A narrative review of genetic factors affecting fluoropyrimidine toxicity

William H. Gmeiner

Department of Cancer Biology, Wake Forest School of Medicine, Winston-Salem, NC, USA
Correspondence to: William H. Gmeiner, PhD. Medical Center Blvd, Winston-Salem, NC 27157, USA. Email: bgmeiner@wakehealth.edu.

Objective: Our objective is to document progress in developing personalized therapy with fluoropyrimidine drugs (FPs) to improve outcomes for cancer patients and to identify areas requiring further investigation.

Background: FPs including 5-fluorouracil (5-FU), are among the most widely used drugs for treating colorectal cancer (CRC) and other gastrointestinal (GI) malignancies. While FPs confer a survival benefit for CRC patients, serious systemic toxicities, including neutropenia, occur in ~30% of patients with lethality in 0.5–1% of patients. While serious systemic toxicities may occur in any patient, patients with polymorphisms in *DPYD*, which encodes the rate-limiting enzyme for pyrimidine degradation are at very high risk. Other genetic factors affecting risk for 5-FU toxicity, including miR-27a, are under investigation.

Methods: Literature used to inform the text of this article was selected from PubMed.gov from the National Library of Medicine while regulatory documents were identified via Google search.

Conclusions: Clinical studies to date have validated four *DPYD* polymorphisms (*DPYD**2A, *DPYD**13, c.2846A>T, HapB3) associated with serious toxicities in patients treated with 5-FU. Genetic screening for these is being implemented in the Netherlands and the UK and has been shown to be a cost-effective way to improve outcomes. Factors other than *DPYD* polymorphisms (e.g., miR-27a, *TYMS*, ENOSF1, p53) also affect 5-FU toxicity. Functional testing for deficient pyrimidine catabolism {defined as [U] >16 ng/mL or [UH2]:[U] <10} is being implemented in France and has demonstrated utility in identifying patients with elevated risk for 5-FU toxicity. Therapeutic drug monitoring (TDM) from plasma levels of 5-FU during first cycle treatment also is being used to improve outcomes and pharmacokinetic-based dosing is being used to increase the percent of patients within optimal area under the curve (AUC) (18–28 mg*h/L) values. Patients maintained in the optimal AUC range experienced significantly reduced systemic toxicities. As understanding the genetic basis for increased risk of 5-FU toxicity becomes more refined, the development of functional-based methods to optimize treatment is likely to become more widespread.

Keywords: Fluoropyrimidine (FP); colorectal cancer (CRC); dihydropyrimidine dehydrogenase (DPD); therapeutic drug monitoring (TDM)

Introduction

Fluoropyrimidine drugs (FPs) have been used for more than 50 years (1-3) and an estimated 2 million cancer patients are treated with FP drugs annually (4-6). In particular, FPs remain among the most effective drugs for treating GI malignancies, including colorectal cancer (CRC) (1.8 million), gastric (1 million), and pancreatic cancer

(n=460,000) (7). Personalized medicine is advancing FP use in two ways: (I) Identifying the patient population for whom FPs constitute the preferred chemotherapeutic option and treating these patients with optimized regimens that include FPs (8); and (II) Identifying patient population for whom standard FP dosing poses a known pre-determined risk of serious toxicities, or even death, and either modifying FP dose or treating these patients with alternative drugs. The

implementation of a personalized medicine approach to FP treatment has already contributed to a decline in mortality due to CRC, and these approaches are likely to become more widespread and applicable to treatment of other malignancies. This article will review tumor characteristics such as deficiencies in DNA mismatch repair and *KRAS* mutation status that are used for stratifying CRC patients, and determining if FP chemotherapy is a preferred option. The occurrence and origin of serious toxicities due to 5-FU treatment is also reviewed as are current measures to identify patients at high-risk for 5-FU toxicity thru pre-treatment screening for *DPYD* polymorphisms, reduced DPD activity, and other genetic factors. Approaches to personalize and optimize treatment thru TDM are also reviewed.

The author presents the following article in accordance with the Narrative Review reporting checklist (available at https://dx.doi.org/10.21037/pcm-21-17).

Methods

Literature used to inform the text of this article was selected from PubMed.gov from the National Library of Medicine. Full length manuscripts and Communications published in the English language between 1997 and 2021 were selected. Regulatory documents were identified via Google search.

Patient populations for whom FP-based regimens are a preferred option

The applicability of FP chemotherapy for treating CRC has evolved over its five decades of use, particularly in Western countries, and many findings are impacting treatment of other GI malignancies, worldwide. For patients with early stage CRC that is localized (stage I/II), or with regional lymph node involvement (stage III), surgery with curative intent is the preferred option, and adjuvant chemotherapy may be administered to reduce risk for disease recurrence (9). For patients with stage I colon cancer, surgery alone results in 95% 5-year disease-free survival (DFS) (10), and adjuvant chemotherapy is not administered. In stage II disease 5-year survival is 82–88%, and use of adjuvant chemotherapy remains controversial (11). An intergroup analysis reported a statistically significant improvement in 5-year DFS for stage II patients treated with chemotherapy, but improvement in overall survival was not statically

significant (12). Follow-up analysis of long-term outcomes demonstrated 5-FU chemotherapy was associated with a 5% absolute improvement in survival at 8 years (13). For patients with high-risk stage II disease (e.g., T4 stage, poorly-differentiated tumors), the benefits of FP chemotherapy for risk reduction are considered by many oncologists to outweigh potential harmful effects due to treatment (14), and adjuvant chemotherapy, frequently FP doublet (5-FU/LV) may be administered (15). However, risks associated with FP-based treatment are real and potentially lethal, and for patients with stage II disease the potential risk-reducing benefits of FP regimens are offset by the potential detrimental effects of treatment (16).

For patients with stage III colon cancer the survival benefit of FP-based chemotherapy regimens outweighs potential risks for serious toxicities and the optimization of FP-based chemotherapy has evolved since initial report of therapeutic benefit in 1988 (17). Infusion dosing of 5-FU/LV (de Gramont schedule) was shown to cause reduced systemic toxicities relative to the Mayo and Roswell Park regimens that used bolus injection, although all schedules displayed similar survival benefit (9). 5-FU triplet therapy (e.g., 5-FU/LV/oxaliplatin—FOLFOX) chemotherapy was demonstrated to improve outcomes for patients with stage III CRC in the MOASIC trial and it is the current standard of care for stage III CRC patients. The exception remains tumors with deficient DNA mismatch repair (dMMR) for which FP-based regimens are ineffective and potentially harmful. Since FP chemotherapy is generally not effective in patients with tumors deficient in MMR (18), the MMR status is generally determined by immunohistochemistry (IHC) and/or by PCR-assessment of increased microsatellite instability (MSI-H) (19). Deficiencies in MMR occur either as the result of germ-line mutation conferring increased risk for colon cancer (e.g., Lynch syndrome; ~15%), or from epigenetic silencing of MMR genes (~85%). The mechanistic basis for lack of efficacy of FPs in dMMR CRC is not clearly understood, however MSI is also associated with elevated thymidylate synthase (TS) (20,21), which is the molecular target of FP chemotherapy (2).

Treatment of metastatic CRC (mCRC) also continues to rely heavily on FP-based chemotherapy regimens (8). However, the use of FPs for mCRC is evolving with refined implementation based on biomarker-based stratification to deliver optimal treatment. For mCRC, FP doublet chemotherapy is widely used in combination with targeted therapies directed at EGFR, such as the

monoclonal antibody Cetuximab. However, tumors that display activating mutations in *KRAS* or *BRAF* negate the efficacy of agents targeting EGFR, and in these cases FP-based chemotherapy (doublet or triplet) is frequently used in combination with anti-VEGF therapy (e.g., Bevacizumab). For highly aggressive mCRC, as occurs with *BRAF* mutations, four component FP-based regimens FOLFOXIRI and FOLFIRINOX are used (22). FOLFIRINOX was also recently shown to be a preferred option for adjuvant chemotherapy of pancreatic cancer (23), and to be an effective option in patients with advanced gastroesophageal adenocarcinoma (24). However, its use was associated with higher incidence of toxic effects (23), demonstrating the need to better understand and manage toxicities related to FP use.

Frequency and types of serious toxicities

Nearly all cytotoxic chemotherapy drugs cause serious systemic toxicities and the side effect profiles for 5-FU and other FPs are considered relatively manageable in most instances. Diarrhea and nausea are common side effects that generally present within 7–10 days after initial treatment and are most often manageable. Dermatological toxicities, such as hand-foot syndrome, are common and generally occur later in treatment (25). Risk for severe toxicities is dependent on the FP and schedule used as well as on the genetic features of individual patients. The Mayo schedule is widely used for 5-FU administration and the primary toxicities associated with its use include neutropenia (69%), mucositis (58%), and leukopenia (46%) (26). Female patients have a higher incidence of severe toxicity and death than male patients and the incidence of severe toxicities is more frequent in elderly patients (27). Cardiotoxicity, while relatively rare, is a potentially lethal occurrence with high risk for long-term morbidity and mortality (28), and its risk with 5-FU is second only to anthracyclines (29). 5-FU crosses the blood-brain barrier and its use has been associated with cognitive impairment (30).

Patient population susceptible to serious toxicities from FPs

FPs are central to clinical management of GI-malignancies (3) (e.g., colorectal, gastric, pancreatic) and are widely used to treat head-and-neck cancer (31), basal cell carcinoma (32), and other malignancies. While their widespread use attests to FPs

clinical activity, serious treatment-related toxicities occur in a sizable minority of patients (33). For example, approximately 30% of CRC patients treated with 5-FU or capecitabine experienced severe (≥ grade 3) toxicities, with 10–20% requiring hospitalization for toxicity management (34). Toxicities ultimately cause death in 0.5–1% of patients. In the U.S. alone, the number of lethalities associated with 5-FU treatment exceeds 1,300 per year (35). Further, the cost of managing toxicities that result from 5-FU treatment is substantial, with one study finding that the expected cost of hospitalization related to 5-FU toxicity was $3,674 per patient, approximately $2,700 higher among 5-FU patients than patients not treated with 5-FU (36).

The occurrence of serious toxicities following 5-FU treatment is much more frequent in patients that are deficient in the catabolism of 5-FU (*Figure 1*). While the anti-cancer activities of FPs result primarily from conversion to FdUMP and inhibition of TS (2,37), up to 90% of 5-FU is degraded (38). The first and rate-limiting step in 5-FU degradation (39,40) is catalyzed by dihydropyrimidine dehydrogenase (DPD). Deficiencies in DPD activity occur in an estimated 3–8% of patients (34,41), and certain populations such as individuals of African descent, are at especially high risk to drug-induced toxicities due to variability in drug metabolism (42) while in Asian populations a distinct spectrum of polymorphisms affecting DPD activity predominates (43). Patients deficient in DPD activity are at very high risk for serious 5-FU-related toxicities.

Although deficiency in DPD activity in most individuals is considered to result from polymorphisms in *DPYD* affecting enzyme expression and function, other causes, such as variability in miR-27a regulation of *DPYD* have been identified (44). miR-27a and miR-27b bind a conserved recognition site on *DPYD* repressing its expression and mouse liver DPD activity was inversely correlated with miR-27a expression. Further a variant of miR-27a was associated with reduced DPD activity in healthy volunteers (44), and miR-27a variants improve predictive values of *DPYD* variants for 5-FU toxicity. Intriguingly, p53 also modulates pyrimidine catabolism by repressing *DPYD* expression (45). Polymorphisms in *TYMS*, which encodes thymidylate synthase (TS), the principal target of 5-FU are also implicated in 5-FU toxicity and combined *DPYD* and *TYMS* genotyping could identify a majority of patients at elevated risk for adverse events (46). An isoform of Enolase Superfamily member 1 (ENOSF1) regulates *TYMS* and is
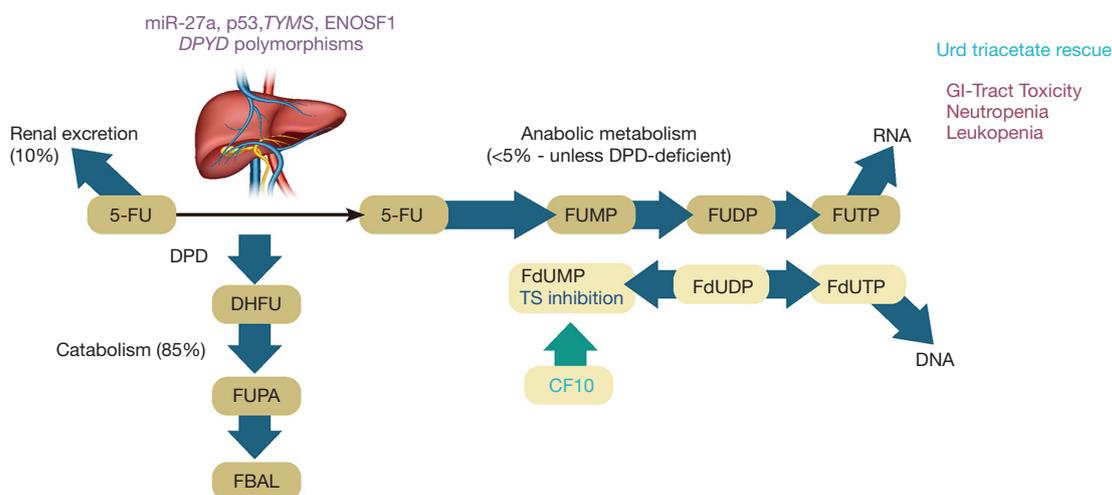
Figure 1 Depiction of fluoropyrimidine metabolism and genetic factors influencing fluoropyrimidine toxicity. In most cancer patients >80% of 5-FU is degraded in the liver. However, for patients with certain *DPYD* polymorphisms reduced catabolism is accompanied by increased anabolic metabolism that perturbs RNA-mediated processes, particularly in the GI-tract and bone marrow, causing serious systemic toxicities. Several factors other than *DPYD* polymorphisms affect 5-FU toxicity including polymorphisms in miR-27a, polymorphisms in *TYMS*, an enzymatic target of 5-FU and levels of ENOSF1, a regulator of *TYMS*. p53 also regulates *DPYD* expression. The fluoropyrimidine polymer CF10 is directly converted to FdUMP and may circumvent issues related to genetic factors affecting 5-FU toxicity.

implicated in capecitabine toxicity (47).

DPYD polymorphisms and increased risk for 5-FU toxicity

DPYD polymorphisms with clinical impact

Deficiencies in DPD activity is causative of increased risk for severe toxicity following FP treatment, with 60–80% of DPD-deficient individuals experiencing dose-limiting and potentially life-threatening toxicities, while the corresponding incidence in DPD-proficient patients is 10–20% (48). Although >160 SNPs in *DPYD* have been reported (49) as outlined by the Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines (6), relatively few are both highly prevalent and have a substantial effect on DPD activity to justify pre-emptive screening. The potential risk for serious toxicities related to the most prevalent *DPYD* polymorphisms associated with reduced or total dysfunction of DPD activity has stimulated national healthcare programs to implement screening programs to reduce serious toxicities in patients treated with 5-FU, capecitabine, or tegafur. The Dutch Pharmacogenetics Working Group (DPWG) identified four *DPYD* variants for which sufficient evidence for

reduced/complete DPD dysfunction (50) and the UK chemotherapy board (51) also considers these same four to have serious clinical impact affecting FP toxicity and since 2020 recommends pre-treatment pharmacogenomic screening for these (*Table 1*): *DPYD**2A (rs3918290), *DPYD**13 (rs55886062), c.2846A>T (rs67376798) and c.1236G>A (*DPYD*-Hap-B3; rs56038477). The clinical relevance of these four SNPs for increased risk of serious toxicities resulting from FP treatment was established by meta-analysis of multiple clinical studies (53). The c.2846A>T and Hap-B3 variants are associated with reduced functionality with modified initial FP treatment regimens recommended (e.g., 50% dose reduction) while the *2A and *13 variants are considered to encode a fully dysfunctional enzyme precluding FP use (50). Case reports document fatal 5-FU toxicity in patients with specific SNPs (54). Neutropenia is the most common toxicity associated with DPD deficiency with one study finding stage IV neutropenia occurring in 55% of patients with decreased DPD activity while the rate in patients with normal DPD activity was only 13% (55) and similar differential toxicity was found in other studies (56). Each of the four *DPYD* SNPs considered to have serious clinical impact by the DPWG and UK chemotherapy

Table 1 *DPYD* polymorphisms with established prognostic value for 5-FU toxicity

DPYD variant	NCBI SNP reference	Nucleotide change	Protein effect	RR[a]
DPYD*2A	rs3918290	1905+1G>A	Exon Skipping	2.87
DPYD*13	rs55886062	1679 T>G	Ile 560 Ser	4.3
c.2846A>T	rs67376798	2846 A>T	Asp 949 Val	3.11
HapB3	s56038477	1236 G>A	Glu 412 Glu, in haplotype B3	1.72

[a], relative risk for overall grade ≥3 fluoropyrimidine-related toxicity compared with non-carriers of this variant (52). CPIC, Clinical Pharmacogenetics Implementation Consortium; HapB3, haplotype B3; RR, relative risk; NCBI, National Center for Biotechnology Information.

board was associated with a significant increased risk for severe neutropenia. Patients with these SNPs display both increased incidence and more rapid onset of serious neutropenia (57).

Importantly, pre-treatment screening for DPD-deficiency based on screening for relatively prevalent *DPYD* polymorphisms significantly improves outcomes when used in conjunction with personalized dose management. The recommendations adopted in the UK parallel results from clinical studies in the Netherlands (58) and other countries that demonstrated prospective screening for clinically-relevant *DPYD* variants was feasible, and that *DPYD* genotype to guide dose improved outcomes. Patient management to reduce risk of toxicities in DPD-deficient individuals includes dose reduction in first-cycle dose of 5-FU or capecitabine followed by dose titration guided by toxicities in subsequent cycles. Collectively these studies established that dose adjustments based on prospective *DPYD* genotyping decreased toxicity while maintaining efficacy (52). Importantly, the data also support that prospective *DPYD* screening not only decreases the incidence of serious toxicities, but reduces overall cost of treatment by an amount that is greater than the cost of screening (59).

Functional implications of DPYD polymorphisms

The effects of SNPs on DPD protein sequence and function have been validated in many cases. For example, the *2A (rs3918290) locus alters a splice recognition sequence leading to a 165 base pair deletion and expression of an inactive DPD protein (60). *DPYD**13 (rs55886062) and rs67376798 result in amino acid changes decreasing DPD activity. Other SNPs that are more prevalent, including *DPYD**9A (~40% heterozygous, 10% homozygous) were implicated in affecting FP toxicity, but are not presently

considered important for pre-treatment screening. A preliminary study indicated *9A was associated with increased risk for serious toxicities, however a later study with larger sample did not reproduce the earlier trend (61). While occurrence of multiple *DPYD* variants in a single individual is rare, it can have serious consequences. For example, a recent study found 4 of 7 patients carrying multiple *DPYD* variants experienced severe toxicity after receiving FP therapy (62). The DPWG considered how combinations of *DPYD* variants impacted overall enzyme activity as indicated by a gene activity score (50). It should be noted that the occurrence of multiple variants (~0.2%) is more common than the *DPYD**13 variant (0.1%) that is widely included in *DPYD* genetic screens and this possibility must be considered (62). Further, it is important to note that the prevalence of *DPYD* SNPs associated with DPD deficiency and severe FP toxicity varies considerably in different patient populations. For example, the HapB3 SNP (rs75017182) is approximately 10-fold more prevalent in Europeans compared to either African or East Asian populations while other SNPs are more prevalent in these populations (48).

Financial implications of DPD testing

The occurrence of serious toxicities related to 5-FU treatment of patients with low DPD activity has serious economic consequences. For example, Murphy *et al.*, calculated the cost of toxicity-related hospitalizations in patients with *DPYD* polymorphisms associated with increased toxicity risk was 10-fold higher than the cost of prospective testing for all patients (63). Martens et al noted an increase in pre-treatment DPD testing from 1% in Q2 of 2017 to 87% in Q4-2018. The availability of clear protocols and evidence of their utility were found to be key factors in increasing testing in the Amsterdam University Medical
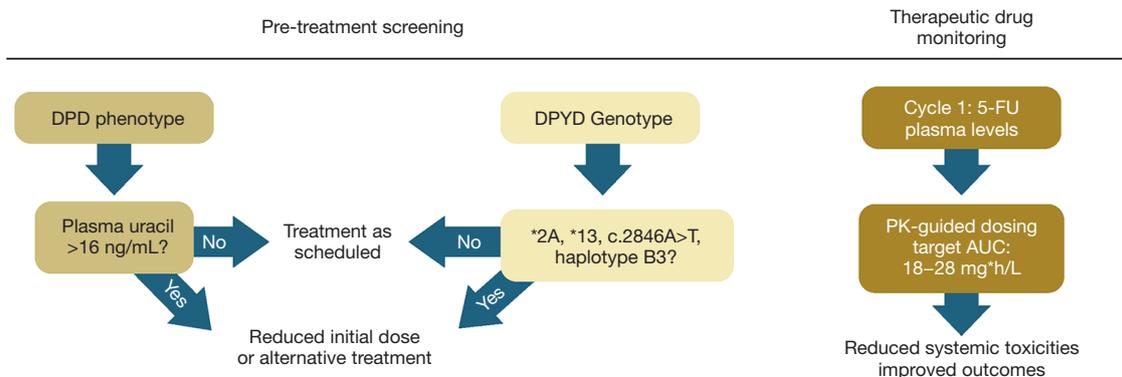
Figure 2 Depiction of strategies to reduce the occurrence of 5-FU-related toxicities. Pre-treatment screening is used to identify patients at elevated risk for 5-FU-related toxicities due to either polymorphisms in the *DPYD* gene associated with reduced DPD functional activity or measuring plasma Uracil values from which deficiencies in pyrimidine metabolism can be inferred. Initial 5-FU dosing may be reduced based on pre-treatment screening results. TDM permits 5-FU dose modulation based on plasma 5-FU levels during cycle 1 treatment such that subsequent cycles are more likely to be in the optimal range.

Centers (64).

Functional testing of DPD activity

Since only 39–61% of patients with serious 5-FU toxicities display polymorphisms associated with reduced DPD activity (41,65) and <20% of patients that experience serious FP-related toxicities are identified by genotyping *DPYD* (34) other factors must contribute to 5-FU toxicity. The need to more generally identify patients with high-risk has resulted in efforts to quantify patient-specific DPD activity prior to initiating 5-FU treatment to identify patients at elevated risk for serious toxicities. While polymorphisms in *DPYD* are a principal contributor to serious 5-FU toxicities, they are not the only cause. For example miR-27 regulation of *DPYD* is another potential causes of reduced DPD activity (*Figure 1*). Also p53 repressively regulates *DPYD* expression (45). Further, there are additional contributing factors including polymorphisms in *TYMS* and variants in *ENOSF1* affecting 5-FU toxicity. Several functional endpoints related to DPD activity and pyrimidine metabolism have been developed and tested clinically including: (I) DPD activity in PBMCs; (II) plasma uracil concentrations; (III) 2-^{13}C breath test; (IV) Uracil test dose (*Figure 2*).

Pallet *et al.* (66) assessed characteristics of 3,680 patients that had completed testing to establish their DPD phenotypes and *DPYD* genotypes. DPD functional activity was assessed both from uracil plasma concentrations ([U]), and the ratio of dihydrouracil to uracil ([UH2]:[U]). Reduced DPD activity is defined as [U] >16 ng/mL or

[UH2]:[U] <10. *DPYD* SNPs associated with reduced DPD activity were detected in 4.5% of patients; however, their detection had only moderate predictive value in identifying patients with functional DPD deficiencies. The authors concluded that a clinical trial comparing toxicity rates after dose adjustments based on genotype vs phenotype testing for DPD deficiency was needed to identify the best strategy for mitigating risk of 5-FU treatment. In another study, plasma uracil concentrations were found to be superior to detection of gene polymorphisms for predicting which patients will experience serious 5-FU-related toxicities. Meulendijks *et al.* (67), conducted testing in 550 patients and elevated plasma uracil concentration was significantly associated with global severe toxicity (P=0.0009), severe gastrointestinal (GI) toxicity (P<0.0001), toxicity-related hospitalization (P<0.0001), and fatal treatment-related toxicity (P=0.001). None of the *DPYD* or *TYMS* variants were significantly associated with severe toxicities in this study.

Several efforts are ongoing to make widespread testing of uracil and dihydrouracil prior to FP treatment more accurate and more economical. To automate testing of uracil and dihydrouracil in plasma, Robin *et al.* developed a LC-MS method with an automated sample preparation system that may enable widespread implementation of pre-treatment testing for DPD functional activity (68). Uracil and dihydrouracil concentrations in saliva may provide a more convenient and less invasive source of biological fluids to identify patients at high risk for 5-FU toxicity. Both fresh saliva and dried saliva spots were shown to

display prognostic value (69). Another approach is use of a breath test to detect uracil degradation. However, this approach, while rapid and reliable, requires use of either radioactive isotopes or mass spectrometry (70), and thus may have less potential for widespread implementation. The French cancer institute and French health authority recommend measurement of uracil blood level and implementation of alternative regimens for cancer patients with elevated levels (71).

Interventions to reduce FP toxicity

Since severe toxicity in response to 5-FU treatment can occur in any patient, regardless of *DPYD* status or DPD activity, pre-treatment screening for these will reduce but not eliminate the occurrence of severe toxic responses. Further, these pre-treatment screening approaches will not enable dose modification for optimal response in the majority of patients that do not display serious DPD deficiencies. For these reasons, approaches to TDM are being implemented to adjust 5-FU dosing for optimal therapeutic response (72). 5-FU is now primarily dosed as an infusion over 46 h and this is used in combination chemotherapy regimens such as FOLFOX and FOLFIRI. Infusion dosing has generally replaced bolus administration and has an improved safety profile although severe toxicities still occur, largely based on large inter-patient variability in 5-FU plasma levels.

As with other cytotoxic agents, 5-FU dose is calculated based on patient body surface area (BSA), however BSA-based dosing does not account for inter-patient variability in plasma clearance which ranged from 0.49 to 4.93 L/h/m^2 in one study (73). Further, this inter-patient variability in plasma clearance correlates with therapeutic response with elevated clearance associated with poor outcomes (74). Multiple studies have now demonstrated that adjusting 5-FU dose based on plasma levels is feasible and can improve outcomes [reviewed in ref (72)]. For example, a recent study showed that implementing an adjusted, PK-guided dose of 5-FU in cycle 2 based 5-FU plasma levels measured during cycle 1 resulted in increasing the percent of patients within optimal area under the curve (AUC; 18–28 mg*h/L) values from 49.3% to 66.9%. Patients maintained in the optimal AUC range experienced significantly reduced systemic toxicities. For example relative risk for neutropenia for overexposed patients based on AUC was 3.05 (1.55–6.01; P=0.004), while patients with at least one AUC below

the target during the first 3 cycles displayed significantly reduced progression-free survival (75).

The serious systemic toxicities such as neutropenia that occur in patients deficient in 5-FU catabolism result from RNA-mediated effects of the drug and not on-target effects related to FdUMP, the metabolite primarily responsible for anti-tumor activity. Among anabolic metabolites, ribonucleotides (e.g., FUTP) are produced at much higher levels than the corresponding deoxyribonucleotides by ~10:1 ratio (76). Under conditions of reduced catabolism, such as in patients with *DPYD* polymorphisms, increased misincorporation of FUTP into RNA causes extensive damage to leukocytes and cells of the GI-tract by an unspecified process. The serious toxicities associated with 5-FU overtreatment can be reversed by treatment with uridine triacetate (77), which increases UTP pools and competes with FUTP for RNA incorporation. Both leukopenia (78) and 5-FU-induced GI-tract toxicity (79) can be reversed with Uridine treatment. An alternative approach to reducing 5-FU's RNA-mediated effects and this its systemic toxicities while increasing levels of FdUMP responsible for anti-tumor activity is use of fluoropyrimidine polymers (80,81). The Gmeiner lab has pioneered testing of FP polymers (e.g., CF10) in pre-clinical models (82-84), and demonstrated these result in sustained high plasma levels of FdU and FdUMP while not causing serious systemic toxicities that occur with 5-FU treatment, in part due to reduced dependence on DPD-mediated degradation (80).

Conclusions

FPs remain the backbone for combination chemotherapy regimens used to treat CRC and are among the most important drugs for treatment of pancreatic cancer and other GI-malignancies. While FP use confers a survival benefit, a significant portion of patients experience serious toxicities that are expensive to manage and may be lethal. Risk/benefit analysis limits 5-FU use in some patient populations that could benefit from treatment if toxicities were not a concern. While serious toxicities may occur in any patient, patients with *DPYD* polymorphisms reducing pyrimidine catabolism are known to be at elevated risk. Genetic screening is being implemented in The Netherlands and UK and other locations to identify these high-risk patients and pre-emptively adjust FP dose. Outcomes and cost-analysis indicates genetic-based screening is effective. A more general approach involving functional screening for

patients with atypical pyrimidine catabolism is also being implemented in France and other locations and may permit improved dosing for a wider spectrum of patients than those with specific *DPYD* polymorphisms. TDM utilizing PK-guided dosing to personalize treatment is demonstrated to increase the percent of patients that achieve an optimal dose range and this approach is both reducing the incidence of serious systemic toxicities and improving outcomes. Since the most frequent serious systemic toxicities of 5-FU appear to be unrelated to the on-target effects of the primary anti-tumor metabolite, FdUMP, next generation FPs, such as FP polymers may reduce the need for identifying patients who are at high risk for toxicities with treatment with the current FP drugs.

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Footnote

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Ethical Statement: The author is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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