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Pharmacogenomic assessment of cisplatin-based chemotherapy outcomes in ovarian cancer

Aim: Cisplatin and its analogs are potent antitumor agents. However, their use is restricted by significant variability in tumor response and toxicity. There is a great need to identify genetic markers to predict the most important adverse events and patient outcomes. **Materials & methods:** We have evaluated the association between polymorphisms in 106 genes involved mainly in xenobiotic metabolism, DNA repair, the cell cycle and apoptosis, and outcomes in 104 ovarian cancer patients receiving cisplatin–cyclophosphamide chemotherapy. Arrayed primer extension technology was used to genotype 228 SNPs. **Results:** Ten SNPs in nine genes were found to be associated with one or more of the assessed clinical end points. SNPs in *TPMT* and *NQO1* were significantly associated with progression-free survival. Polymorphisms in *ERCC5*, *RAD52*, *MUTYH* and *LIG3* correlated with the occurrence of severe neutropenia. SNPs in *NAT2* and *EPHX1* were associated with anemia and nephrotoxicity, respectively. A SNP in *ADH1C* was correlated with complete tumor response. **Conclusion:** The results obtained suggest that SNPs in different genes involved in drug metabolism can be important in identifying patients at risk for nonresponse to or toxicity from cisplatin-based treatment.

Original submitted 2 August 2013; Revision submitted 25 November 2013

KEYWORDS: cisplatin ■ ovarian cancer ■ pharmacogenomics

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Cisplatin and its analogs are among the most potent agents used in antitumor chemotherapy [1–3]. However, their clinical use is limited by the development of severe side effects [4]. Moreover, in most solid tumors, including ovarian cancer, significant interpatient variability in disease response is observed, ranging from complete tumor disappearance to continued progression. Being difficult to foresee, these interindividual variations require identification of prognostic markers that may distinguish those patients who would have no benefit from platinum-based treatment. The results of numerous studies have suggested a cause at the DNA sequence level for the heterogeneity of patients' responses and interindividual variations of toxicity of different drugs [5–7].

Several genetic polymorphisms have been associated with platinum-treatment efficacy and toxicity, including those in DNA repair (*ERCC1*, 8092C>A and 19007T>C; *ERCC2*, Asp312Asn and Lys751Gln) and GST (e.g., *GSTP1*, Ile105Val) genes [8,9]. Although these polymorphisms have been explored by many investigators and are still among the most cited [10,101], the overall contribution of these genetic markers to predictions of response to platinum-based therapy is not yet well established. This situation may be at least partially attributed to the differences in agents used for treatments together with the platinum drugs. Another cause may be the involvement of additional genes. The relevance of this latter attribution comes from an examination of the metabolism of cisplatin and its analogs. Not actually being prodrugs, which would require specific production pathways (more active aquated platinum species are generated under physiological conditions of lower concentration of chloride ions inside the cell [11]), platinum agents have complex metabolic pathways, all of which can contribute to overall clearance. Therefore, integrated approaches are warranted to identify genetic variants associated with interindividual differences in response to treatment. Here, we report the results of a systematic investigation of more than 200 polymorphisms in 106 genes related to major chemotherapy drug action pathways, including DNA repair, the cell cycle, apoptosis and xenobiotic metabolism, in a cohort of ovarian cancer patients treated with a cisplatin–cyclophosphamide regimen.

Materials & methods

■ Patients

Detailed procedures of patient enrollment and data collection have been described previously [12]. Briefly, native born Russian women with morphologically confirmed epithelial ovarian carcinoma who were chemotherapy and

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radiation-therapy naive and not older than 65 years of age entered this study. To ascertain ethnicity, women completed a questionnaire about their ancestry; only patients with two generations of Russian ancestors were recruited. Before the first course of chemotherapy, blood samples were obtained for DNA testing. The regimen used was cisplatin (100 mg/m^2) and cyclophosphamide (600 mg/m^2), which were both administered intravenously on day 1 of the 21-day cycle, for up to six cycles. Adverse events were recorded using standard criteria. All patients were assessed for the maximal grades of nephrotoxicity, ototoxicity, neurotoxicity, nausea/vomiting, as well as neutropenia, anemia and thrombocytopenia. Tumor response was measured every two cycles. After chemotherapy, patients were observed for disease recurrence and survival. Patients with progressive disease were treated with second-line chemotherapy, mostly taxane/platinum doublets.

Before the initiation of study-related procedures, all patients gave written informed consent. The study protocol and informed-consent form were approved by the Ethics Committee of the N. N. Blokhin Cancer Research Centre (Moscow, Russia).

Genotyping

DNA was isolated from the blood samples (leukocytes) using an approach that included proteinase K treatment and phenol–chloroform extraction [13]. All polymorphisms were analyzed simultaneously using a microarray DNA repair SNP detection test (version 2, Asper Biotech, Tartu, Estonia). This test genotypes 228 SNPs in 106 genes and is an extension of the previously developed microarray 'MetaboChip' that included less than 100 polymorphisms [14]. Despite its name, the microarray also includes SNPs located in genes involved in DNA repair, cell cycle control, apoptosis and xenobiotic metabolism. The number of SNPs varied from one to seven per gene (the list of genes and 228 polymorphisms analyzed is given in Supplementary Table 1; www.futuremedicine.com/doi/suppl/10.2217/pgs.13.237).

To check the quality of genotyping, we compared the results of the microarray with the SNP results that had been obtained previously in our department for the same DNA sample set using an RFLP method in which regions of SNPs were amplified and digested with appropriate endonucleases [12]. The comparison revealed concordance between the two methods, similar to that reported by Kweekel *et al.*, who showed that 0.0–3.6% of the samples yielded results that were discordant [15].

Statistical analysis

The purpose of this work was to explore the associations between gene polymorphisms and the outcomes of cisplatin-based treatment (i.e., tumor response, overall survival [OS], progression-free survival [PFS] and side effects). A permutation exact test, a two-sided Fisher exact test and a χ^2 test were used to assess the relationship between the variables and genotypes tested. To calculate homozygous and heterozygous odds ratios (ORs), the 'wild-type' genotype was used as a reference (in the case of empty cells, 0.5 was added to each cell to allow calculation). Survival curves were obtained using a Kaplan–Meier product limit analysis. Differences between the survival curves were assessed using the Mantel–Cox log-rank test. The relative risk of progression and death associated with the different polymorphisms was estimated using the Cox proportional hazards regression model, with adjustment for treatment variant (adjuvant, i.e., patients without residual disease after surgery, vs nonadjuvant, i.e., all other patients). The significance of associations was set at $p < 0.00022$ (threshold of 0.05 adjusted for the number of SNPs tested). The statistical analyses were performed using the IBM SPSS statistics software package (version 19, SPSS, Inc., IBM Company, IBM Corporation, NY, USA), GraphPad InStat (version 3.00, GraphPad Software, CA, USA), and the PowerMarker software (version 3.25) [16].

Results

A total of 104 women with ovarian cancer were enrolled in the study (the last patient was entered in June 2007). The median age of the patients was 52 years (range: 23–65 years). The majority of them (72 women) had stage III disease. Stages I, II and IV were detected in 14, six and ten patients, respectively. In total 506 cycles of chemotherapy were delivered, and 60 patients received all six cycles. The chemotherapy administered to 21 patients, including all patients with stage I, five patients with stage II and two patients with stage III disease, was adjuvant. At the time of analysis (September 2012), 100 patients were available for survival analysis (four patients were lost to follow-up). Fifty-seven of the 75 patients who exhibited progression died. The median PFS was 12 months, and the median OS was 55 months. Only 80 patients were evaluable for their responses to treatment (21 of the

104 patients enrolled initially underwent radical operation and had no residual disease before the initiation of chemotherapy, and another three patients were not available for assessment). There were 36 complete and 32 partial responses; the disease of eight patients was stable and the tumors of four patients continued to grow.

From the SNPs genotyped, ten were found to be significantly associated with one or more of the assessed clinical end points.

Associations between polymorphisms & tumor response

As there were no differences in OS between patients with partial response and those who had stable or progressive disease, partial responders were combined with nonresponders in further analyses. A significant difference in complete response was observed according to the *ADH1C* SNP (rs689; χ^2 = 16.903, p = 0.00021). The proportion of patients who achieved a complete response was higher among carriers of wild-type genotype AA and homozygous variant genotype GG (72.7 and 61.1%, respectively) compared with patients with a heterozygous genotype AG (22.5%). The difference was less powerful, but still evident, after the exclusion of patients with stable and progressive disease (p = 0.003).

Correlation between polymorphisms & survival

The genotypes of two SNPs of *NQO1* (rs1131341 and rs1800566) and one SNP of *TPMT* (rs1142345) were significantly associated with differences in the PFS of patients (Table 1). The patients with a homozygous wild-type genotype for any of the three SNPs had a longer progression-free interval compared with carriers of the corresponding heterozygous genotype or homozygous variant genotype. After adjustment for treatment variant, which was strongly correlated with time to progression, *NQO1* genotypes ceased to be significantly associated with PFS. By contrast, the association between PFS and the *TPMT* SNP remained significant.

SNPs in *NQO1* were also associated with differences in OS (Table 2). However, similar to the case of PFS, the associations became nonsignificant after adjustment for variations in treatment.

Correlation between polymorphisms & toxicity

To assess the association between genotype and the toxicity of the treatment used, patients were classified as having good or poor tolerance to treatment (grades 3–4 of neutropenia, grades 2–4 of anemia, grades 2–4 of neuropathy, grades 3–4 of emesis, in addition to any grade of thrombocytopenia, nephrotoxicity or ototoxicity were considered as clinically significant toxicities). There were no statistical differences in toxicity between the patients who received adjuvant chemotherapy and the remaining patients.

Genotypes of four SNPs were associated with an incidence of severe neutropenia (Table 3). The most significant was SNP rs3219484 in *MUTYH*. It was represented by only two genotypes, and patients who carried a heterozygous genotype AG had very low (actually unobserved) risk of developing severe neutropenia: OR: 0.013, 95% CI: 0.000–0.220. Combined analysis of the SNPs did not identify a genotype combination that would be more powerful (in terms of ORs) compared with genotypes at rs3219484 that seemed to be the result of moderate-to-strong linkage disequilibrium (D′ = 0.8–1.0) between SNP rs3219484 and other SNPs. At the same time, the analysis revealed a subgroup of patients with a higher incidence of severe neutropenia who were carriers of the combined wild-type genotype GG at rs3219484 plus variant genotype CC at rs1052536. Twenty-one out of 22 of those patients (95.5%) had grade 3 or 4 neutropenia.

Anemia was associated with SNP rs1801280 of *NAT2* and was more prevalent among patients with the heterozygous CT genotype (78.4%) compared with the homozygous wild-type (OR: 5.655, 95% CI: 2.231–14.335, p = 0.0002) and variant genotypes (Table 3). SNP rs1051740 in *EPHX1* was associated with nephrotoxicity. Like anemia, nephrotoxicity was more frequent among patients with a heterozygous genotype (OR: 9.524, 95% CI: 3.621–225.520, p = 0.000003; Table 3).

No significant associations were found between the polymorphisms and ototoxicity, thrombocytopenia, emesis and neurotoxicity.

Discussion

In this study, we systematically investigated the associations between SNPs in more than 100 genes and outcomes in ovarian cancer patients receiving cisplatin-based chemotherapy. Like similar genotyping panels, the list of genes tested comprised candidate genes involved in key pathways of cellular response to different drugs [17–19], including many genes that are related to the cisplatin pathway [102]. Although such panels generally compare poorly to genome-wide genotyping approaches, they are still useful for pharmacogenomic testing, as our knowledge of genetic variants in even the most intensively studied

candidate genes remains fragmentary [19]. A good example of the latter is recently described associations between SNPs in *TPMT* and *COMT* genes and ototoxicity of cisplatin chemotherapy in children; these were detected using a SNP genotyping assay designed to capture the genetic variation of 220 key drug metabolism genes [20]. This finding subsequently motivated the FDA to update the Platinol (cisplatin) product label in 2011 [103].

In the current study, no associations were found between SNPs in *TPMT* and *COMT* and ototoxicity, which could at least be the result of less frequent cisplatin-related hearing loss in adults compared with children [21]. At the same time, SNPs, particularly rs1142345 in *TPMT*, showed effects on PFS. TPMT is a cytosolic enzyme with a physiological role that remains unclear [22]. It is only known that this enzyme catalyzes the *S*-methylation of aromatic and heterocyclic compounds, preferentially thio compounds (e.g., 6-mercaptopurine and 6-thioguanine). Ross *et al.* have hypothesized that TPMT can affect cisplatin toxicity through inactivation of cisplatin–purine compounds that form cytotoxic DNA cross-links, and cause cell death [20]. The assumption was based on identification of known loss-of-function *TPMT* alleles, including the abovementioned rs1142345 (*TPMT*3C*, Tyr240Cys), found only among individuals with cisplatin ototoxicity. As might be expected from the data, those patients in our study who had the loss-of-function genotype AG at rs1142345 should demonstrate a better outcome as a result of decreased inactivation of cisplatin–purine compounds, but such a correlation was not observed. Moreover, the patients with an AG genotype had a shorter PFS compared with the carriers of the functionally normal AA variant.

Although the associations between *NQO1* genotypes and survival were statistically nonsignificant after adjustment for variations in treatment, they remained in good agreement with the results of other studies, in which individuals with a variant allele, especially those who were homozygous for *NQO1*2*, exhibited reduced survival compared with individuals who were homozygous for *NQO1*1* [23,24]. The numbers 2 and 3 denote the two characteristic polymorphic variants of *NQO1*: *NQO1*2* (rs1800566 C>T; Pro187Ser) and *NQO1*3* (rs1131341 C>T; Arg139Trp) [25]. Moreover, if the Arg-to-Trp substitution leads to a change in the rate of the metabolism of some substrates by NQO1, the Pro-to-Ser substitution results in a rapidly ubiquitinated and degraded protein. Individuals who are homozygous for *NQO1*2* have essentially no active enzyme. Taking into account the key role of NQO1 in preventing the formation of reactive semiquinone radicals and generating reactive oxygen species (ROS) via redox cycling, one can propose that the worse survival observed for *NQO1*2* carriers is at least the consequence of a chronically elevated level of ROS, which results in intensified ROS-mediated DNA damage, enhanced genetic instability and further cancer progression [24].

Despite the role *TPMT* and *NQO1* polymorphisms play in survival, no significant correlation was observed between them and complete tumor response. Complete tumor response was associated with the SNP in the *ADH1C* gene. This gene encodes the class I alcohol dehydrogenase γ subunit, playing a vital role in ethanol metabolism (oxidation of ethanol to acetaldehyde) [26]. The SNP of interest (rs689) results in an amino acid substitution (Ile350Val) that affects the ethanol-oxidizing capacity of ADH1C. It has been associated with a risk of alcoholism and ethanol-related cancers [26–28]. However, to our knowledge, the current study is the first to associate the SNP with a chemotherapy outcome (i.e., complete tumor response).

Some SNPs were found to be associated with different side effects of the chemotherapy used. Four out of six associations were related to severe neutropenia. The most prominent association was with SNP rs3219484 in *MUTYH*. This gene encodes a DNA glycosylase involved in oxidative DNA damage repair, in particular the glycosylase removes adenines misincorporated opposite the 7,8-dihydro-8-oxoguanines. Such mispairs are promutagenic, and if left unrepaired, they can give rise to CG>AT transversion mutations [29]. The observed association may reflect the substantial role of oxidative stress (i.e., ROS) in cisplatin-induced cytotoxicity. On the other hand, the quite high level of linkage disequilibrium (D′ = 0.8–1.0) between this SNP and SNPs in other genes, particularly *RAD52* and *ERCC5*, may also indicate a role of *MUTYH* in the repair of cisplatin-induced DNA damage [30].

SNP rs1051740 in *EPHX1* was associated with nephrotoxicity. This gene encodes EPHX1. EPHX1 metabolizes various compounds, including polycyclic aromatic hydrocarbons, which are carcinogens [31]. However, there is little information about the role of epoxide

Table 1. Polymorphisms associated with progression-free survival.

Gene	SNP ID in dbSNP (rs)	Genotype	Patients (n)	PFS (median, months)	Log rank Mantel–Cox test p-value	Cox regression analysis†				
						β *regression coefficient*	*Standard error (β)*	*Exp (β)*	*95% CI exp (β)*	*p-value*
TPMT	rs1142345	AA	87	14	3×10^{-8}	Reference				
		AG	11	6		1.688 (1.401)	0.352 (0.350)	5.407 (4.059)	2.713–10.774 (2.043–8.065)	2×10^{-6} (6×10^{-5})
NQO1	rs1131341	CC	88	13	2×10^{-6}	Reference				
		CT	10	6		1.507 (1.215)	0.358 (0.357)	4.512 (3.370)	2.238–9.095 (1.676–6.679)	2×10^{-5} (7×10^{-4})
NQO1	rs1800566	CC	76	15	9×10^{-11}	Reference				
		CT	21	7		1.336 (0.966)	0.274 (0.274)	3.803 (2.628)	2.222–6.509 (1.536–4.498)	1×10^{-6} (4×10^{-4})
		TT	2	4		2.979 (1.975)	0.790 (0.790)	19.678 (14.304)	4.181–92.614 (3.043–67.230)	2×10^{-4} (8×10^{-4})

†*The results of adjustment for treatment variant are in brackets.*
PFS: Progression-free survival.

Table 2. Polymorphisms associated with overall survival.

Gene	SNP ID in dbSNP (rs)	Genotype	Patients (n)	OS (median, months)	Log rank Mantel–Cox test p-value	Cox regression anlysis†				
						β *regression coefficient*	*Standard error (β)*	*Exp (β)*	*95% CI exp (β)*	*p-value*
NQO1	rs1131341	CC	89	49	2×10^{-5}	Reference				
		CT	10	18		1.447 (1.155)	0.371 (0.371)	4.249 (3.173)	2.053–8.796 (1.534–6.566)	1×10^{-4} (2×10^{-3})
NQO1	rs1800566	CC	77	55	1×10^{-4}	Reference				
		CT	21	22		1.153 (0.828)	0.291 (0.293)	3.169 (2.290)	1.793–5.601 (1.290–4.062)	7×10^{-5} (7×10^{-3})
		TT	2	38		0.440 (0.108)	1.015 (1.016)	1.552 (1.114)	0.212–11.346 (0.152–8.154)	0.665 (0.916)

†*The results of adjustment for treatment variant are in brackets.*
OS: Overall survival.

Table 3. Polymorphisms associated with toxicity of chemotherapy.

Toxicity	Gene	SNP ID in dbSNP (rs)	Genotype	Total patients (n)	Patients with toxicity (n)	Genotype exact test, p-value	OR	95% CI (OR)	p-value
Neutropenia grades 3–4	*ERCC5*	rs17655	GG	49	39	3×10^{-5}	1.00		
			CG	47	19		0.174	0.070–0.431	0.0001
			CC	6	1		0.051	0.005–0.490	0.0043
	LIG3	rs1052536	TT	27	6	1×10^{-6}	1.00		
			CT	53	32		5.333	1.845–15.416	0.0019
			CC	22	21		73.500	8.126–664.84	0.0000002[†]
	RAD52	rs11226	CC	46	38	1×10^{-5}	1.00		
			CT	41	15		0.122	0.045–0.328	0.00002[†]
			TT	15	6		0.140	0.039–0.507	0.0028
	MUTYH	rs3219484	GG	85	59	4×10^{-8}	1.00		
			AG	17	0		0.013	0.001–0.220	0.00000004[†]
Anemia grades 2–4	*NAT2*	rs1801280	TT	64	25	7×10^{-5}	1.00		
			CT	37	29		5.655	2.231–14.335	0.0002
			CC	2	0		0.310	0.014–6.725	0.5221
Nephrotoxicity grades 1–4	*EPHX1*	rs1051740	TT	57	9	2×10^{-6}	1.00		
			CT	39	25		9.524	3.621–25.52	0.000003[†]
			CC	6	1		1.067	0.111–10.248	1.0000

[†]*Two-sided exact p-values calculated using an interactive calculation tool for Fisher's exact probability test for 2 × 2 tables* [104].
OR: Odds ratio.

hydrolases in anticancer treatment, particularly related to cisplatin-induced toxicity. It is only known that inhibition of the cytosolic partner of EPHX1, EPHX2, can attenuate cisplatin-induced kidney injury [32,33]. This effect has been associated with the abilities of epoxyeicosatrienoic acids that are metabolized by EPHX2 to interfere with renal inflammation, which occurs before cisplatin-induced acute kidney injury. EPHX1 also accepts epoxyeicosatrienoic acids, although generally to a much lesser extent than EPHX2. Therefore, a role for *EPHX1* in cisplatin nephrotoxicity should not be excluded, particularly because of its high expression in kidneys [31].

Similar to EPHX1, there are no experimental data demonstrating any relationships between NAT2 and the metabolism or toxicity of cisplatin or cyclophosphamide (the second component of our regimen). One can propose that the association found here is a result of linkage with a functional SNP(s) in other gene(s) [34].

The limitations of our study were: the small sample size, which lowered the statistical power of our analyses and rendered subgroup analysis less informative, and the candidate gene approach, which omitted other potentially important polymorphisms, including those located in the same gene but not included in the microarray.

Conclusion

In summary, we have studied the importance of SNPs in more than 100 genes involved in key chemotherapeutic drug action pathways in ovarian cancer patients treated with a cisplatin–cyclophosphamide regimen. The results obtained, examined in the context of data from the literature, suggest a potential role for polymorphisms of various genes coding for metabolic enzymes in identifying patients at risk of nonresponse to or toxicity from cisplatin-based treatment. However, because of the relatively small sample size and limited number of markers tested, further studies are required to confirm these findings.

Future perspective

Although our knowledge of the genes that affect the efficacy and toxicity of platinum drugs has evolved substantially, pharmacogenetic markers (polymorphisms) that can be translated into clinical practice are still largely missing. It is anticipated that, over the next few years, intensive research in the field of the complex metabolism of cisplatin and its analogs will generate additional data regarding the associations between genetic variants and treatment outcomes. After proper evaluation in experimental and clinical settings, some of those variations may became a basis for individualized platinum-based anticancer therapy.

Financial & competing interests disclosure

This study was supported by grants from The Ministry of Education and Science of Russian Federation (project 8805) and Russian Basic Research Foundation (grant 11-04-00913); the Programs 'Molecular and Cell Biology' and 'Fundamental Science for Medicine' of the Russian Academy of Sciences; the Federal Support of Leading Scientific Schools (grant 4294.2012.4). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Executive summary

- Platinum-containing drug treatment is associated with unpredictable variability in patient outcomes, including treatment-related response and toxicity.
- Attempts to associate the observed variability in patient reactions with polymorphisms in a limited number of candidate genes (e.g., *GSTP1, ERCC1* and *ERCC2*) have provided inconsistent results.
- Platinum agents have complex metabolic pathways, all of which can contribute to overall clearance.
- Using arrayed primer extension genotyping technology we have evaluated the association between polymorphisms in 106 genes, involved mainly in drug metabolism, DNA repair, the cell cycle and apoptosis, and outcomes in a cohort of ovarian cancer patients receiving cisplatin-based (combination with cyclophosphamide) chemotherapy.
- Ten SNPs in nine genes were found to be associated with one or more of the assessed clinical end points.
- SNPs in *TPMT* and *NQO1* were associated with progression-free survival.
- SNPs in *NAT2, ERCC5, RAD52, MUTYH*, *LIG3* and *EPHX1* were associated with side effects of the treatment used.
- A SNP in *ADH1C* was correlated with complete tumor response.
- The resulting spectrum of associations suggests a potential role for polymorphisms of various genes coding for metabolic enzymes in identifying patients at risk of nonresponse to or toxicity from cisplatin-based treatment.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

References

Papers of special note have been highlighted as:
▪ of interest
▪▪ of considerable interest

1 Kelland L. The resurgence of platinum-based cancer chemotherapy. *Nat. Rev. Cancer* 7(8), 573–584 (2007).

2 Muggia F. Platinum compounds 30 years after the introduction of cisplatin: implications for the treatment of ovarian cancer. *Gynecol. Oncol.* 112(1), 275–281 (2009).

3 Hogberg T. Chemotherapy: current drugs still have potential in advanced ovarian cancer. *Nat. Rev. Clin. Oncol.* 7(4), 191–193 (2010).

4 Rabik CA, Dolan ME. Molecular mechanisms of resistance and toxicity associated with platinating agents. *Cancer Treat. Rev.* 33(1), 9–23 (2007).

5 Evans WE, McLeod HL. Pharmacogenomics – drug disposition, drug targets, and side effects. *N. Engl. J. Med.* 348(6), 538–548 (2003).

6 Eichelbaum M, Ingelman-Sundberg M, Evans WE. Pharmacogenomics and individualized drug therapy. *Annu. Rev. Med.* 57, 119–137 (2006).

7 Ma Q, Lu AY. Pharmacogenetics, pharmacogenomics, and individualized medicine. *Pharmacol. Rev.* 63(2), 437–459 (2011).

▪ **Comprehensive review of recent progress in the understanding of genetic impact on individual variability in drug therapy with focus on the role of gene polymorphisms.**

8 Shimoyama S. Pharmacogenetics of fluoropyrimidine and cisplatin. A future application to gastric cancer treatment. *J. Gastroenterol. Hepatol.* 24(6), 970–981 (2009).

9 Marsh S. Pharmacogenomics of taxane/platinum therapy in ovarian cancer. *Int. J. Gynecol. Cancer.* 19(Suppl. 2), 30–34 (2009).

10 Yu W, Gwinn M, Clyne M, Yesupriya A, Khoury MJ. A navigator for human genome epidemiology. *Nat. Genet.* 40(2), 124–125 (2008).

11 Jung Y, Lippard SJ. Direct cellular responses to platinum-induced DNA damage. *Chem. Rev.* 107(5), 1387–1407 (2007).

▪ **Exhaustive review of different mechanisms used by cells to protect themselves from platinum-induced DNA lesions.**

12 Milligan BG. Total DNA isolation. In: *Molecular Genetic Analysis of Populations.* Hoelzel AR (Ed.). Oxford University Press, London, UK, 29–60 (1998).

13 Khrunin AV, Moisseev A, Gorbunova V, Limborska S. Genetic polymorphisms and the efficacy and toxicity of cisplatin-based chemotherapy in ovarian cancer patients. *Pharmacogenomics J.* 10(1), 54–61 (2010).

14 Landi S, Gemignani F, Gioia-Patricola L, Chabrier A, Canzian F. Evaluation of a microarray for genotyping polymorphisms related to xenobiotic metabolism and DNA repair. *Biotechniques* 35(4), 816–827 (2003).

15 Kweekel DM, Antonini NF, Nortier JW, Punt CJ, Gelderblom H, Guchelaar HJ. Explorative study to identify novel candidate genes related to oxaliplatin efficacy and toxicity using a DNA repair array. *Br. J. Cancer* 101(2), 357–362 (2009).

16 Liu K, Muse SV. PowerMarker: an integrated analysis environment for genetic marker analysis. *Bioinformatics* 21(9), 2128–2129 (2005).

17 Dai Z, Papp AC, Wang D, Hampel H, Wolfgang S. Genotyping panel for assessing response to cancer chemotherapy. *BMC Med. Genomics* 1, 24 (2008).

18 Deeken J. The Affymetrix DMET platform and pharmacogenetics in drug development. *Curr. Opin. Mol. Ther.* 11(3), 260–268 (2009).

19 Sissung TM, English BC, Venzon D, Figg WD, Deeken JF. Clinical pharmacology and pharmacogenetics in a genomics era: the DMET platform. *Pharmacogenomics* 11(1), 89–103 (2010).

20 Ross CJ, Katzov-Eckert H, Dube MP *et al.* Genetic variants in *TPMT* and *COMT* are associated with hearing loss in children receiving cisplatin chemotherapy. *Nat. Genet.* 41(12), 1345–1349 (2009).

▪▪ **Association study that discovered and validated SNPs in *TPMT* and *COMT* genes associated with cisplatin-induced hearing loss in children.**

21 Li Y, Womer RB, Silber JH. Predicting cisplatin ototoxicity in children: the influence of age and the cumulative dose. *Eur. J. Cancer* 40(16), 2445–2451 (2004).

22 Fotoohi AK, Coulthard SA, Albertioni F. Thiopurines: factors influencing toxicity and response. *Biochem. Pharmacol.* 79(9), 1211–1220 (2010).

23 Dinkova-Kostova AT, Talalay P. NAD(P)H:quinone acceptor oxidoreductase 1 (NQO1), a multifunctional antioxidant enzyme and exceptionally versatile cytoprotector. *Arch. Biochem. Biophys.* 501(1), 116–123 (2010).

24 Fagerholm R, Hofstetter B, Tommiska J *et al.* NAD(P)H:quinone oxidoreductase 1 *NQO1*2* genotype (P187S) is a strong prognostic and predictive factor in breast cancer. *Nat. Genet.* 40(7), 844–853 (2008).

25 Kolesar JM, Dahlberg SE, Marsh S *et al.* The *NQO1*2/*2* polymorphism is associated with poor overall survival in patients following resection of stages II and IIIa non-small cell lung cancer. *Oncol. Rep.* 25(6), 1765–1772 (2011).

26 Cederbaum AI. Alcohol metabolism. *Clin. Liver Dis.* 16(4), 667–685 (2012).

27 Xue Y, Wang M, Zhong D *et al. ADH1C* Ile350 Val polymorphism and cancer risk: evidence from 35 case–control studies. *PLoS ONE* 7(5), e37227 (2012).

28 Chang JS, Straif K, Guha N. The role of alcohol dehydrogenase genes in head and neck cancers: a systematic review and meta-analysis of ADH1B and ADH1C. *Mutagenesis* 27(3), 275–286 (2012).

29 Markkanen E, Dorn J, Hübscher U. MUTYH DNA glycosylase: the rationale for removing undamaged bases from the DNA. *Front. Genet.* 4, 18 (2013).

30 Jansson K, Alao JP, Viktorsson K, Warringer J, Lewensohn R, Sunnerhagen P. A role for Myh1 in DNA repair after treatment with strand-breaking and crosslinking chemotherapeutic agents. *Environ. Mol. Mutagen.* 54(5), 327–337 (2013).

31 Decker M, Arand M, Cronin A. Mammalian epoxide hydrolases in xenobiotic metabolism and signalling. *Arch. Toxicol.* 83(4), 297–318 (2009).

32 Parrish AR, Chen G, Burghardt RC, Watanabe T, Morisseau C, Hammock BD. Attenuation of cisplatin nephrotoxicity by inhibition of soluble epoxide hydrolase. *Cell Biol. Toxicol.* 25(3), 217–225 (2009).

33 Liu Y, Webb HK, Fukushima H *et al.* Attenuation of cisplatin-induced renal

injury by inhibition of soluble epoxide hydrolase involves nuclear factor κB signaling. *J. Pharmacol. Exp. Ther.* 341(3), 725–734 (2012).

34 Elkiran T, Harputluoglu H, Yasar U *et al.* Differential alteration of drug-metabolizing enzyme activities after cyclophosphamide/adriamycin administration in breast cancer patients. *Methods Find. Exp. Clin. Pharmacol.* 29(1), 27–32 (2007).

Websites

101 HuGE Navigator: An integrated, searchable knowledge base of genetic associations and human genome epidemiology. http://hugenavigator.net/HuGENavigator/home.do (Accessed 27 February 2013)

102 Platinum pathway. www.pharmgkb.org/pathway/PA150642262 (Accessed 31 October 2013).

103 PLATINOL® (cisplatin). www.accessdata.fda.gov/drugsatfda_docs/label/2011/018057s081lbl.pdf

104 Preacher KJ, Briggs NE. Calculation for Fisher's Exact Test: an interactive calculation tool for Fisher's exact probability test for 2 × 2 tables (Computer software). http://quantpsy.org (Accessed 30 October 2013)