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Abstract

Excision repair cross-complementation group 1 (ERCC1) is a key component in DNA repair mechanisms and may influence the tumor DNA-targeting effect of the chemotherapeutic agent oxaliplatin. Germline ERCC1 polymorphisms may alter the protein expression and published data on their predictive and prognostic value have so far been contradictory. In the present article we review available evidence on the clinical role and utility of ERCC1 polymorphisms. No consistent associations with efficacy outcomes were found, while an increased oxaliplatin-related toxicity in patients carrying minor ERCC1 variants has been repeatedly documented

Keywords	ERCC1; oxaliplatin; single nucleotide polymorphisms; colorectal cancer
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1.INTRODUCTION

ERCC1 (Excision repair cross-complementation group 1) is a key protein involved in the repair of DNA alterations, including those induced by classic chemotherapeutic agents [1]. High activity of ERCC1-related pathways has been associated in some reports to reduced efficacy as well as increased toxicity of chemotherapeutic drugs, such as platinum salts (cisplatin and oxaliplatin) [2]. These correlations, however, have not been consistently confirmed in other datasets, especially when ERCC1 protein expression was analysed [3].

In cancer biomarker discovery, the assessment of germline genetic polymorphisms that regulate the protein expression and activity (functional polymorphisms) of a candidate gene has been sometimes deemed as more robust methodology to appreciate their real prognostic/predictive value.[4,5] Germline polymorphisms of the ERCC1 gene have also been identified [6].They may influence the protein expression and have been investigated as biomarkers in cancer patients treated with oxaliplatin.

In the present article we first review ERCC1 structure and function and the evidence of ERCC1 polymorphisms assessment to predict the toxicity and efficacy of oxaliplatin. Then, we confirm results from the literature with our local experience by genotyping for ERCC1 rs3212986 and rs11615 loci consecutive patients treated between April 2015 and October 2015 with the widely used oxaliplatin-based chemotherapy adjuvant regimen FOLFOX (fluorouracil/leucovorin/oxaliplatin) in radically resected colorectal cancer patients [7].

2.ERCC1 STRUCTURE AND PHYSIOLOGICAL FUNCTION

ERCC1 is a 32 KDa protein (~300 aminoacid) that in humans operates in tandem with the nearly three-fold heavier protein XPF (xeroderma pigmentosum group F).
It is a crucial component of an important cellular machinery that presides over the genome integrity, the nucleotide excision repair (NER) system (figure 1). NER in human cells is a sophisticated process which involves dozens of proteins that assemble in a stepwise fashion. Most important NER proteins are: XP (xeroderma pigmentosum) group C (or XPC), RNAP (RNA polymerase), Cockayne syndrome group A and group B (CSA and CSB), transcription/DNA repair factor IIH (TFIIH), ERCC1, XPF (or ERCC4), XP group G (XPG), DNA polymerase, DNA ligase.
Overall, NER recognizes several types of DNA damages, and repairs them by excising the DNA oligonucleotide segments containing the damaged spot (usually a 25-30 bp segment).[8]
ERCC1 lacks of intrinsic nuclease activity, however it is indispensable for the cleavage of altered DNA sequences carried out by the eterodimer ERCC1/XPF endonuclease.
ERCC1 is constituted by a central domain, which interacts with both proteins and DNA (in particular single strand DNA, ssDNA), and by a HhH (helix–hairpin–helix) domain, which secures the interaction with both the XPF subunit (the one that contains the nuclease catalytic region) and double strand DNA (dsDNA) segments. The DNA cleavage by ERCC1/XPF is performed in 5' at junctions between ssDNA and dsDNA structures such as those seen in the DNA bubbles created during NER (figure 2). [9]
ERCC1 and XPF are usually found in the nucleus as a dimer and are reciprocally essential in terms molecular stability, so that ERCC1 defective cells are also XPF-deficient and vice versa.[10,11]
Four isoforms of ERCC1 generated by alternative splicing have been identified, namely isoform 201, 202, 203 and 204, however only isoform 202 seems to have DNA repairing potential [12].
Steps encountered in NER are now well-defined and are in common with those observed in prokaryotes (e.g. E. coli) [13]. First, proteins sensing the DNA damage are activated. Two types of complexes can be involved in this phase: the XPC-based complexes or the RNAP/CSA/CSB-based complexes. The latter are involved in the repair of lesions in the transcribed strand of active genes (Transcription-coupled repair, TCR). It has been demonstrated that lesions that occur in the transcribed strand of active genes are repaired more rapidly than those occurring in the opposite strand of the same genes [14]. XPC-based complexes are involved in all the other DNA lesions (Global genomic repair, GGR). These NER multiproteins that at first recognize the genomic insult are responsible for an initial melting of the DNA around the lesion and favour the access of a second-step multiprotein complex: the TFIIH-based complex.
TFIIH-based complex creates a 20-30 bp 'bubble' around the damaged area by separating the DNA double strand.
A third step is carried out by the ERCC1/XPF(ERCC4) complex, which is responsible for the incision in 5' of the altered strand, and by XPG that completes the incision in 3'.
A DNA polymerase complex is then activated that replaces the damaged 20-30 bp segment with the normal nucleotide sequence on the base of the intact opposite ssDNA template. Finally a ligase complex seals the newly generated repaired segment (figure 1).

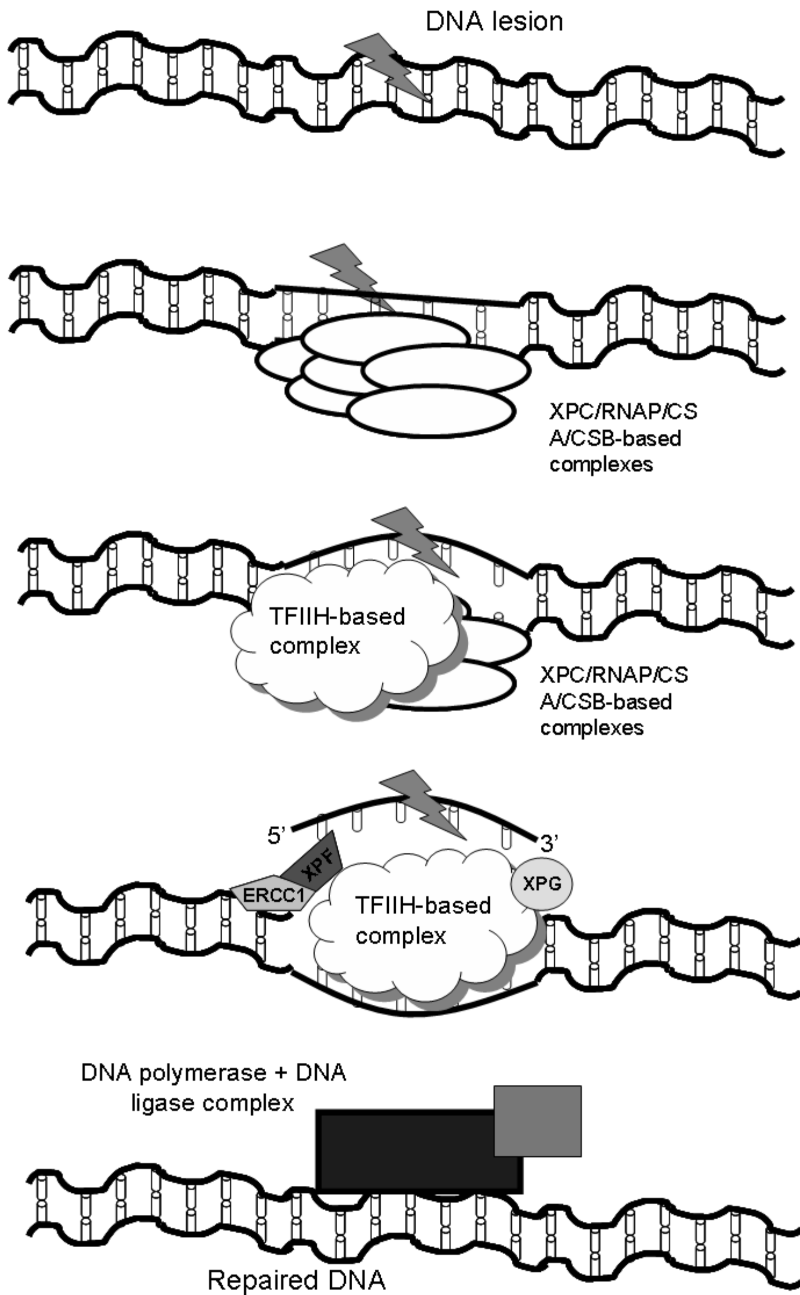

Figure 1. Schematic representation of the activity of the NER (nucleotide excision repair) system.

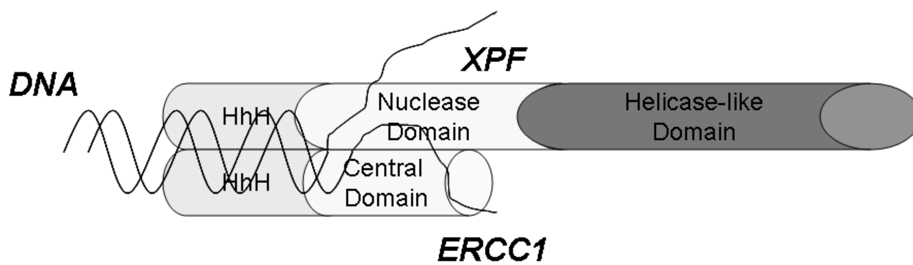

Figure 2 Structure and function of the ERCC1/XPF complex

Although it is recongnized that ERCC1 has a principal role in the NER machinery, other important functions of this protein have been identified.
The dimer ERCC1–XPF has functions in the direct repair of interstrand crosslinks and intervenes in some aspects of the homologous recombination and of the non-homologous end-joining (NHEJ) for the repair of DSB (double strand breaks). Finally, it also takes part in the Base Excision Repair (BER) and in the Telomere Length regulation. [15]
Preclinical and clinical models of defective ERCC1 function have helped clarify the physiological role of this protein and its possible use as pharmacological target.
Up to now one patient with the Cockayne syndrome, a syndrome characterized by microcephaly and delayed development (patient CS20LO), and one patient with a cerebro-oculo-facio-skeletal syndrome (patient 165TOR), have been found with inherited mutations of ERCC1 gene, suggesting that genome integrity guaranteed by this pathway is essential in human development.[16,17]
Several mouse models of mutated ERCC1 have also been created to confirm the differential role of ERCC1 domains (in particular of the central domain and the HhH domain) [18]. Many of these mutations have been proven incompatible with life and with early development. On the contrary, deletion of the first 91 amino acids of protein sequence does not affect ERCC1 function.[19,20]

3.ERCC1 AND CHEMOTHERAPY

Classic chemotherapeutic drugs, such as platinum salts, are known to execute their tumoricidal action by directly injuring DNA integrity, an effect that invariably induces cell apoptosis. DNA repair systems, such those driven by ERCC1, counteract the DNA-damaging effect of chemotherapy and therefore may be associated with drug resistance.
In 1990s first reports on the reduced efficacy of cisplatin in patients bearing tumors with high expression of ERCC1 were published.[21-23]
In a seminal work by Dabholkar et al [24], 26 patients with ovarian cancer treated with cisplatin were analyzed for the expression of ERCC1 mRNA in the fresh tumor tissue.
They found a nearly 3-fold higher expression of ERCC1 mRNA in non responding patients as compared to platinum-sensitive patients (p 0.015).
After Dabholkar's report, a number of other studies have investigated the potential effect of ERCC1 expression on cisplatin efficacy with contradictory results.
Metzger et al evaluated the combinatorial test of ERCC1 and TS (Thymidilate Synthase) mRNA expression in 36 gastric cancer patients undergoing neo-adjuvant cisplatin/fluororuracil chemotherapy[25]. They found that response rate was 85% for patients with both ERCC1 and TS mRNA below the median as compared to 20% for patients with high ERCC1 and TS (p 0.003)

Olaussen et al used immunohistochemistry to assess ERCC1 protein expression in a large number of patients with lung cancer, n=761, undergoing adjuvant chemotherapy with cisplatin within the International Adjuvant Lung Cancer Trial (IALT). ERCC1 was positive in 44% of patients. The benefit of adiuvant cisplatin, as compared to the no-chemotherapy arm, was observed only for patients with ERCC1 negative tumors (median survival 56 vs 42 months, respectively, P=0.002) Patients with ERCC1 positive tumors derived no benefit from adjuvant cisplatin-based chemotherapy (median survival 50 vs 55 months, respectively, P=0.40) [26]
More recently, however, a clear discrepancy was found in the frequency of protein expression in lung tumor tissue and doubts were raised on the technical reliability of ERCC1 immunohistochemical assessment. In fact, although using the same antibody, the mouse anti-human ERCC1 8F1, in a validation set of about 500 samples from other two randomized adjuvant trials (JBR.10 and CALGB 9633), the ERCC1-positivity rate was found to be 77%, sensibly higher than previously reported [27]. The same authors eventually demonstrated a significant sensitivity difference between the 8F1 batch originally used in 2006 and the new 8F1 used in 2011, with the predictive and prognostic role of ERCC1 no longer confirmed. Other 15 anti-ERCC1 were also tested, with none of them proven of prognostic/predictive value. Moreover, none of them was able to recognize the ERCC1 isoform with effective DNA-repairing activity (isoform 202). The authors concluded that further optimization of ERCC1 immunhistochemical assessement was needed to validate this protein as a biomarker.

Oxaliplatin is a sencond-generation platinum salt, chemically modified to reduce renal toxicity. It is widely used for the treatment of tumors of the gastrointestinal tract and is a key component of FOLFOX, the most utilized adjuvant regimen in treating colorectal cancer patients [28].
Mechanism of action of oxaliplatin is similar to that of cisplatin and ERCC1 has also been assessed as biomarker in oxaliplatin-treated patients.
Shirota et al, analysed ERCC1 mRNA expression, togheter with TS mRNA expression, in 50 colorectal cancer patients candidate for a second-line FOLFOX after progression to fluorouracil/irinotecan. Patients with high ERCC1 mRNA (number of patients: 10 out of 50), based on an investigator-defined cutoff, had shorter survival as compared to patients with low ERCC1 mRNA levels, median survival 10 vs 2 months, $p < 0.001$. However, ERCC1 did not predict for radiologic response [29].

ERCC1 immunhistochemical expression was evaluated in 1,197 colorectal cancer patients enrolled in the FOCUS trial, that compared the sequential use of active chemotherapeutic drugs (fluorouracil, irinotecan and oxaliplatin) with their concomitant use in polychemotherapy regimens (FOLFIRI or FOLFOX). ERCC1 protein expression had no predictive value for the efficacy of combination chemotherapy vs sequential monotherpy [30].

Given that the contradictory data on the role of ERCC1 as cancer biomarker is due, at least in part, to the technical difficulties in assessing ERCC1 mRNA or protein in a reproducible and robust fashion, it has been hypothesized that germline functional ERCC1 polymorphisms that ultimately influence the protein expression might be a more 'stable' methodology to assess ERCC1 influence as putative biomarker for patient outcome. ERCC1 gene has known functional polymorphisms that have been tested as predictor of oxaliplatin efficacy and toxicity.

4.ERCC1 POLYMORPHISMS AND OXALIPLATIN

Currently, no known non-synonymous SNPs in the coding region of the ERCC1 gene with potential clinical utility have been identified. There are two valuable synonymous germline ERCC1 gene polymorphisms not altering the final product aminoacid sequence/structure that have been actively studied for their predictivity in platinum-treated cancer patients: rs3212986 (also named ERCC1 C8092A or *197G>T) and rs11615 (also named ERCC1 T19007C or ERCC1 C118T or ERCC1 Asn118Asn). The former lies in the 3' untranslated region (3'-UTR), the latter is a synonymous point mutation in exon 4. Both of them are supposed to influence the stability and the nuclear levels of ERCC1 mRNA and hence the ERCC1 protein expression.[31-34] In particular 118T allele of rs11615, even if not determining a change in the amino acid (Asn 118 Asn), was hypothesized to reduce the translation efficiency of ERCC1 mRNA with consequent reduced ERCC1 protein expression [35]. However, some authors found no change in mRNA and protein levels with either genetic variant [36], and suggested that the possible clinical impact of these SNPs would reside in a genetic linkage with still unknown haplotypes of proximal genes conferring resistance/sensitivity to chemotherapeutic drugs.

Other 3 SNPs have been indentified lying in intron 3, 4, and 5: IVS3 +74C>G (rs3212948), IVS4 +86T>C (rs3212955) and IV5 +33C>A (rs3212961), respectively, but these are less commonly investigated because of their rarity.

A systematic pubmed search was performed using the following search terms: ERCC1 polymorphisms AND oxaliplatin. As for October 2016, 52 article in English were retrieved, the following sections will review published findings on the impact of ERCC1 polymorphisms on oxaliplatin efficacy and toxicity. Henceforth wild type ERCC1 alleles will be abbreviated as WT and the minor/mutated alleles as MUT.
ERCC1 polymorphisms as predictive markers for oxaliplatin-treated cancer patients have been evaluated in gastroesophageal cancer, colorectal cancer and nasopharyngeal cancer.

4.1.ERCC1 SNPs and oxaliplatin in gastroesophageal cancer

A number of reports have been published on the usefulness of ERCC1 polymorphisms in gastroesophageal cancer patients, in different disease settings: adjuvant, neo-adjunvant and metastatic setting.
Among the others, a study by Huang et al evaluated the role of ERCC1 rs11615 in 89 patients treated, after radical resection, with at least four cycles of oxaliplatin-based adjuvant chemotherapy. No significant difference was detected in terms of either relapse free survival or overall survival. In particular WT/WT patients (50% of patients) had a median survival of 29 months as compared to

26 months for patients with the WT/MUT genotype (43% of patients) or MUT/MUT genotype (7% of patients), P = 0.214. In the same study authors evaluated the effect of ERCC1 mRNA expression level in tumor tissue, which was available for 62 patients. A great inter-patient variability was found and no significant difference in ERCC1 mRNA level was observed across different rs11615 genotypes. ERCC1 mRNA, however, significantly correlated with survival, with a median survival of 29.6 months for patients with low ERCC1 mRNA as compared to 18.7 months for patients with high ERCC1 mRNA. [37]

Li et al [38] evaluated ERCC1 rs3212986 SNP (Single Nucleotide Polymorphism), together with other 23 SNPs involved in chemotherapeutic drug metabolism, in 103 gastric cancer patients undergoing neoadjuvant oxaliplatin+fluropirimidine-based chemotherapy (either FOLFOX, XELOX or SOX regimen). They found no clear association between ERCC1 rs3212986 SNP and either short-term activity (tumor regression grade) or long-term activity (overall survival) of the chemotherapy. Among all analyzed SNP, rs717620 SNP of ABCC2 gene (a membrane transporter responsible for chemotherapy drug cell efflux) was significantly associated with both overall survival and tumor regression (i.e. TGR 1a or 1b).
The authors made available the database of enrolled patients including data on ERCC1 rs3212986 SNP and respective TRG which was tested on 101 patients out of 103. Percentage of TRG 1 response was 19.5%, 12.5% and 25%, for ERCC1 rs3212986 WT/WT, WT/MUT and MUT/MUT genotype, respectively, chi square p-value 0.4902.

Goekkurt et al analyzed germline polymorphisms in 11 key genes (namely TS, MTHFR, MTR, OPRT, XPD, ERCC1, XRCC1, XPA, GSTP1, GSTT1, and GSTM1) in patients with metastatic gastroesophageal adenocarcinoma enrolled in a phase III AIO (Arbeitsgemeinschaft Internistische Onkologie) study comparing fluorouracil/leucovorin/cisplatin (FLP) to fluorouracil/leucovorin/oxaliplatin (FLO). [39]
In the patients eligible for the pharmacogenomic study (total number 134 pateints, FLO = 71 patients, FLP = 63 patients), they found polymorphisms of TS, GSTT1, OPRT and XRCC1 genes to be significantly associated with overall survival, while TS, MTR , GSTP1 and ERCC1 were associated with haematological toxicity and with neuropathy.
For ERCC1, rs11615 and rs3212986 SNPs were analysed. They were found to be in linkage disequilibrium (P<.0001) and frequency of the commonest haplotype (ERCC1 118T/8092C) was 55%. There was no significant association of the two ERCC1 SNPs with either overall survival or progression free survival. However, the response rate for ERCC1 rs3212986 WT/WT, WT/MUT and MUT/MUT genotypes were 44%, 27% and 71%, and the higher response associated with the rare MUT/MUT genotype (found in only 7 patients) was statistically significant, p 0.029.
ERCC1 rs11615 MUT/MUT genotype was associated with a non-significant superior grade 3-4 incidence of anemia, leukopenia and neutropenia as compared to WT/WT genotype (13% vs 5%, 13% vs 5%, 26% vs 10%, respectively). ERCC1 rs3212986 MUT/MUT genotype was also associated with a non-significant superior incidence of grade 3-4 anemia as compared to WT/WT (14% vs 9%, respectively). When considered as haplotype, the effect of ERCC1 SNPs on Grade 3-4 neutropenia became significant (P .042).

4.2.ERCC1 SNPs and oxaliplatin in colorectal cancer
The largest study with a pre-planned prospectively performed analysis of ERCC1 gene variants in colorectal cancer patients was the TOSCA trial [40].
The TOSCA trial randomized patients in the adjuvant setting to either six or three months of fluoropyrimidine/oxaliplatin chemotherapy (either FOLFOX-4 or CAPOX).
There were 517 patients assessable for the pharmacogenetic substudy (256 and 261 in the six and three months arm, respectively). The genes investigated were involved in drug pharmacokinetics/pharmacodynamics and had demonstrated promising results in small retrospective

reports (17 polymorphisms in 11 genes, including ERCC1, GST, MTHFR and ABCC). So far, only the impact of genotypes on toxicity has been reported.
Overall the study could not find any significant association between pharmacogenetic markers and toxicity. There was no substantial difference in grade 3-4 neutropenia for carriers of ERCC1 rs11615 MUT/MUT (17% of patients) vs WT/WT (38% of patients), odds ratio 1.17, p 0.584. Similar results were observed for incidence of peripheral neuropathy (odds ratio 0.75, p 0.356). Details in the incidence of other haematological toxicities, such as anemia, were not reported. Some drawbacks could be observed in the study. Half of the TOSCA patients (261 out of 517 patients) received, as per protocol, only three months of chemotherapy, which is not the present standard of care. Moreover, Grade 2 hematologic toxicities were neglected in the TOSCA pharmacogenetic analysis, though they were also clinically important since, according to the protocol, grade 2 toxicities imposed treatment delay, and if persistent, dose reduction.

For other four randomized trials, that included the use of oxaliplatin in colorectal cancer, the role of ERCC1 polymorphisms has been retrospectively investigated.

The FFCD 2000-05 was a phase III randomized trial in the pre-biologics era, comparing two different sequential strategies in the metastatic setting [41]. Arm A: first-line FOLFOX followed at progression by second-line FOLFIRI vs Arm B: first-line 5FU-only regimen followed at progression by second-line FOLFOX followed at progression by third-line FOLFIRI.
ERCC1 rs11615 polymorphism was tested in 174 patients receiving FOLFOX as first line (arm A) and in 130 patients receiving FOLFOX as second line (arm B). Other three polymorphisms of ERCC1 (rs3212948, rs3212955 and rs3212961) and polymorphisms of other genes of interest (DPD, TS, MTHFR, ERCC2, GSTP1, GSTM1, GSTT1, and UGT1A1) were also tested.
Overall, grade 3-4 hematologic toxicity was seen in 71 out of 183 patients (39%) carrying the ERCC1 rs11615 MUT allele (MUT/MUT or WT/MUT genotypes) and in 37 out of 121 patients (30%) with rs11615 WT/WT genotype (chi-square p-value 0.14). Differences in toxicity were similarly non significant also for the other ERCC1 SNPs. Also gastrointestinal toxicity and peripheral neuropathy were not associated with ERCC1 SNPs. However, the K751Q polymorphism of ERCC2 gene (another gene involved in NER) was significantly associated with grade 3-4 hematologic toxicity, with a significantly shorter time to hematologic toxicity occurrence for ERCC2 K751Q C/C genotype as compared to A/A and A/C genotypes. Response rate to first-line FOLFOX was fairly high (60-70%) with no difference across ERCC1 genotypes. ERCC1 rs11615 MUT allele was associated to 27% response rate to second-line FOLFOX as compared to 19% for the ERCC1 rs11615 WT/WT genotype (chi-square p value 0.35). GSTT1 and MTHFR genotypes were significantly associated with response to FOLFOX.
As for survival, in the FFCD 2000-05 trial it was not possible to demonstrate an overall survival benefit with the upfront use of FOLFOX as compared to a 5FU-only first-line regimen, median survival 16.2 vs 16.4 months [42]. However, ERCC1 rs11615 was of border-line statistical significance as predictive marker of upfront use of FOLFOX. First-line use of FOLFOX was associated with a favourable Hazard Ratio of 0.77 for carriers of the ERCC1 rs11615 MUT/MUT genotype, p 0.07 at the multivariate analysis.

The N9741 trial randomized colorectal cancer patients to three first-line options: FOLFOX, IROX or IFL. In a post-hoc analysis of 520 patients, germline DNA was tested for the genotype of 15 candidate genes. For the FOLFOX arm, 290 patients were assessed for ERCC1 rs11615 SNP. The ERCC1 rs11615 WT/WT genotype was significantly associated with lower grade 4 neutropenia as compared to the other genotypes, 13% vs 25%, respectively, p value 0.05. Febrile neutropenia was also less frequent in ERCC1 rs11615 WT/WT subjects (0% v 10%, p 0.02).
These results were not reproduced for the 107 patients treated in the IROX arm.
ERCC1 genotype was not associated with tumor response (p 0.86) and overall survival (p 0.62) in both FOLFOX-treated patients and in IROX-treated patients (p 0.07 and p 0.55, respectively).

Authors found other polymorphisms to be predictive of toxicity or outcome. In particular mutations in the GTSM1 gene were predictive of neutropenia and mutations in GSTP1 were predictive of neurotoxicity in patients treated with FOLFOX. [43]

A third randomized trial for which a pharmacogentic analysis was performed is the NORDIC-VII trial. Analysis of 17 germline SNPs in 10 key genes involved in drug biotransformation, transport and DNA repair was carried out in 519 enrolled patients. The NORDIC-VII trial tested the addition of cetuximab to a standard first-line oxaliplatin/fluorouracil-based chemotherapy (the FLOX regimen) in metastatic colorectal cancer patients mainly from north Europe regions (Norway and Sweden). ERCC1 SNPs were associated with neither survival nor toxicity. However, ERCC2 rs23840 minor allele was associated with favourable progression free survival.[44]

More recently, the pharmacogenomic analysis of patients enrolled in the COIN and COIN-B studies (that included treatment with fluoropyrimidine, oxaliplatin and cetuximab) was presented as a poster at the 2013 ASCO annual meeting.[45,46] Genes of drug metabolism, EGFR pathway or DNA repair (overall 259 allelic variants in 143 genes) were investigated in 2183 patients. In particular, data on ERCC1 genetic variants were not reported. However, PIK3R2, EXO1 and XRCC1 were polymorphic genes with significant associations with 12-week response, and DPYD was a gene with significant association with toxicity [47].

4.3.Meta-analyses in patients with cancer of the gastrointestinal tract

Several authors have tried to meta-analyze available data of the effect of ERCC1 polymorphisms on the outcome of oxaliplatin-treated colorectal and gastric cancer patients [48].
Overall, no clear association with clinical outcome (radiologic response rate and survival) has been shown.
A trend to worse outcome associated with the mutant/minor variant (the T allele) of ERCC1 rs11615 has been observed in some studies, especially on Asian population [49]. This evidence is somehow contradictory of the biologic rationale, as it is supposed that the mutant allele is associated with less expression of ERCC1, less DNA repair efficiency and hence superior oxaliplatin activity. The possible explanation could be a linkage disequilibrium in some ethnic groups of ERCC1 with other key genes remaining still unknown. In studies with Caucasian populations the opposite trend has been observed, with better outcome associated with the T allele of ERCC1 rs11615 [50].
The largest meta-analysis was conducted by Qian et al, who selected 22 studies for a total of 2,846 colorectal cancer patients. The mutant ERCC1 rs11615 T allele (homozygous or heterozygous) was associated with a significantly favourable progression free and overall survival in the Caucasian population (Hazard Ratios 0.58 and 0.38, respectively) and significantly worse survival in the Asian population (Hazard Ratios 2.49 and 2.63, respectively). No difference in terms of radiologic response was observed [51].

4.4.ERCC1 SNPs and oxaliplatin in Nasopharingeal cancer
A single report has been published on the effect of ERCC1 SNPs in oxaliplatin-treated nasopharyngeal cancer patients [52].
A phase II study enrolling 42 patients with recurrent nasopharyngeal cancer, mainly metastatic disease (60% of patients), was conducted by Ma et al. ERCC1 rs11615 was genotyped in 29 patients. Due to the small sample size, no significant difference in terms of survival was observed: median overall and progression free survival for rs11615 CC vs CT+TT, 19.6 vs 22 months and 10 vs 9.6 months, respectively, p values 0.76 and 0.82.

5. A UNIVERSITY HOSPITAL MONOCENTRIC EXPERIENCE

5.1.Patients and Methods

Between April 2015 and November 2015, all consecutive patients radically resected in our hospital (the Tor Vergata University Hospital of Rome, Italy) for a node-positive colon cancer and deemed eligible, as per standard practice, for adjuvant chemotherapy with the FOLFOX regimen were included in the present pharmacogenomic study. Enrolled patients were systematically tested for eight polymorphisms in seven key genes proved to influence outcome and toxicity of fluoropyrimidine and oxaliplatin in previous reports.
The seven selected genes and respective polymorphisms were: GSTP1 (rs1695), XRCC1 (rs25487), ERCC1 (rs3212986 and rs11615), UGT1A1 (rs8175347), CYP3A5 (rs776746), MTHFR (rs1801131) and DPYD (rs3918290).
An EDTA peripheral blood sample (3 mL) was used to extract white blood cell DNA and assess for germline polymorphisms. Genetic variants were determined by a first step target sequence amplification using standard PCR and a second step of pyrosenquencing according to standard procedure (see manufacturer instructions at www.diatechpharmacogenetics.com). In table 1 are summarized the allelic variants and the functional significance of minor alleles of analyzed polymorphisms.

Gene	*polymorphism*	*wild type*	*minor variant*	*Position in the gene and chromosome*	*Significance*
GSTP1	rs1695	A	G	exon 5, position 562	substitution of Ile with a Val at position 105 of the amino acid sequence of the protein. Diminishes GSTP1 enzyme activity
XRCC1	rs25487	A	G	exon 10, position 1316	substitution of Gln with an Arg at position 399 of the amino acid sequence of the protein.Defects in the detection of the DNA damage by XRCC1 and hence the activation of the BER pathway.
ERCC1	rs3212986	C	A	UTR-3, position 1165	reduced mRNA stability
ERCC1	rs11615	T	C	exon 4, position 500	synonimous mutation at position 118 of the protein. Reduced mRNA translation
UGT1A1	rs8175347	[TA]6	[TA]7	TATA box of the promoter, position	reduced expression
CYP3A5	rs776746	A	G	intron 3, position 12083	Splice variant with premature termination codon and non functional protein
MTHFR	rs1801131	A	C	exon 8, position 1515	substitution of glu to ala at position 429 of the protein. Reduced enzyme activity with reduced conversion of conversion of MTHF to BH4 (tetrahydrobiopterin)
DPYD	rs3918290	G	A	Intron 14, position 476002	exon-skipping mutation. Altered non-functional protein

Table 1 polymorphisms assessed in the Tor Vergata monocentric study

Patients were managed as per standard practice, in particular routine blood tests, including blood cell count were performed at each of the 12 planned cycles. Chemotherapy-related toxicities were recorded according to Common Terminology Criteria for Adverse Events (CTCAE) v4.0 (https://www.nih.gov/).
Study endpoint was to correlate genotypes with early changes of haematological variables, in particular with early decline in white blood cell count, neutrophil count, lymphocyte count, haemoglobin concentration and platelet count.
Given the small sample size included so far, no formal analysis of CTCAE toxicity and patient survival has been conducted. The effect of genotypes on haematological variables changes in the present pilot study has been considered as a potential useful marker of subsequent more profound myelotoxicity over the course of the adjuvant treatment.
Univariate and Multivariate multiple regression analyses, setting as dependent variable the percentage change of haematological variables after two cycles of chemotherapy and as independent variable the analyzed genotypes, were performed to screen for potentially valuable biomarkers of toxicity.
The value of 0, 1 or 2 for each genotype was assigned depending on the number of minor alleles found at the analysis. Polimorphisms found to be significantly associated with haematological variable changes at univariate analysis were selected to be run at the multivariate analysis.
Mann-Whitney test were used to confirm statistically significant differences found at the regression analysis.

The present research has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. Informed consent from participating patients was obtained before study entry. The privacy rights of human subjects have been observed.

5.2.Results

Sixteen patients were enrolled in the study. In the table 2 are summarized patients' characteristics. At univariate analysis ERCC1 polymorphisms were associated with the decline of all type of analyzed blood cells but platelets (table 3). UGT1A1 and GSTP1 were also associated with Leucocyte decline at both univariate and multivariate regression analysis, however at the multivariate analysis ERCC1 rs11615 was the polymorphism with the highest statistical significance (p values 0.002 vs 0.02 vs 0.03, respectively). The DPYD polymorphism was also associated with lymphocyte count changes together with ERCC1 rs3212986. However, also in this case, the ERCC1 polymorphism displayed the highest statistical significance (p value 0.002 vs 0.004, respectively).

According to the subsequent Mann-Whitney analysis, ERCC1 rs3212986 was significantly associated with percentage change in haemoglobin concentration after 2 cycles of FOLFOX. Patients heterozygous for rs3212986 (WT/MUT) experienced a median reduction of Hb concentration of 4%, which was significantly different from the Hb change of patients WT homozygous who experienced an increase of Hb concentration by 7%, p 0.0011 (figure 3). No other significant associations were detected with the Mann-Whitney tests.

Out of 16 enrolled patients, three patients experienced significant treatment delay, dose reduction or even early discontinuation because of grade 3-4 anemia (HB < 8gr/dL). All those three patients were ERCC1 rs3212986 eterozygous. Because of the immature follow-up and the limited sample size, no analysis on outcome measures (disease free and overall survival) or comprehensive toxicity data have been performed.

Number of patiens = 16			
Characteristic	Value		
sex, male:female	12:6		
Age	61years (range 52-75)		
hematological variables	*Baseline*	*% change after 2 cycles*	
Hb	12.3 g/dL	+3%	
WBC	5.7	-13%	
Neutrophil	3.5	-29%	
Lymphocyte	1.8	+5%	
Platelets	261	-13%	
Genotypes	*wt/wt*	*wt/mut*	*mut/mut*
GSTP1 A313G	4	10	2
XRCC1 G28152A	10	6	0
ERCC1 C8092A rs3212986	10	6	0
ERCC1 C118T rs11615	6	8	2
UGT1A1*28	2	12	2
CYP3A5*3	12	4	0
MTHFR A1298C rs1801131	4	8	4
DPYD IVS14+1G>A rs3918290	12	4	0

Table 2 Tor Vergata patients' characteristics, Hb: haemoglobin; WBC: white blood cells; GSTP1: glutathione S-transferase P1; XRCC: X-ray repair cross complementing 1; ERCC1: Excision repair cross-complementation group 1; UGT1A1 UDP glucuronosyl transferase family 1 member A1; CYP3A5: cytochrome P450 family 3 subfamily A member 5; MTHFR: methylene-tetra-hydro-folate reductase; DPYD dihydro-pyrimidine dehydrogenase

		UNIVARIATE					MULTIVARIATE				
Dependent variable	*Independent variables*	*Coefficient*	*Std. Error*	*rpartial*	*t*	*P*	*Coefficient*	*Std. Error*	*rpartial*	*t*	*P*
WBC 1-month-change	CYP3A5	-0,34	0,1805	-0,4497	-1,884	0,0806					
	DPYD	-0,1533	0,1979	-0,2028	-0,775	0,4513					
	ERCC1_C8092A	0,02933	0,1806	0,04337	0,162	0,8733					
	ERCC1_T19007C	***-0,2657***	***0,1116***	***-0,5368***	***-2,381***	***0,032***	*-0,2811*	*0,07272*	*-0,7447*	*-3,865*	*0,0022*
	GSTP1	***0,2835***	***0,1248***	***0,519***	***2,272***	***0,0394***	*0,2148*	*0,08828*	*0,5749*	*2,434*	*0,0315*
	MTHFR	0,025	0,1236	0,05399	0,202	0,8426					
	UGT1A1	***0,375***	***0,1435***	***0,5727***	***2,614***	***0,0204***	*0,2676*	*0,1055*	*0,5907*	*2,536*	*0,0261*
	XRCC1	0,328	0,1581	0,485	2,075	0,0569					
Lymphocyte 1-month-change	CYP3A5	-0,1283	0,1659	-0,2025	-0,774	0,4521					
	DPYD	***0,3917***	***0,1332***	***0,6179***	***2,94***	***0,0107***	*0,3382*	*0,09818*	*0,6908*	*3,445*	*0,0044*
	ERCC1_C8092A	***0,366***	***0,1157***	***0,6456***	***3,163***	***0,0069***	*0,3209*	*0,08781*	*0,7119*	*3,654*	*0,0029*
	ERCC1_T19007C	0,085	0,1086	0,2048	0,783	0,4467					
	GSTP1	0,1291	0,1174	0,282	1,1	0,2899					
	MTHFR	0,055	0,1027	0,1417	0,536	0,6007					
	UGT1A1	0,015	0,1467	0,02732	0,102	0,92					
	XRCC1	-0,05533	0,1508	-0,0976	-0,367	0,7192					
Neutrophil 1-month-change	CYP3A5	-0,3867	0,2167	-0,4305	-1,785	0,096					
	DPYD	-0,26	0,2298	-0,2895	-1,132	0,2768					
	ERCC1_C8092A	0,09067	0,2133	0,1129	0,425	0,6773					
	ERCC1_T19007C	***-0,4343***	***0,1059***	***-0,7386***	***-4,1***	***0,0011***					
	GSTP1	0,1843	0,1662	0,2842	1,109	0,2861					
	MTHFR	0,08	0,1454	0,1455	0,55	0,5909					
	UGT1A1	0,345	0,1863	0,4435	1,852	0,0853					
	XRCC1	0,2773	0,2015	0,3452	1,376	0,1903					
Hb 1-month-change	CYP3A5	0,07833	0,04874	0,3947	1,607	0,1303					
	DPYD	-0,075	0,04911	-0,3779	-1,527	0,149					
	ERCC1_C8092A	***-0,13***	***0,03231***	***-0,7323***	***-4,024***	***0,0013***					
	ERCC1_T19007C	0,03929	0,0331	0,3024	1,187	0,255					
	GSTP1	0,03174	0,03736	0,2214	0,849	0,4099					
	MTHFR	0,015	0,03223	0,1234	0,465	0,6488					
	UGT1A1	0,075	0,04133	0,4363	1,814	0,0911					
	XRCC1	0,08333	0,04189	0,4694	1,989	0,0666					
PLT 1-month-change	CYP3A5	-0,4117	0,2955	-0,349	-1,393	0,1853					
	DPYD	-0,3183	0,3036	-0,2698	-1,049	0,3121					
	ERCC1_C8092A	0,2953	0,2707	0,2799	1,091	0,2937					
	ERCC1_T19007C	-0,2821	0,1921	-0,3653	-1,468	0,1641					
	GSTP1	0,3335	0,2096	0,3914	1,591	0,1339					
	MTHFR	-0,145	0,1891	-0,2007	-0,767	0,456					
	UGT1A1	0,1	0,2717	0,09788	0,368	0,7184					
	XRCC1	0,1567	0,2789	0,1485	0,562	0,5831					

TABLE 3 Univariate and Multivariate regression analysis. Hb: haemoglobin; WBC: white blood cells; PLT: platelets; GSTP1: glutathione S-transferase P1; XRCC: X-ray repair cross complementing 1; ERCC1: Excision repair cross-complementation group 1; UGT1A1 UDP glucuronosyl transferase family 1 member A1; CYP3A5: cytochrome P450 family 3 subfamily A member 5; MTHFR: methylene-tetra-hydro-folate reductase; DPYD dihydro-pyrimidine dehydrogenase

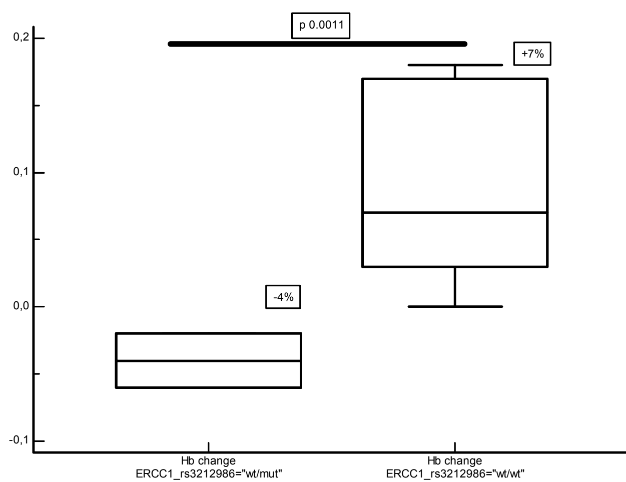

Figure 3 Mann-Whitney test comparing the percentage change of haemoglobin concentration after 2 cycles of chemotherapy by rs3212986 genotype. Hb: haemoglobin; ERCC1: Excision repair cross-complementation group 1; wt: wild type; mut: minor/mutant allele.

6.CONCLUSIONS

In the present article we reviewed the evidence on the role of ERCC1 polymorphisms in the prediction of outcome and toxicity of patients treated with oxaliplatin. Moreover, in the absence of a virtual perfect trial (what we call a 'sliding doors' trial) we assessed the clinical utility of systematically genotyping key genes in our local practice.

In clinical practice the primacy of two medical goals has to be acknowledged, namely overall survival and quality of life. Physicians, with their treatment and management, must aim, above all, at helping patients to live as long and as well as possible. The tools with the highest methodological quality to find the best way to manage patients are randomized clinical trials on patients with homogeneous characteristics (i.e. same distribution of gender, age and other important characteristics).
But, how do we define homogeneity thoroughly in a patient cohort? The ideal methodological setting would be to have two 'identical' groups of subjects to whom two (or more) distinct types of protocol could be administered, so that the net management effect is 'isolated'. The co-primary endpoints must be overall survival and quality of life and the only variable influencing the outcome should be the administered therapy/protocol itself.
In this regard, the perfect trial is a 'sliding doors' trial, to quote the popular 1998 Gwyneth Paltrow movie, where the same character lives two parallel lives, thanks to a sort of magic. In a 'sliding doors' trial every single patient would be made to live two parallel disease histories, with two alternative management strategies, to assess which one has the best outcome for 'that individual patient'.
In the absence of magic, a high degree of patient cohort homogeneity is the best approximation to a perfect sliding doors trial.
In this context, how does the 'monocentric' nature of a study contribute to cohort homogeneity?. Although a major flaw of monocentric studies is the absence of external validation, they guarantee 'geographical' homogeneity, that is to say more homogeneous genetic backgrounds, routine practices and environmental factors.
Therefore, monocentric studies that are carried out in order to confirm locally, in a limited geographical area, the results of large international trials can be regarded as important tools to measure inter-'clinical environment' heterogeneity, including differences in clinical practices, in patient characteristics or the availability of medical facilities. A similar utility of monocentric studies is that they enable us to validate in a given clinical 'habitat' contradictory results that have been published on a specific topic.
Hypothetically an 'x' correction factor measuring the distance between the perfect sliding doors trial and the degree of patient homogeneity of a published clinical trial, and an 'y' correction factor measuring the distance between the patient characteristics of a published trial and the characteristics of an individual patient that is being treated in a given local area, could be introduced into the decision making algorithms (for example, in online software for adjuvant chemotherapy) (figure 4).

The present article is an example of the utility of confirming in a single clinical center contradictory results previously published on important clinical issues.

Published data on ERCC1 polymorphisms and efficacy of oxaliplatin have been inconsistent. Overall, ERCC1 SNPs cannot be considered as standard marker of survival or radiologic response in oxaliplatin-treated patients. The functional effect of ERCC1 SNPs on the protein expression has also been questioned, even though in vitro experiments cannot always reproduce the complex mechanisms regulating the transcription and translation of human genes.

Meta-analyses including more than 2000 patients, specifically looking at radiological response, progression free survival and overall survival, demonstrated in some subgroups a trend in favour of better outcome for the wild type alleles (especially in asian population), while the opposite has been observed in other patient populations (e.g. Caucasian patients), thus confirming the inconsistency of these findings.

However, the effect of ERCC1 polymorphisms on toxicity has been under-reported, and when analysed a more consistent effect could be observed, in the sense that the presence of mutated variants was associated to an increased risk of common chemotherapy-related side effects, possibly because of the reduced DNA repair efficiency and increased toxic effect of platinum adducts in normal tissues and cells, such as hematopoietic cells.

No meta-analyses has been so far conducted on this issue, and comprehensive safety data including also minor toxicities (i.e. grade 2 toxicity), which still can significantly impair full treatment delivery, are clearly lacking in the literature.

Such a lacking aspect, prompted us to systematically assess in our Unit the effect of ERCC1 (and other key genes) polymorphisms in a homogenous group of patients treated with oxaliplatin, specifically looking at the association with changes in haematological variables.

Even though in only 16 patients, we could confirm a predictive value of ERCC1 rs3212986 for the risk of haemoglobin decline and anemia during adjuvant FOLFOX. In our 'real life' research, rs3212986 heterozygous patients treated with standard adjuvant chemotherapy seem to be at increased risk of treatment delay and early discontinuation because of substantial haemoglobin reduction (three patients with a rs3212986 MUT allele out of 16 had to discontinue/delay the treatment because of chemotherapy-induced anemia). It is well known that maintaining dose intensity in adjuvant treatment is crucial for reducing the risk of disease relapse and hence overall survival.[53,54] Moreover, chemotherapy toxicity is a key determinant of quality of life in cancer patients [55].
If this finding is confirmed with further patient enrolment, it will be likely that a practice changing amendment is introduced in our treatment policy, and rs3212986 heterozygous patients will start chemotherapy with upfront reduced doses of oxaliplatin.
In fact, in this case of ERCC1 genotyping, a 'sliding doors' trial would be a trial when the very same patient is treated either with an unknown genotype at standard chemotherapy doses or with an upfront dose reduction according to a known pre-chemotherapy ERCC1 genotype showing the risk of anemia, to see if a pharmacogenetic-oriented dose adjustment will reduce the risk of adjuvant chemotherapy delay or early discontinuation and eventually influence survival.
In conclusion, even though we acknowledge that these results have to be confirmed after an adequate sample size enhancement, we think that confirmatory monocentric studies are always desirable, in the absence of an ideal 'sliding doors' trial.

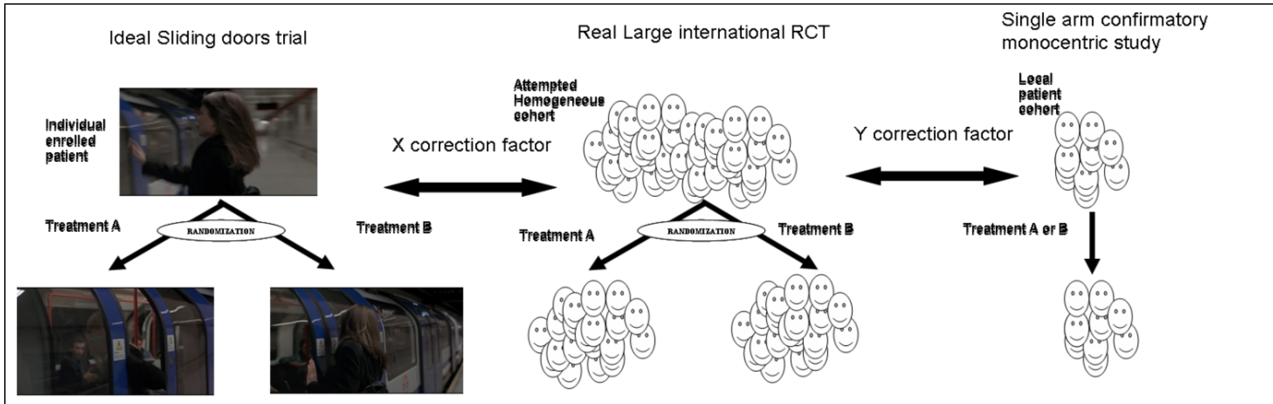

Figure 4 schematic representation of differences between a hypothetical 'sliding doors' trial, a large randomized clinical trial (RCT) and monocentric single arm study, with associated correction factors to be introduced in the decision making process.

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- A review of the literature on the effect of ERCC1 polymorphisms on oxaliplatin efficacy and safety is proposed
- Available evidence does not support ERCC1 genotyping to predict survival or radiologic response in oxaliplatin-treated patients
- ERCC1 minor alleles of rs3212986 and rs11615 polymorphisms have been consistently associated with increased oxaliplatin toxicity and we confirmed this finding in our clinical centre

ERCC1 polymorphisms and oxaliplatin. A review of the literature and a monocentric experience in the absence of a 'sliding doors' trial.

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ABSTRACT
Excision repair cross-complementation group 1 (ERCC1) is a key component in DNA repair mechanisms and may influence the tumor DNA-targeting effect of the chemotherapeutic agent oxaliplatin. Germline ERCC1 polymorphisms may alter the protein expression and published data on their predictive and prognostic value have so far been contradictory. In the present article we review available evidence on the clinical role and utility of ERCC1 polymorphisms and, in the absence of a 'perfect' trial, what we call the 'sliding doors' trial, we present the data of ERCC1 genotyping in our local patient population finding a useful predictive value for oxaliplatin-induced risk of anemia.

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