During laboratory proceedings, the clinical status and outcome of patients were not known by the investigators.

Samples. Blood samples were collected in ethylenediaminetetraacetic acid (EDTA) containing tubes. Genomic DNA was extracted from the white blood cell fraction of each case, using a salting-out method [35].

TP53 genotype analysis. TP53 codon 72 polymorphism was evaluated using the allele specific-polymerase chain reaction (AS-PCR) method to amplify the DNA from all of the 114 samples, as previously performed by Santos et al. [10]. Each PCR was performed separately for each variant, using specific primers for the ARG allele: ARG− (5′-CTGGTGCAG GGGCCACGC-3′) and TP53+ (5′-TCCCCCTTGCCGTCCCAA-3′) amplifying a 141 base-pair (bp) fragment and specific primers for the PRO allele: PRO+ (5′-GCCAGAGGCTGCTCCCCC-3′) and TP53− (5′-CGT GCAAGTCACAGACTT-3′) amplifying a 177 bp fragment. Both PCRs were carried out in a 50 μL mixture, including 1× *Taq* Buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.25μM from each primer, and 1 U *Taq* DNA polymerase. Cycling parameters were as follows: 95 °C for 5 min; 45 cycles of 94 °C for 60 s, 60 °C/55 °C (Arg allele or Pro allele, respectively) for 45 s, and 72 °C for 60 s; and a final extension step at 72 °C for 5 min. The specific PCR amplification products were analysed by electrophoresis in a 1.5% agarose gel stained with ethidium bromide and visualised under UV light (Fig. 1).

$P21^{WAF1/CIP1}$ exon 3 genotype analysis. Amplification of a 300 bp DNA fragment was obtained after a polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method adapted from a previously described protocol [36]. The primers used were E3A: 5′-CCCAGGGAAGGGTGTCCTG-3′ and E3B: 5′-GGGCGGCCAGGGT ATGTAC-3′. DNA was amplified in a 50 μL mixture, including 1× *Taq* Buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.3 μM from each primer, and 1 U *Taq* DNA polymerase. Thermocycler parameters were as follows: 94 °C for 5 min; 30 cycles of 94 °C for 60 s, 60 °C for 45 s, and 72 °C for 60 s; and a final extension step at 72 °C for 5 min. The 300 bp fragment, verified by electrophoresis on a 1.5% agarose gel, was digested overnight at 37 °C with 1 U of *Pst*I. This enzyme recognises the C → T polymorphism leading to the loss of a *Pst*I site. In the presence of the C allele, two fragments of 174 and 126 bp were expected. The restriction-digested products were analysed by electrophoresis in 2% agarose gels stained with ethidium bromide and visualised under UV light (Fig. 2).

Five percent of the results were re-analysed and confirmed on a random analysis.

Statistical analysis. Data analysis was performed using the computer software Statistical Package for Social Sciences (SPSS) (version 11.5) for Windows.

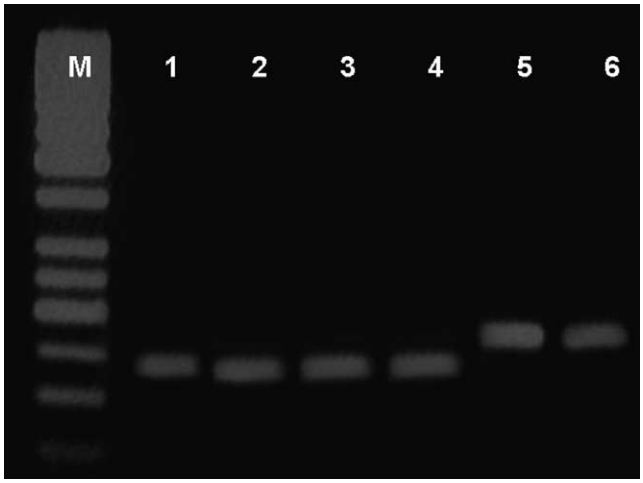

Fig. 1. AS-PCR products of the *TP53* codon 72 polymorphism: M, 50 bp ladder; lanes 1–4, Arg allele (141 bp); lanes 5 and 6 Pro allele (177 bp).

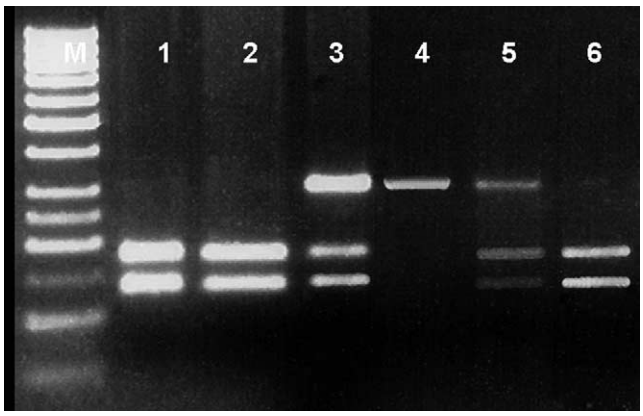

Fig. 2. Analysis of the *P21* 3′UTR genotypes by RFLP: M, 50 bp ladder; lanes 1, 2, and 6, homozygous CC; lanes 3 and 5 heterozygous CT; and lane 4, homozygous TT.

Chi-square analysis was used to compare categorical variables. A 5% level of significance was used in the analysis. Probabilities of survival and progression-free survival were calculated and the means and life-tables were computed using the product-limit estimate of Kaplan and Meier. The curves were analysed by the log-rank test, a statistical test for equality of survival distributions. A level of $P < 0.05$ was considered statistically significant. Survival duration was defined as the time between diagnosis and either death or the time of the last clinical evaluation of the patient. The cause of death was determined from the patient's records. Progression-free interval survival was defined as the time interval between the date of diagnosis and the date of increased parameters of disease or the date of the last clinical evaluation of the patient.

Results

We analysed *TP53* codon 72 and *P21* 3′UTR genotypes in 114 Portuguese women diagnosed with OC using the AS-PCR and PCR-RFLP methods, respectively.

Clinicopathological information of women with OC (median age 52 years) who underwent a platinum-based chemotherapy are described in Table 1. According to this, most patients were diagnosed in an advanced stage, so that stages III and IV were the most frequent among our population.

The clinicopathological parameters of the OC cases analysed according to the *TP53* and *P21* genotypes are shown in Table 2. We observed that the AA genotype was the most frequent among the *TP53* codon 72 variants and that the CC *P21* genotype was the most prevalent among all OC samples analysed. There were no statistical significant differences between the AA and the P carrier (AP/PP) genotypes of the *TP53* codon 72 polymorphism, and between the CC and the T carrier (CT/TT) genotypes of the *P21* 3′UTR polymorphism, regarding age at diagnosis, histological type, and clinical stage ($P > 0.05$), with an exception in the analysis between *P21* polymorphism and the histological type ($P = 0.048$). Similarly, no statistical significant differences were found when evaluating the influence of the *TP53* genotypes among the *P21* wild-type genotypes (CC) (Table 3).

As shown in Fig. 3, there were no significant statistical differences between the AA and CC genotypes in *TP53* codon 72 and *P21* 3′UTR polymorphism ($P = 0.082$ and $P = 0.860$) on the overall survival. However, the most frequent genotype AA showed statistical significant differences on the progression-free interval with a better response to cisplatinum/paclitaxel chemotherapy ($P = 0.011$). Also, besides non-statistical results on the overall survival, when we investigate the influence of the *TP53* genotypes within the *P21* CC genotype, we verified statistical differences on the progression-free interval for chemotherapy treated women ($P = 0.020$) (Fig. 4).

Table 1
Patient's complete clinicopathological parameters

Characteristic	No. of patients ($N = 114$)	%
Age		
⩽52 years	51	44.7
>52 years	63	55.3
Histologic type		
Serous	63	55.3
Mucinous	19	16.7
Endometrioid	11	9.6
Clear-cell	20	17.5
Others	1	0.9
Grade of differentiation		
Well differentiated	26	22.8
Moderate differentiated	23	20.2
Poorly differentiated	37	32.5
Undifferentiated	12	10.5
Undetermined	4	3.5
FIGO stage		
I and II	44	38.6
III and IV	70	61.4

FIGO—International Federation of Gynaecology and Obstetrics.

Table 2
Association of *TP53* codon 72 and the *P21* 3′UTR genotypes with clinicopathological parameters in ovarian cancer women

	TP53 polymorphism		P[a]	*P21* polymorphism		P[a]
	AA (%)	AP/PP (%)		CC (%)	CT/TT (%)	
Age (years)						
⩽52 years	52.2	47.8		88.2	11.8	
>52 years	47.2	52.8	0.619	94.6	5.4	0.336
Histological type						
Serous	50.0	50.0		97.4	2.6	
Others	50.0	50.0	1.000	83.9	16.1	0.048
FIGO stage						
I + II	56.8	43.2		87.1	12.9	
III + IV	45.2	54.8	0.264	95.0	5.0	0.235

FIGO—International Federation of Gynaecology and Obstetrics.
[a] Chi-square test.

Table 3
Influence of *TP53* codon 72 genotypes in *P21* CC genotype among ovarian cancer women who underwent chemotherapy treatment

	N	*P21* CC genotype				P[a]
		AA		AP/PP		
		n	%	*n*	%	
Age (years)	63					
⩽52 years	30	13	43.3	17	56.7	
>52 years	33	14	42.4	19	57.6	0.942
Histological type[b]	62					
Serous	37	16	43.2	21	56.8	
Others	25	11	44.0	14	56.0	0.953
FIGO stage	63					
I + II	25	14	56.0	11	44.0	
III + IV	38	13	34.2	25	65.8	0.087

[a] Chi-square test.
[b] Data not available in 1 case.

Discussion

Ovarian cancer is the most lethal cause of death among gynaecologic cancers. However, as it is frequently an asymptomatic malignancy it is often diagnosed in an advanced stage of disease and the standard treatment is a

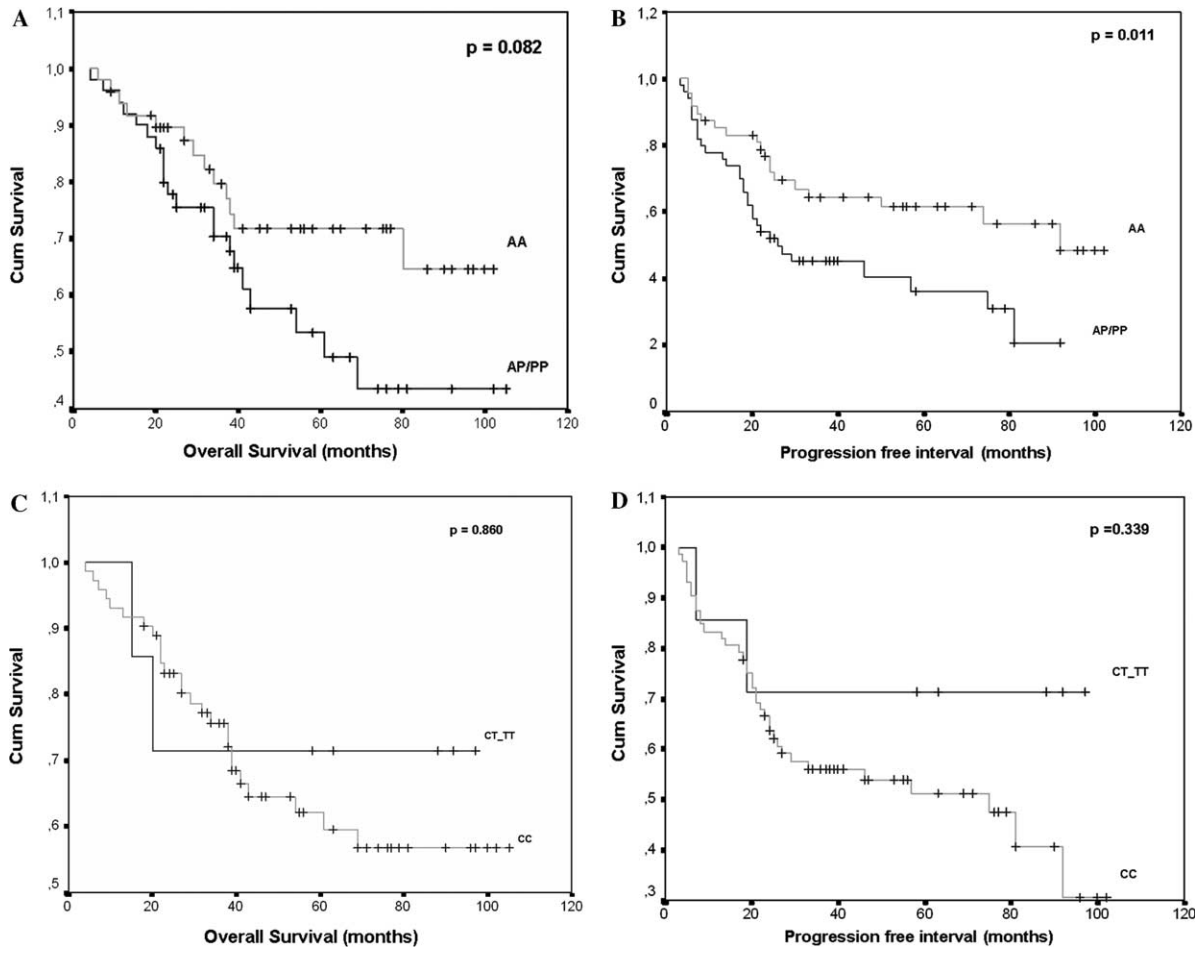

Fig. 3. Kaplan–Meier curves and log-rank test on overall survival and progression-free interval after platinum/paclitaxel chemotherapy in ovarian cancer patients: influence of *TP53* codon 72 polymorphism (A,B); influence of *P21* 3′UTR polymorphism (C,D).

platinum-based chemotherapy after cytoreductive surgery [5,8,9].

Individual characteristics, such as genetic variations, are believed to be associated with an altered response to a chemotherapeutical treatment. Therefore, as predictive factors, it becomes important to study and understand the role of polymorphisms in cancer susceptibility and also in drug response [6,7,17,27]. Some studies have investigated the effect of *TP53* mutations on platinum-based chemotherapy in OC cancer describing that sensitivity of tumour cells to anticancer therapy seems to depend on the efficient induction of apoptosis mediated by a functional p53 protein [8,9,37,38].

TP53 gene encodes p53 protein that plays a critical role on cell cycle regulation, activating the transcription of DNA repair genes or inducing apoptosis. *P21*, located at chromosome 6, is a downstream effector of p53. When p53 is activated due to some damage on DNA, it binds to the *P21* promoter stimulating its expression. Consequently, p21, a cyclin kinase inhibitor, binds to and inhibits all CDK complexes that become inhibited and are unable to phosphorylate Rb proteins, arresting cell cycle progression and DNA replication. If *TP53* alleles are lost or mutated, p21 cannot be activated and the cell cycle keeps on progressing despite DNA damage. Therefore, alterations in the *TP53* and *P21* genes can affect the regulation of cellular proliferation and can be associated with an increased susceptibility for cancer.

The study of the polymorphisms on these two genes seems to be important for the prediction of treatment response with platinum–taxanos chemotherapy [8,9,27].

Sullivan and co-workers indicated that cells expressing the wild-type Arg variant on codon 72 of *TP53* and exposed to anticancer drugs induced higher apoptosis. Moreover, patients with head and neck carcinoma expressing the wild-type Arg variant had the highest complete response rate, while patients lacking a wild-type allele had shorter survival and progression-free survival [27]. In agreement with these results, Pim and Banks [19] demonstrated that the Arg variant is significantly more efficient in inducing apoptosis than the Pro variant that appeared to induce higher levels of G1 arrest.

Furthermore, our results demonstrate that the *TP53* AA genotype is associated with a higher progression-free interval (log-rank test, $P = 0.011$), conferring a better prognosis than the AP/PP genotype. We suggest that this polymorphism plays an important role in response to platinum-based chemotherapy, conferring a better response to therapy

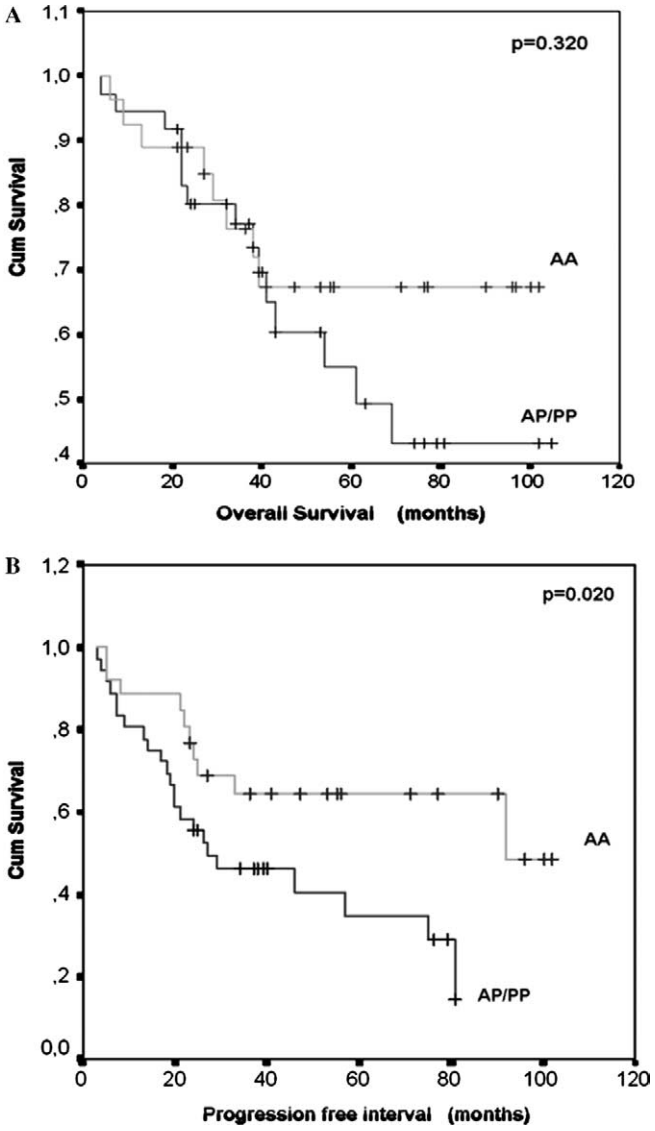

Fig. 4. Kaplan–Meier curves and log-rank test considering the influence of *TP53* codon 72 genotypes among the *P21* CC genotype on overall survival (A) and progression-free interval (B) after platinum/paclitaxel chemotherapy in ovarian cancer patients.

probably due to higher induction of apoptosis, as corroborated by other investigators [27,19].

Polymorphisms in untranslated regions have been shown to be important for cellular proliferation, differentiation, tumour suppression, and metastasis suppression on several genes [39–41]. For this reason, *P21* polymorphism at the 3′ UTR region can have an important role on protein expression and stability, as well as on its response to therapy. There are few reports that studied this polymorphism [36,42,43], however as Mousses et al. [44] investigated, the 3′UTR polymorphism always occurs together with a more common *P21* polymorphism on codon 31. Thus, there are several studies that have investigated the effects of the codon 31 polymorphism on lung [45,46], breast [29,30], gastric [28], endometrial [31], cervix [33,47], and prostate cancers [32]. However, to the best of our knowledge, there is no study that evaluates the effect of the *P21* polymorphism on the efficiency of platinum-based chemotherapy. Our results suggest that the *P21* 3′UTR genotypes may not be relevant as a predictive factor for the platinum-based agent treatment on OC patients ($P > 0.05$).

As p53 and p21 are two proteins involved in cell cycle regulation, the *TP53* and *P21* polymorphisms could interact to an altered response to cisplatinum/paclitaxel chemotherapy, therefore, we analysed the overall survival and progression-free interval that showed that among the CC *P21* genotype, the AA *TP53* genotype has a better response to anticancer agent's ($P = 0{,}020$). However, future studies will be needed, with a larger population, to validate this interaction and to be able to understand if this interaction is relevant for chemotherapy response in ovarian cancer patients.

As Sullivan et al. [27] reported the in vivo clinical response and outcome to chemo-radiotherapy in head and neck carcinoma are more favourable in cells expressing the Arg variant of the p53. Taking this into account and considering that the treatment success also depends on cellular sensitivity to the drug concentration and its time of exposure, future studies using cell lines will be needed to determine the *TP53* polymorphism effect on drug absorption, metabolism, and excretion, and, therefore, playing a role in drug-induced apoptosis and cytotoxicity.

As far as we are concerned, this was the first study evaluating the *TP53* codon 72 and *P21* 3′UTR polymorphisms together in response to platinum-based chemotherapy in OC patients. We believe that the polymorphism on codon 72 of the *TP53* gene interferes with the efficacy of the cisplatinum/paclitaxel chemotherapy for OC. We also believe that the understanding of polymorphisms' effects on anticancer agent's response seems to play a relevant role on cancer treatment, becoming important as predictive factors.

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