# Pharmacokinetic and Metabolism Determinants of Fluoropyrimidines and Oxaliplatin Activity in Treatment of Colorectal Patients

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**Abstract:** Fluoropyrimidines and oxaliplatin continued to be the mainstay of therapeutic regimens in the treatment of colorectal cancer (CRC). For this reason, pharmacokinetic and metabolism of these drugs were analyzed and the identification of accurate and validated predictive, prognostic and toxicity markers became necessary to develop an effective therapy adapted to the patient's molecular profile, while minimizing life-threatening toxicities. In this review, we discuss literature data, defining predictive and prognostic markers actually identified in the treatment of CRC. We analyzed predictive markers of fluoropyrimidines effectiveness, principally for 5-Fluorouracil (5-FU) and also for oral fluoropyrimidines, as thymidylate Synthase (TS), dihydropyrimidine dehydrogenase (DPD), orotate phosphoribosyl transferase (OPRT), methylenetetrahydrofolate reductase (MTHFR), deoxyuridine triphosphate nucleotidohydrolase (dUTPase), microsatellite instability. DPD represent the more studied 5-FU toxicity marker, followed by TS and OPRT. Oxaliplatin effectiveness is principally regulated by nucleotide excision repair (NER) pathway, including excision repair cross-complementation group 1 (ERCC1), X-ray cross-complementing group 1 (XRCC1) and xeroderma pigmentosum group D (XDP). The major oxaliplatin toxicity marker is represented by glutathione S-transferase (GST). All these results are based principally on retrospective studies. The future challenge became to validate molecular markers and their association with clinical outcomes in prospective trials, refining technologic platforms and bioinformatics to accommodate the complexity of the multifaceted molecular map that may determine outcome, and determining CRC patients most likely to benefit from therapeutic interventions tailored specifically for them.

**Keywords:** 5-Fluorouracil, dihydropyrimidine dehydrogenase, glutathione S-transferase, nucleotide excision repair, oxaliplatin, predictive marker, thymidylate synthase, toxicity marker.

## 1. INTRODUCTION

From several years research on a global scale has attempted to define subsets of biochemical markers that may be useful predictors of response to treatment, in particular evaluating clinical response, toxicity, and time to disease progression, prognostic markers to determine the aggressiveness of the disease and the likelihood of recurrence after surgery. Also pharmacogenomics is emerging as an increasingly useful molecular tool to investigate the drugs efficacy by analysis of patient variables (i.e. genetic polymorphisms, metabolizing enzymes, transporters). For this reason, the identification of accurate and validated predictive and prognostic markers will provide the clinician with the knowledge and the means of tailoring a targeted and effective therapy to the patient's molecular profile while minimizing life-threatening toxicities.

## 2. FLUOROPYRIMIDINES: 5-FLUOROURACIL (5-FU)

Since its introduction in 1957, the fluoropyrimidine 5-FU has been the mainstay of therapeutic regimens in the treatment of CRC. Several steps are present in its complex pharmacokinetic. Upon entry to the cell, 5-FU is converted to its active metabolite, 5-fluoro-2-deoxyuridine monophosphate (FdUMP), whose primary mechanism of action is inhibition of thymidylate synthase (TS) by formation of a ternary complex. This blocks the de novo synthesis of thymidine, essential component for DNA synthesis, and initiates DNA damage (Fig. **1**) [1].

### 2.1. Predictive Markers of 5-FU Effectiveness

Adjuvant treatment with 5-FU has been shown to improve the absolute survival rate of stage III colon carcinoma patients by ~10%–15%. Increasing evidence indicates that stage II CRC patients also gain benefit from 5-FU-based therapies [2]. Because only a relatively small proportion of patients appear to benefit from this treatment, considerable effort has been directed towards finding biomarkers that can accurately predict tumour response [1]. These have included initially molecular factors such as microsatellite instability [3] and chromosomal deletions [4], subsequently described.

There is, however, currently insufficient evidence to justify the incorporation of these or any other candidate predictive markers into routine clinical practice for the selection of CRC patients to receive 5-FU. In this condition, the levels of TS expression became a strong candidate marker for the prediction of 5-FU response [5].

#### *2.1.1. Thymidylate Synthase (TS) in CRC patients treated with 5-FU*

A significant number of studies initially have correlated intratumoral TS levels with response to fluoropyrimidine-based therapy [6-8]. The study by Salonga *et al*. demonstrated for the first time that patients with CRC who responded to 5-FU therapy could be segregated by analysis of three genes in the 5-FU pathway. Those patients with low expression levels of TS, thymidine phosphorylase (TP), and dihydropyrimidine dehydrogenase (DPD) (these last two factors implicated also in 5-FU metabolism) all responded, and those with elevated expression in at least one of the three genes did not [8]. It is unclear whether this occurs because such tumours have better prognosis or they are more sensitive to 5-FU treatment. The success of these preliminary studies conducted to the analysis by Kornmann M *et al*. [9]. In this Germany study, an mRNA quantization technique using real-time RT-PCR and determining TS and DPD levels from paraffin-embedded primary CRC tissue sections was used. Patients with colon cancer UICC stage IIb (T4pN0) and III (pT1–4pN positive) and patients with rectal cancer UICC stage II (pT3–4pN0) and III (pT1–4pN positive) receiving adjuvant 5-

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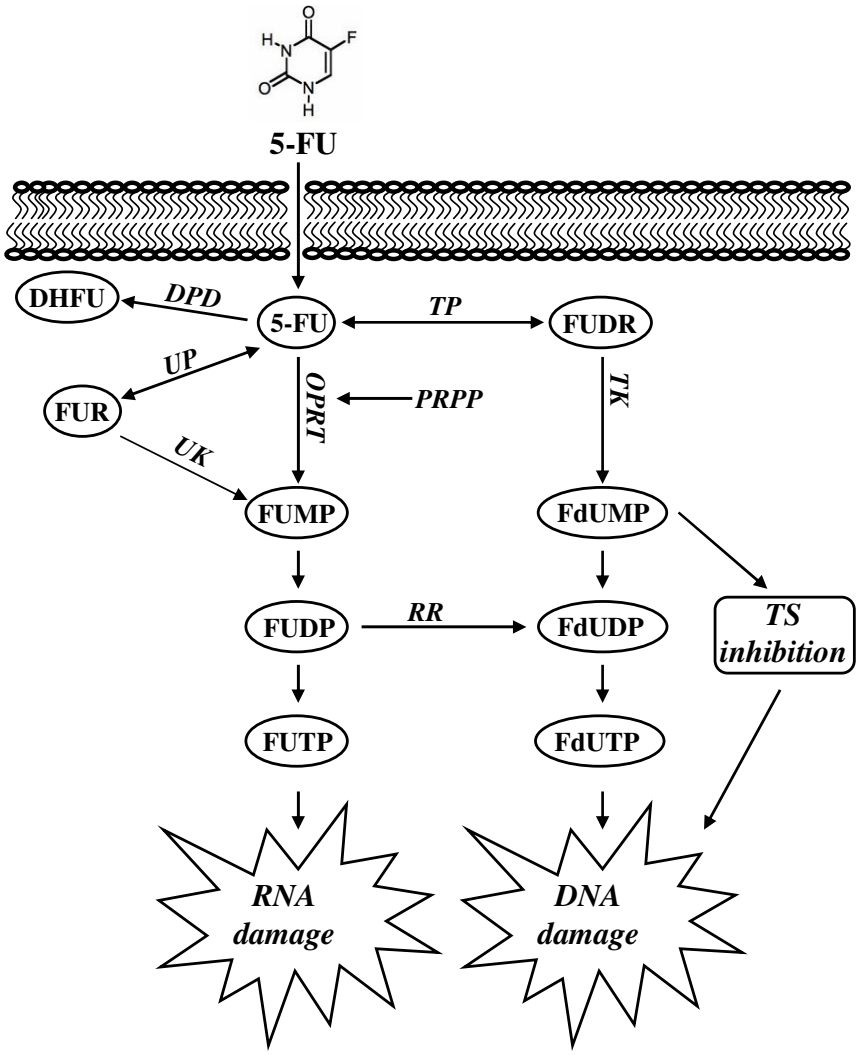


**Fig. (1).** Mechanisms of action of 5-FU.

FU-based chemotherapy who participated in FOGT-1 colon (i.v. 5-FU 450 mg/m2 in 60–120 min weekly) in combination with oral levamisol (3 x 50 mg/day for 3 days every 14 days for a total of 1 year) or FOGT-2 rectal cancer (FOGT-1 plus postoperative radiation of the pelvis with 50.4 Gy) trial were enrolled. The results explain that longer disease free survival was correlated with low levels of DPD and surprisingly with high levels of TS, while the TS and DPD levels were identified as not important prognostic factors for tumor recurrence. In conclusion, TS and DPD play key roles in 5-FU resistance, and intratumoral TS mRNA level for the first time appeared to be a prognostic marker for disease-specific survival. Several other larger studies, as described by Edler D *et al*, have concluded that elevated TS levels are more likely to benefit from 5-FU–based chemotherapy [10].

While Salonga *et al*. used tumour response and disease-specific survival evaluating mRNA levels of predictive factors**,** Soong R *et al*. analyzed the associations between TS, DPD and TP protein levels, determined by tissue microarrays and immunohistochemistry, and survival in 945 CRC patients according to treatment status [11]. The results showed that low TS and DPD expression were associated with worse prognosis in stage II (HR 1.69 and 1.92, respectively) and stage III CRC patients treated by surgery alone (HR 1.39 and HR 1.49, respectively). No correlation was demonstrated with TP levels. Low TS, DPD and TP were associated with trends for better outcome in stage III patients treated with 5-FU (HR 0.81, 0.70 and 0.66, respectively]. These results confirmed that low TS and DPD expression were prognostic for worse outcome in CRC patients treated by surgery alone, whereas low TS, DPD and TP expression are prognostic for better outcome in patients treated with 5-FU chemotherapy. On these basis, we can presuppose that the results proven by Soong R and colleagues can provide an indirect evidence that low expression of all three proteins are predictive of good response to 5-FU chemotherapy. A possible explanation for these conflicting observations is that TS levels detected by immunohistochemistry (IHC) can demonstrate significant variation as a result of antibody specificity and tissue handling/preparation. For this reason, TS became the 5-FU pathway that is more studied around all the last 15 years. Several subsequently studies have analyzed is role as prognostic and predictive factor for CRC treated with 5-FU and derivates.

The greater meta-analysis was performed by Popat *et al*. [12], which analyzed 20 studies of over 3,000 patients, stratifying overall survival and/or progression-free survival in CRC patients by TS expression status. The principal examined outcome measure was the Hazard Ratio (HR). Thirteen studies of 30 investigated outcome in a total of 887 cases with advanced CRC, and seven studies investigated outcome in a total of 2.610 patients with localized CRC. A number of methods were used both to assess TS expression and to assign TS status. Methods used to determine TS expression and assign expression status were IHC, reverse transcriptase polymerase chain reaction (RTPCR), and enzyme assay. The median proportion of cases expressing high TS levels was similar in both advanced

and adjuvant settings; 53% (range, 14%- 80%) and 50% (range, 19%-77%, respectively. The combined HR estimate for overall survival (OS) was 1.74 (95% CI, 1.34 to 2.26) and 1.35 (95% CI, 1.07 to 1.80) in the advanced and adjuvant settings, respectively, but there was evidence of important heterogeneity among these data. Restricting analysis to the three eligible studies in which patients received surgery only, the pooled HRs were 1.92 (95% CI, 1.12 to 3.32) and 1.90 (95% CI, 1.35 to 2.67) for OS and Progression Free Survival (PFS), respectively. On the other hand, evaluating studies in which patients received surgery and adjuvant 5-FU chemotherapy, the pooled HRs for OS and PFS were 0.93 (95% CI, 0.69 to 1.24) and 1.00 (95% CI, 0.92 to 1.08), respectively. However, these results, for the authors, should be interpreted with caution due to the small number of contributing studies. There was the evidence of many publication biases, including heterogeneity between studies (sample sizes varied greatly), unclear optimal method of assessing TS expression and many number of differing scoring methods used. These results can demonstrate that in patients with both local and advanced CRC, TS expression is predictive for survival. TS retains its prognostic significance whether expression is measured from metastasis or primary CRC. In patients treated with both surgery and adjuvant 5-FU, TS expression does not seem to predict outcome. The final result of the study is that CRC patients with elevated TS expression demonstrated poorer overall survival compared with those whose tumors expressed low levels [12].

We are agree with the authors of this metanalysis, when they suggest that several small and early studies report a positive relationship between high TS levels and poor prognosis, while the corresponding subsequent larger studies fail to replicate these data. However, it is actually accepted the hypothesis that variation in TS expression is a determinant of prognosis and it is an attractive mechanism for explaining any inter-individual variation in clinical outcome after fluoropyrimidines therapy.

After these evidences, proving the determinant and critical role of regulation of TS expression for the efficacy of fluoropyrimidines, therefore identifying genetic alterations that regulate TS gene expression could became crucial for developing predictive markers. Functional genomic polymorphisms have been demonstrated, interesting within the 5′ region and the 3′-UTR of the TS gene [13]. The 5′ polymorphic variant results in the majority of patients possessing a series of 28bp repeats termed TS2R (2 repeats) or TS3R (3 repeats). TS2R is associated with lower TS enzyme expression *in vitro* and *in vivo* and has been associated with increased clinical benefit in fluoropyrimidine treatment. Conversely, the 3R/3R genotype has been associated with increased TS mRNA expression, a significantly reduced response rate, and increased toxicity during 5-FU therapy [14]. Subsequently, additional polymorphisms within this 28bp repeat have been identified. Mandola *et al* described a G–C nucleotide transition located only in the TS3R allele, which disrupts transacting factors from binding a functional E-Box element and leads to reduced TS mRNA expression [15].

All these experiences show the possibility that an elevated level of TS alone is unlikely to be sufficient to predict response to 5-FU. A subset of patients with low TS expression do not respond to 5-FU–based therapy and may have additional mechanisms of resistance explained by modulation of other 5-FU–metabolizing enzymes, such as dihydropyrimidine dehydrogenase (DPD), methylenetetrahydrofolate reductase (MTHFR), orotate phosphoribosyl transferase (OPRT), and deoxyuridine triphosphate nucleotidohydrolase (dUTPase).

#### *2.1.2. Dihydropyrimidine Dehydrogenase (DPD) in CRC Patients Treated with 5-FU*

DPD catalyses the rate-limiting step in the catabolism of fluoropyrimidines with more than 80% of 5-FU administered degraded by DPD in the liver. Variation in expression levels of DPD therefore has a direct effect on the bioavailability of 5-FU and its role, principally in the toxicity of therapy, as described subsequently. However, DPD has demonstrated also a prognostic significance in several studies. It was demonstrated that patients with DPD low expression had longer disease-free recurrence and increased survival than those with high expression [16, 17]. However, its role in predicting response to 5-FU is complicated by widely variable expression levels in tumor and normal tissues, as expressed in the study of van Kuilenburg *et al*. [18].

#### *2.1.3. Orotate Phosphoribosyl Transferase (OPRT) in CRC Patients Treated with 5-FU*

Additional enzymes involved in fluoropyrimidine metabolism have been implicated in determining drug sensitivity, including OPRT, the enzyme catalyzing the reduction of FUDP to the active metabolite FdUMP which irreversibly binds to TS. Ichikawa W *et al*. in their study directly linked OPRT expression to 5-FU sensitivity, concluding that increased OPRT mRNA expression (P=.0008) and high OPRT/DPD mRNA ratio (P = .003) predicted response to fluoropyrimidine- based therapy in a small cohort of patients with metastatic CRC [19]. In a more recent revaluation, Ishikawa *et al*. investigated TS, DPD and OPRT activities and their association with clinicopathological variables in cancer tissue and adjacent normal tissue from 40 CRC patients who had undergone curative surgery and were orally administered adjuvant tegafur/uracil (UFT) chemotherapy. While there was no evidence of clear relationship between pathological findings and TS or DPD activity, the authors concluded that OPRT activity was significantly lower in tumors with lymph node metastasis than in tumors lacking lymph node metastasis (with P<0.01). Postoperative survival was significantly better in the groups with low TS activity and/or high OPRT activity [20].

This result open the possibility that OPRT activity levels in tumor tissue may be important prognostic factors for survival in Dukes' B and C CRC with radical resection and adjuvant chemotherapy with UFT. Really, these conclusions are drawn from a limited retrospective study. The limit of this study can also be explained by bias patients and treatment data. Patients enrolled were of Eastern and Asiatic Population (with their genetic variability) and the treatment schedule used (UFT) was different from European and Western formulation.

#### *2.1.4. Methylenetetrahydrofolate Reductase (MTHFR) in CRC Patients Treated with 5-FU*

Only few data are available about the role of others fluoropyrimidines pathways correlated with 5-FU response. MTHFR is the enzymatic determinant of intracellular folate levels. A polymorphic region of the MTHFR gene termed 677T results in decreased enzymatic activity and subsequent increased levels of 5,10 methylenetetrahydrofolate and is associated with a significantly improved response to 5-FU [21]. More recently, Fernández-Peralta AM *et al*. analyze the relationship of *MTHFR* C677T and A1298C polymorphisms with biological, clinicopathological, genetic and epigenetic features of tumors, and the patient outcome after treatment with 5-FU-based chemotherapy. One hundred and forty-three Spanish sporadic CRC and 103 controls were analyzed by polymerase chain reaction/restriction fragment length polymorphism and sequencing. The C677T polymorphism has protective effect on CRC showing TT genotype with an odds ratio of 0.06 and a CT of 0.51, while MTHFR A1298C polymorphism is not associated with CRC risk. Patients with 1298CC and AC genotypes exhibit worse survival than those with the wild genotype (p = 0.001), whereas C677T genotypes do not affect patient survival (p = 0.92). MTHFR 677T allele carriers responded better to 5-FU-based chemotherapy than patients with the wild CC genotype (p = 0.05), and the variant C allele of A1298C affects negatively the response to 5-FU-based chemotherapy (p = 0.009) [22].

On these results, principally the variant allele of the C677T has a protective effect on CRC development, showing a possible role

for predictive response factor. Further studies also in this application are necessary.

### 2.2. Deoxyuridine Triphosphate Nucleotidohydrolase (dUTPase).

**dUTPase** is a key regulator of intracellular dUTP pools and has been shown to be an important determinant of cytotoxicity mediated by TS inhibitors by regulation of dUTP pools and prevention of detrimental uracil misincorporation into genomic DNA in the absence of thymine. Only a small retrospective study determined that elevated expression of dUTPase was associated with resistance to 5-FU, shorter time to progression, and reduced overall survival. In the last years this markers has been examined for its correlation with worse prognosis in CRC [23]. However, no other studies explain the exact role of this pathway in 5-FU therapy as predictive or prognostic factor. Kawahara A *et al*. analyzed 55 patients with colorectal cancer, 20 without metastasis and the other 35 with distant metastasis with immunochemistry evaluation for dUTPase levels. The numbers of metastatic and non metastatic patients with expression of dUTPase (54% versus 15%, respectively; p=0.005) were significantly different in those with primary tumours with metastasis compared with those with non-metastasis. It is suggested that dUTPase may be a predictive biomarker for the metastatic potential of colorectal cancer.[24]. Further evaluations must confirm these data.

### 2.3. Microsatellite Instability: Background from the Literature

As previously described, microsatellite instability is the biomarker that could accurately predict tumour response in colorectal cancer treated with fluoropyrimidines. The majority of colorectal cancers display aneuploidy appearing as chromosomal anomalies, whereas the remainder that constitutes 15–20% of these cancers is characterized by microsatellite instability (MSI) [3, 25, 26]. Microsatellites are DNA sequences in which a short motif of 1–5 nucleotides is tandem repeated ten to hundred times. Microsatellites are prone to mutation during replication due to transient split of the two helical strands and slippage of the DNA polymerase complex at reannealing, which generate an insertion or deletion loop depending on slippage direction. Unless such mismatch is corrected, the loss or gain of repeated units on the daughter strand results in length variation termed MSI [26]. To reduce this instability, the activity of mismatch repair (MMR) started and it is performed by the proteins hMSH2 heterodimerized with hMSH6. Upon assemblage, this complex interacts with another heterodimeric complex, composed of hMLH1 and hPMS2 [27]. Deficient MMR that arise in sporadic colorectal cancer is nearly always due to an epigenetic biallelic hypermethylation of the hMLH1 gene promoter, and from genetic disorders by an acquired alteration of the wild-type allele leading to inactivation of one of the three main MMR genes (MLH1, MSH2, and MSH6) [28].

MSI characterises a particular subset of colorectal cancers with specific characteristic biology and chemosensitivity. Several previous studies demonstrated in patients with local and advanced CRC that an high-frequency MSI (MSI-H) was associated with a favourable prognosis compared to microsatellite stable/low-frequency MSI (MSS/MSI-L), independently of chemotherapy [29, 30]. On the other hand, patients in treatment with 5-FU with MSS/MSI-L tumors had improved overall survival, whereas no similar benefit in outcome pertained to MSI-H tumors [30]. Initial investigations into the predictive role of microsatellite instability showed similar improvement in outcome from adjuvant 5-fluorouracil therapy irrespective of microsatellite status of the resected adenocarcinomas [31]. According all these data, there was an evidence of improved outcome from adjuvant 5-fluorouracil in terms of reduced recurrence rate and better OS related to patients with microsatellite stable tumors only [32, 33], whereas the subset having MSI cancers gained no similar beneficial effect from chemotherapy [29]. According these data, we can suggest that 5-fluorouracil therapy should not be given to patients with MSI colorectal cancer.

In a report of Watanabe T *et al*., the presence of a mutation in transforming growth factor-beta-RII (TGF-beta-RII) was shown to improve survival in patients who also possess MSI-H. The 5-year survival rate for patients with MSI-H tumors and the TGF-beta-RII mutation was 74% following adjuvant 5-FU-based therapy, compared to 46% in patients with MSI-H tumors lacking the mutation in TGF-beta-RII. Interestingly, 61% of stage III colon cancers in this study exhibited the TGF-beta-RII mutation, indicating that this high frequency mutation may be useful in combination with MSI status as a prognostic marker for adjuvant therapy [34].

Jensen SA *et al*. measured the relationship between MSI and that biomarker. MSI in five reference loci, MMR enzymes (hMSH2, hMSH6, hMLH1 and hPMS2), TS and DPD expression were assessed in paraffin embedded tumor specimens, and associated with outcome in 340 patients completely resected for colorectal cancer stages II-IV and subsequently receiving adjuvant 5-fluorouracil therapy [35]. Microsatellite status was assessed in 311 (92%) of 340 tumors and MSI tumors with instability in at least three and in most cases four markers were found. MSI was found in 43 (13.8%) tumors and MSI tumors were prevalently in more elderly patients, (P = 0.02), in poorly differentiated tumor types (P = 0.001), in tumor mainly located proximally to the splenic flexure in ascending and transverse colon (P = 0.001). No statistically significant differences (P > 0.05) were found according to microsatellite status regarding gender, stage, vascular tumor invasion, perineural tumor invasion, or tumors complicated by perforation. In multivariate analysis, MMR deficient compared to MMR proficient tumors were significantly associated with lower risk of recurrence (HR = 0.4; P = 0.003) and death (HR = 0.5; P = 0.02), while MSI compared to MSS tumor patients had significantly lower risk of recurrence (HR = 0.3; P = 0.0007) and death (HR = 0.4; P = 0.02) [35]. The distribution of biomarkers according to microsatellite status in the study indicated a direct relationship between MSI/MMR and increasing TS staining intensity (P = 0.001), recurrence (P = 0.002) and overall survival (P = 0.02), while there was no evidence of an association with DPD in all these variables (P = 0.1) [35].

On these premises, the outcome according to microsatellite status in the present study can therefore be ascribed mainly to the biology of MSI colorectal cancer and to a lesser extent to antitumor response to 5-fluorouracil therapy. The correlation between high TS expression and microsatellite instability should be interpreted cautiously. There are no evidence to suggest direct influence of microsatellite instability on DPD or TS expression, nor that differential expression of these enzymes mediates the features for tumor biology or 5-FU resistance of MSI carcinomas. The reason of this association failure is due to technical reasons: the most significant limitation of immunohistochemistry is the semiquantitative nature of immunostaining. For these reasons, Microsatellite instability due to MMR deficiency remain one of the main biomarkers in colorectal cancer, as it not only indicates the pathogenesis, but also provides information on prognosis and prediction of response to chemotherapy. Future investigations into gene targets for microsatellite instability-driven deregulation are necessary to clarify the exact molecular foundation for the distinct clinico-pathological characteristics of MSI tumors.

### 2.4. Loss of Heterozygosity of 18q and 17p

It is reported in literature that allelic deletions involving chromosomes 18q and 17p occur in more than 70% of CRC [36]. Such deletions are thought to signal the existence of a tumor suppressor gene in the affected region. The tumor suppressor gene p53, often referred to as the "guardian of the genome" due to its central role in detection of genotoxic stress, is located on 17p and is mutated in 40% to 60% of colorectal cancers [36]. The p53 status has been rigorously analyzed as both a prognostic and predictive marker in

CRC, with conflicting results. Retention of 18q alleles in MSS cancers points to a favourable outcome after adjuvant chemotherapy with 5-FU–based regimens for stage III colon cancer [37]. The 18q chromosome contains DCC (deleted in colon cancer), a cell adhesion molecule, whose elevated expression can lead to enhanced tumor growth and metastatic potential. Several studies have determined that cancers with chromosome 18q loss appear to be associated with worse disease-free and overall survival [29, 37]. Further studies are necessary to determine the prognostic value of these genetic molecular markers.

### 2.5. Oral Fluoropyrimidines: Pharmacology and Predictive Markers

Even if 5-FU remains one of the most commonly prescribed anticancer drugs with significant activity against CRC, repeated and protracted intravenous administrations are heavy for patients. In the last years new oral 5-FU prodrugs, with new pharmacological characteristics, are emerging in the clinical area for the treatment in oncology [38]. New 5-FU prodrugs differ markedly in their mode of activation, their pharmacokinetic behaviour, particularly related to DPD inhibition, and their pharmacologic modulation. The 5-FU prodrugs currently at more or less advanced stage of clinical development are Eniluracil (5-Ethynyluracil), UFT (Uracil + tegafur), S-1 (5-Chloro-2, 4 dihydropyrimidine + tegafur + potassium oxonate) and the most used Capecitabine (N4-pentyloxycarbonyl-5'-deoxy-5-fluorocytidine) [39] (Table **1**). Their activity is related to the pharmacologic benefits conferred by the inhibition of 5-FU catabolism during gastrointestinal absorption and first pass in the liver, by DPD inhibition. DPD inhibition improves pharmacokinetic behaviour of delivered 5-FU by reducing interpatient variability and by increasing 5-FU half-life, particularly useful in limiting repeated oral administrations of the drug which is uncomfortable for patients. At one extreme, eniluracil has no cytotoxic activity but constitutes the most efficacious DPD inhibitor, through the formation of a covalent bond with DPD. UFT, including uracil (U), is a simple competitive inhibitor of DPD activity since it is the natural substrate of DPD. S-1 incorporates another DPD competitor, 5-chloro-2,4-dihydroxypyridine which is 200-fold more potent than U [39]. At the other extreme, Capecitabine does not incorporate a DPD inhibitor and is converted to the cytotoxic moiety 5-FU in target tissue through a series of three metabolic steps [40].

Oral fluoropyrimidines differ particularly as concerns their pharmacokinetic profile. Eniluracil was initially administered with 5-FU in a 10 : 1 ratio and produces 5-FU directly in the blood compartment. Baker *et al*. reported the pharmacokinetic of enluracil, with elimination half-life of 5-FU around 4.0 hours and constant pharmacokinetic parameters between day 2 and day 29 [41]. The promising results of this DPD inactivator disappeared due to its high toxicity, as explained in subsequently clinical studies. Hirata *et al* first reported the pharmacokinetics of 5-FU following the administration of S-1 at a standard dose of 80 mg/m$^2$ on day 1, with evidence of relative stability in the pharmacokinetics of 5-FU during S-1 treatment. Elimination half-life of 5-FU during S-1 treatment was in the range of 1.9–2.9 hours [42]. Also the pharmacokinetic of UTF explain the stability of levels of 5-FU in plasma. A steady state was attained for UTF and 5-FU at least on day 8 and there was no further cumulative increase in the AUC of these compounds after 1 week of treatment [43]. In colorectal patients treated by UFT, Sadahiro *et al* (2001) examined the respective concentrations of 5-FU in serum, tumour and normal mucosa at various intervals after the final dose of UFT. While the serum 5-FU concentration decreased to very low levels by 24 h following the UFT dose, the intratumour concentration of 5-FU had been lowered to only about half, and drug levels in normal mucosa were maintained at least 48 h after the final dose [44].

The clinical pharmacokinetics of capecitabine has been initially studied by Reigner *et al* . The preferential delivery of 5-FU into the tissues through the intermediary of thymidine phosphorylase (TP) is responsible for its much lower presence (approximately 10 times lower) in plasma than its prodrugs capecitabine, 50DFCR, 50DFUR or its catabolites FUH2 and FBAL. The authors noted that the AUC of capecitabine, 50-DFCR and 50-DFUR did not increase in plasma after long-term administration [45]. The influence of various covariables including gender, biological functions, food intake and coadministration of other drugs has been examined for the pharmacokinetics of oral fluoropyrimidines. In particular, pharmacokinetic changes due to food have been particularly well studied for UFT and capecitabine, with evidence that food had marked effects on the AUC of capecitabine, as 50% reduction. Consequently, it was recommended that capecitabine be administered with food [45]. In contrast, Damle *et al*. demonstrated that food significantly decreased the maximal plasma concentrations and UFT AUC values. These observations led to the conclusion not to administer UFT simultaneously with food [46]. In patients with renal dysfunction and hepatic dysfunction, treated by capecitabine there was a significant increase in 50DFUR. This led to specifically recommend a dose modification of capecitabine for patients with very low creatinine clearance and increase levels of liver failure markers [47].

**Table 1. Oral Fluoropyrimidines Under Clinical Evaluation**

| Compound | Chemical Name | 5-FU Prodrug | Effect on DPD |
|---|---|---|---|
| ***Eniluracil*** (Clinical development stopped) | 5-Ethynyluracil | No | Inactivator (complete DPD inhibition) |
| ***UFT*** (Orzels®, Bristol-Myers Squibb, contains UFT plus leucovorin) | Uracil + tegafur | Yes | Inhibitor |
| ***S-1*** (Bristol-Myers Squibb) | 5-Chloro-2, 4 Dihydropyrimidine + tegafur + potassium oxonate | Yes | Inhibitor |
| ***Capecitabine*** (Xeloda®, Roche) | N4-pentyloxycarbonyl-5'-deoxy-5-fluorocytidine | Yes | No DPD inhibitor |

Whatever the oral fluoropyrimidine considered all release 5-FU which is the final cytotoxic prodrug. Consequently, our clinical knowledge of the predictive markers for 5-FU-based treatment efficacy should apply to oral fluoropyrimidines. Ichikawa *et al.* [19] examined the relative tumoural expression of DPD and TS for advanced colorectal patients to be treated by UFT, as previously described. Capecitabine ultimately delivers 5-FU at the cellular level through the action of TP; for this reason, the flux of 5-FU production can be counterbalanced by a more or less marked opposite flux of 5-FU intracellular catabolism mediated by DPD. This view was confirmed by Tsukamoto *et al* (2001), who measured, *in vitro*, the enzyme kinetic parameters of each of the enzymes involved in the activation of capecitabine to 5-FU and elimination [48]. The authors constructed a physiologically based pharmacokinetic model which revealed that the most important factors determining the selective production of 5-FU in tumour tissue after capecitabine administration were activation by TP, nonlinear elimination of 5-FU by DPD and the tumour blood flow rate. These data confirmed those previously reported by Ishikawa *et al*. [20] showing the efficacy of capecitabine correlated to the tumour TP/DPD ratio.

Overall, our current understanding of predictive markers for oral fluoropyrimidine treatment is limited and, with the exception of UFT, remains based on extrapolations from 5-FU clinical studies. Further studies are necessary in the future, in consideration of the widely use especially of capecitabine in adjuvant and first line treatment of CRC.

### 2.6. Fluoropyrimidines and Markers of Toxicity

Although the fluoropyrimidines (5-FU and derivates) are in clinical use for almost 50 years, there is still no clear methodology to identify patients who are likely to benefit most from the treatment. Therefore, there is a clear need to identify several markers to predict the treatment toxicity and provide a rational basis for treatment selection.

Numerous serious adverse side effects have been reported with fluoropyrimidine treatment, including myelosuppression, cardiac toxicity, mucositis, hand-foot syndrome (HFS), and diarrhoea [49, 50]. The oral derivate capecitabine in the last years has been favoured because of the convenience of its administration. In addition, capecitabine is better tolerated by patients, who reported fewer cases of stomatitis, alopecia, neutropenia, diarrhoea, and nausea, but more cases of HFS, compared to 5-FU [51]. These toxicities can have significant effects on treatment time and cost, including dose reductions, treatment delays, changes in or discontinuation of therapy, extra interventions to address potentially harmful side effects, fluid- and nutritional-replacement therapies, hematopoietic-stimulating therapies, use of antibiotics, hospitalization, and poor quality of life.

#### *2.6.1. Dihydropyrimidine Dehydrogenase (DPD)*

In contrast with the not well defined 5-FU and derivates mechanism of activation, the 5-FU elimination pattern is univocal. Catabolism and deactivation of fluoropyrimidine drugs depend on a single and exclusive enzymatic step driven by dihydropyrimidine dehydrogenase (DPD). With the increasing number of patients with cancer likely to be treated with fluoropyrimidines, predicting and preventing the occurrence of such toxicities is now a major issue in clinical oncology [51]. The drug displays an extensive first-pass metabolism, because > 95% of an administered dose of 5-FU is quickly dehydrogenated in the liver by DPD to dihydrofluorouracil (5-FU-H2). Dihydrofluorouracil is subsequently converted to beta-fluoro-beta-ureido-propionic acid, then fluoro-beta-alanine (FbetaAL) by dihydropyrimidinase and beta-ureidopropionase, respectively, and conjugated FbetaAL derivatives are eventually eliminated in urine [52]. DPD is the initial and rate-limiting enzyme in the pathway. For the past 20 years, a causative link between deficiency in DPD activity and severe toxicity in response to 5-FU and derivates treatment, including grade 4 symptoms and death, has been extensively studied. It has been shown that DPD activity was slightly lower in women (< 15%) than in men [39]. The pivotal role of DPD in 5-FU-based chemotherapy has been shown in CRC patients with a complete or partial deficiency of this enzyme. These patients suffered from severe, possibly fatal multi-organ toxicity following the administration of 5-FU [39, 53, 54].

DPYD, the gene encoding DPD, located within human chromosomal region 1p22, is composed of 23 exons encompassing approximately 950 kb [55, 56]. Over 30 single nucleotide polymorphisms (SNPs) and deletion mutations have been identified within DPYD, although most of these variants have no functional consequences on enzymatic activity. Of particular interest is the IVS14 + 1 G > A variant (DPYD*2A), which has been found in up to 40–50% of people with partial or complete DPD deficiency [57].

Aberrant methylation of the DPYD promoter was found to cause a partially DPD deficient phenotype [58-60]. Meta-analysis of over 1200 patients suggested that more than 30% of patients treated with 5-FU experienced severe drug-related toxicity [61]. The frequency of low DPD enzymatic activity, indicating partial DPD deficiency, in the general population was initially estimated at between 3% and 5% [62]. Additional studies have shown significant variability among different ethnic subpopulations, showing that the prevalence of partial DPD deficiency is higher in Asian [63], Southwest Asian [64], African [65], American [66] than European and Caucasian Group [67, 68].

Several possible methods are used for testing DPD levels. The most common test, used as control system in several comparative tests, involves the ex vivo incubation of a patient's peripheral blood mononuclear cells (PBMCs) with radiolabelled 5-FU and measuring the resulting rate of catabolite formation by high-performance liquid chromatography (HPLC) [69]. An alternative method of measuring DPD enzymatic activity is the Real-time quantitative PCR of DPD [70]. A recent report, using a small number of patients, suggested measuring plasma levels of fluoro-β-alanine (FBAL), the final metabolite of 5-FU in the catabolic pathway, by HPLC to assess DPD enzyme activity [70]. More promising alternate assays involve measuring uracil levels to determine DPD activity, prior to starting on a 5-FU or capecitabine therapeutic regimen. Several recent studies found that elevated levels of uracil in plasma were significantly associated with impaired clearance of 5-FU and development of 5-FU-related toxicity [70]. A new test for uracil catabolism was developed. Forty-two subjects ingested an aqueous solution of 2-13C-uracil, and exhaled levels of $13CO_2$ and $12CO_2$ were measured using infrared spectrophotometry [71]. At last, several groups have reported tests, using the WAVETM DNA Fragment Analysis System, developed by Transgenomic, connected to a denaturing HPLC (DHPLC) for the detection of DNA mutations in DPYD [72].

Many reports have studied the correlation between specific DPYD mutations and DPD activity. In particular, the studies of Largillier explore the development of grade 3 or 4 adverse side effects correlated with DPYD gene mutations [73] and concluded that genetic mutation in DPYD does not always translate into severe 5-FU toxicity.

On this basis, subsequently, various strategies have been proposed to screen for patients with a DPD deficiency, including genotyping [74, 75]. The evidence that DPYD heliotype not containing any no synonymous or splice-site mutations was associated with 5-FU toxicity suggested the presence of additional genetic variations in the nonbonding region of DPYD [74]. No deep intrinsic mutations in DPYD have been described affecting the splicing of DPD pre-mRNA, as evidenced in 7% of paediatric patients with a complete DPD deficiency [76]. Therefore, it is conceivable that genomic deletions encompassing a part or the entire DPYD gene might also provide a molecular basis for cancer patients with a phenotypic ally established DPD deficiency. For this reason, van Duis-

enberg *et al*. [77] recently investigated the presence or absence of genomic rearrangements in DPYD, using multiplex ligation-dependent probe amplification (MLPA), in 92 patients with a reduced DPD activity and/or grade III/IV toxicity. This German study characterized a novel missense mutation K874R based on the crystal structure of DPD and identified the first deep intronic mutation in DPYD affecting the regulation of pre-mRNA splicing. Analysis of the prevalence of the various DPYD mutations, in cancer patients experiencing severe toxicity, showed that the splice-site mutation c.1905 + 1G/A (IVS14 + 1G/A) and the c.2846A/T (D949V) were the most common pathological mutations, data confirmed also in the study of Loganayagam *et al.* [78]. The deleted region, encompassing exons 21–23, was located outside the common fragile site FRA1E which extends over 370 kb within DPYD [79]. In a subgroups analysis, conspicuous finding was a deep intronic mutation affecting DPD pre-mRNA splicing in five individuals. The c.1129–5923C/G mutation in intron 10 created a cryptic splice donor site and as a consequence, a 44 bp fragment of intron 10 was inserted in the mature DPD mRNA, reducing its activity. Really, conflicting data exist as to weather the c.1236G/A mutation is associated with an increased risk of development of severe 5-FU-associated toxicity [75, 80], because of its apparently high mutation prevalence in the normal population. These data refer the necessity to confirm the genetic screening for this mutation in cancer patients prior to the start of 5-FU-containing chemotherapy

In another study, Amstutz *et al.* [81] analized the hypermethylation of the DPYD promoter region as an alternative mechanism for DPD deficiency and severe 5-FU toxicity. The ipermethylation was assessed in 27 cancer patients (22 gastrointestinal cancer patients), receiving 5-FU based chemotherapy, including 17 patients experiencing severe toxic side effects following drug administration. None of which were carriers of a known deleterious DPYD mutation, and ten control patients. The final results showed no evidence of DPYD promoter methylation, whereas in a control experiment, as little as 10% methylated genomic DNA could be detected.

Various studies nevertheless suggest epigenetic factors as an alternative explanation for the occurrence of severe 5-FU toxicity where no other molecular basis was found in DPYD [82, 83]. Probably because only of minor importance as a predictive factor for severe toxic side effects in 5-FU based chemotherapy

#### *2.6.2. Tymidilate Sinthetase (TYMS) and Methylene Tetrahydrofolate Reductase (MTHFR)*

Various polymorphisms in genes other than DPYD have recently been shown to be correlated with the occurrence of severe adverse side effects to 5-FU.

Previous results on tymidilate sinthetase (TYMS) and methylene tetrahydrofolate reductase (MTHFR) polymorphism showed by Pullakart *et al*. [14] were tested in subsequent studies, in particular by Schwab *et al.* The authors evaluated the *TYMS* gene, with his variations in certain regions, which alter its expression and relative enzyme, a target of 5-FU-based therapy. Low levels of enzyme (2R/2R) are associated with a 1.4 - 2.5-fold risk of toxicity to 5-FU-based therapy, in particular diarrhea [80].

#### *2.6.3. Orotate Phosphoribosyltransferase (OPRT)*

Although the importance of the value of TYMS and DPD in the cytotoxicity of 5-FU is recognized, the contribution of phosphorylation is necessary to activate 5-FU into its nucleotides [19]. The preferential use of the pathway directly to FUMP by orotate phosphoribosyltransferase (OPRT) was revealed to correlate with the cytotoxicity of 5-FU [83]. FUMP is then phosphorylated to fluorouridine diphosphate, which can be either converted to FdUMP or phosphorylated to the active metabolite fluorouridine triphosphate. Fluorouridine triphosphate is extensively incorporated into RNA (F-RNA), disrupting normal RNA processing and function [83].

Using this concept, Ichikawa *et al.* enrolled 69 patients with CRC B2-C Dukes from January 2000 to August 2002, who underwent radical surgery and received bolus 5-FU. All eligible patients were treated between 14 and 35 days after surgery with the Roswell Park regimen (500 mg/m$^2$ 5-FU i.v. bolus given 1 hour after L-leucovorin infusion weekly for 6 weeks, combined with 250 mg/m2 L-leucovorin by 2-hour infusion; four treatment cycles were given, each consisting of six weekly treatments followed by a 2-week rest period). OPRT Gly213Ala polymorphism was successfully assessed for all 69 patients, utilizing samples genotyped for OPRT Gly213Ala polymorphism by Assay-by-Design by Applied Biosystems (ABI, Foster City, CA) [83], and finally verifying analysis of OPRT activity and mRNA expression. The authors concluded that there was no statistical association of OPRT Gly213Ala promoter polymorphisms with the clinicopathologic features, such as age, gender, performance status, location of primary tumor (colon, rectum), tumor depth of invasion, and Dukes' classification.

The OPRT Gly213Ala promoter polymorphisms were demonstrated factors to predict grade 3 to 4 diarrhea. The frequency of the Ala allele of OPRT Gly213Ala polymorphism was 27.5%. The Ala allele was also associated with increased OPRT mRNA level in normal tissue, leading to the hypothesis that this polymorphism could play a role in mRNA stability and translation. Patients with the Ala allele were 16 times more likely to have severe toxicity compared with those normal (Gly/Gly genotype) [83].

Moreover, a conspicuous finding was that the onset of severe toxicity occurred earlier from the start of chemotherapy in patients with the Ala/Ala genotype than those with other genotypes. For this case, OPRT Gly213Ala polymorphism might predict not only the risk of toxicity but also the time of the occurrence of severe toxicity. Prospective translational treatment trials, including larger number of patients, are needed to corroborate these results and resolve these questions.

### 2.7. 5-Fluorouracil Test Dose

Reduced test dose strategies with subsequent sampling for pharmacokinetic evaluation could provide valuable information on patients with impaired DPD and increased risk of iatrogeny upon 5-FU administration. Monitoring of 5-FU and 5-FU catabolites such as dihydrofluorouracil [84] in plasma has been proposed as a marker for DPD function. A recently demonstrated pharmacokinetic-based test to prevent severe toxicities upon 5-FU administration showed that using a reduced 5-FU test dose with 5-FU/5-FU-H2 monitoring permitted one to detect approximately 2% of patients with marked alterations in 5-FU pharmacokinetic profiles. These patients were subsequently selected for alternative treatments without fluoropyrimidine drugs, thus preventing life-threatening toxicities [80].

On this basis, a recent Test (Thera Guide, Myriad Genetic Laboratories, Inc. of Salt Lake City, Utah, USA) was performed to provide comprehensive analysis of the *DPYD* and *TYMS* genes to identify high-risk individuals and help prevent toxicity in patients being considered for 5-FU-based therapy. The classification is defined in three class of risk (high: together mutated, moderate: only TYMS mutated, low: no mutations) to whom is based the final decision to include in therapy 5-FU and derivates.

In conclusion, choosing a method for identifying DPD-deficient patients at risk with fluoropyrimidine drugs remains an uneasy task. No method has stood out as a standard that would me*et al*l the requirements (e.g., time- and cost-effectiveness, availability, and relevance) of large-scale screening. We can explain that, in the absence of new better alternative chemotherapies, screening patients for conditions that would predispose them to being unable to tolerate 5-FU, such as DPD deficiency, remains the best solution for improving patient outcomes and the techniques for assessing DPD deficiency, more than OPRT polymorphism, must be finally refined and should include a search for genomic rearrangements and aberrant splicing to permit that screening will become practicable in the future.

## 3. OXALIPLATIN

After the previously described 5-FU efficacy as therapeutic options in the treatment of advanced CRC (RR 20-25%), the introduction of newer agents, such as oxaliplatin in combination with 5-FU, has increased response rates to 40% to 50% in advanced disease and improved overall survival [85, 86]. Selection of the most beneficial treatment regimens in CRC remains a challenge and is hindered by a lack of predictive and prognostic markers. In addition, drug resistance remains a major stumbling block to effective cancer treatment.

Oxaliplatin is a third-generation platinum analogue in which the 1,2-diaminocyclohexane (DACH) ligand substitutes the amine groups of cisplatin. Oxaliplatin also exhibits a relatively favourable toxicity profile and in many instances is clinically more favourable than cisplatin, with reduced toxicity and substantial activity against cisplatin- resistant tumors platinum compounds form positively charged species that cause DNA-damaging crosslinks blocking both DNA replication and transcription and initiating apoptosis [87].

Several distinct mechanisms are proposed to mediate response to oxaliplatin, including increased drug inactivation and efflux, decreased cellular uptake/accumulation, enhanced tolerance to pt-DNA damage, and an increase in the efficiency of DNA repair mechanisms [88].

### 3.1. Nucleotide Excision Repair (NER) Pathway: ERCC1

The nucleotide excision repair **(**NER) pathway represents the resistance mechanism best described to date for removal of the bulky pt-DNA adducts induced by oxaliplatin treatment (see above). The more studied and conserved member of the NER pathway implicated in mediating response to oxaliplatin is excision repair cross-complementation group 1 (ERCC1). ERCC1 forms a complex with xeroderma pigmentosum group F (XPF), which recognizes and cleaves the 5′ damaged DNA strand in lesion repair [88] (Fig. **2**).

Several studies in the last 20 years have correlated ERCC1 gene expression levels with clinical outcome in patients receiving a platinum-based regimen, from data related to ovarian carcinoma [89] to analyses in CRC patients [90, 91]. Shirota Y *et al*. analyzed ERCC1 mRNA expression level in a study of 50 patients with advanced disease refractory to 5-FU/irinotecan chemotherapy, and it results to be an independent predictive marker of survival in 5-FU/oxaliplatin chemotherapy ($P < .001$) [90]. The influence of polymorphisms in *ERCC1* and *XPD* genes on outcomes in patients with metastatic CRC (mCRC) receiving first-line therapy with 5-FU/LV/oxaliplatin (FOLFOX4) was examined using peripheral blood samples from 166 patients. Genotypes independently associated with shorter progression-free survival (PFS) included ERCC1-118 T/T, XPD-751 A/C, and XPD-751 C/C. The median PFS was 11.2 months for patients without any of the 3 genotypes, 9.8 months for those with 1 of the high-risk genotypes, and 8 months for those with both the ERCC1-118 T/T and either XPD-751 A/C or C/C genotypes (hazard ratio, 2.84; $P = .002$) [92].

The ERCC1-118 T allele has been associated with higher ERCC1 mRNA levels, which supports the profile of oxaliplatin resistance. Polymorphic variants within the ERCC1 gene have been identified and associated with clinical outcome in patients receiving 5-FU/oxaliplatin [91]. Partly different from fluoropyrimidines genes previously described, the frequency of these polymorphisms varies with race and may account for reduced response rates in black patients when compared with white patients, as expressed by Goldberg RM *et al*. and confirmed in more recent studies, as in the subgroup of patients of CAIRO study [93, 94].

Subsequently, recent studies investigated the ERCC1 role alone and together with other factors. Kim SH *et al*. evaluated whether the expression of ERCC1, together with TS and glutathione S-transferase pi (GSTpi), examined using immunochemistry, predict clinical outcome in 70 patients with advanced colorectal cancer treated with 5-FU/oxaliplatin chemotherapy. The results showed that ERCC1, TS, and GSTpi were positive in 55.7%, 68.6%, and 71.4% of cases, respectively. While it was confirmed that TS lower levels correlate with best response to chemotherapy (P = 0.009), there was no significant correlation between response to treatment and the ERCC1 or GSTpi expression pattern (P = 0.768, P = 0.589, respectively). The median OS, however, was significantly longer in patients without ERCC1 expression (P = 0.0474). Patients who were simultaneously ERCC1 and TS positive had a poor OS (P = 0.0017). Multivariate analysis revealed and confirmed that both ERCC1 and TS expression significantly impacted OS (HR 1.72, P = 0.023), justifying the dosage of these factor for the prediction of clinical outcome in this patients group [95].

### 3.2. Nucleotide Excision Repair (NER) Pathway: XRCC1

Another member of the NER pathway is X-ray cross-complementing group 1 (XRCC1) and together with ERCC1 was analyzed in the study of Liang J *et al* [96]. The authors evaluated the effect of the two gene polymorphism, ERCC1 codon 118C/T and XRCC1

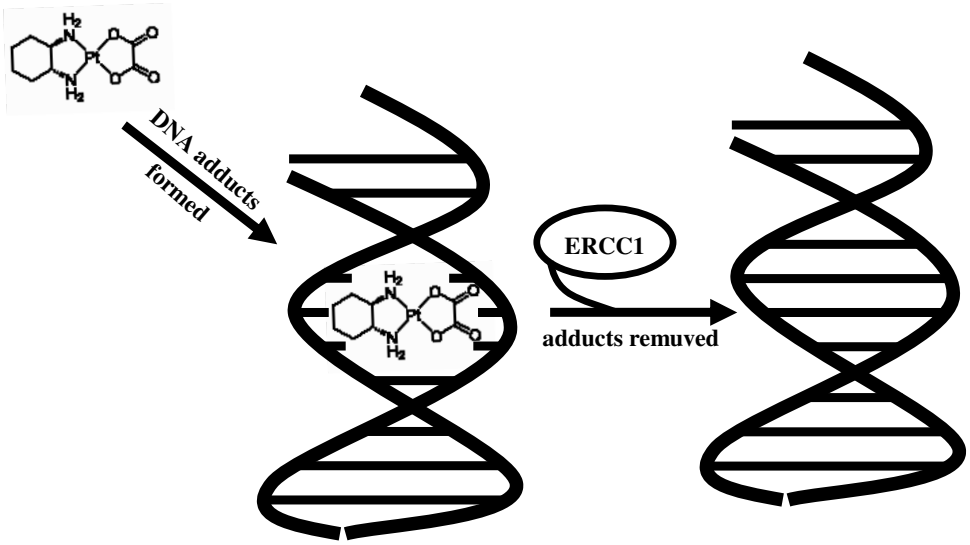


**Fig. (2).** Oxaliplatin Mechanism of Action and Role of ERCC1.

codon 399A/G, tested by RT-PCR method in peripheral blood lymphocytes, on treatment outcome in 113 patients with a diagnosis of metastatic colorectal cancer receiving oxaliplatin-based regimens. Analyses of the patterns of the polymorphism located at ERCC1 codon 118 showed that 55 (48.67%) patients were homozygous for C/C genotype, 15 (13.27%) were homozygous for the T/T genotype, and 43 (38.06%) were heterozygous for C/T genotype. Analyses of the polymorphism located at XRCC1 codon 399 showed that 61 (53.98%) patients were homozygous for A/A genotype, 13 (11.50%) were homozygous for the G/G genotype, and 39 (34.52%) were heterozygous for A/G genotype. Adjusted for some clinical factors, ERCC1 and XRCC1 polymorphisms alone lost their roles in predicting disease control rates (DCR) and OS (P = 0.662 and 0.631, respectively), while the combination of two genes polymorphisms was significantly associated with DCR (P = 0.01) and OS (P = 0.001), independently, becoming the first study to demonstrate a possible selection of patients who would benefit from oxaliplatin-based chemotherapy for metastatic colorectal cancer [96].

### 3.3. Nucleotide Excision Repair (NER) Pathway: XDP

Interestingly, xeroderma pigmentosum group D (XPD) is another gene that codes for an important protein in the NER pathway. A polymorphic variant at position 751 results in a lysine to glutamine substitution and has been linked with significantly lower response rates in a study of 73 patients receiving 5-FU/oxaliplatin. Patients with the Lys/Lys genotype demonstrated a median survival of 17.4 months whereas those possessing the Lys/Gln and Lys/Gln demonstrated 12.8 and 3.3 months respectively (P = 0.02) [97]. X-ray repair cross complementing 1 (XRCC1) is involved in the repair of single strand breaks following base excision repair and has been demonstrated to mediate repair of alkylating agent-induced DNA damage [98]. A study by Stoehlmacher *et al* analyzed gene expression of the XRCC1 polymorphic variant found at codon 399 in a 61-patient study [99]. Seventy-three percent of patients with the favourable Arg/Arg genotype responded to treatment, and patients who possessed at least one Gln allelic polymorphism in XRCC1 were 5.2-fold more likely to fail 5-FU/oxaliplatin chemotherapy.

This polymorphic was also identified as demonstrating significant heterogeneity according to race-again a possible determinant of race-specific response [93] . One study demonstrated the potential benefits of performing multivariate analysis of multiple gene polymorphisms in patients with refractory colorectal cancer and identified a gene dosage effect on 5-FU/oxaliplatin treatment response with patients with two or more unfavourable XPD, TS, ERCC1, and GST-P1 polymorphisms having a significantly reduced OS [100] .

### 3.4. DNA Repair Pathways

The precise clinical effect of modulation of components of the DNA repair pathways in platinum drug efficacy remains to be clearly established. The majority of oxaliplatin that enters cells never becomes associated with DNA. A possible explanation for this is inactivation via formation of conjugates between glutathione, detoxification by the glutathione-S-transferase (GST) family, and exportation of the complexes via the ABC membrane transporter superfamily [101, 102]. Studies have identified polymorphisms in GST enzymes that have been correlated with response to platinum agents. One polymorphic variant results in an amino acid substitution at position 105 of GSTP1, which is reported to diminish enzymatic activity. A study of 107 patients determined that those homozygous for the val/val genotype demonstrated significantly longer survival compared with those heterozygous or homozygous for the wild type [103] and an additional study linked the GST-P1 105V polymorphism to drug activity, efficacy and sensory neurotoxicity in patients receiving FOLFOX, this last aspect subsequently described [104].

### 3.5. Oxaliplatin Toxicity

Oxaliplatin frequently causes neutropenia, fatigue, gastrointestinal symptoms (nausea, vomiting, and diarrhea), ototoxicity (hearing loss), and saltuary hypokalemia. However, the primary toxicity, dose limited in many cases, is a purely sensory neuropathy, which seems to be cumulative and, at least in a large part, reversible with drug cessation. CRC patients treated with oxaliplatin discontinue therapy more often because of peripheral neuropathy than tumor progression, potentially compromising patient benefit. The Food and Drug Administration noted that more than 70% of the patients receiving oxaliplatin are affected by some degree of sensory neuropathy [105]. Most importantly, neurotoxicity, and not tumor progression, is often the cause of treatment discontinuation. An additional recent study examining 383 patients treated with oxaliplatin and irinotecan showed that 52% of patients required dose reduction due to adverse events, including neurotoxicity, and that 26% required hospitalization because of these negative events [106]. There are two patterns of neuropathy: an acute cold-aggravated but transient condition and a more chronic form that has onset after multiple exposures to the drug and that often improves but does not disappear with drug cessation. Acute oxaliplatin neurotoxicity can occur within hours of dosing as this may be precipitated or exacerbated by exposure to cold temperature or cold objects and typically resolves within hours to days [107]. This acute neurotoxicity is dose related and reversible. The more chronic pattern of sensory neuropathy was observed in 50% of study patients who received oxaliplatin with infusional 5-fluorouracil/leucovorin [108]. Oxaliplatin, less than cisplatin but more than carboplatin, undergoes aquation, which is a key step in the drug forming a complex with the target DNA. The result of this hydrolysis is the formation of a positively charged molecule that then cross-links to DNA, forming the DNA/platinum adducts. The amount of DNA crosslinks in DRG neurons at a given cumulative dose was significantly correlated with the degree of neurotoxicity [109].

Although the mechanism of the transition from a platinum-DNA adduct to neuronal apoptosis is not fully understood, one proposal has suggested that the DNA repair machinery is unable to repair the damaged DNA. Polymorphisms in the DNA repair genes, including genes in base excision repair, nucleotide excision repair, mismatch repair, and double-strand break repair pathways, cause the individuals to be less proficient in repairing carcinogen-induced damage. It has also been proposed that the platinum-DNA adducts interfere with the normal function of cellular proteins such as binding or interactions with other proteins [110].

In addition to efforts to identify a successful neuroprotective agent, there have been numerous studies attempting to establish the role of various phenotypic markers for chemotherapy-induced neurotoxicity. An accurate marker of neurotoxicity that would enable a quantitative monitoring of progress of neurotoxicity or provide a prediction of the ultimate severity would prove valuable in controlling this toxicity. Cavaletti *et al*. proved initially a highly significant correlation between the decrease in circulating levels of nerve growth factor and the severity of chemotherapy-induced neurotoxicity in patients treated with platinum drugs and taxanes, however, it did not predict the final neurologic outcome and further studies are request to provide an effective benefit for this class of patients [111]. Subsequently, many efforts have been made to determine genetic linkages as a cause of platinum-based toxicity in order to ultimately diminish these effects and augment the beneficial antitumor qualities. A great number of genome-wide studies have been pursued.

#### *3.5.1. Glutathione S-transferase (GST)*

The more important data were directed to Glutathione S-transferase (GST) enzymes, which are responsible, in part, for oxaliplatin detoxification. Glutathione S-transferases (GST) are a

family of enzymes that catalyze the conjugation of glutathione to electrophilic toxins to inactivate them and aid in their excretion from the body [92]. The GST genes encode metabolizing enzymes that decrease the reactivity of toxins with substrates in the body. Genetic polymorphisms have been found, including GSTM1, GSTT1, and GSTP1. Two independent studies in advanced CRC patients treated with oxaliplatin looked at the GST genes for patients who experienced grade 3 cumulative neuropathy. Ruzzo *et al*. described the association between the GSTP1 105 Val G/G allele and the development of grade 3 neuropathy from oxaliplatin treatment [92]. Additionally, Lecomte and colleagues indicated that in a cohort of 64 patients, there was a significant association between the GSTP1 105 Val G/G allele and risk of developing neurotoxicity [112]. Other studies are ongoing and further evaluations are necessary.

#### *3.5.2. Cell Entry Process*

A cell entry mechanism is another process under evaluation for its role in platinum toxicity. As a heavy metal, platinum drugs must have a particular method of entering their cell of interest. Metal transporters, such as the copper transporters CTR1, ATP7A, and ATP7B, have been of particular interest [113]. Forced overexpression of human CTR1 in ovarian cancer cells increased cisplatin uptake. CTR1 mediates cellular accumulation of platinum-containing drugs used in patients. For neurotoxicity to develop, the drug must be capable of entering its target cells to cause damage. Therefore, any genes or proteins involved in the transport of platinum into or out of cells could play a role in neurotoxicity development. However, there are no clinical studies assessing the influence of genetic variation in platinum transporters on patient toxicity or outcome.

#### *3.5.3. DNA Repair: ERCC1*

DNA repair is an important mechanism for resistance to platinum-based therapy and possibly the development of neurotoxicity. The capacity of the cells to repair their DNA attacked by the platinum agent determine probably the unsuccessful in inducing apoptosis. After an initial no association shown between NER and chemotherapy neurotoxicity, ERCC1, previously described as probably predictive response factors, have been hypothesized to play also a role in the efficacy of platinum-based drugs. Among the numerous studies done assessing the association between nucleotide excision repair genes and chemotherapy clinical outcome, many have provided concrete evidence of an association, as described by Park DJ *et al*. Patients, whose response to oxaliplatin therapy was better, presented a lower level of neutropenia and neurotoxicity [104]. We need for other studies investigating a molecular process to prove it.

Until the concrete phenotype/genotype associations have been established to enable individualized therapy, we should be aware of the risk of the severe, life-altering side effect of toxicity until suspension of treatment. Time to optimal treatment is one of the key factors in determining the success of therapy in patients with cancer. Hence, the use of biomarkers to guide current clinical practice is imperative to improve disease prognosis and patient tolerability. Though intriguing, further studies are needed before translating these findings into the clinical setting.

## 4. CONCLUSION

Current data strongly supports the use of some of the biomarkers discussed in this article to guide CRC therapy based on inter-individual variability. These results represents a good start toward the goal of bringing personalized fluoropyrimidines and oxaliplatin regimens to each patient with CRC, but more comprehensive and integrated studies are needed to make this a reality. In fact, many of the biomarker studies suffer from small sample sizes, retrospective design, ambiguous study endpoints, and non-uniform acquisition of source tumor material and bioassays. To make progress, it becomes necessary a more coordinated evaluation of these markers before genetic information, as a routine part of clinical CRC treatment. Molecular diagnostics capable of a more precise prognosis based on individual biomarkers are imperative to the successful clinical adaptation of the field of pharmacogenetics. Use of predictive biomarkers should be made an integral part of current clinical practice more frequently, and be used as an aid to clinical experience and expertise in making CRC patient therapy decisions. Follow-up studies are also required to identify the functional significance of the many mutations and polymorphic variants for response and toxicity that exist in the patient population during treatment. Such functional information will inevitably assist in unravelling the complex and multifaceted mechanisms of drugs metabolism and cytotoxicity. The continuing evolution of highthroughput technologies such previous described microarray gene profiling, proteomic profiling, and the newly developed metabolomics field will improve the resolution and sensitivity with which we can detect such markers. The inclusion of pharmacogenetic biomarkers, such as those reviewed in this article, in the paradigm of CRC therapy will enable the determination of patients most likely to benefit from therapeutic interventions tailored specifically for them.

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