Genetic markers for toxicity of adjuvant oxaliplatin and fluoropyrimidines in the phase III TOSCA trial in high-risk colon cancer patients

Genetic markers for toxicity of adjuvant oxaliplatin and fluoropyrimidines in the phase III TOSCA trial in high-risk colon cancer patients

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We investigated 17 polymorphisms in 11 genes (TS, MTHFR, ERCC1, XRCC1, XRCC3, XPD, GSTT1, GSTP1, GSTM1, ABCC1, ABCC2) for their association with the toxicity of fluoropyrimidines and oxaliplatin in colorectal cancer patients enrolled in a prospective randomized trial of adjuvant chemotherapy. The TOSCA Italian adjuvant trial was conducted in high-risk stage II–III colorectal cancer patients treated with 6 or 3 months of either FOLFOX-4 or XELOX adjuvant chemotherapy. In the concomitant ancillary pharmacogenetic study, the primary endpoint was the association of polymorphisms with grade 3–4 CTCAE toxicity events (grade 2–4 for neurotoxicity). In 517 analyzed patients, grade 3 neutropenia and grade 2 neurotoxicity events occurred in 150 (29%) and 132 patients (24.8%), respectively. Diarrhea grade 3 events occurred in 34 (6.5%) patients. None of the studied polymorphisms showed clinically relevant association with toxicity. Hopefully, genome-wide association studies will identify new and more promising genetic variants to be tested in future studies.

Adjuvant chemotherapy is the standard of care for stage III colorectal cancer patients and an accepted treatment option for high-risk stage II patients. Standard regimens include oxaliplatin combined with bolus/infusional 5-fluorouracil (FOLFOX) or capcitabine (XELOX). Unfortunately, several patients experience mild or moderate side effects at some point during treatment. Most frequently reported adverse events of these regimens in randomized adjuvant trials in Western populations are neutropenia (≥grade 3 in 40% to 56% of patients), neurotoxicity (≥grade 3 in 10% to 20% of patients), and diarrhea (≥grade 3 in 10% to 15% of patients). Therefore, the safety profile may be suboptimal and causing treatment delay, reduction, cessation and even death in a minority of patients. This is very important in the adjuvant setting, where potentially cured patients undergo an effective prophylactic treatment strategy. Prediction of an individual patients’ risk of severe toxicity could allow for an adequate monitoring and improve overall management and quality of care. Host non-genetic factors such as medical comorbidity and organ dysfunction may account for differences in the safety profile of adjuvant chemotherapy across populations. However, genetic variability among individuals may play a key role. Functional germline polymorphisms may contribute to inter-individual differences in the...
Table 1 | Genotype and allele frequencies

| Gene (site) | Polymorphism | Genotype | ID number | N pts | Genotype Frequency | Allele Frequency |
|-------------|--------------|----------|-----------|-------|-------------------|-----------------|
| TYMS (5’UTR) | VNTR advisable U 3 or 2R | rs34743033 | 516 | 174 | 240 | 102 | 0.57 | 0.43 |
| TYMS (5’UTR) | SNP p | G > C in 3R | rs2853542 | 414 | 108 | 45 | 251 | 0.34 | 0.66 |
| TYMS (3’UTR) | 6 bp deletion | ins/del | rs11280036 | 516 | 189 | 240 | 87 | 0.60 | 0.40 |
| MTHFR (exon 4) | SNP q | C > T (Ala222Val) | rs1801133 | 515 | 162 | 250 | 103 | 0.56 | 0.44 |
| MTHFR (exon 7) | SNP g | A > G (Glu299Ala) | rs1801131 | 515 | 256 | 213 | 46 | 0.70 | 0.30 |
| ERCC1 (exon 4) | SNP H | T > G (Asn118Asn) | rs11615 | 517 | 198 | 230 | 89 | 0.60 | 0.40 |
| XRCC1 (exon 10) | SNP j | G > A (Gln399Arg) | rs25487 | 511 | 210 | 243 | 58 | 0.65 | 0.35 |
| XPD (exon 10) | SNP k | G > A (Asp312Asn) | rs1799793 | 499 | 212 | 217 | 70 | 0.54 | 0.46 |
| XPD (exon 23) | SNP l | T > G (Lys751Gln) | rs13181 | 513 | 193 | 238 | 82 | 0.59 | 0.41 |
| XRCC3 (exon 7) | SNP m | C > T (Thr241Met) | rs861539 | 509 | 174 | 245 | 90 | 0.59 | 0.41 |
| GST-P1 (exon 5) | SNP n | A > G (Ile105Val) | rs1695 | 515 | 246 | 228 | 41 | 0.70 | 0.30 |
| GST-T1 (exon 7) | SNP o | Deletion yes/no | - | 516 | 252 | - | 264 | 0.49 | 0.51 |
| GST-M1 (exon 10) | SNP p | Deletion yes/no | - | 516 | 463 | - | 93 | 0.82 | 0.18 |
| ABCB1 (intronic) | SNP q | G > C | rs2074087 | 484 | 344 | 129 | 11 | 0.84 | 0.16 |
| ABCB2 (exon 28) | SNP r | G > A (Ile1324Ile) | rs3740066 | 514 | 192 | 244 | 78 | 0.61 | 0.39 |
| ABCB2 (5’ flanking) | SNP s | G > A | rs1885301 | 507 | 159 | 238 | 110 | 0.55 | 0.45 |
| ABCB2 (intronic) | SNP t | G > A | rs4148386 | 516 | 166 | 244 | 106 | 0.44 | 0.56 |

Legend: p: major allele frequency; q: minor allele frequency; VNTR: variable number of tandem repeats; SNP: single nucleotide polymorphism; bp: base pair; ins: insertion; del: deletion; pts: patients.

Table 2 | Study sample characteristics

| Age (years) | N [%] |
|-------------|-------|
| ≤70 | 378 (73.1%) |
| >70 | 139 (26.9%) |

| Sex | N [%] |
|-----|-------|
| Male | 298 (57.6%) |
| Female | 219 (42.4%) |

| Tumor Ascending colon | 137 (26.5%) |
| Transverse colon | 37 (7.2%) |
| Splenic flexure | 22 (4.3%) |
| Descending colon | 59 (11.4%) |
| Sigmoid colon | 237 (45.8%) |

| Tumor stage | N [%] |
|-------------|-------|
| Stage II | 188 (36.4%) |
| Stage III | 329 (63.6%) |

| Adjuvant therapy | N [%] |
|-----------------|-------|
| 3-month Folfox-4 | 189 (36.6%) |
| 6-month Folfox-4 | 188 (36.4%) |
| 3-month Xelox | 72 (13.9%) |
| 6-month Xelox | 68 (13.1%) |

N: number of patients.
(positive or null) were assayed as previously reported\(^1\). \textbf{Table S1.} Briefly, all amplification reactions were performed in a volume of 25\(\mu\)l.

\(\text{Primer sequences and preparative PCR conditions are reported in the supplementary instructions (Diatheva). All laboratory analyses were performed blind to the patients'}

\(\text{Primary outcome was defined as the occurrence of a grade 3–4 toxicity (grade 2, 3, 4 clinically relevant degrees of both hematologic and non-hematologic toxicity. To achieve such a toxicity event at the time of analysis were censored at the date they were last known to be event-free while on treatment. The treatment compliance was described in terms of treatment interruption and dose intensity, defined as the dose given in mg/m\(^2\) per week. Logistic regression and Cox proportional hazard models were used to assess the effects of genotypes on MGT and TTT, respectively, adjusting for treatment duration (6 or 3 months). For each polymorphism, toxicity analysis was performed across the three group genotypes (\(p^2\), \(pq\), \(q^2\)) and after grouping carriers of the heterozygous and homozygous risk genotypes.}

\(\text{Sample size calculation was based on an expected prevalence of at higher risk allele of at least 30% and assuming a 25% risk of toxicity. Accordingly, 440 patients (105 events) would allow the detection of an odds ratio (OR) of at least 2.0 associated to the group with unfavorable genotypes with a power of 90% and a I type error of 5%, for a bilateral test. Deviation from the Hardy-Weinberg equilibrium was assessed using the}\)

\[\text{p value} = 0.05\text{ considered statistically significant.}

\(\text{Analysis plan, sample size, and statistics. According to the planned management of toxicity in TOSCA trial, we chose outcome measures and endpoints, which reflects clinically relevant degrees of both hematologic and non-hematologic toxicity. Primary outcome was defined as the occurrence of a grade 3–4 toxicity (grade 2, 3, 4 for neurotoxicity) considering in each patient the maximum grade of toxicity (MGT) reported during treatment. Secondary outcome was the time to toxicity (TTT), defined as the time from date of randomization in TOSCA trial to the date of first grade \(\geq 2\) event for neurotoxicity and \(\geq 3\) event for other toxicities. Subjects without such a toxicity event at the time of analysis were censored at the date they were last known to be event-free while on treatment. The treatment compliance was described in terms of treatment interruption and dose intensity, defined as the dose given in mg/m\(^2\) per week. Logistic regression and Cox proportional hazard models were used to assess the effects of genotypes on MGT and TTT, respectively, adjusting for treatment duration (6 or 3 months). For each polymorphism, toxicity analysis was performed across the three group genotypes (\(p^2\), \(pq\), \(q^2\)) and after grouping carriers of the heterozygous and homozygous risk genotypes. Sample size calculation was based on an expected prevalence of at higher risk allele of at least 30% and assuming a 25% risk of toxicity. Accordingly, 440 patients (105 events) would allow the detection of an odds ratio (OR) of at least 2.0 associated to the group with unfavorable genotypes with a power of 90% and a I type error of 5%, for a bilateral test. Deviation from the Hardy-Weinberg equilibrium was assessed using the Pearson \(x^2\) test. Analyses were performed with SAS 9.2 (SAS Institute, Cary, NC). All reported \(p\) values are two-sided, and confidence intervals (CIs) are at the 95% level. A \(p\) value < 0.05 was considered statistically significant.}

\(\text{Table 3 | Maximum Grade of Toxicity (MGT)}\)

| Toxicity   | \(N\) | \(\%\) | \(N\) | \(\%\) | \(N\) | \(\%\) | \(N\) | \(\%\) | \(N\) | \(\%\) |
|------------|------|-------|------|-------|------|-------|------|-------|------|-------|
| Anemia     | 287  | 55.5  | 189  | 36.6  | 39   | 7.5   | 2    | 0.4   | 0    | 0.0   |
| Leukopenia | 263  | 50.9  | 166  | 32.1  | 77   | 14.9  | 10   | 1.9   | 1    | 0.2   |
| Neutropenia| 203  | 39.3  | 58   | 11.2  | 106  | 20.5  | 117  | 22.6  | 33   | 6.4   |
| Thrombocytopenia | 223  | 43.1  | 240  | 46.4  | 48   | 9.3   | 5    | 1.0   | 1    | 0.2   |
| Anemia     | 281  | 54.4  | 136  | 26.3  | 83   | 16.1  | 17   | 3.3   | 0    | 0.0   |
| Diarrhea   | 289  | 55.9  | 143  | 27.7  | 51   | 9.9   | 31   | 6.0   | 3    | 0.6   |
| Nausea     | 253  | 48.9  | 173  | 33.5  | 77   | 14.9  | 14   | 2.7   | 0    | 0.0   |
| Vomiting   | 414  | 80.1  | 64   | 12.4  | 28   | 5.4   | 11   | 2.1   | 0    | 0.0   |
| Stomatitis | 447  | 90.3  | 39   | 7.5   | 9    | 1.7   | 3    | 0.6   | 0    | 0.0   |
| Mucositis  | 436  | 84.3  | 61   | 11.8  | 16   | 3.1   | 3    | 0.6   | 0    | 0.0   |
| Hepatic    | 357  | 69.1  | 120  | 23.2  | 33   | 6.4   | 7    | 1.4   | 0    | 0.0   |
| Cutaneous  | 468  | 90.5  | 31   | 6.0   | 17   | 3.3   | 0    | 0.0   | 1    | 0.2   |
| Neurological| 159  | 30.8  | 226  | 43.7  | 110  | 21.3  | 22   | 4.3   | 0    | 0.0   |

\(N:\) number of patients.

\(\text{Table 4 | Dose Intensity)}\)

| Dose intensity | Follox-4 | Xelox |
|----------------|----------|-------|
| Median (Q1–Q3) | 3 months N = 189 | 6 months N = 188 |
| Oxaliplatin   | 41.7 [39.2–42.5] | 38.8 [34.2–42.0] |
| Leucovorin    | 50.0 [48.8–50.0] | 50.0 [44.1–50.0] |
| 5-FU, bolus   | 197.6 [176.8–200.0] | 184.8 [156.3–200.0] |
| 5-FU, intravenous | 296.5 [272.7–300.0] | 279.2 [245.5–300.0] |
| Capecitabine  | -        | -     |
|                | 333.3 [291.7–333.3] | 320.9 [273.0–333.3] |

\(\text{Table 5 | Treatment interruptions)}\)

| Treatment interruptions | Follox-4 | Xelox |
|-------------------------|----------|-------|
| N (%)                   | 3 months N = 189 | 6 months N = 188 | 12 weeks N = 72 | 24 weeks N = 68 |
| Completed without time or dose changes | 46 [24.3] | 12 [6.4] | 31 [43.1] | 11 [16.2] |
| Completed with time or dose changes | 130 [68.8] | 114 [60.6] | 31 [43.1] | 37 [54.4] |
| Interrupted:            | 13 [6.9] | 62 [33.0] | 10 [13.8] | 20 [29.4] |
| - Interrupted for toxicity* | 7 [53.8] | 36 [58.1] | 8 [80.0] | 12 [60.0] |

*Percentages calculated on patients who interrupted treatment.
### Table 6 | Pharmacogenetic associations with neutropenia

| Genotype                        | Maximum Grade of Toxicity | Time To Toxicity |
|---------------------------------|---------------------------|------------------|
|                                 | Odds Ratio (95% CI)       | p-value          |
|                                 | Hazard Ratio (95% CI)     | p-value          |
| **TS-5 UTR**                    |                           |                  |
| 3R/3R {reference}               | 1.00                      | 1.00             |
| 2R/3R                           | 1.11 (0.72–1.71)          | 0.633            | 1.13 (0.79–1.62) | 0.505 |
| 2R/2R                           | 0.93 (0.54–1.61)          | 0.799            | 0.97 (0.61–1.54) | 0.895 |
| 2R allele                       | 1.06 (0.70–1.58)          | 0.795            | 1.08 (0.77–1.52) | 0.654 |
| **TS-5 UTR**                    |                           |                  |
| 3G allele carriers (reference)   | 1.00                      | 1.00             |
| 3C allele carriers              | 0.79 (0.54–1.15)          | 0.221            | 0.81 (0.59–1.12) | 0.212 |
| **TS-3 UTR**                    |                           |                  |
| SS {reference}                  | 1.00                      | 1.00             |
| SL                              | 0.95 (0.55–1.65)          | 0.868            | 0.96 (0.61–1.52) | 0.864 |
| LL                              | 1.13 (0.65–1.97)          | 0.673            | 1.06 (0.66–1.70) | 0.802 |
| LL/SL vs SS                     | 1.03 (0.62–1.72)          | 0.912            | 1.01 (0.65–1.55) | 0.979 |
| **MTHFR (exon 4)**              |                           |                  |
| CC {reference}                  | 1.00                      | 1.00             |
| CT                              | 0.88 (0.57–1.36)          | 0.560            | 0.86 (0.59–1.24) | 0.432 |
| TT                              | 1.18 (0.69–2.01)          | 0.541            | 1.16 (0.74–1.80) | 0.518 |
| TT/CT vs CC                     | 0.96 (0.64–1.45)          | 0.846            | 0.94 (0.67–1.33) | 0.733 |
| **MTHFR (exon 7)**              |                           |                  |
| AA {reference}                  | 1.00                      | 1.00             |
| AC                              | 1.16 (0.78–1.73)          | 0.459            | 1.10 (0.79–1.53) | 0.576 |
| CC                              | 0.72 (0.34–1.53)          | 0.397            | 0.80 (0.41–1.56) | 0.515 |
| CC/AC vs AA                     | 1.08 (0.73–1.58)          | 0.705            | 1.05 (0.76–1.45) | 0.770 |
| **ERCC1 (exon 4)**              |                           |                  |
| CC {reference}                  | 1.00                      | 1.00             |
| TC                              | 1.47 (0.84–2.58)          | 0.174            | 1.42 (0.88–2.30) | 0.149 |
| TT                              | 1.17 (0.66–2.09)          | 0.584            | 1.14 (0.69–1.87) | 0.609 |
| TT/TC vs CC                     | 1.33 (0.78–2.25)          | 0.291            | 1.28 (0.82–2.02) | 0.281 |
| **XRCC1 (exon 10)**             |                           |                  |
| AA {reference}                  | 1.00                      | 1.00             |
| GA                              | 1.11 (0.58–2.13)          | 0.760            | 1.17 (0.67–2.05) | 0.583 |
| GG                              | 1.39 (0.72–2.68)          | 0.331            | 1.45 (0.83–2.54) | 0.196 |
| GG/AG vs AA                     | 1.23 (0.66–2.30)          | 0.511            | 1.30 (0.76–2.21) | 0.344 |
| **XPD (exon 10)**               |                           |                  |
| GG {reference}                  | 1.00                      | 1.00             |
| GA                              | 1.16 (0.76–1.77)          | 0.479            | 1.18 (0.83–1.69) | 0.352 |
| AA                              | 1.22 (0.68–2.11)          | 0.507            | 1.20 (0.73–1.96) | 0.470 |
| AA/GA vs GG                     | 1.18 (0.79–1.75)          | 0.416            | 1.19 (0.85–1.66) | 0.313 |
| **XPD (exon 23)**               |                           |                  |
| TT {reference}                  | 1.00                      | 1.00             |
| TG                              | 1.33 (0.87–2.04)          | 0.184            | 1.31 (0.91–1.87) | 0.145 |
| GG                              | 1.14 (0.64–2.03)          | 0.655            | 1.13 (0.70–1.85) | 0.612 |
| GG/TG vs TT                     | 1.28 (0.86–1.91)          | 0.226            | 1.26 (0.90–1.77) | 0.184 |
| **XRCC3 (exon 7)**              |                           |                  |
| TT {reference}                  | 1.00                      | 1.00             |
| CT                              | 0.93 (0.60–1.44)          | 0.757            | 0.95 (0.66–1.37) | 0.778 |
| CC                              | 1.33 (0.77–2.30)          | 0.306            | 1.33 (0.85–2.09) | 0.210 |
| CC/CT vs TT                     | 1.03 (0.69–1.55)          | 0.879            | 1.04 (0.74–1.47) | 0.807 |
| **GST-PI (exon 5)**             |                           |                  |
| GG {reference}                  | 1.00                      | 1.00             |
| AG                              | 1.44 (0.67–3.10)          | 0.356            | 1.35 (0.70–2.63) | 0.370 |
| AA                              | 1.23 (0.57–2.64)          | 0.602            | 1.22 (0.63–2.36) | 0.562 |
| AA/AG vs GG                     | 1.32 (0.63–2.78)          | 0.460            | 1.28 (0.67–2.44) | 0.448 |
| **GST-T1/M1 deletion**          |                           |                  |
| Yes/Yes {reference}             | 1.00                      | 1.00             |
| Yes/Null                        | 1.12 (0.73–1.72)          | 0.599            | 1.07 (0.74–1.54) | 0.709 |
| Null/Yes                        | 1.21 (0.58–2.55)          | 0.612            | 1.15 (0.62–2.15) | 0.653 |
| Null/Null                       | 1.99 (1.06–3.73)          | 0.032            | 1.70 (1.03–2.78) | 0.036 |
| Null vs Yes/Yes                 | 1.26 (0.85–1.87)          | 0.243            | 1.18 (0.85–1.65) | 0.317 |
| **ABCC1 (intron)**              |                           |                  |
| CC {reference}                  | 1.00                      | 1.00             |
| GC                              | 0.77 (0.21–2.81)          | 0.695            | 0.82 (0.29–2.29) | 0.705 |
| GG                              | 0.65 (0.18–2.27)          | 0.497            | 0.67 (0.25–1.82) | 0.429 |
| GG/GC vs CC                     | 0.68 (0.19–2.37)          | 0.545            | 0.71 (0.26–1.91) | 0.495 |
| **ABCC2 (exon 28)**             |                           |                  |
| AA {reference}                  | 1.00                      | 1.00             |
| AG                              | 1.11 (0.61–2.00)          | 0.738            | 1.10 (0.66–1.84) | 0.712 |
Results

Patient characteristics and toxicity. From July 2007 to October 2011, 534 patients from 26 experimental centers entered the study. This figure represents 81% of patients randomized in the same period and by the same centers in the main TOSCA trial study. Seventeen patients were not assessable; five patients who were never treated, two patients because of unavailability of treatment data, and ten due to technical problems about blood sampling. Therefore, the analysis was conducted on 517 patients.

Characteristics of the 517 patients are shown in Table 2. Patients’ baseline characteristics were consistent with those of the whole trial population (data not reported). Most patients were randomized to FOLFOX-4 because option for XELOX regimen was introduced only during the late phase of accrual of this ancillary study. Toxicity caused by adjuvant chemotherapy is reported in Table 3. Again, the spectrum and the frequency of toxicities did not differ from those observed in whole trial population (data on file). The target number of events was reached for neutropenia (150/517 patients, 29%) and neurotoxicity (132/517, 25.5%), only. Dose intensity and treatment interruptions were shown in Table 4 and Table 5, respectively. Dose intensity for patients randomized in 6 months arms is slightly lower than that reported for patients randomized in 3 months arms.

Genetic assessments. Table 1 lists the studied genetic variants and the distribution of genotypes of patients. Consistent with previous observations, genotype frequency did not differ from those observed in Caucasian population. Allele frequencies of all polymorphisms were consistent with the Hardy-Weinberg equilibrium (in Caucasian population. Allele frequencies of all polymorphisms showed some statistically significant association. In presence of the ABCC2 (rs 4148386) GG genotype, there was a greater occurrence of grade 3–4 leukopenia (OR 9.82, 95% CI 1.16–83.02; p = 0.036) and the time to leukopenia was shorter (HR 9.40, 95% CI 1.13–78.10; p = 0.038) in comparison to ABCC2 AA genotype. TS 5’UTR L allele showed a protective effect for mucositis for MGT (OR 0.07, 95% CI 0.01–0.65; p = 0.020) and TTT (HR 0.07, 95% CI 0.01–0.67; p = 0.021). Risk of vomiting (MGT) was increased in carriers of the TS 5’UTR 2R2R genotype (OR 8.83, 95% CI 1.01–76.91; p = 0.049) compared to TS 5’UTR 3R3R genotype.

Discussion

This study assessed 17 polymorphisms in 11 genes thought to be associated with toxicity of fluoropyrimidines or oxaliplatin. To the best of our knowledge this is the first and the largest prospective pharmacogenetic analysis in a randomized trial of adjuvant chemotherapy in colorectal cancer. Candidate polymorphisms were selected on the basis of previous promising data from retrospective or single arm studies. The prospective accrual of patients achieved the required number of events for neutropenia and neurotoxicity, however only GST-T1/M1 was statistically associated to neutropenia and the strength of this association was very low. Therefore, no polymorphism showed a clinically relevant association with neurotoxicity and neutropenia. The results on the other toxicities should be looked at with caution because of the low number of events.

To date, five randomized clinical trials in colorectal cancer have incorporated pharmacogenetic analysis8–10, but only one study in the adjuvant setting11. In the US Intergroup N9741 pharmacogenetic analysis there were 114 patients treated with IFL chemotherapy, 299 patients treated with FOLFOX-4 regimen and 107 patients who received IrOX chemotherapy12. Therefore, despite the 520 initial patients assessed for pharmacogenetic analyses, this remarkable study population was diluted among three treatments arms, with a small number of patients assessable for an oxaliplatin-based regimen. In this study, ≥grade 3 neutropenia, neurotoxicity and diarrhea occurred in the 27%, 13% and 13% of patients respectively. In the FOLFOX-4 regimen analysis, the GST-P1 TT genotype carriers were more likely to suffer from febrile neutropenia and to discontinue the treatment because of neurotoxicity, carriers of the GST-M1 null genotype were at increased risk of neutropenia. In the Fédération Francophone de Cancérologie Digestive 2000-05 trial, metastatic colorectal cancer patients were randomized to receive 5-FU plus leucovorin followed by FOLFOX-6, followed by FOLFIRI (arm A), or FOLFOX-6 followed by FOLFIRI (arm B). The pharmacogenetic analysis included 346 patients who received more regimens in a different sequence13. There was a remarkable frequency of ≥grade 2
neurotoxicity (about half of the patients) and grade 3 myelotoxicity in about one-third of the patients. The XPD C allele (rs13181) was significantly associated with an increased risk of FOLFOX-induced hematologic toxicity (p < 0.01). In the pharmacogenetic analysis associated with the randomized FOCUS UK trial, 1,188 patients were assessed. In this study, metastatic colorectal cancer patients were randomized to receive three treatment strategies according to a different sequence of the following regimens: 5-FU alone, irinotecan alone, 5-FU with irinotecan and 5-FU with oxaliplatin. Only 280 patients were assessable for first- or second-line oxaliplatin-based chemotherapy. No significant pharmacogenetic association was found in this study. The most recently published analysis in metastatic colorectal cancer patients depicts the results of a large panel of genetic variants in a robust sample of more than 2,000 patients enrolled in the COIN trials in UK. Again, this study ruled out clinically relevant associations between pharmacogenetics and clinical outcomes of patients treated with fluoropyrimidine/oxaliplatin with or without cetuximab.

As far as the adjuvant setting is concerned, the recently published pharmacogenetic study from the QUASAR2 trial has investigated the role of fluoropyrimidine-related polymorphisms in 927 patients who were randomized between capecitabine and capecitabine with bev-

| Table 7 | Pharmacogenetic associations with neurotoxicity |
|---------|----------------------------------|
| Genotype | Maximum Grade of Toxicity | Time To Toxicity |
|          | Odds Ratio (95% CI) | p-value | Hazard Ratio (95% CI) | p-value |
| ERCC1 (exon 4) |                     |
| CC (reference) | 1.00 | 1.00 |
| TC | 0.95 (0.53–1.71) | 0.863 | 0.85 (0.53–1.35) | 0.483 |
| TT | 0.75 (0.41–1.37) | 0.356 | 0.71 (0.44–1.15) | 0.165 |
| TT/TC vs CC | 0.85 (0.49–1.46) | 0.560 | 0.78 (0.51–1.20) | 0.258 |
| XRCC1 (exon 10) |                     |
| AA (reference) | 1.00 | 1.00 |
| GA | 0.76 (0.39–1.49) | 0.418 | 0.77 (0.45–1.30) | 0.323 |
| GG | 0.90 (0.45–1.77) | 0.754 | 0.89 (0.53–1.52) | 0.681 |
| GG/AG vs AA | 0.82 (0.43–1.55) | 0.543 | 0.82 (0.50–1.36) | 0.447 |
| XPD (exon 10) |                     |
| GG (reference) | 1.00 | 1.00 |
| GA | 1.12 (0.70–1.78) | 0.646 | 1.06 (0.73–1.55) | 0.755 |
| AA | 0.94 (0.49–1.83) | 0.861 | 1.02 (0.60–1.75) | 0.929 |
| AA/GA vs GG | 1.07 (0.69–1.66) | 0.764 | 1.05 (0.74–1.50) | 0.776 |
| XPD (exon 23) |                     |
| TG | 1.03 (0.64–1.66) | 0.897 | 0.96 (0.65–1.41) | 0.825 |
| GG | 1.26 (0.68–2.31) | 0.462 | 1.28 (0.79–2.07) | 0.313 |
| GG/TG vs TT | 1.09 (0.70–1.70) | 0.697 | 1.04 (0.73–1.49) | 0.833 |
| XRCC3 (exon 7) |                     |
| TT (reference) | 1.00 | 1.00 |
| CT | 1.22 (0.75–1.98) | 0.430 | 1.29 (0.86–1.92) | 0.215 |
| CC | 1.47 (0.79–2.75) | 0.226 | 1.52 (0.92–2.51) | 0.100 |
| CC/CT vs TT | 1.28 (0.81–2.03) | 0.295 | 1.35 (0.92–1.96) | 0.124 |
| GST-PI (exon 5) |                     |
| GG (reference) | 1.00 | 1.00 |
| AG | 0.63 (0.29–1.36) | 0.237 | 0.66 (0.36–1.19) | 0.167 |
| AA | 0.72 (0.34–1.53) | 0.390 | 0.71 (0.40–1.27) | 0.255 |
| AA/AG vs GG | 0.68 (0.33–1.40) | 0.292 | 0.69 (0.40–1.20) | 0.186 |
| GST-T1/M1 deletion |                     |
| Yes/Yes (reference) | 1.00 | 1.00 |
| Yes/Null | 1.08 (0.67–1.72) | 0.761 | 0.97 (0.66–1.41) | 0.860 |
| Null/Yes | 1.40 (0.63–3.12) | 0.414 | 1.48 (0.79–2.77) | 0.224 |
| Null/Null | 0.85 (0.40–1.80) | 0.666 | 0.74 (0.39–1.38) | 0.339 |
| Null vs Yes/Yes | 1.07 (0.70–1.64) | 0.759 | 0.97 (0.69–1.38) | 0.871 |
| ABCC2 (exon 28) |                     |
| AA (reference) | 1.00 | 1.00 |
| AG | 0.92 (0.49–1.73) | 0.796 | 0.97 (0.59–1.60) | 0.905 |
| GG | 0.77 (0.40–1.48) | 0.430 | 0.79 (0.47–1.33) | 0.378 |
| GG/AG vs AA | 0.85 (0.47–1.54) | 0.594 | 0.88 (0.55–1.43) | 0.615 |
| ABCC2 (5 flank) |                     |
| AA (reference) | 1.00 | 1.00 |
| AG | 1.24 (0.70–2.21) | 0.458 | 1.21 (0.75–1.96) | 0.429 |
| GG | 1.66 (0.91–3.06) | 0.101 | 1.43 (0.87–2.35) | 0.164 |
| GG/AG vs AA | 1.40 (0.82–2.40) | 0.222 | 1.30 (0.83–2.04) | 0.256 |
| ABCC2 (intron) |                     |
| AA (reference) | 1.00 | 1.00 |
| AG | 0.78 (0.48–1.26) | 0.306 | 0.87 (0.60–1.28) | 0.483 |
| GG | 0.60 (0.33–1.12) | 0.107 | 0.68 (0.41–1.12) | 0.130 |
| GG/AG vs AA | 0.72 (0.46–1.13) | 0.158 | 0.81 (0.57–1.16) | 0.251 |

CI: Confidence Interval. Abbreviation: CI = Confidence Interval.
acizumab. Of the 36 assessed polymorphisms only four TS and DPYD genetic variants were associated with grade ≥ 3 global toxicity, but with modest predictive power14.

Considering the characteristics of the above mentioned studies, we would emphasize the remarkable sample size in the adjuvant setting of our oxaliplatin-based study population, as well as the quality of pharmacogenetic analyses in a prospective and controlled collection of clinical data15. It seems that we recorded a lower frequency of grade ≥ 2 neurotoxicity and grade > 3 neutropenia than previously reported in the literature6. Generally, we observed a global lower incidence of toxicity events than expected. This finding is likely related to the accuracy of physicians in the monitoring of patients with early detection of signs of side-effects and consequently, their conservative attitudes towards treatment delays and dose-reductions.

However, this did not jeopardize the study plan of the ancillary pharmacogenetic study and a sufficient number of events for neurotoxicity and neutropenia was observed. Unfortunately, given the low rate of other severe toxicities, we cannot rule out the risk of observing false-negative associations in these cases.

A number of drug- and host-related variables contribute to pharmacodynamic and pharmacokinetic changes of chemotherapy drugs. Therefore, because of the moderate functional effects of polymorphism in the enzyme/target activity, their clinical impact may be masked according study populations and clinical settings. This may also explain the heterogeneity of results across pharmacogenetic studies. On the whole, we highlight the necessity for large-scale validation trials before pharmacogenetic findings from small studies are incorporated into clinical practice12–15. In fact, our findings, together with the results of the analyses in metastatic colorectal cancer9–11 and other malignancies12, mitigate the positive expectations for the growing burden of small, retrospective published studies on the predictive/prognostic role of polymorphisms in colorectal cancer patients, only UDP glucuronosyltransferase 1 family, polypeptide A1 (UGT1A1) and dihydrophrymidine dehydrogenase (DPYD) genetic variants have shown a promising level of evidence for clinical practice16. However, we did not study the UGT1A1**28 genotype analysis since it is typically associated with Irinotecan pharmacokinetic and toxicity16. As far as the DPYD IVS14 + 1G > A splice mutation is concerned, we did not include this variant for 5-fluorouracil toxicity analysis because of its very low frequency16. In fact, there were 2 heterozygous carriers in the 346 patients (0.5%) of the French trial6, 4 heterozygous carriers in the 520 patients (0.7%) of US trial7 and 12 heterozygous carriers in the 1088 patients (1.1%) of FOCUS trial7.

Pharmacogenetics may still offer a unique opportunity for tailoring the administration of chemotherapy and novel biologic agents to cancer patients. Hopefully, new sophisticated techniques such as SNP arrays and genome-wide association studies (GWAS) will identify new and more promising genetic variants to be tested in future studies17–18.

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Author contributions
A.R., Francesco G., E.G. and M.M. conceived and performed the study design, performed the manuscript preparation and data interpretation. Fabio G. performed coordination study. Francesca G., L.F., Fabio G. and E.R. performed statistical analysis, data interpretation and manuscript preparation. S.L., M.R., R.M., V.Z., N.P., C.M., R.L., M.T.T., E.V., P.S., S.B., V.R., L.F., M.N., E.B., A.B., D.T., S.L., C.V., F.B., A.S. and L.F., collected samples and patients’ data, and commented the manuscript. R.L., L.F. and A.S. participated in the study design and data interpretation, and helped to draft the manuscript. All authors reviewed the manuscript.

Additional information
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