# 'Genetic profiling' and ovarian cancer therapy (Review)

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Received May 27, 2011; Accepted June 16, 2011

DOI: 10.3892/mmr.2011.512

**Abstract.** High variability observed among ovarian cancer patients in response to the same therapy and the related toxicity may be correlated to gene polymorphisms and genetic alterations affecting the metabolism of drugs commonly used to treat this tumor. Recent studies have shown a correlation between the polymorphisms characterizing GSTM1-T1 detoxifying enzymes and poor outcome in advanced ovarian cancer patients treated with platinum/paclitaxel-based chemotherapy. Multidrug resistance 1 (mdr-1) polymorphisms were found to be associated with resistance to paclitaxel treatment. Polymorphisms of MRP2, a protein involved in methotrexate, cisplatin and irinotecan active metabolite glucuronide transport, negatively affect platinum-based chemotherapy response. A similar occurrence has been observed with CYP1A1 Ile462Val and ercc1 C118T polymorphisms while patients who were carriers of MTHFR C677T polymorphism had a better response to methotrexate therapy, but an elevated risk of toxicity. Biological therapy with Bevacizumab, the anti-vascular endothelial growth factor has been shown to be less efficient in ovarian cancer patients carrying the polymorphism of the Interleukin-8 gene. Instead, polymorphisms in the XPD gene (Lys751Gln and Asp312Asn), a member of the nucleotide excision repair pathway, positively affects the response to therapy with carboplatin/paclitaxel. Therefore, the study of 'genetic profiling' is crucial to improving the clinician's ability to tailor effective therapy to the molecular profile of the patient while minimizing toxicities. This review describes clinical applications of the above genetic polymorphisms in ovarian cancer patients treated with platinum/paclitaxel-based chemotherapy.

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*Key words:* ovarian cancer, gene polymorphisms, biological therapy

**Contents**



## 1. Introduction

Ovarian cancer is a tumor with a low prevalence but high mortality and it is the sixth most frequently occurring cancer in women. Incidence rates differ according to geographical location. Ovarian cancer is more prevalent in Caucasians of the eastern and north western United States, whereas it occurs less frequently in populations of Asia, Africa, Latin America and Japan.

Developed countries have incidence rates higher than 10/100,000, whereas in Africa and India the incidence rate is 3-4/100,000. In Europe, in 1990, ovarian cancer accounted for 5% of all cancers of the female gender, but the incidence was highly variable in different countries.

Approximately 25,000 new cases of ovarian cancer developing in later life are diagnosed annually, and at least 14,000 of these patients succumb to the disease each year. The high rate of mortality for ovarian cancer is due to the difficulty of making an early diagnosis as ovarian cancer frequently develops without well-defined symptoms. In most cases, the early symptoms include an increase in volume of the lower abdomen, or a heaviness/tension sense, or a vague abdominal and pelvic discomfort. Gastrointestinal symptoms may be present. A total amount of 70% of patients present an advanced cancer (stage III) at diagnosis.

Given the insidious development of the ovarian cancer, it is a common observation that patients with advanced disease report symptoms of recent onset. In approximately half of the patients the interval between the beginning of symptoms and histological diagnosis is 2-3 months and in approximately 30% of patients this interval is less than 1 month. As a result, ovarian cancer has a low prevalence, but one which is higher than that of uterine and cervical cancers, although the latter tumors are more prevalent (but easier to diagnose).

Multiparity, breast feeding and long-term use of progestogens contraceptive reduce the risk of developing ovarian cancer and are protective factors, while a family history for ovarian cancer, the length of the ovulatory period (early menarche and late menopause) and junk infertility in women who ovulate regularly are risk factors for ovarian cancer (1).

## 2. Current therapy of ovarian cancer

The treatment of ovarian cancer should be based on an agreement between various strategic and operational specialists. Surgery is the first step of treatment planning (diagnostic or therapeutic time). Surgery may be conservative or radical, debulking, palliative or only exploratory. The extent and type of surgery varies in relation to the spread of the disease. Intraperitoneal radioisotopes and external radiation with high energy have been widely used in the past. Both modes, and in particular the latter, were almost entirely replaced by chemotherapy. Ovarian cancer is the most chemosensitive gynecologic neoplasia after gestochoriocarcinoma and extraembryonal teratomas (1).

*First-line therapy*. Commonly used drugs included alkylating agents and anthracycline. The most frequently used alkylating compound was melphalan. Other commonly used drugs included cyclophosphamide, thio-TEPA and chlorambucil.

Platinum and taxanes have been shown to be the most active drugs. Cisplatin has numerous adverse reactions, such as neurotoxicity, nephrotoxicity, ototoxicity and gastrointestinal toxicity which limit its use and require antiemetic drugs and renoprotectives. A platinum derivative, carboplatin, has a similar activity to cisplatin, but is different and generally less toxic (absence of nephrotoxicity, ototoxicity and neurotoxicity, but substantial myelotoxicity).

The introduction of chemotherapy regimens containing cisplatin or platinum derivates to clinical practice generated a higher number of responsive patients, increasing the duration of therapeutic response and the progression-free interval. The literature showed that carboplatin has the same therapeutic effect as cisplatin, with a lower toxicity. Therefore, carboplatin in monochemotherapy is an appropriate, effective and safe treatment.

Taxanes are also considered to be effective drugs. The combination of platinum (cisplatin or carboplatin) and paclitaxel improves disease-free and overall survival compared to conventional monochemotherapy with platinum. For this reason, the combination of paclitaxel and carboplatin or paclitaxel and cisplatin is the treatment of choice as first-line therapy (paclitaxel/carboplatin is better tolerated and causes fewer side effects than cisplatin/paclitaxel). Finally, the combination of cisplatin and paclitaxel has a therapeutic effect greater than that of cisplatin with cyclophosphamide (1).

*Maintenance/consolidation therapy*. The efficacy of prolonged chemotherapy in ovarian cancer patients was evaluated in three randomized trials conducted in the nineties (GOG, 1992; Danish ovarian study group 1993 and North Thames ovary group 1997). The three studies showed that the continuous use of chemotherapy does not present significant therapeutic benefits. A recent study (2) by the SWOG-GOG conducted in 222 patients, who obtained a complete clinical remission following treatment with paclitaxel and cisplatin, compared long-term administration of paclitaxel (12 cycles) vs. only three cycles of paclitaxel. The long-term administration resulted in an improvement in the median of disease-free survival (28 vs. 21 months). However, long-term administration did not improve overall survival, but caused more side effects and deterioration in quality of life.

Two recent studies have reported that the maintenance of monochemotherapy with topotecan following treatment with paclitaxel and carboplatin is not able to improve the therapeutic results (1,3).

*Second-line chemotherapy*. A high percentage of patients responsive to first-line chemotherapy relapsed. Additionally, second-line chemotherapy poses a significant clinical problem in that the effective drugs constituted part of the first-line therapy. Thus, the efficacy of a second-line therapy depends on the response to first-line therapy.

There are three prognostic groups: a) patients with a documented complete response in first-line therapy with platinum or platinum-containing regimens who relapse at a variable distance of time, b) patients with partial response to first-line regimens, and c) patients who are unresponsive to first-line therapy.

In the first group of patients, if relapse does not occur early (minimum free interval higher than 6 months), the disease is likely to be sensitive to platinum. If the free interval is high then there is a greater chance of obtaining a response using the same drugs as those of first-line therapy. In this first group of patients, complete clinical regressions have been documented with platinum or platinum-containing regimens in more than 25% of cases.

In the second group of patients, the question is raised as to whether it is more useful to continue with the same regimen that had led to a partial response or change to a new regimen. In the third group of patients, a second-line therapy is mandatory.

Despite improvements in complete clinical remission and progression-free survival, ovarian cancer is a disease with a high risk of relapse. For this reason, it is necessary to study the efficacy of more novel chemotherapy or anticancer agents (1).

## 3. Genetic alterations

Pharmacogenetics studies in cancer therapy have shown an association between specific genetic variants of drug metabolizing enzymes (pharmacogenetic determinants of response) and adverse drug reactions (ADR) or toxicity. Primarily, genetic abnormalities are considered to affect treatment response (4-6).

Studies correlating current ovarian cancer therapies with genetic polymorphisms responsible for improved response or non-response to treatment and increased toxicity are described in Table I.

*GSTM1-T1 polymorphisms and platinum/paclitaxel*. A recent study emphasized the correlation between the polymorphisms characterizing GSTM1-T1 enzymes and disease outcome in advanced ovarian carcinoma patients, following platinum/paclitaxel-based chemotherapy (7). GST are a family of detoxification enzymes. The general reaction catalyzed by all isoforms is the conjugation of glutathione (GSH) to a molecule

Table I. Genetic alterations associated with current ovarian cancer therapies.

| Genetic alteration | Result of alteration | Anticancer agent(s) | Effect | Refs. |
|---|---|---|---|---|
| GSTM1/T1 | Reduction or absence of GST enzyme synthesis | Platinum | Increase of mean survival time | (5) |
| G2677T/A mdr-1 SNPs | Alteration of P-glycoprotein expression and phenotype | Paclitaxel | Better response to paclitaxel therapy | (6-16) |
| MRP2 Cyt24Thy | Active metabolite glucuronide transport | Cisplatin | Development of chemoresistance to cisplatin | (17-20) |
| MTHFR C677T | Reduced enzyme activity, Impaired remethylation of Hcy to methionine, Hyperhomocysteinemia | Methotrexate | Increased MTX toxicity<br>Increased response to MTX<br>Reduction of response to chemotherapy | (21-27)<br>(21-27)<br>(21-27) |
| ercc1 N118N | Lower levels of ercc1 mRNA | Platinum | Higher risk of disease progression | (28-38) |
| CYP1A1 Ile462Val | Functional changes | Platinum | Development of platinum resistance | (39) |
| IL-8 251 T>A | Increased IL-8 production | Cyclophosphamide | Lower response to cyclophosphamide and bevacizumab | (40-50) |
| GSTP1 Ile105Val | Increase enzymatic activity | Platinum | Worse progression-free survival | (51) |
| XPD Asp312Asn<br>XPD Lys751Gln | Lower DNA repair capacity | Carboplatin<br>Paclitaxel | Reduction of death risk | (52-54) |

GST, glutathione S-transferase; SNP, single nucleotide polymorphism; Hcy, homocysteine; MTX, methotrexate; XPD, xeroderma pigmentosum groud D.

containing an electrophilic group. This reaction facilitates the elimination of the molecule in the conjugate form as it is more soluble and likely to be excreted. In addition, GST are endogenous modulators of the proteins that regulate programmed cell death (apoptosis). This review suggests that characterization of the drug-metabolizing genetic individual profile is of great interest in clinical oncology. This profile is capable of defining the optimal chemotherapy for each patient, improve efficacy, and reduce the incidence of drug toxicity and poor drug response.

*mdr1 and CYP2C8 polymorphism and paclitaxel*. The mdr1 gene encodes for P-glycoprotein, a 170 kDa plasmatic membrane protein that acts as an ATP-dependent drug export pump. P-glycoprotein extracts taxanes and other cytotoxic drugs of natural origin through the cell membranes.

A high expression of P-glycoprotein on tumor cells leads to chemoresistance (8,9) and appears to be correlated with a poor response to paclitaxel treatment (10,11).

The first well-characterized polymorphism of the mdr1 gene was the single nucleotide polymorphism (SNP) in exon 26, C3435T (11). Hoffmeyer *et al* found that patients who are homozygous for this polymorphism had a lower P-glycoprotein expression and concomitant higher plasma levels of digoxin (the P-glycoprotein substrate) (12). Approximately 25 SNPs for the mdr1 gene were identified (13-15). However, the most important SNPs in Caucasians are G2677T/A and C3435T as these SNPs have been shown to be involved in P-glycoprotein expression and phenotype (13,16). A non-functioning P-glycoprotein may affect the pharmacokinetics and pharmacodynamics of paclitaxel. Cancer cells with mutant or non-functional drug export pump should respond better to chemotherapy. Furthermore, paclitaxel is administered intravenously and excreted in the feces, thus an altered transport of paclitaxel from the blood circulation to the intestine would increase the exposure of the chemotherapeutic regimen.

Gréen *et al* studied the effect of the G2677T/A and C3435T mdr1 SNP response to paclitaxel treatment in ovarian cancer. These authors found that patients with ovarian cancer who are homozygous for the G2677T/A mdr1 SNP responded better to paclitaxel treatment and that homozygosity may be considered a favorable predictive factor for paclitaxel treatment, whereas the C3435T SNP appears not to have any impact on the treatment outcome (17). Gréen *et al* also studied the relationship between neurotoxicity in paclitaxel therapy and genetical alterations of the mdr1 gene and the genotypes of CYP2C8*1B, *1C, *2, *3, *4, *5, *6, *7 and *8.

These investigators found that patients heterozygous for G2677T/A mdr1 SNP had an important higher clearance of

paclitaxel than other mdr1 polymorphisms, whereas patients heterozygous for CYP2C8*3 had a lower clearance of paclitaxel. Consequently, mdr1 and CYP2C8 genotypes may be used to predict inter-patient variability in paclitaxel pharmacokinetics and provide essential information for tailored therapy (18).

*MRP2 polymorphism and cisplatin*. MRP2, also known as cMOAT (canalicular multispecific organic anion transporter) is thought to be involved in methotrexate, cisplatin and irinotecan active metabolite glucuronide transport (18).

Certain *in vitro* studies correlated MRP2 mRNA expression with the development of pharmaco-resistance to anticancer agents, such as cisplatin. This correlation was demonstrated in particular in a set of clear cell carcinomas of the ovary cell lines (20). Yokoyama *et al* (21) reported that MRP2 expression may be a potential predictor of the response to standard chemotherapy in ovarian cancer. Nonetheless, other *in vivo* studies failed to confirm this association in a population of epithelial ovarian carcinoma patients treated with combination therapies, including cisplatin (22).

*MTHFR C677T polymorphism and methotrexate*. Methylene tetrahydrofolate reductase (MTHFR) catalyses the reduction of 5,10-methylenetetrahydrofolate (5,10-methyleneTHF) to 5-methyltetrahydrofolate (5-methylTHF), which is the methyl donor for the methionine synthesis from homocysteine (Hcy).

A common thermolabile genetic variant (677C>T) in the MTHFR gene, resulting in an alanine/valine amino acid substitution is associated with reduced enzyme activity, impaired remethylation of Hcy to methionine, and subsequent hyperhomocysteinemia. This variant is associated with vascular disease, spina bifida, diabetic nephropathy and human cancers (23). Inhibition of methylation responses by methotrexate (MTX) may result in toxicity. Therefore, patients with an impaired intracellular methylation process, such as those with a 677C>T mutation, may be predisposed to MTX toxicity.

Preliminary reports indicate that the MTHFR C677T polymorphism increases the toxicity and response to MTX and the level of hyperhomocysteinemia subsequent to drug administration (24). Our group previously demonstrated in a population of 43 ovarian cancer patients treated with MTX, that the homozygous 677TT carriers were more exposed to the risk of developing G3-4 toxicity after chemotherapy and hyperhomocysteinemia than the heterozygous 677AC or wild-type 677AA carriers (24). Although our findings should be considered as exploratory due to the limited sample size, they strongly suggest that the lower MTHFR enzymatic activity associated with the TT MTHFR genotype, increases the pharmacologic effects of MTX, leading to toxicity. However, the results are consistent with those of other studies, indicating that patients with a low activity of MTHFR (TT genotype) appear at risk of MTX toxicity (26,27). Hcy plasma level is a sensitive and responsive indicator of MTX cytotoxicity, which is also affected by MTFR activity (23,28,29).

*ERCC-1 C118T polymorphism and platinum/paclitaxel therapy*. ERCC-1 is a protein encoded by the excision repair cross-complementation group 1 gene. The ERCC1 protein belongs to the nucleotide excision repair (NER) pathway and is activated when DNA is damaged by platinum-based chemotherapeutic agents such as cisplatin and carboplatin (30,31).

Although platinum is largely used in ovarian cancer, approximately 20% of patients do not respond to treatment, and early responders present risk for disease progression (32,33). Genetic alterations of the cellular repair system modify the patient response to chemotherapy.

Increased NER activity and a high expression of ercc1 mRNA have been found to play a crucial role in cisplatin-resistant ovarian cancer cell lines (34). An elevated expression of ERCC-1 in ovarian cancer has also been correlated with the development of resistance to platinum-based therapy (35,36).

An SNP in codon 118 of the ercc1 gene is responsible for a C to T transition that can be used as a marker of ERCC-1 expression.

Previously, it was found that the T substitution in ovarian cancer cells is associated with lower levels of ercc1 mRNA and consequently a reduced DNA repair capability (37).

Another study on ovarian cancer patients has stressed the association between the C/C genotype and increased resistance to platinum-based chemotherapy, consistent with a higher transcription activity of the AAC codon (38).

Smith *et al* investigated the effect of ercc1 SNP 118 on ovarian cancer treatment and survival (39). Consistent with previous studies regarding the C/C genotype and its relationship with poor treatment response (38), these authors found that ovarian cancer patients harboring C/C genotypes were less responsive to chemotherapy and maintained a higher risk for disease progression and death in contrast to those harboring C/T or T/T genotypes. Findings of Smith *et al* showed that ovarian cancer patients with a high ERCC-1 expression or the C/C genotype at codon 118 may benefit from a platinum/paclitaxel combination, whereas patients with a low ERCC-1 expression or the C/T or T/T genotype may respond well to platinum without paclitaxel.

This finding may be explained by the results of an *in vitro* experiment by Toiyama *et al* (40). Their research group has shown that radiations activate ERCC-1 expression in gastrointestinal cancer cells, but not in the same cells previously treated with paclitaxel, suggesting that paclitaxel blocks ERCC-1 activity, inhibits the DNA repair system and prevents cellular resistance to drug-induced DNA damage. If ERCC-1 expression in ovarian cells is arrested by paclitaxel, then paclitaxel treatment may offset the chemoresistance to platinum therapy in ovarian cancer patients.

*CYP1A1 Ile462Val polymorphism and platinum*. Cytochrome P450 is involved in estrogen metabolism, and polymorphisms have been associated with functional changes and risk for ovarian cancer. Heubner *et al* found a statistically significant association between the 462Val allele and platinum resistance, which was defined as a time interval of less than 6 months to disease progression following the administration of a platinum-based primary chemotherapy (OR=5.9; 95% CI, 1.5-23.2; p=0.005). Their findings suggest an association between the 462Val allele and the development of platinum resistance in ovarian tumors, although the potential involvement of CYP1A1 in the metabolism of platinum-containing agents remains to be determined (41).

*IL-8 polymorphism and cyclophosphamide and bevacizumab.* VEGF is crucial in both physiological and pathological angiogenesis regulation as it is also produced by cancer cells when they are in a strong state of hypoxia. Polymorphisms of the VEGF gene have been identified and correlated with variations in VEGF protein production. Preclinical studies have shown that VEGF-related angiogenesis is crucial in initiating and increasing the growth of ovarian cancers (42-44). Therefore, VEGF may be important as a marker of poor prognosis in these patients (45,46).

Certain chemotherapeutic drugs, such as cyclophosphamide, are not specific for tumor cells. Instead, these drugs interfere with the cell cycle and target endothelial cells. This shortcoming of cyclophosphamide probably leads to an anti-angiogenic effect that is increased with the addition of specific antiangiogenic drugs, such as monoclonal antibodies against VEGF (bevacizumab).

A Californian study (47) analyzed genes encoding for proangiogenic molecules and/or their receptors. The aim of this study was to evaluate the significance of genetic variations of genes involved in the angiogenesis mechanism and their impact on treatment efficacy. Authors of this study examined a germline polymorphism in the IL-8 gene (consistent in T to A substitution at position -251). IL-8 acts on endothelial cells by binding the receptors CXCR1 and CXCR2 (46-51) and probably mediates angiogenesis independently of VEGF (52). Patients with at least one A allele were found to have an increased IL-8 production, and a statistically significant lower response to cyclophosphamide and bevacizumab chemotherapy rate than those patients who were homozygous for the wild-type (T-allele), suggesting that the overexpression of IL-8 causes resistance to bevacizumab-based chemotherapy (53).

*GSTP1 Ile105Val polymorphism and cisplatin.* Findings of a Russian study showed the relationship between the GSTP1 Ile105Val polymorphism and cisplatin efficacy in ovarian cancer patients. These authors observed a better progression-free survival in patients with the homozygous genotype (Ile/Ile) as compared to patients with one or two Val alleles. On the other hand, overall survival did not present significant differences, although ovarian cancer patients carrying the Ile/Ile genotype exhibited a longer lifespan than those with the Ile/Val genotype (54).

*XPD Asp312Asn and Lys751Gln polymorphisms and carboplatin/paclitaxel.* Xeroderma pigmentosum groud D (XPD) is a member of the NER family and plays an important role in the repair of DNA damage caused by alkylating drugs, platinum analogues and radiation. On the other hand, protein levels were demonstrated to correlate with resistance to these agents in human tumor cell lines (55). Notably, Khrunin *et al* (54) studied the relationship between XPD Asp312Asn and Lys751Gln polymorphisms (both responsible for lower DNA repair capacity) and survival in ovarian cancer patients treated with carboplatin/paclitaxel. These authors found that patients carrying at least one variant allele (312 Asn or 751 Gln) had a significant reduction of death risk compared to patients with the Asp/Asp or Lys/Lys genotype (56).

*BRAF and chemoresistance in ovarian cancer.* Dysregulation of the Ras/Raf/MEK/ERK pathway plays a key role in the pathogenesis of a variety of human cancers. Mutations at membrane receptors, upstream signaling transducers, such as Ras and B-Raf, as well as in genes regulating Raf activity (e.g., PI3K, PTEN and Akt), promote constitutive ERK signaling, stimulate proliferation and survival, and provide essential tumor growth and maintenance functions. In ovarian cancer, we have observed that the V600E B-Raf mutation is associated with a poor outcome. To explore this possibility, functional experiments have also been performed by analyzing a number of ovarian cell lines transfected with mutated B-Raf (V600E). In agreement with our clinical observations, transfected clones showed a higher chemo-resistance, supporting the hypothesis that alteration of the Ras/Raf/MEK/ERK pathway is associated with resistance to chemotherapy. Our data therefore indicate that in ovarian cancer the Ras/Raf/MEK/ERK pathway may be a promising therapeutic target involved in the reversal of chemoresistance.

## 4. Conclusion

The majority of women present with advanced disease at diagnosis due to the insidious onset of this disease and the lack of effective early detection methods. Current treatments, consisting of debulking surgery and subsequent platinum-based chemotherapy, yield a response rate of over 80%, but almost all patients relapse or develop resistance to therapy and ultimately succumb to their cancer. This review reports the growing importance of a tailored therapy in cancer patients in whom the neoplastic disease is already devastating in itself.

Therefore, it is imperative to gain a better understanding of the molecular pathways involved in ovarian cancer progression. Thus, further studies should be conducted on all possible genetic variables, such as polymorphisms, mutations, leading to a change in the individual response to a certain chemotherapeutic agent in order to control/reverse these variables and develop more tailored therapies. The aim of this review was to show the manner in which some polymorphisms act in favor of certain drugs while others greatly enhance their toxicity.

A study of the 'genetic profile' of cancer patients is crucial to better design an effective therapy and reverse chemoresistance. Therefore, chemotherapy regimens may not be identical for all cancer patients, an essential point of consideration for anticancer therapy.

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