European Review for Medical and Pharmacological Sciences 2012; 16: 1211-1217

# Pharmacogenomics panel test for prevention toxicity in patient who receive Fluoropirimidine/ Oxaliplatin-based therapy

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**Abstract. – AIM: Both Fluoropirimidine and Oxaliplatin (FluOx) are the most common anticancer drugs used to treat lung, colorectal, ovarian, breast, head/neck, and genitourinary cancers. However, the efficacy of FluOx-based therapy is often compromised because of the severe risk of toxicity. Stratification of patients for multidrug response is a promising strategy for cancer treatment and personalized therapy.**

**METHODS: Here, we review the late findings on the most appropriate gene variants related to the toxicity in patients receiving FluOx chemotherapy. Several criteria were used to select a genotyping panel tests, including dihydropyrimidine dehydrogenase (DPYD), thymidylate synthase (TYMS), Glutathione S-transferase (GSTP1), and ATP-binding cassette, subfamily C member 2 (ABCC2).**

**RESULTS: Results of allelic status from 7 validated polymorphism assays, allow the stratification of the patients who are most likely to respond to FluOx treatments. Also, we will take in consideration the usefulness and costs of the methods used to detect these polymorphisms.**

**CONCLUSIONS: With these pharmacogenomics markers, the oncologists will have new means based on the genetic profile of the individual, to make treatment decisions for their patients in order to maximize benefits and minimize toxicity.**

*Key Words:*
Pharmacogenomics, Toxicity, Fluoropirimidine, Oxaliplatin, Genotyping methods.

## Introduction

Toxicity profile of FluOx is well documented and often this adverse reaction leads to the suspension of therapy and potentially compromises patient benefit. Primarily toxicities, include severe gastrointestinal and hematologic events linked to the fluoropirimidine administrations, and peripheral neuropathy linked to acute and cumulative doses of oxaliplatin[1]. Several strategies to prevent toxicity have been so far investigated with modest success. Some adverse drug response due to the administration of FluOx can be predicted through validated pharmacogenomics (PGx) markers[3]. Current evidences of pharmacogenomics, have reported different polymorphisms associated to genes involved with fluoropyrimidine[4] and oxaliplatin biotransformation[5].

This report reviews the late findings on the validated gene variants that are related to the toxic effect in patients receiving FluOx therapy. In order to prevent toxicity/resistance we suggest a validated genotyping panel of the most relevant pharmacogenomics markers, including dihydropyrimidine dehydrogenase (DPYD), thymidylate synthase (TYMS), glutathione S-transferase (GSTP1), and ATP-binding cassette, subfamily C member 2 (*ABCC2*).

So far, a multitude of methods have been applied to assess the mutational status of these genes, without defining a golden standard for the daily diagnostic routine. We will also take in consideration the usefulness and the costs of the methods used to detect these genetic alterations.

Furthermore, trials assessing the pharmacoeconomic impact of genotyping testing in FluOx-based therapy will likely provide answers for policy making in the internalization of PGx testing into clinical practice. The primary aim of a cost-effectiveness analysis is to provide accurate information for decision-makers to allocate resources to personalized care interventions. Overviews of cost-effectiveness studies on PGx technologies are now available[6,7]. A relevant example is the National Institute for Health and Clinical Excellence (NICE). NICE forms a Diagnostic Advisory com-

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mittee, which is willing to stimulate Pharma and Academic communities to produce a comprehensive set of data, including design and data sources in economic models of healthcare[8].

### Toxicity of Fluoropyrimidines

Several dose and schedules of 5-Fluorouracil (5-FU) and other fluoropirimidine (capecitabine, raltitrexed, tegafur-uracil, etc)[9,10] are currently used in clinical practice as bolus and infusional regimens (short-term and chrono-modulated).

The toxicity profile differs between bolus and infusional 5-FU. Bolus 5-FU mono-therapy has limited activity; only 10% of patients achieve an objective response. Higher response rates can be achieved with infusional regimens, but the survival impact is minimal[11]. While rates of gastrointestinal toxicity are similar, grade 3-4 neutropenia is more common with bolus 5-FU (31% bolus vs. 4% infusional), as is hand-foot skin syndrome (34% vs. 13%, respectively). Compared to bolus 5-FU alone, FU plus (LV) is associated with a twofold higher response rate (21% vs. 11%)[12].

### Toxicity of Oxaliplatin

Despite a modest activity as single agent, oxaliplatin exerts a significant activity in combination with other drugs (especially used in combination with fluoropirimidines)[13]. Treatment in conjunction with 5-FU/LV (FOLFOX) has shown improved survival in the adjuvant setting among Stage III patients compared to 5-FU/ LV and 5-FU/irinotecan treatments[14]. Importantly, the prevalence of low neurotoxicity associated with 5-FU, is increased with the addition of Oxaliplatin[15]. The *Food and Drug Administration* (FDA) noted that over 70% of the patients receiving oxaliplatin are affected by some degree of sensory neuropathy[16], including ototoxicity and dysphonic syndrome[17]. Notably, neurotoxicity, and not tumor progression, is often the cause of treatment discontinuation[18]. Despite these adverse events, FluOx association could have a key role for the treatment choice in a large setting of patients, including in the so called frail patients (i.e. elderly and HIV-positive patients)[19-21] for whom the efficacy and especially the toxicity profile are important aspects[20,22].

### Genotyping Panel Assay

Several criteria were used to select polymorphisms for pharmacogenomics panel tests (Table I):

**Table I.** Selection of validated pharmacogenomics markers influencing Fluoropirimidine/oxaliplatin-based therapy.

| Genetic variants (codons) | db SNPrs | Activities | Annotation | Ref |
|---|---|---|---|---|
| **DPYD** | | | | |
| IVS14+1G > A | rs3918290 | Mucosites severe Leukopenia | Heterozygous for A has been associated with low DPYD enzyme activity, while homozygous A is related to complete DPYD deficiency. | 52 |
| A1627G | rs1801159 | Severe nausea vomiting | The elimination constant (Ke) for 5-FU was significantly lower in patients homozygous for the G allele. | 53 |
| **TYMS** | | | | |
| 28bp tandem repeat | rs34743033 | Neutropenia grade 3-4 | Allele with the triple tandem repeat (3R) has increased TYMS expression compared with those with the double repeat (2R). Low TYMS levels are postulated to be markers of more favourable therapeutic response in advanced colorectal cancer | 54 |
| **GSTP1** | | | | |
| 313A > G (Ile105Val) | rs1695 | Neurotoxicity, Neutropenia | Patients homozygous for the G (Val) allele were associated to a lower toxicity and tumour progression compared to the homozygous for the A (Ile) allele | 32 55 |
| **ABCC2** | | | | |
| 3591A > G<br>-24C > T<br>3972C > T | rs 1885301<br>rs717620<br>rs3740066 | Grade 3 or higher neurotoxicity grade 3 to 4 neutropenia | ABCC2 polymorphisms taken together, are associated with increased risk of neurotoxicity. rs 717620 allele TT was also associated to a 5-fold increased risk of severe leukopenia | 3 |

1. Searching the most validated genetic variants known to influencing the Pharmacokinetics/ pharmacodynamics of fluoropirimidine and oxaliplatin (www.pharmagkb.org);
2. Reviewing the most recent studies upgrading in clinical research, in particular, trials including pharmacogenomics profile tests;
3. Issues evaluating the pharmacoeconomic impact of genotyping testing, likely providing answers for policy making in the incorporation of PGx markers into clinical practice.

### Selection of Candidate Polymorphisms and Reviews of the Most Recent Study Upgrading in Clinical Research

Low expression of DPYD enzyme has been associated with accumulation of 5-FU, thereby exposing patients to increased risk of severe or lethal toxicities, while high expression of DPYD has been associated with poor response to 5-FU. The frequency of low DPYD enzymatic activity has also been shown to vary significantly among different ethnic subpopulations[23]. The most known *DPYD Single Nucleotide Polymorphisms (*SNPs) associated with grade 3 and 4 toxicities are intronic variant IVS14 + 1 G > A (also named *DPYD*2A*), and mutation A1627G[24]. Important results have previously demonstrated that a homozygote *DPYD*2A* genotype has resulted in complete deficiency (high-risk patients) while the heterozygous *DPYD*2A* genotype has resulted in partial deficiency of DPYD enzyme[25]. Various genotyping methods to screen the known *DPYD* gene polymorphism have been developed, without defining better platforms for their use in the daily diagnostic routine. Current methodologies includes: conventional polymerase chain reaction (PCR) followed by sequencing, single-strand conformational polymorphism (SSCP)[26], pyrosequencing[27], fluorescent resonance energy transfer (FRET) probes[25,28].

Furthermore, a less pronounced genetic contribution of TYMS polymorphism has been demonstrated in a various large prospective study, in whom the TYMS 3R/3R genotype was found to increase the risk of toxicity 1.6 fold (rate of 43% of patients treated with 5-FU), compared with the TYMS 3R/2R genotype; whereas only 3% of patients who had the TYMS 3/3 genotype developed 3 or 4 grade of toxicity[29].

Polymorphism *GSTP1* Ile105Val (313A>G in exon 5, sometimes labelled GSTP1*B) has been associated with reduced enzyme activity and anticancer drug resistance, and toxicity[30]. The allele frequency of the Ile105Val polymorphism varies widely among populations[31]. However, in 166 colorectal cancer patients receiving oxaliplatin and 5-FU, the GSTP1 Ile105Val heterozygous allele was associated with increased risk of neutropenia[32] and neurotoxicity[33], while patients homozygous to Val/Val tended to a lower risk of neurotoxicity and tumour progression compared to Ile/Ile phenotypes[34]. This SNP in position 313 of GSTP1 gene could be detected by allelic discrimination methods such as germline mutation[35,36].

For ABCC2, three genetic polymorphisms (rs1885301, rs717620 and rs3740066) have been associated with grade 2-3 neurological toxicity and one of them have been also related to severe neutropenia. The functional effect of these variants is unknown. In particular, ABCC2 rs717620, has been previously associated to decreased protein expression *in vitro*[37]. Also, it has been associated with a 13-fold increased risk of grade 2-3 neurological toxicity and to a 5-fold increased risk of severe leukopenia. In addition, ABCC2-rs717620 and rs3740066 have had a combined effect in increasing platinum-related toxicity in lung cancer[38] and colon cancer patients[3].

Additional candidate gene variants influencing oxaliplatin-based chemotherapy have been well documented[39,40]. They included "ATP-binding cassette 1" (ABCC1), X-ray repair complementing defective repair in Chinese hamster cells 3 (XCCR3) and "DNA repair cross-complementation group 1 (ERCC1).

Overexpression of the ABCC1 protein has been related to resistance to 5-fluorouracil *in vitro*. This could be due to the ability of ABCC1 to extrude folates and thus depleting their intra-cellular availability for the activity of 5-fluorouracil. This might explain, in part, the effect of ABCC1 rs35587 on both neutropenia and neurological toxicity, suggesting that ABCC1-rs35587 might increase the function or expression of the ABCC1 transporter. More confirmatory studies (both at the clinical and molecular level) should be conducted to confirm the clinical associations. XRCC3 is a DNA repair protein that is part of the double strand break repair machinery. Its reduced activity is associated with significantly higher levels of bulky DNA adducts. Polymorphism XRCC3 rs1799794 is associated with severe non-hematological toxicity. DNA repair is an important mechanism for resistance to platinum-based therapy. If the cell is able to repair the DNA being attacked by the platinum agent, then the agent

will be unsuccessful in inducing apoptosis. Park et al have described an association between the *ERCC1* codon 118 polymorphism and clinical output in colorectal cancer patients treated with platinum-based chemotherapy. This genotype could be a useful predictor of clinical outcome for colorectal cancer[41], ovarian cancer[42], and new issues like stress and fatigue in cancer patients[43].

However, the fine molecular function of these SNPs remains unclear, and controversial. Furthermore, there are many genes whose effects on neurotoxicity to FluOx have yet to be studied. In addition, emerging new evidences in nutrigenomic field suggesting an accurate evaluation between diet during therapy[44].

### Early Outline Evaluation of Genotyping Costs

Few studies have addressed the cost-effectiveness of pharmacogenomics testing implication in clinical practice[7]. For example van den Akker et al, included thiopurine S-methyltransferase (TPMT*)* genotyping prior to 6-mercaptopurine treatment in paediatric Acute Lymphoblastic Leukaemia (ALL); the mean calculated cost from 4 European countries was € 2100,00 per life-year considering low myelosuppression-related hospitalization; the cost for genotyping of TMPT mutation averaged around €150,00[45]. In other study, early outline of genotyping cost for "home brew" tests (based on Fluorescent allele discrimination assay), averaged about € 20,00 per SNP[46]. The technology platforms needed for detecting the described SNPs are able to address allelic discriminations (detection of DNA mutant between the two alleles). Rational selection of the best method to detect them is dependent from the specifics aims of different laboratories[47].

Furthermore, the major issues to consider for the clinical laboratories (who are responsible for providing PGx services), are: (1) the availability of FDA-cleared tests; (2) the current absence of public reimbursement; (3) the need for genotyping accuracy; and iv) the need to find clinical expertise to interpret laboratory data results[48].

### Conclusions and Future Outlook

Genetic variants and predictive markers allow physicians to improve the efficacy of cancer therapy. The clinical utility of the described polymorphisms involved in FluOx based-therapy is in part limited by: (1) less wide diffusion of genotyping methods in routine clinical diagnostics; (2) the evidence that PGx testing improves clinical outcomes is still an open question; and (3) the cost-effectiveness of the testing is unknown.

The usefulness of the described genetic variants for clinical practice will depend on their improving diagnostic prediction or fostering changes in prevention or treatment strategies[49]. Particularly, the molecular testing for mutation in DPYD, TYMS, GSTP1and ABCC2 genes, could help the oncologist in stratifying patients who are most likely to respond to FluOx. In order to assess a basic profile of good/bad responding patients, a panel test of 7 SNPs is proposed (Table II). Despite our efforts to make an precise and comprehensive list of polymorphisms, the limitation of our proposed tests need to be addressed. This issues cause same bias in our estimation but conclusion criteria could help the clinicians to stratify patients called FluOx1 (homozygous) profile, showing a favourable biotransformation machinery for fluoropyrimidine and lower neurotoxicity for oxaliplatin because of a protective genetic profile (GSTP1 Val/Val and ABCC2 phenotype). While FluOx3 pharmacogenomic profile (homozygous), is predisposed to very high risk of mucosites and neutropenia due to 5-FU administrations, and neurotoxicity due to oxaliplatin, FluOx2 (heterozygous) have variable effects, making it unhelpful to stratify a good/bad responder.

Over the next few years, the emergence of molecular resistance/toxicity in the new therapies as results of the genomic alterations in cancer will drive diagnostics companies to develop new tests able to produce results for tailoring patient's treatment. Hopefully, the future implementation of the methods for genotyping the variants influencing fluoropyrimidine/oxaliplatin-based therapy will result in personalized treatments. Therefore, it is fundamental that pharmaceutical and biotechnology companies join together, in order to develop an extensive study on the standardization method to validated tests suitable for routine diagnostics in pharmacogenomics of FluOx.

In summary, with the increasing number of novel PGx markers being identified and validated, oncologists will have new means based on the individual genetic profile to make treatment decisions, as well as correlation between nutrition and cancer[50,51], and may eventually be personalized on the patients in order to minimize toxicity.

Based on these purpose, the clinician and the lab manager may join together to evaluate advantages and limitation, in terms of costs and applicability, of the most appropriate methods to setting molecular diagnostics of oxaliplatin pharmacogenomics tests.

**Table II.** Basic profile of good/bad patients responding to FluOx treatment.

| PGx profile | DPYD | | TYMS | GSTP1 | ABCC2 | | | Effects |
|---|---|---|---|---|---|---|---|---|
| | rs3918290 G > A | rs1801159 A > G | rs34743033 28 tandem repeat | rs1695 A > G | rs1885301 G > A | rs717620 C > T | rs374006 C > T | |
| FluOx1 homozygous protectives | GG | AA | 2R/2R | GG (Val/Val) | GG | CC | CC | Favourable biotransformation machinery for fluoropyrimidine administration. Lower neurotoxicity and tumour progression for oxaliplatin, protected by GSTP1 (Val/Val) and ABCC2 phenotype |
| FluOx2 hetrozygous | GA | AG | 2R/3R | AG (Ile/Val) | GA | CT | CT | Divergent effects |
| FluOx3 homozygous toxicity risk | AA | GG | 3R/3R | AA (Ile) | AA | TT | TT | Very high risk of mucosites and neutropenia due to 5-FU administrations. Acute and cumulative europathy |


## Acknowledgements

The Authors are grateful to Dr. O. Barletta from the "Italian Association of Pharmacogenomics and Molecular Diagnostics" for the invaluable bibliography research, and Mrs Paola Favetta for her expert assistant in the preparation and correction of the manuscript.


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