

Research Paper

# ERCCI, XRCCI and GSTPI Single Nucleotide Polymorphisms and Survival of Patients with Colon Cancer Receiving Oxaliplatin-Based Adjuvant Chemotherapy

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## Abstract

**Background:** While single nucleotide polymorphisms (SNP) in genes involved in DNA repair or drug metabolism have been shown to influence survival of metastatic colon cancer patients treated with FOLFOX, data on adjuvant setting are scarce.

**Methods:** This study evaluated the correlation between disease-free survival (DFS) of 210 unselected stage III colon cancer patients receiving FOLFOX chemotherapy, and ERCCI-118 (rs11615, c.354T>C), XRCCI-399 (rs25487, c.1196G>A) and GSTPI-105 (rs1695, c.313A>G) polymorphisms. SNP were determined on tumor DNA using a PCR-based RFLP technique.

**Results:** In univariate analysis, a trend towards longer DFS was observed for ERCCI (C/T + T/T) versus (C/C) (HR=2.29; p=0.06), and XRCCI (A/A) versus (G/G + G/A) (HR=1.61; p=0.16), but not for GSTPI genotypes; a statistically significant p value was obtained when combining ERCCI and XRCCI favorable genotypes (0 versus ≥ 1 favorable genotypes, HR=2.42; p=0.02). After adjustment on tumor stage, lymph node ratio and differentiation grade, multivariate analysis showed that combining ERCCI and XRCCI genotypes gave a p value slightly above the threshold for statistical significance (HR=2.03; p=0.06), which was lower than for tumor stage, lymph node ratio or differentiation grade.

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**Conclusion:** The association of *ERCC1* and *XRCC1* polymorphisms may influence the prognosis of stage III colon cancer patients treated with FOLFOX adjuvant chemotherapy. Yet, these findings need to be confirmed in independent prospective studies.

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Key words: colon cancer, adjuvant FOLFOX, polymorphism, *ERCC1*, *XRCC1*, *GSTP1*.

## Introduction

Oxaliplatin is a platinum-based chemotherapeutic agent that carries 1,2-diamino-cyclohexane ring. This chemotherapeutic agent has shown *in vitro* and *in vivo* antitumor activities in colorectal cancer (CRC) (1). The administration of oxaliplatin in combination with 5-fluorouracil (5FU) (FOLFOX regimen) significantly improved progression-free survival and response rate for patients with metastatic CRC (2). More recently, the addition of oxaliplatin to 5FU was demonstrated to improve the adjuvant treatment of stage III colon cancer by reducing the risk of recurrence and increasing overall survival (3, 4). However, oxaliplatin failed to eradicate micrometastatic disease in approximately one third of stage III colon cancer patients. Oxaliplatin exerts its action by forming DNA-platinum mono-adducts, primarily with guanines, which inhibits DNA replication and transcription and induces apoptosis (1). Unlike cisplatin, oxaliplatin-induced adducts are apparently not recognized by the mismatch repair (MMR) system, but are predominantly repaired by the nucleotide excision repair (NER) and base excision repair (BER) pathways (5, 6). An enhanced DNA repair efficiency or a decreased accumulation of the cytotoxic agent may contribute to resistance to platinum drugs.

Several studies reported that single nucleotide polymorphisms (SNP) in genes involved in DNA repair, as *Excision Repair Cross-Complementing group 1* (*ERCC1*) (7) and *X-Ray Repair Cross-Complementing group 1* (*XRCC1*) (8, 9), or involved in drug metabolism, as *glutathione S-transferases P1* (*GSTP1*) (10-12), may predict the clinical outcome for patients receiving oxaliplatin-based chemotherapy for metastatic CRC treatment. *ERCC1* forms a heterodimeric complex with Xeroderma Pigmentosum Group F, that has an important role in the incision process of NER (13). *XRCC1* interacts with many proteins involved in single-strand break repair and in BER, participating in some of the rate-limiting reactions (14). Finally, glutathione S-transferases are implicated in drug detoxification through the conjugation of glutathione to electrophilic xenobiotics. The *GSTP1* isoenzyme that participates in the detoxification of platinum drugs is highly expressed in human CRC tissues and could possibly influence resistance to platinum-based chemotherapy (15). To our knowledge, the impact of polymorphisms in these genes on the survival of pa-

tients treated with oxaliplatin-based adjuvant chemotherapy has been scarcely explored yet. Thus, in the current study, we have examined the influence of *ERCC1* (Asn118Asn), *XRCC1* (Arg399Gln) and *GSTP1* (Ile105Val) polymorphisms, separately or in combination, on disease-free survival (DFS) of patients with stage III colon cancer receiving FOLFOX adjuvant chemotherapy.

## Methods

### Study population

We retrospectively collected data of all consecutive patients treated with FOLFOX adjuvant chemotherapy after surgical resection of a stage III colon cancer between 06/2003 and 12/2007 in 3 University centers (Ambroise Paré, Georges Pompidou, and Saint-Antoine hospitals) and 1 private hospital (Mutualiste Montsouris Institute). Patients were eligible for this retrospective multicenter study if they met the following criteria: older than 18 years, histologically proven colon cancer, at least 1 pathologically involved lymph node, R0 resection, available tumor specimen in the pathology departments, adjuvant FOLFOX chemotherapy starting within 8 weeks after surgery and no history of sensitive peripheral neuropathy. Fourteen stage III colon cancer patients enrolled in the MOSAIC trial with available tumor and treated in these centers with FOLFOX between 11/1998 and 10/2000 were also included. Patients with rectal cancer or treated with abdominopelvic radiotherapy have been excluded. This retrospective pharmacogenetic study has been approved by the ethics committee of the Pitié-Salpêtrière Hospital (Paris, France).

### Treatment and follow-up

The chemotherapy cycle consisted of a 2-hour infusion of 85 mg of oxaliplatin per square meter on day 1, in addition to the standard LV5FU2 regimen (FOLFOX4) or the simplified LV5FU2 regimen (modified FOLFOX6) (3, 16). Patients were scheduled to receive one cycle of chemotherapy every two weeks for 6 months. After treatment, a control visit was performed every 3 to 6 months for 3 years, then every 6 months for 2 years, then annually, to assess potential disease recurrence. Physical examination, serum carcinoembryonic antigen assay, and abdominal plus thoracic imaging were performed at each visit.

## DNA extraction and genotyping

Genomic DNA was extracted from 20- $\mu$ m-thick tissue sections of frozen tumors (111 patients) or from 7- $\mu$ m tissue sections of paraffin-embedded tumors when frozen samples were not available (99 patients). Areas of tumor tissues were previously delineated by microscopic examination of a reference slide stained with haematoxylin and eosin. Extraction was done using the NucleoSpin Tissue Kit (Macherey-Nagel) for frozen samples and NucleoSpin DNA FFPE Kit (Macherey-Nagel) or KAPA Express DNA Extraction Kit (Kapa Biosystems) for paraffin-embedded tissues, according to the manufacturers' instructions.

SNP genotyping analyses were done on amplified tumor DNA followed by digestion with the corresponding restriction enzyme: BsrD1 (New England Biolabs) for *ERCC1* (rs11615, c.354T>C, p.Asn118Asn) (17), HpaII (Fermentas) for *XRCC1* (rs25487, c.1196G>A, p.Arg399Gln) and Alw26I (Fermentas) for *GSTP1* (rs1695, c.313A>G, p.Ile105Val) polymorphisms. For *ERCC1-118*, a 239 bp fragment was amplified using 5'-GTGCGAGGAGGCAGGAGGTGTGGG-3' and 5'-GAGCTCACCTGAGG AACAGG-3', as primers. BsrD1 cleaves the *ERCC1* T

allele into two fragments of 155 and 84 bp. For *XRCC1-399*, a 242 bp was amplified using 5'-CCCCAAGTACAGCCAGGTCC-3' and 5'-CCGCTCCTCTCAGTAGTCTG-3' as primers. The *XRCC1* G allele is cleaved by HpaII into two fragments of 149 and 93 bp. For *GSTP1-105*, a 176 bp was amplified using 5'-ACCCCAGGGCTCTATGGGAAG-3' and 5'-TGAGGGCACAAGAAGCCCCT-3' as primers. Alw26I cleaves the G allele into two fragments of 89 and 87 bp. After digestion, the PCR fragments were electrophoresed in a 2% agarose gel in the presence of GelRed™ (1/10000, Biotium Inc) and visualized using a G: BOX gel imaging system (Syngene, Ozyme).

## Statistical analysis

Differences in distributions between the clinicopathological variables listed in Table 1 were assessed with the Chi2 or the Fisher's exact test, as appropriate. The cut-off value of lymph node ratio (LNR) between metastatic and examined lymph nodes corresponded to the median. The number of FOLFOX cycles according to the 3 possible genotypes of each gene was compared using a non-parametric Kruskal-Wallis test.

**Table 1.** Clinicopathological characteristics of patients with stage III colon cancer according to *ERCC1-118*, *XRCC1-399* and *GSTP1-105* polymorphisms.

| Characteristics                        | All patients<br>(n = 210) | <i>ERCC1-118</i> (rs11615)<br>(n = 202) |             |             | p    | <i>XRCC1-399</i> (rs25487)<br>(n = 207) |                     |             | p    | <i>GSTP1-105</i> (rs1695)<br>(n = 209) |                     |             | p    |
|--|---------------------------|---|-------------|-------------|------|---|---------------------|-------------|------|--|---------------------|-------------|------|
|  | No. of patients (%)       | No. of patients (%)                     |             |             |      | No. of patients (%)                     | No. of patients (%) |             |      | No. of patients (%)                    | No. of patients (%) |             |      |
|  |                           | C/C                                     | C/T         | T/T         |      |   | G/G                 | G/A         |      |  | A/A                 | A/A         |      |
| <b>Age</b>                             |                           |   |             |             |      |   |                     |             |      |  |                     |             |      |
| Median                                 | 65.9                      | 64.3                                    | 64.4        | 66.3        |      | 65.9                                    | 64.9                | 64.5        |      | 66.8                                   | 63.4                | 61.7        |      |
| Range                                  | 30.5 - 82.8               | 30.5 - 82.8                             | 31.1 - 81.0 | 37.8 - 82.8 |      | 30.8 - 82.1                             | 37.8 - 82.8         | 30.5 - 77.4 |      | 30.8 - 81.0                            | 30.5 - 82.8         | 40.9 - 81.0 |      |
| < 65 yr                                | 101 (48.1)                | 26 (53.1)                               | 44 (50.0)   | 29 (44.6)   | 0.65 | 44 (46.8)                               | 40 (50.0)           | 16 (48.5)   | 0.92 | 39 (40.2)                              | 50 (54.3)           | 12 (60.0)   | 0.08 |
| <b>Sex</b>                             |                           |   |             |             |      |   |                     |             |      |  |                     |             |      |
| Female                                 | 99 (47.1)                 | 23 (46.9)                               | 40 (45.4)   | 31 (47.7)   | 0.96 | 39 (41.5)                               | 39 (48.8)           | 19 (57.6)   | 0.26 | 42 (43.3)                              | 48 (52.2)           | 9 (45.0)    | 0.46 |
| Male                                   | 111 (52.9)                | 26 (53.1)                               | 48 (54.6)   | 34 (52.3)   |      | 55 (58.5)                               | 41 (51.2)           | 14 (42.4)   |      | 55 (56.7)                              | 44 (47.8)           | 11 (55.0)   |      |
| <b>Tumor location</b>                  |                           |   |             |             |      |   |                     |             |      |  |                     |             |      |
| Proximal                               | 67 (31.9)                 | 16 (32.7)                               | 28 (31.8)   | 22 (33.8)   | 0.97 | 31 (33.0)                               | 27 (33.8)           | 9 (27.3)    | 0.79 | 27 (27.8)                              | 34 (37.0)           | 6 (30.0)    | 0.40 |
| Distal                                 | 143 (68.1)                | 33 (67.3)                               | 60 (68.2)   | 43 (66.2)   |      | 63 (67.0)                               | 53 (66.2)           | 24 (72.7)   |      | 70 (72.2)                              | 58 (63.0)           | 14 (70.0)   |      |
| <b>Differentiation grade</b>           |                           |   |             |             |      |   |                     |             |      |  |                     |             |      |
| Well / moderate                        | 182 (86.7)                | 45 (91.8)                               | 76 (86.4)   | 55 (84.6)   | 0.50 | 82 (87.2)                               | 69 (86.2)           | 29 (87.9)   | 1.00 | 82 (84.5)                              | 81 (88.0)           | 18 (90.0)   | 0.76 |
| Poor                                   | 28 (13.3)                 | 4 (8.2)                                 | 12 (13.6)   | 10 (15.4)   |      | 12 (12.8)                               | 11 (13.8)           | 4 (12.1)    |      | 15 (15.5)                              | 11 (12.0)           | 2 (10.0)    |      |
| <b>Bowel perforation / obstruction</b> | 16 (7.6)                  | 6 (12.2)                                | 6 (6.8)     | 2 (3.1)     | 0.16 | 9 (9.6)                                 | 5 (6.3)             | 2 (6.1)     | 0.73 | 8 (8.2)                                | 7 (7.6)             | 0 (0.0)     | 0.58 |
| <b>Stage</b>                           |                           |   |             |             |      |   |                     |             |      |  |                     |             |      |
| III A (T1-T2, N1)                      | 23 (11.0)                 | 9 (18.4)                                | 6 (6.8)     | 7 (10.8)    | 0.07 | 14 (14.9)                               | 5 (6.3)             | 4 (12.1)    | 0.37 | 9 (9.3)                                | 11 (12.0)           | 3 (15.0)    | 0.90 |
| III B (T3-T4, N1)                      | 108 (51.4)                | 29 (59.2)                               | 43 (48.9)   | 33 (50.8)   |      | 46 (48.9)                               | 46 (57.5)           | 15 (45.5)   |      | 51 (52.6)                              | 47 (51.0)           | 9 (45.0)    |      |
| III C (Tx, N2)                         | 79 (37.6)                 | 11 (22.4)                               | 39 (44.3)   | 25 (38.4)   |      | 34 (36.2)                               | 29 (36.2)           | 14 (42.4)   |      | 37 (38.1)                              | 34 (37.0)           | 8 (40.0)    |      |
| <b>Lymph node ratio</b>                |                           |   |             |             |      |   |                     |             |      |  |                     |             |      |
| < 0.100                                | 102 (48.6)                | 30 (61.2)                               | 43 (48.9)   | 29 (44.6)   | 0.20 | 44 (46.8)                               | 43 (53.8)           | 15 (45.5)   | 0.59 | 50 (51.5)                              | 41 (44.6)           | 11 (55.0)   | 0.53 |
| $\geq$ 0.100                           | 108 (51.4)                | 19 (38.8)                               | 45 (51.1)   | 36 (55.4)   |      | 50 (53.2)                               | 37 (46.2)           | 18 (54.5)   |      | 47 (48.5)                              | 51 (55.4)           | 9 (45.0)    |      |
| <b>MMR status</b>                      |                           |   |             |             |      |   |                     |             |      |  |                     |             |      |
| dMMR                                   | 17 (8.1)                  | 6 (12.2)                                | 3 (3.4)     | 7 (10.8)    | 0.09 | 9 (9.6)                                 | 7 (8.8)             | 1 (3.0)     | 0.59 | 5 (5.2)                                | 11 (12.0)           | 1 (5.0)     | 0.21 |
| pMMR                                   | 193 (91.9)                | 43 (87.8)                               | 85 (96.6)   | 58 (89.2)   |      | 85 (90.4)                               | 73 (91.2)           | 32 (97.0)   |      | 92 (94.8)                              | 81 (88.0)           | 19 (95.0)   |      |
| <b>No. of FOLFOX cycles</b>            |                           |   |             |             |      |   |                     |             |      |  |                     |             |      |
| Median                                 | 11                        | 11                                      | 10          | 11          | 0.90 | 12                                      | 10                  | 11          | 0.09 | 11                                     | 10                  | 12          | 0.40 |

Abbreviations: ERCC1, Excision repair cross-complementing group 1; XRCC1, X-ray repair cross-complementing group 1; GSTP1, glutathione-S-transferase P1; dMMR, defective DNA Mismatch Repair; pMMR, proficient DNA Mismatch Repair. The cut-off value of LNR between metastatic and examined lymph nodes corresponded to the median. The p values have been determined using the Chi<sup>2</sup> test or Fisher's exact test, as appropriate. The number of FOLFOX cycles according to the 3 possible genotypes of each gene was compared using a non-parametric Kruskal-Wallis test.

Unknown data: *ERCC1* polymorphism for eight patients; *XRCC1* polymorphism for three patients; *GSTP1* polymorphism for one patient.

The primary endpoint was DFS, defined as the time between the date of surgery and the first event (local or distant disease recurrence or death from any cause, whichever occurred first). Patients who were alive and relapse-free at the last contact were censored at the last follow-up date.

Univariate and multivariate Cox proportional hazard regression models were used to estimate the hazard ratio (HR) and 95% confidence interval (CI). The following variables were examined in univariate analyses for their relation with DFS: age, sex, tumor location, differentiation grade, bowel perforation or obstruction, tumor stage, LNR, MMR status and polymorphisms in *ERCC1-118*, *XRCC1-399* and *GSTP1-105*, alone or in combination for *ERCC1* and *XRCC1*. Yet, the *ERCC1* and *XRCC1* combining variable was included in the multivariate model in order to take into account the significant interaction ( $p < 0.05$ ) found between *ERCC1-118* and *XRCC1-399* polymorphisms. MMR status was defined by immunohistochemistry as previously described (18). All variables potentially associated with DFS and having a univariate  $p$  value  $\leq 0.10$  were included in multivariate analyses. The multivariate Cox model was constructed according to the "one variable for 10 events" rule. The discriminatory capacity was tested using the Harrell's concordance index. Harrell's C statistic was used to estimate the proportion of correct predictions. Harrell's C index ranges from 0.5 (no discrimination) to 1 (perfect discrimination).  $P$  values  $< 0.05$  were considered as statistically significant. All analyses were performed using Stata V11 software (StataCorp LP, College Station, TX). The cutoff date for this analysis was December 2010.

## Results

### Patient characteristics

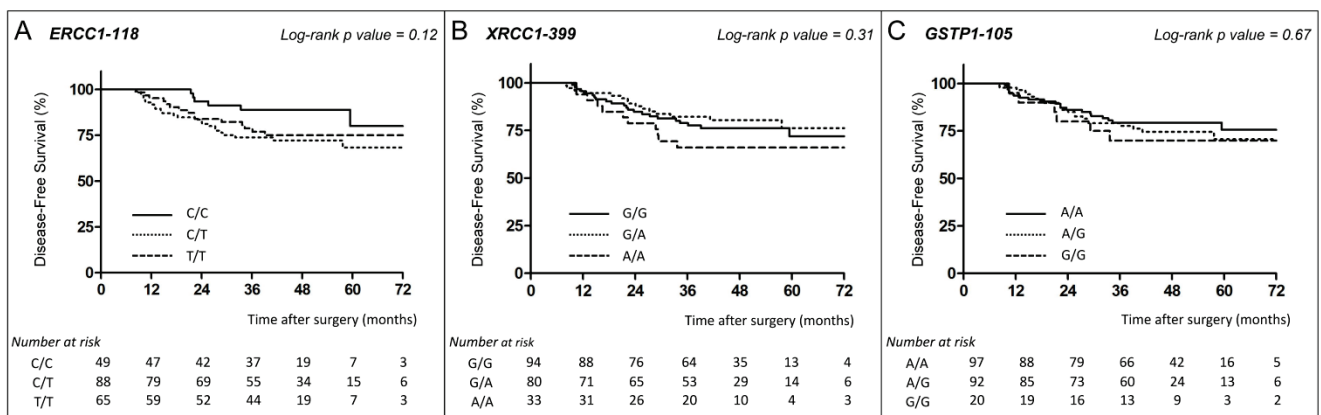
A total of 210 patients were included in this

study. The median number of FOLFOX cycles received was 10 for the whole population and there was no statistically significant difference in the median number of cycles received by patients according to the genotypes of any of the 3 genes analyzed (Table 1). The median follow-up was 47.3 months (95% CI, 42.8 – 48.7). At the end of follow-up, 48 patients (22.8%) had relapsed or died in the whole study population. The 3-year DFS rate was 78.3% (95% CI, 71.8 – 83.4). The clinicopathological characteristics of patients according to *ERCC1-118*, *XRCC1-399* and *GSTP1-105* polymorphisms are summarized in Table 1. The *ERCC1-118*, *XRCC1-399* and *GSTP1-105* genotypes were successfully determined for 202, 207 and 209 patients, respectively. There was no significant correlation between clinicopathological characteristics and *ERCC1-118*, *XRCC1-399* or *GSTP1-105* polymorphisms (Table 1).

### Association between the *ERCC1*, *XRCC1* and *GSTP1* polymorphisms and DFS

***ERCC1-118*.** Among the 202 patients whose *ERCC1* genotype was defined, 45 (22.3%) had relapsed or died (6 with the C/C genotype, 24 with C/T, and 15 with T/T). The 3-year DFS rate was 88.9% (95% CI, 75.3 – 95.2), 73.8% (95% CI, 63.0 – 81.9), and 78.8% (95% CI, 66.3 – 87.1) in patients with homozygous C/C, heterozygous C/T and homozygous T/T genotypes, respectively (log-rank test,  $p=0.12$ ) (Fig.1A).

***XRCC1-399*.** Among the 207 patients whose *XRCC1* genotype was defined, 48 (23.2%) had relapsed or died (22 with the G/G genotype, 15 with G/A, and 11 with A/A). The 3-year DFS rate was 78.9% (95% CI, 68.9 – 86.0), 82.2% (95% CI, 71.3 – 89.3), and 66.0% (95% CI, 47.0 – 79.6) in patients with G/G, G/A and A/A genotypes, respectively (log-rank test,  $p=0.31$ ) (Fig. 1B).



**Figure 1.** DFS of stage III colon cancer patients receiving FOLFOX according to *ERCC1*, *XRCC1* and *GSTP1* polymorphisms. Disease-free survival curves of stage III colon cancer patients receiving FOLFOX adjuvant chemotherapy according to *ERCC1-118* (rs11615, c.354T>C) (A), *XRCC1-399* (rs25487, c.1196G>A) (B) and *GSTP1-105* (rs1695, c.313A>G) (C) polymorphisms. Survival curves were estimated with the Kaplan–Meier method and compared using the log-rank test.

**GSTP1-105.** For the 209 patients whose *GSTP1* genotype was defined, 48 (23%) had relapsed or died (20 with the A/A genotype, 22 with A/G, and 6 with G/G). The 3-year DFS rate was 79.4% (95% CI, 69.5 – 86.3), 79.0% (95% CI, 68.8 – 86.3), and 70.0% (95% CI, 45.1 – 85.3) in patients with A/A, A/G and G/G genotypes, respectively (log-rank test,  $p=0.67$ ) (Fig. 1C).

**Table 2.** Univariate analyses between covariates of interest and disease-free survival.

|                                       | n   | Events | HR             | IC 95%         | p    | Harrell's |
|---------------------------------------|-----|--------|----------------|----------------|------|-----------|
| <b>Age</b>                            |     |        |                |                |      | 0.51      |
| < 65 years                            | 101 | 22     | 1 <sup>R</sup> |                |      |           |
| ≥ 65 years                            | 109 | 26     | 1.17           | [0.67 - 2.07]  | 0.58 |           |
| <b>Sex</b>                            |     |        |                |                |      | 0.53      |
| Female                                | 99  | 21     | 1 <sup>R</sup> |                |      |           |
| Male                                  | 111 | 27     | 1.25           | [0.7 - 2.21]   | 0.45 |           |
| <b>Tumor location</b>                 |     |        |                |                |      | 0.52      |
| Distal                                | 143 | 32     | 1 <sup>R</sup> |                |      |           |
| Proximal                              | 67  | 16     | 1.13           | [0.62 - 2.05]  | 0.70 |           |
| <b>Differentiation grade</b>          |     |        |                |                |      | 0.55      |
| Well / Moderate                       | 182 | 39     | 1 <sup>R</sup> |                |      |           |
| Poor                                  | 28  | 9      | 1.87           | [0.91 - 3.87]  | 0.09 |           |
| <b>Bowel perforation/ obstruction</b> |     |        |                |                |      | 0.52      |
| Absent                                | 194 | 44     | 1 <sup>R</sup> |                |      |           |
| Present                               | 16  | 4      | 1.47           | [0.53 - 4.1]   | 0.46 |           |
| <b>Stage</b>                          |     |        |                |                |      | 0.58      |
| III A / III B (Tx, N1)                | 131 | 23     | 1 <sup>R</sup> |                |      |           |
| III C (Tx, N2)                        | 79  | 25     | 2.03           | [1.15 - 3.57]  | 0.01 |           |
| <b>Lymph node ratio</b>               |     |        |                |                |      | 0.60      |
| < 0.100                               | 102 | 16     | 1 <sup>R</sup> |                |      |           |
| ≥ 0.100                               | 108 | 32     | 2.21           | [1.21 - 4.03]  | 0.01 |           |
| <b>MMR status</b>                     |     |        |                |                |      | 0.53      |
| dMMR                                  | 17  | 1      | 1 <sup>R</sup> |                |      |           |
| pMMR                                  | 193 | 47     | 4.11           | [0.57 - 29.76] | 0.16 |           |
| <b>ERCC1-118 (rs11615)</b>            |     |        |                |                |      | 0.59      |
| C/C                                   | 49  | 6      | 1 <sup>R</sup> |                |      |           |
| C/T                                   | 88  | 24     | 2.48           | [1.02 - 6.08]  | 0.14 |           |
| T/T                                   | 65  | 15     | 2.04           | [0.79 - 5.25]  |      |           |
| <b>XRCC1-399 (rs25487)</b>            |     |        |                |                |      | 0.56      |
| G/G                                   | 94  | 22     | 1 <sup>R</sup> |                |      |           |
| G/A                                   | 80  | 15     | 0.81           | [0.42 - 1.57]  | 0.33 |           |
| A/A                                   | 33  | 11     | 1.47           | [0.71 - 3.04]  |      |           |
| <b>GSTP1-105 (rs1695)</b>             |     |        |                |                |      | 0.53      |
| A/A                                   | 97  | 20     | 1 <sup>R</sup> |                |      |           |
| A/G                                   | 92  | 22     | 1.21           | [0.66 - 2.22]  | 0.67 |           |
| G/G                                   | 20  | 6      | 1.46           | [0.59 - 3.64]  |      |           |

Abbreviations: R, reference; ERCC1, Excision repair cross-complementing group 1; XRCC1, X-ray repair cross-complementing group 1; GSTP1, glutathione-S-transferase P1; dMMR, defective DNA Mismatch Repair; pMMR, proficient DNA Mismatch Repair. The cut-off value of LNR between metastatic and examined lymph nodes corresponded to the median.

**Univariate and multivariate analyses of DFS**

In univariate analysis, tumor stage (stage IIIC vs IIIA/B: HR=2.03;  $p=0.01$ ), LNR ( $\geq 0.100$  vs  $< 0.100$ : HR=2.21;  $p=0.01$ ) and differentiation grade (poor vs well/moderate: HR=1.87;  $p=0.09$ ) were potentially associated with improved DFS (threshold, 10 %) (Table 2). The genotype distribution of *ERCC1-118*, *XRCC1-399* and *GSTP1-105* polymorphisms did not show any significant correlation with DFS (Table 2).

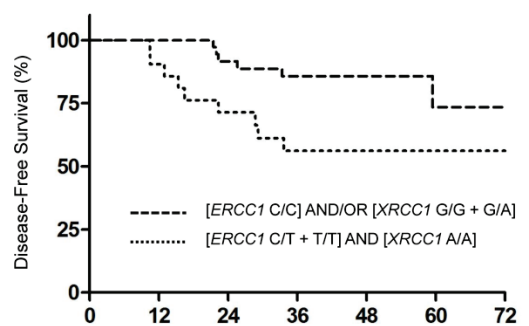
When grouping the heterozygous subjects with the homozygous genotype whose behavior was closest, we observed a trend toward improvement in DFS for *ERCC1-118* (C/T + T/T vs C/C: HR=2.29;  $p=0.06$ ), and for *XRCC1-399* (A/A vs G/G + G/A: HR=1.61;  $p=0.16$ ) (Table 3). With regard to *GSTP1-105* polymorphism, no tendency could be ascribed for the heterozygous group; yet, pooling the A/G patients with the G/G or with the A/A group did not unravel any influence of *GSTP1-105* polymorphism on DFS (data not shown).

*ERCC1-118* [C/C] and *XRCC1-399* [G/G + G/A] were identified as favorable genotypes, whereas *ERCC1-118* [C/T + T/T] and *XRCC1-399* [A/A] represented unfavorable genotypes. In univariate analysis, patients with at least one favorable genotype had a significantly longer DFS compared to patients with no favorable genotype (0 vs  $\geq 1$  favorable genotype: HR=2.42;  $p=0.02$ ) (Table 3; Fig. 2).

Multivariate analyses included all variables associated with DFS and having a  $p$  value  $\leq 0.10$  in the univariate analysis, i.e. tumor stage, LNR, differentiation grade, and the combination of *ERCC1-118* and *XRCC1-399* polymorphisms because of the significant interaction between *ERCC1-118* and *XRCC1-399* ( $p < 0.05$ ). Multivariate analysis showed that none of these four variables was significantly associated with DFS (Table 4). Nevertheless, the combination of *ERCC1-118* and *XRCC1-399* polymorphisms was the variable associated with the lowest  $p$  value slightly above the threshold for statistical significance (0 vs  $\geq 1$  favorable genotype: HR=2.03;  $p=0.06$ ) (Table 4).

**ERCC1 / XRCC1**

Log-rank  $p$  value = 0.015



| Number at risk         | 0   | 12  | 24  | 36  | 48 | 60 | 72 |
|------------------------|-----|-----|-----|-----|----|----|----|
| ≥ 1 favorable genotype | 179 | 164 | 146 | 123 | 68 | 26 | 10 |
| 0 favorable genotype   | 21  | 19  | 15  | 11  | 3  | 2  | 2  |

**Figure 2.** DFS of stage III colon cancer patients receiving FOLFOX according to combined *ERCC1/XRCC1* polymorphisms. Disease-free survival curves of stage III colon cancer patients receiving FOLFOX adjuvant chemotherapy according to the combination of *ERCC1-118* (rs11615, c.354T>C) and *XRCC1-399* (rs25487, c.1196G>A) polymorphisms. Survival curves were estimated with the Kaplan–Meier method and compared using the log-rank test.

**Table 3.** Univariate analyses between *ERCC1*-118 and *XRCC1*-399 polymorphisms, separately or in combination, with disease-free survival.

|  | n   | Events | HR             | IC 95%        | P    | Harrell's |
|--|-----|--------|----------------|---------------|------|-----------|
| <b><i>ERCC1</i>-118 (rs11615)</b>  |     |        |                |               |      | 0.57      |
| C/C  | 49  | 6      | 1 <sup>R</sup> |               |      |           |
| C/T + T/T  | 153 | 39     | 2.29           | [0.97 - 5.41] | 0.06 |           |
| <b><i>XRCC1</i>-399 (rs25487)</b>  |     |        |                |               |      | 0.54      |
| G/G + G/A  | 174 | 37     | 1 <sup>R</sup> |               |      |           |
| A/A  | 33  | 11     | 1.61           | [0.82 - 3.12] | 0.16 |           |
| <b><i>ERCC1</i>-118 (rs11615) AND <i>XRCC1</i>-399 (rs25487)</b>             |     |        |                |               |      | 0.56      |
| ≥ 1 genotype favorable: [ <i>ERCC1</i> C/C] AND/OR [ <i>XRCC1</i> G/G + G/A] | 179 | 36     | 1 <sup>R</sup> |               |      |           |
| 0 genotype favorable: [ <i>ERCC1</i> C/T + T/T] AND [ <i>XRCC1</i> A/A]      | 21  | 9      | 2.42           | [1.16 - 5.03] | 0.02 |           |

Abbreviations: R, reference; *ERCC1*, Excision repair cross-complementing group 1; *XRCC1*, X-ray repair cross-complementing group 1.

**Table 4.** Prognostic factors for disease-free survival in multivariate analysis.

|  | n   | Events | HR             | IC 95%        | P    |
|--|-----|--------|----------------|---------------|------|
| <b>Differentiation grade</b>   |     |        |                |               |      |
| Well / Moderate  | 174 | 36     | 1 <sup>R</sup> |               |      |
| Poor   | 26  | 9      | 1.58           | [0.74 - 3.40] | 0.24 |
| <b>Stage</b>   |     |        |                |               |      |
| III A / III B (Tx, N1)   | 127 | 23     | 1 <sup>R</sup> |               |      |
| III C (Tx, N2)   | 73  | 22     | 1.28           | [0.62 - 2.64] | 0.51 |
| <b>Lymph node ratio</b>  |     |        |                |               |      |
| < 0.100  | 102 | 16     | 1 <sup>R</sup> |               |      |
| ≥ 0.100  | 98  | 29     | 1.62           | [0.74 - 3.56] | 0.23 |
| <b><i>ERCC1</i>-118 (rs11615) AND <i>XRCC1</i>-399 (rs25487)</b>             |     |        |                |               |      |
| ≥ 1 genotype favorable: [ <i>ERCC1</i> C/C] AND/OR [ <i>XRCC1</i> G/G + G/A] | 179 | 36     | 1 <sup>R</sup> |               |      |
| 0 genotype favorable: [ <i>ERCC1</i> C/T + T/T] AND [ <i>XRCC1</i> A/A]      | 21  | 9      | 2.03           | [0.96 - 4.28] | 0.06 |

Abbreviations: R, reference; *ERCC1*, Excision repair cross-complementing group 1; *XRCC1*, X-ray repair cross-complementing group 1. The cut-off value of LNR between metastatic and examined lymph nodes corresponded to the median.

A multivariate Cox model was constructed according to the "one variable for 10 events" rule. The discriminatory capacity was tested using the Harrell's concordance index. Harrell's C statistic was used to estimate the proportion of correct predictions. Harrell's C index ranges from 0.5 (no discrimination) to 1 (perfect discrimination).

Ten patients were excluded from the multivariate model because they had at least one missing polymorphism data (Harrell's C=0.65).

## Discussion

The present study indicates that pharmacogenetic profiling of genes involved in DNA repair may influence clinical outcome of patients receiving FOLFOX adjuvant treatment for stage III colon cancer. This study focused on *ERCC1*-118, *XRCC1*-399 and *GSTP1*-105 genes polymorphisms because previously published data suggested a potential association between their SNPs and clinical outcome of patient treated with oxaliplatin-based chemotherapy for advanced CRC (7-12, 17). In our study, patients with no favorable genotype among *ERCC1*-118 and *XRCC1*-399 combination polymorphism have a shorter DFS upon FOLFOX adjuvant chemotherapy. However, the prognostic effect of this combined genotype analysis did not reach statistical significance in multivariate analysis, possibly because the number of patients in our series was not large enough, notably for the group of patients having no favorable genotype. Nevertheless, regarding the association with DFS in multivariate analysis, the p value obtained when combining *ERCC1* and *XRCC1* genotypes was lower than for the other well-recognized prognostic factors (differentiation grade, LNR or tumor stage). A

second possible explanation is that the polymorphism might be in linkage disequilibrium with another factor influencing survival to platinum-based chemotherapy within the same gene or in a gene nearby, such as *ERCC2*, as previously suggested (11).

Several studies reported that SNP in *ERCC1* could predict the clinical outcome for patients receiving platinum-based chemotherapy for treatment of various tumors, including colorectal, gastric or lung cancers (19). Previous data suggested that T allele compared to C allele at codon 118 of *ERCC1* gene, both coding for asparagine, was associated with higher *ERCC1* mRNA level resulting in resistance to platinum drugs (20). DFS benefit for colon cancer patients with *ERCC1*-118 C/C in our study may support these findings. However, other analysis performed in ovarian cancer cells lines did not confirm these results (21), and the relationship between *ERCC1*-118 SNP and clinical outcome of patients receiving oxaliplatin-based chemotherapy for advanced CRC remains controversial. Regarding patients with metastatic colorectal cancer, several studies reported that *ERCC1*-118 C/C homozygote genotype was associated with better clinical outcome (7, 9, 11, 12, 22-25), whereas others showed no correlation between gen-

otype and survival (26, 27). In adjuvant setting, there is to date only one published study that evaluated the impact of *ERCC1*-118 SNP on survival of patients with stage III colon cancer (28). This retrospective study included 98 patients with stage III colon cancer receiving oxaliplatin-based (n = 53) or Mayo regimen (leucovorin and bolus fluorouracil) (n = 45) adjuvant chemotherapy. In univariate and multivariate survival analyses, *ERCC1*-118 polymorphism did not influence DFS, whatever the treatment administered (28).

Several authors attempted to identify combination of genomic variants occurring in several genes involved in DNA repair or metabolism drugs that may help predicting efficacy of platinum-based chemotherapy for CRC patients (11, 12, 22). Liang *et al* evaluated the prognostic value of *ERCC1*-118 and *XRCC1*-399 SNP, separately and in combination, in 113 patients receiving oxaliplatin-based chemotherapy for metastatic CRC (26). This retrospective study reported that the favorable genotypes of *ERCC1*-118 and *XRCC1*-399 polymorphisms, in combination but not individually, were an independent prognostic factor for disease control rate and overall survival (26). However while favorable genotypes for *ERCC1*-118 SNP were identical in both studies (C/C homozygous), the *XRCC1*-399 favorable genotype (A/A homozygous) differed from ours (G/G + G/A) (26).

The first study evaluating the value of *XRCC1*-399 as a pharmacogenetic marker was performed in 61 patients with advanced CRC receiving 5FU/oxaliplatin chemotherapy (8). The authors showed that 73% (8/11) of responders had a G/G genotype and 27% (3/11) were G/A, while 66% (33/50) of non-responders showed A/A or G/A genotypes (p=0.038) (8). Patients carrying at least one A allele were at a 5.2 fold increased risk to be resistant to oxaliplatin-based chemotherapy (8). More recently, another study reported similar results in 62 patients treated with oxaliplatin-based chemotherapy for metastatic CRC (9). In keeping, we observed in our study that DFS of patients with *XRCC1*-399 A/A genotype was shorter. However, other studies including between 61 and 166 patients failed to detect a significant prognostic impact of *XRCC1*-399 SNP in metastatic setting (11, 22, 24, 29). This indicates that *XRCC1* genotype is probably not strongly associated with clinical outcome, though subtle differences have been reported in some studies.

*GSTP1* is a member of a superfamily of metabolic enzymes involved in the detoxification of platinum compounds. Stoehlmacher *et al* have reported in two studies that *GSTP1*-105 G allele coding a Valine was associated with increased survival of patients with advanced CRC receiving 5-FU/oxaliplatin chemo-

therapy (10, 11). More recently, Chen *et al* confirmed these results in a series of Asian metastatic CRC patients, with higher tumor response and longer survival seen in the group of patients with at least one *GSTP1*-105 G allele (12). However, the association between *GSTP1*-105 polymorphism and efficacy in the context of oxaliplatin-based treatment remains controversial. Indeed, some studies did not confirm the prognostic value of *GSTP1*-105 SNP in metastatic (22, 25) or adjuvant (28) oxaliplatin-based treatment.

In conclusion, this study suggests that pharmacogenetic profiling of genes involved in DNA repair may have an impact on clinical outcome of patients receiving FOLFOX adjuvant treatment for stage III colon cancer. In univariate analysis, combination of *ERCC1*-118 and *XRCC1*-399 emerged as promising prognostic markers for DFS, although this combined genotype factor did not reach statistical significance in multivariate analysis. Future prospective studies with large sample sizes are required to confirm and extend our findings.

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## Competing Interests

The authors have declared that no competing interest exists.

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