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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 in 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[1]. Standard regimens include oxaliplatin combined with bolus/infusional 5-fluorouracil (FOLFOX) or capecitabine (XELOX)[1]. 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)[2]. 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[1]. 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[3]. 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	
					*p^2	pq	q^2	p	q
TYMS (5′UTR)	VNTR§	3R or 2R	rs34743033	516	174	240	102	0.57	0.43
TYMS (5′UTR)	SNP§	G > C in 3R	rs2853542	414	108	45	251	0.34	0.66
TYMS (3′UTR)	6 bp deletion	ins/del	rs11280056	516	189	240	87	0.60	0.40
MTHFR (exon 4)	SNP	C > T (Ala222Val)	rs1801133	515	162	250	103	0.56	0.44
MTHFR (exon 7)	SNP	A > C (Glu429Ala)	rs1801131	515	256	213	46	0.70	0.30
ERCC1 (exon 4)	SNP	T > C (Asn118Asn)	rs11615	517	198	230	89	0.60	0.40
XRCC1 (exon 10)	SNP	G > A (Gln399Arg)	rs25487	511	210	243	58	0.65	0.35
XPD (exon 10)	SNP	G > A (Asp312Asn)	rs1799793	499	212	217	70	0.64	0.36
XPD (exon 23)	SNP	T > G (Lys751Gln)	rs13181	513	193	238	82	0.59	0.41
XRCC3 (exon 7)	SNP	C > T (Thr241Met)	rs861539	509	174	245	90	0.59	0.41
GST-PI (exon 5)	SNP	A > G (Ile105Val)	rs1695	515	246	228	41	0.70	0.30
GST-T1‡	Deletion	yes/no	-	516	252	-	264	0.49	0.51
GST-M1‡	Deletion	yes/no	-	516	423	-	93	0.82	0.18
ABCC1 (intron)	SNP	G > C	rs2074087	484	344	129	11	0.84	0.16
ABCC2 (exon 28)	SNP	G > A (Ile1324Ile)	rs3740066	514	192	244	78	0.61	0.39
ABCC2 (5′flank)	SNP	G > A	rs1885301	507	159	238	110	0.55	0.45
ABCC2 (intron)	SNP	A > G	rs4148386	516	166	244	106	0.44	0.36

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.
*The first allele indicated in the Genotype column produces the p^2 homozygous genotype.
§TYMS VNTR is a tandem repeat polymorphism. Results are stated as three copies of the repeat (p^2) or two copies of the repeat (q^2). The VNTR polymorphism is reanalyzed according to a SNP in 3R carriers. In this case, only 3G allele carriers are considered high TS producers (q^2).
‡GST-T1 and -M1 are deletion polymorphisms. Results are stated as the number of patients with at least one copy of the gene (p^2) versus patients with a homozygous gene deletion (q^2).

pharmacokinetic and pharmacodynamics of anti-cancer drugs and this may contribute to the differences in toxicity among patients[3].

In the last decades, some genetic variants involved in the oxaliplatin and the fluoropyrimidines pathways were identified as potential predictors of toxicity[4,5]. However, the majority of clinical data have been obtained from retrospective analyses including a limited number of patients. In fact, none of the studied polymorphisms showed sufficient evidence for use in clinical practice[4,5]. Prospective analyses from randomized clinical trial represent a unique opportunity for evaluating association between genetic variants and clinical outcomes and are necessary for confirming the predictive role for toxicity of candidate polymorphisms[6–9].

TOSCA (Three Or Six Colon Adjuvant) is a large randomized trial addressing the role of a shorter duration of an adjuvant oxaliplatin/fluoropyrimidines regimen in surgically resected stage III and high-risk stage II colorectal cancer[10]. We adopted this clinical trial for planning a robust pharmacogenetic assessment for toxicity focusing on candidate polymorphisms, which had showed promising associations in previous studies[6–8,11–13].

Patients, Materials and Methods

TOSCA trial. Patients included in this study represent a subgroup of the 3.759 patients with surgically-resected, stage III and high-risk stage II colorectal cancer recruited in TOSCA trial between 2007 and 2011[10]. This is an Italian intergroup, multicentre, randomized, non-inferiority phase III study in high-risk stage II and stage III colon cancer patients treated with 3 or 6 months of either FOLFOX-4 or XELOX adjuvant chemotherapy, sponsored by GISCAD (Italian Group For The Study Of Gastrointestinal Cancer) and supported by Italian Medicines Agency (AIFA)[10]. Patients eligible for the TOSCA study were asked to give further and specific written informed consent for the pharmacogenetic study. All experiments were performed in accordance with relevant guidelines and regulations and the Local Ethics Committee of each institution approved the Study.

Assessment and management of chemotherapy toxicity. Selected hematologic and non-hematologic toxicities (anemia, leukopenia, neutropenia, thrombocytopenia, asthenia, diarrhea, mucositis stomatitis, vomiting, nausea, hepatic toxicity, skin toxicity, neurotoxicity) were assessed at the start of each cycle using Common Toxicity Criteria for Adverse Events (CTCAE) version 2.0. All adverse events at any time were monitored and reported. Toxicity was managed as follows: in case of grade $\geq$ 3 hematologic toxicity or persistent grade 2 the dose of all drugs was reduced by 25%. In case of grade $\geq$ 3 non-hematologic toxicity the dose of the related drugs was reduced by 50%. In case of grade $\geq$ 3 or persistent grade 2 neurotoxicity, oxaliplatin dose was reduced by 20%. Oxaliplatin was definitely stopped if grade $\geq$ 2 neurosensory symptoms persisted between cycles.

Molecular assessments. This prospective study was planned as a confirmatory analysis of genetic variants, which had shown some putative predictive effect for toxicity in previous studies[6–8,11–13]. Seventeen polymorphisms in eleven genes involved in DNA repair and drug metabolism and as drug targets, were selected from various reports as being potentially predictive of 5-fluorouracil (5-FU) or oxaliplatin toxicity (Table 1) in colorectal cancer patients. For each polymorphism, patients were considered in three groups: homozygous wild type (p2); heterozygous (pq); homozygous variant (q2). We also considered the model with merged heterozygous and homoxygous risk variant carriers.

Blood samples were taken before starting adjuvant chemotherapy. Genomic DNA was extracted by means of QIAmp DNA Blood kit (Qiagen, Valencia, CA). Polymorphisms in *TS* (rs34743033, rs2853542, rs11280056), *XRCC1* (rs25487), *XRCC3* (rs861539), *XPD* (rs1799793, rs13181), *GSTT1* (positive or null), *GSTM1*

Table 2 | Study sample characteristics

	N (%)
Age (years)	
≤70	378 (73.1%)
>70	139 (26.9%)
Sex	
Male	298 (57.6%)
Female	219 (42.4%)
Tumor site	
Ascending colon	137 (26.5%)
Hepatic flexure	25 (4.8%)
Transverse colon	37 (7.2%)
Splenic flexure	22 (4.3%)
Descending colon	59 (11.4%)
Sigmoid colon	237 (45.8%)
Tumor stage	
Stage II	188 (36.4%)
Stage III	329 (63.6%)
Adjuvant therapy	
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.

Table 3 | Maximum Grade of Toxicity (MGT)

	Maximum Grade Toxicity									
	0		1		2		3		4	
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
Thrombocitopenia	223	43.1	240	46.4	48	9.3	5	1.0	1	0.2
Asthenia	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	467	90.3	39	7.5	9	1.7	2	0.4	0	0.0
Mucositis	436	84.3	61	11.8	16	3.1	3	0.6	1	0.2
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.

(positive or null) were assayed as previously reported[11]. *ABCC1* (rs2074087) and *ABCC2* (rs3740066, rs1885301, rs4148386) genetic variants were analyzed using HRM (Rotor-Gene 6000®, Corbett Research, Sydney, Australia) or Pyrosequencing (PSQ 96MA®, Biotage AB) technique according to the manufacturer's instructions. Primer sequences and preparative PCR conditions are reported in the supplementary Table S1. Briefly, all amplification reactions were performed in a volume of 25 ul using 2× PCR Master Mix® (Diatheva, Fano, Italy) kit, 25 ng of gDNA and 200 nM of each primer. The intercalating dye EvaGreen® (Biotium Inc, CA, USA) was added for the HRM analyses. HRM conditions are listed in Table S1. *ERCC1* (rs11615), *MTHFR* (rs1801133, rs1801131) and *GSTP1* (rs1695) were genotyped with HRM analyses by means of kits containing reagents, enzymes and genotype controls: *ERCC1* Asn118Asn HRM kit, *MTHFR* C677T HRM kit, *MTHFR* A1298C HRM kit and *GSTP1* Ile105Val HRM kit respectively, according to the manufacturer's instructions (Diatheva). All laboratory analyses were performed blind to the patients' treatment and clinical outcomes. Genetic data were then transferred to IRCCS Istituto di Ricerche Farmacologiche "Mario Negri" for statistical analysis.

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 ≥ 2 event for neurotoxicity and ≥ 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 χ^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.

Table 4 | Dose Intensity

	Folfox-4		Xelox	
Median (Q1–Q3)	3 months N = 189	6 months N = 188	12 weeks N = 72	24 weeks N = 68
Oxaliplatin	41.7 (39.2–42.5)	38.8 (34.2–42.0)	43.3 (38.3–43.3)	37.9 (30.4–43.3)
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 continous	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)

Table 5 | Treatment interruptions

	Folfox-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

	Maximum Grade of Toxicity		Time To Toxicity	
Genotype	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 (0.74–1.801)	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 (0.68–2.21)	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

Table 6 | Continued

Genotype	Maximum Grade of Toxicity		Time To Toxicity	
	Odds Ratio (95% CI)	p-value	Hazard Ratio (95% CI)	p-value
GG	1.53 (0.84–2.79)	0.163	1.44 (0.86–2.40)	0.167
GG/AG vs AA	1.28 (0.73–2.24)	0.381	1.24 (0.77–2.02)	0.374
ABCC2 (5′flank)				
AA (reference)	1.00		1.00	
AG	1.19 (0.71–2.00)	0.508	1.13 (0.73–1.77)	0.581
GG	1.63 (0.94–2.81)	0.081	1.49 (0.94–2.36)	0.093
GG/AG vs AA	1.36 (0.83–2.20)	0.219	1.27 (0.84–1.93)	0.263
ABCC2 (intron)				
AA (reference)	1.00		1.00	
AG	0.70 (0.45–1.08)	0.105	0.74 (0.52–1.06)	0.097
GG	0.73 (0.43–1.25)	0.255	0.78 (0.50–1.23)	0.283
GG/AG vs AA	0.71 (0.47–1.06)	0.094	0.75 (0.54–1.05)	0.091

CI: Confidence Interval.
Abbreviation: CI = Confidence Interval.

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 are 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 (χ^2; $p > .05$) and with values in the published literature.

Pharmacogenetics for neutropenia and neurotoxicity. The results of pharmacogenetic analyses in the 517 patients for neutropenia and neurotoxicity are shown in Table 6 and Table 7, respectively. A weak association was observed between the *GST-T1/M1 null/null* genotype (presence of homozygous deletion in both genes) and neutropenia according to MGT (OR 1.99, 95%CI 1.06–3.73; p = 0.03) and TTT (HR 1.70, 95%CI 1.03–2.78; p = 0.04), when compared with wild-type genotype. As far as the *ABCC1* rs2074087 genotype and neurotoxicity is concerned, the planned statistical analyses could not be performed due to low model convergence. Regarding neurotoxicity no evidence of association was found between polymorphisms and MGT and TTT.

Other toxicities. Despite the number of events was less than required, therefore decreasing the power of the tests, the analyses 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* 3′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 analysis[6–9], but only one study in the adjuvant setting[14]. 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 chemotherapy[6]. 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[6]. 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 sequence[7]. There was a remarkable frequency of ≥grade 2

Table 7 | Pharmacogenetic associations with neurotoxicity

	Maximum Grade of Toxicity		Time To Toxicity	
Genotype	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)				
TT (reference)	1.00		1.00	
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.

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[8]. 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[8]. 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[9]. Again, this study ruled out clinically relevant associations between pharmacogenetics and clinical outcomes of patients treated with fluoropyrimidine/oxaliplatin with or without cetuximab[9].

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-

acizumab. Of the 36 assessed polymorphisms only four TS and DPYD genetic variants were associated with grade ≥ 3 global toxicity, but with modest predictive power[14].

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 data[10]. It seems that we recorded a lower frequency of grade ≥ 2 neurotoxicity and grade > 3 neutropenia than previously reported in the literature[2]. 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 practice[12–15]. In fact, our findings, together with the results of the analyses in metastatic colorectal cancer[6–9] and other malignancies[3], mitigate the positive expectations for the routine use of pharmacogenetics. It is a matter of fact that in spite of 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 dihydrophyrimidine dehydrogenase (*DPYD*) genetic variants have shown a promising level of evidence for clinical practice[16]. However, we did not study the *UGT1A1*28* genotype analysis since it is typically associated with Irinotecan pharmacokinetic and toxicity[16]. 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 frequency[16]. In fact, there were 2 heterozygous carriers in the 346 patients (0.5%) of the French trial[6], 4 heterozygous carriers in the 520 patients (0.7%) of US trial[7] and 12 heterozygous carriers in the 1088 patients (1.1%) of FOCUS trial[8].

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 studies[17,18].

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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., I.F., Fabio G. and E.R. performed statistical analysis, data interpretation and manuscript preparation. S.L., M.R., B.M., V.Z., N.P., C.M., R.L., M.T.I., 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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