Abstract. We aim to present a comprehensive review of the molecular basis of 5-fluorouracil (5-FU) toxicity, of which dihydropyrimidine dehydrogenase (DYPD) deficiency is a well-known mechanism. The prevalence of partial DYPD deficiency is fairly common, ranging between 3-5% in the general population, whereas it can be as high as 12% in African-American females. More than 50 genetic polymorphisms have been described as being associated with decreased enzymatic activity, whereas the c.1905+1G>A point mutation is the most commonly found (52% of cases), with a prevalence of heterozygosity in the general population ranging between 1-2%. Several methods have been utilized to identify reduced DYPD activity; functional tests are expensive and only available in specialized centers. Genotyping alone is not reliable enough, as some of the polymorphisms may not result in significantly reduced DYPD activity. The rate of cardiotoxicity associated with 5-FU or capecitabine does not seem to be related to DYPD deficiency, and has been estimated to range between 1.2-8%. Several pathophysiological mechanisms seem to contribute to 5-FU cardiotoxicity, including coronary spasm, increased endothelial thrombogenicity and myocardial inflammation. Tegafur/uracil and raltitrexed may be alternative options for patients with partial DYPD deficiency and previous manifested 5-FU cardiotoxicity, respectively. Pharmacogenetics is expected to further identify and clarify the mechanisms associated with 5-FU-related toxicity, thus aiding the oncology societies to formulate specific guidance on pre-treatment testing.

Capecitabine is an orally administered pro-drug of 5-fluorouracil (5-FU), which is a widely used chemotherapeutic agent incorporated in the treatment of several malignancies, i.e. colorectal, gastric, pancreatic, breast and head and neck cancer. It is absorbed through the gastrointestinal tract as an intact molecule (1, 2). It is subsequently activated via a triple enzymatic process, by the sequential catalytic activity of carboxylesterase (liver), cytidine deaminase, and thymidine phosphorylase. This cascade results in the formation of 5-FU (1, 2). Of note, higher levels of thymidine phosphorylase have been detected in cancerous cells, as opposed to healthy tissues (3). 5-FU is further metabolized to 5-fluorodeoxyuridine monophosphate which inhibits thymidylate synthase (TYMS). The latter converts deoxyuridine monophosphate into thymidine monophosphate, which is a key molecule for DNA synthesis (4). Additionally, 5-FU can be directly incorporated into RNA, interfering with RNA transcription, and, less often, into DNA, inhibiting its replication (5).

Dihydropyrimidine dehydrogenase DYPD consists of the initial step in the catabolism of the physiological pyrimidines, as well as 5-FU and capecitabine. It is predominantly expressed in the liver and de-activates more than 80% of administered 5-FU. Up to 20% of 5-FU is excreted in the urine (6). Reduced activity of DYPD (DYPD deficiency) results in increased half-life of 5-FU and therefore severe toxicity. DYPD activity is influenced by several factors, including circadian rhythms, possibly gender, drug interactions, but most importantly genetic polymorphisms (7).

There is a well-described autosomal recessive syndrome in pediatric patients where complete deficiency manifests as thymine-uraciluria, convulsions and psychomotor retardation (8). In 5-FU/capecitabine-treated patients with DYPD deficiency, toxicity can be potentially lethal, and there has been a similarly well-described syndrome consisting of severe and prolonged pancytopenia, often associated with sepsis, as well as serious gastrointestinal toxicity with refractory diarrhea, vomiting and severe mucositis (9, 10).
Approximately 3-5% of the general population have been reported to have partial DYPD activity (11). However, there seems to be great variability, with up to 12% of African-American females reported to exhibit partial DYPD deficiency (12). DYPD deficiency has been reported to account for at least 50% of severe toxicity relating to 5-FU treatment. However, the frequency of the deleterious gene polymorphisms appears to be relatively low, leaving a substantial number of severe 5-FU toxicity cases unexplained (13-16). The large variation between reported frequencies of DYPD gene polymorphisms associated with severe 5-FU toxicity across studies can be attributed to several factors and is explained in depth by Amstutz et al. (17): i) the large variability in the frequency of potentially deleterious polymorphisms amongst different populations; ii) the use of combination treatments in several studies as opposed to fluoropyrimidine monotherapy, allowing for the possibility of the contributory effect of non-fluoropyrimidines in the toxicity observed; iii) the relatively small number of patients developing toxicity and subsequently undergoing genetic analysis; and iv) differences in toxicity inclusion criteria, with some studies including less than grade 2 toxicities, while others allowed for toxicity observed only within the first two cycles of chemotherapy.

The DYPD gene (843 kb) is located on chromosome 1p22, and consists of 23 exons. Several thousands of genetic polymorphisms have been described, however, most of them refer to non-coding areas of the gene (17-19). Three of them have been directly associated with 5-FU-related toxicity: c.1679T>G and c.2846A>T result in decreased enzyme activity, but are very rare (17). More commonly found, c.1905+1G>A polymorphism results in a 165-bp mRNA deletion, as exon 14 is subsequently skipped, and the product is a non-functional protein (20). The frequency of c.1905+1G>A in the proportion of patients suffering from severe 5-FU toxicity has been reported to range from 5.5-14% (14, 15). The prevalence of heterozygosity of the c.1905+1G>A polymorphism in the general population has been estimated to be 0.5-1% (21, 22). Compound heterozygote genotypes associated with severe 5-FU toxicity, such as concurrence of c.1905+1G>A with c.1679T>G, as well as c.1905+1G>A with c.2846A>T, have been reported, indicating that a combination of polymorphisms may contribute to the observed toxicity (23, 24). On the contrary, carriers of the c.1905+1G>A, as well as other polymorphisms, have been found to have normal DYPD activity, allowing for the postulation of functional enzymatic compensation via increased activity of the wild-type alleles (25).

The large number of unexplained toxicities has led to the identification of new molecular mechanisms, other than those previously described, associated with increased toxicity from 5-FU treatment. For example, there was initial evidence that methylation of the DYPD gene promoter can result in DYPD deficiency, however, subsequent analysis of a larger number of patients concluded that this epigenetic mechanism is unlikely to contribute to DYPD deficiency (26-28). Furthermore, polymorphisms in intronic areas of the DYPD gene were recently identified and result in aberrant pre-mRNA splicing and a non-functional protein; the deep intronic variant in intron 10 (c.1129-5923C>G) resulted in the inclusion of 44 additional base pairs in the mature DYPD mRNA, thus causing a shift in the reading frame, resulting in a pre-mature stop codon. The frequency of this variant was subsequently identified in 9% of a study population showing severe 5-FU toxicity (29). Alternatively, polymorphisms involving genes other than DYPD, i.e. TYMS, have also been associated with increased toxicity; patients homozygous for the TS 3RG allele, compared with heterozygous or those not carrying the 3RG allele, exhibited significantly higher toxicity from capecitabine treatment, as well as a lower response duration in patients with breast cancer (30). Similar results were presented from another study where the TYMS 2R/2R variant was associated with a relative risk of toxicity of 1.66, and the methyleneteratetrahydrofolate reductase c1298 C/C variant was associated with a relative risk of toxicity of 1.77 (31).

Several approaches have been utilized to detect DYPD deficiency in an effort to identify individuals not suitable for treatment with 5-FU or capecitabine: genotyping, radio-immunoassays measuring DYPD activity in peripheral blood lymphocytes, as well as measuring the concentration of uracil in serum, urine or exhaled air. However, functional tests are usually expensive, often time consuming (peripheral blood lymphocyte test), patient-unfriendly (uracil breath test) and have shown low correlation, especially in patients with partial deficiency. Furthermore, DNA sequencing tests on their own are not considered to be reliable enough, as many of the DYPD polymorphisms are not associated with functional DYPD deficiency. Measuring uracil and dihydouracil in plasma as a ratio appears to be a relatively reliable method with a high prognostic significance of severe toxicity (7, 32).

The oral combination of another 5-FU pro-drug (tegafur) with uracil (competitive inhibitor of DYPD) in a 1:4 molar ratio has been previously reported to be an alternative option for patients with partial DYPD deficiency. The proposed mechanism of action is that oral co-administration of both agents allows for the intact absorption of fluoropyrimidine (tegafur), while uracil depletes DYPD, therefore leading to an artificial state of partial DYPD deficiency. Its protective role against severe 5-FU-related toxicity relies on the assumption that the dose of tegafur is already calculated for the above state, and therefore overdose is avoided. The above proposed mechanism of action has received criticism. There are two large clinical trials comparing uracil/tegafur (UFT) and oral leucovorin versus fluorouracil and leucovorin (LV) in patients with previously untreated metastatic colorectal cancer; the first reported a median time to progression of 3.4 months [95% confidence interval (CI)=2.6 to 3.8 months] with UFT/LV and 3.3 months (95% CI=2.5 to 3.7 months) with 5-FU/LV (p=0.591). There were no statistically significant
differences in survival, tumor response, duration of response, and
time to response. Patients experienced significantly less
stomatitis/mucositis ($p<0.001$) and myelosuppression, resulting
in fewer episodes of febrile neutropenia ($p<0.001$) and fewer
infective episodes ($p=0.04$) (33). The second study reported a
median survival of 12.4 months (95% CI=11.2 to 13.6 months)
with UFT/LV and 13.4 months (95% CI=11.6 to 15.4 months)
with 5-FU/LV ($p=0.630$). The hazard ratio for survival was
0.964 (95.6% CI=0.826 to 1.125), supporting equivalent survival.
The overall response rate did not differ between treatment arms
(UFT/LV, 11.7%; 5-FU/LV, 14.5%; $p=0.232$). Median time to
progression favored 5-FU/LV (UFT/LV, 3.5 months; 5-FU/LV,
3.8 months; $p=0.011$), with similar significantly lower toxicity
with UFT/LV compared to the first trial (34). We recognize the
limited use of UFT around the world, however, it is worth
mentioning that based on the above results, the National Institute
of Clinical Excellence has approved its use in the UK. Finally,
UFT is still contraindicated in cases of complete DYPD
deficiency, and a reduced initial dose has been suggested in cases
of partial deficiency (12, 35, 36).

Fluoropyrimidines are associated with a risk of
cardiotoxicity. In a recent excellent literature review by Kelly et al.,
the overall cardiotoxicity incidence was found to range
between 0.55-19%, with a mean of 5% and a median of 3.85% (37).
Angina appears to be the commonest manifestation, with
up to 19% patients developing chest pain during treatment (38).
Saif et al. reported that angina occurred in 45% of patients with
5-FU-associated cardiotoxicity, whereas myocardial infarction
was seen in 22%, arrhythmia in 23%, acute pulmonary edema
in 5%, cardiac arrest and pericarditis in 1.4% and heart failure
in 2% (39). Furthermore, bolus regimes have been reported to
be associated with a smaller risk of cardiotoxicity (1.6-3%), as
opposed to prolonged infusions, where cardiac events have
been reported as high as 7.6-18% (40, 41).

The mechanism by which 5-FU provokes cardiac toxicity is
largely unknown. One of the most common explanations in the
literature is via 5-FU-induced coronary spasm (42, 43).
Furthermore, 5-FU appeared to induce direct vasoconstriction
on smooth muscle cells which was shown to be protein kinase
C-dependent (44). Increased endothelial thrombogenicity has
been identified in animal models treated with 5-FU, indicating
a toxic effect of 5-FU on endothelial cells (45). Furthermore,
there is evidence to support a direct toxic effect on myocardial
cells, with different patterns of pathology caused in animal
models, depending on the dose and frequency of administration
of 5-FU; in this study, a single bolus 5-FU dose induced a
massive hemorrhagic myocardial infarct with spasms of the
proximal coronary arteries, whereas repeated lower doses of 5-
FU mimicked toxic myocarditis (46). Finally, there has been a
suggested role for 5-FU metabolites in the pathogenesis of
cardiotoxicity; 5-FU is catabolized to alpha-fluoro-beta alanine
and subsequently to fluoroacetate, the latter being an inhibitor of
the Krebs cycle and known cardiotoxic substance (47).

There has been no direct association of fluoropyrimidine-
associated cardiotoxicity with DYPD deficiency, with small
case series showing no DYPD polymorphisms in the patients
investigated (48). The latter indicates that the mechanisms
involved in cardiotoxicity are likely to be independent of the
level of function of DYPD.

Raltitrexed is another TYMS inhibitor that could potentially
serve as an alternative therapeutic approach upon demonstrated
fluoropyrimidine-related cardiotoxicity. It is a water-soluble
molecule that enters cells via the reduced folate carrier (RFC)
and folate receptors (FR) (49). RFC is expressed by both
normal and cancerous cells, whereas some FR isoforms are
predominantly expressed by tumor cells (50, 51). Once
raltitrexed has entered the cell, it is poly-glutamated, resulting
in a more potent inhibition of TYMS and intracellular retention
of the molecule for a longer period, allowing for 3-weekly
administration of the drug (52).

Raltitrexed has been compared with 5-FU in several clinical
trials, either as monotherapy or in combinations with other
cytotoxics. Median survival with raltitrexed has been reported
to range between 9.7-10.9 months, which was comparable
with the survival observed with 5-FU and leucovorin
monotherapy (10-12.7 months) (53). Furthermore, in
combination with oxaliplatin, raltitrexed has demonstrated
superior response rates (45%), and similar overall (15.6
months) and progression-free survival, when compared to
5-FU, LV, oxaliplatin (FOLFOX) chemotherapy (36%, 17.2
months and 8.7 months, respectively). However, two trials
have demonstrated superior time-to-progression with 5-FU
plus LV compared to raltitrexed monotherapy (53, 54).

Raltitrexed was well-tolerated and demonstrated a different
toxicity profile from that of 5-FU; it was associated with
significantly lower risk of hematological toxicity, diarrhea and
mucositis, but higher incidence of elevated transaminases and
asthenia. Cardiac events were not associated with raltitrexed,
and there have been reported cases where re-challenging
patients previously suffering from 5-FU-related cardiotoxicity
with raltitrexed proved to be a safe approach (53-55).

**Conclusion**

It is clear that understanding and further clarifying the
molecular mechanisms behind 5-FU-induced toxicity is central
to identifying patients at increased risk, aiming to select an
appropriate alternative safe regimen for them. Pharmacogenetics
is expected to play a significant role in identifying genetic
polymorphisms associated with increased toxicity. Given the
example of thiopurine methyltransferase activity measurement
prior to initiating treatment with azathioprine or 6-
mercaptopurine, it is expected that with the emerging evidence
of efficacy and toxicity biomarkers, international oncology
societies will formulate specific guidance to aid the clinician in
tailoring each individual treatment regimen.
Conflict of Interest

The Authors declare that there is no conflict of interest.

References


