Risk of Toxicity From Topical 5-Fluorouracil Treatment in Patients Carrying *DPYD* Variant Alleles

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Patients carrying *DPYD* variant alleles have increased risk of severe toxicity from systemic fluoropyrimidine chemotherapy. There is a paucity of data regarding risk of toxicity from topical 5-fluorouracil (5-FU) treatment in these patients, leading to inconsistent guideline recommendations for pretreatment testing and topical 5-FU dosing. The objective of this retrospective cohort study was to investigate whether *DPYD* variant allele carriers have increased risk of toxicity from topical 5-FU. Treatment and toxicity data were retrospectively abstracted from the electronic medical records. Genotypes for the five *DPYD* variants that are associated with increased toxicity from systemic fluoropyrimidine chemotherapy (*DPYD*2A*, *DPYD*13*, *DPYD p.D949V*, *DPYD HapB3*, and *DPYD p.Y186C*) were collected from a genetic data repository. Incidence of grade 3+ (primary end point) and 1+ (secondary end point) toxicity was compared between *DPYD* variant carriers vs. wild-type patients using Fisher's exact tests. The analysis included 201 patients, 7% (14/201) of whom carried a single *DPYD* variant allele. No patients carried two variant alleles or experienced grade 3+ toxicity. *DPYD* variant allele carriers did not have a significantly higher risk of grade 1+ toxicity (21.4% vs. 10.2%, odds ratio = 2.40, 95% confidence interval: 0.10–2.53, $P = 0.19$). Given the low toxicity risk in patients carrying a single *DPYD* variant allele, there is limited potential clinical benefit of *DPYD* genetic testing prior to topical 5-FU. However, the risk of severe toxicity in patients with complete DPD deficiency remains unknown and topical 5-FU treatment should be avoided in these patients.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Risk of severe toxicity from systemic fluoropyrimidine chemotherapy is ~2-4 times greater in the 5-7% of patients who carry a polymorphism in the *DPYD* gene that reduce activity of the dihydropyrimidine dehydrogenase (DPD) enzyme responsible for 5-FU catabolism. *DPYD* genetic or DPD phenotypic activity testing prior to systemic fluoropyrimidine treatment is standard practice in Europe and increasingly conducted in the USA. There is a case report of life-threatening systemic toxicity from topical 5-FU in a patient with complete DPD deficiency; however, there is a paucity of data on the risk severe toxicity from topical 5-FU in *DPYD* variant carriers, leading to inconsistent testing and dosing guidelines.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ The objective of this study was to determine whether patients carrying *DPYD* variant alleles have increased risk of severe toxicity from topical 5-FU treatment, and to determine whether testing and dosing recommendations should also apply to topical 5-FU administration.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ Our results indicate the risk of severe toxicity from topical 5-FU treatment is extremely low, even in patients with partial DPD deficiency. We also did not observe a significant increase in mild, dermatological toxicity in *DPYD* variant carriers. These findings suggest there may limited potential clinical benefit of *DPYD* or DPD testing prior to topical 5-FU treatment.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ Clinical guidelines should not routinely recommend *DPYD* genotype or DPD activity phenotype testing prior to topical 5-FU treatment, though topical 5-FU treatment should be avoided or used with extreme caution in patients who are known to have complete DPD deficiency.

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5-fluorouracil (5-FU) cream (5%) is administered as a topical treatment for dermatologic conditions, including actinic keratosis.[1,2] Use of topical 5-FU causes minor local toxicity (e.g., erythema, crusting, and ulceration) in 60%–80% of patients[3,4] and there are case reports of rare, severe systemic toxicity.[5–9] Intravenous 5-FU and the oral pro-drug capecitabine are systemically administered fluoropyrimidine chemotherapy used to treat colorectal and other solid tumors[10] that cause severe (> 30%), and, in some cases, fatal (< 1%) toxicity.[11]

Risk of severe toxicity from fluoropyrimidine chemotherapy is ~2–4 times greater in the 5%–7% of patients who carry a polymorphism in the *DPYD* gene that reduces activity of the dihydropyrimidine dehydrogenase (DPD) enzyme responsible for 5-FU catabolism.[12,13] The ~0.4% of patients who carry 2 *DPYD* variants have dramatically increased the risk of severe and fatal toxicity from fluoropyrimidine chemotherapy.[11]

DPYD genetic testing and/or DPD phenotypic activity testing prior to systemic fluoropyrimidine chemotherapy treatment is standard practice in Europe[14] and is increasingly conducted in the United States.[15,16] The Dutch Pharmacogenetics Working Group (DPWG) and the Clinical Pharmacogenetics Implementation Consortium (CPIC) have developed an activity score (AS) system to translate a patient's *DPYD* genotype into a DPD activity phenotype.[17,18] Alleles associated with null activity receive an AS = 0.0 (e.g., *DPYD*2A* and *DPYD*13*) and those with diminished activity receive an AS = 0.5 (e.g., *DPYDp.D949V*, *DPYD HapB3*, and *DPYD p.Y186C*). Fluoropyrimidine dosing guidelines from these organizations recommend 50% dose reduction in patients with cumulative AS = 1.0–1.5 and avoidance of fluoropyrimidine in patients with AS = 0.0–0.5.

There is a single case report of life-threatening systemic toxicity from topical 5-FU in a patient with complete DPD deficiency.[5] However, there is a paucity of data on the risk of severe toxicity from topical 5-FU in patients who carry *DPYD* variant alleles, leading to inconsistent testing and dosing guidelines as to whether[18] or not[19] recommendations apply to topical 5-FU treatment. The objective of this study was to determine whether patients carrying *DPYD* variant alleles have increased risk of severe toxicity from topical 5-FU treatment, and to determine whether testing and dosing recommendations should also apply to topical 5-FU administration.

METHODS

Study setting and patient population

This retrospective analysis included adult patients who received topical 5-FU treatment at Michigan Medicine and had genetic data available in the Michigan Genomics Initiative (MGI) institutional genetic data repository. Patients who received other fluoropyrimidine treatments, including systemic 5-FU or capecitabine, tegafur-uracil, or floxuridine via hepatic arterial infusion pump, were excluded. The study protocol was approved by the Institutional Review Board (IRB# HUM00161844) and conducted in accordance with the Helsinki Declaration of 1975.

Clinical and genetic data

Clinical data were abstracted from the University of Michigan electronic health record (MiChart) by an investigator blinded to genotype data. MiChart was searched using Electronic Medical Record Search Engine (EMERSE).[20] Abstracted data included demographics, treatment indication, and prescribed topical 5-FU regimen. Toxicities occurring during the first cycle of topical 5-FU treatment that were attributable to topical 5-FU treatment, based on provider notes, were retrospectively abstracted from MiChart and graded according to National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. The *a priori*-defined primary end point was grade 3 or higher (grade 3+) toxicity; whereas grade 1 or higher (grade 1+) toxicity was a prespecified secondary end point. A grade 3 toxicity was defined as any systemic toxicity or dermatological toxicity, including dry skin and erythema multiforme covering 30% body surface area or erythroderma primary, all hindering activities of daily living. A grade 2 toxicity could include similar signs but less body surface area or without limitations to activities of daily living.

MGI genotyping was conducted on Illumina Infinium CoreExome-24 bead arrays and genetic data was cleaned as previously described in detail.[21] The current analysis focused on carriers of the 5 *DPYD* alleles (*DPYD*2A* (rs3918290), *DPYD*13* (rs55886062), *DPYD p.D949V* (rs67376798), *DPYD HapB3* (rs56038477), and *DPYD p.Y186C* (rs115232898)) that are validated to be associated with increased risk of systemic fluoropyrimidine toxicity; these same alleles were included in our prior analysis demonstrating that MGI participants who carried these *DPYD* variants had increased risk of severe toxicity from systemic fluoropyrimidine chemotherapy.[22]

Statistical analysis

The *a priori* defined primary analysis was the comparison of the rate of grade 3+ toxicity in carriers of any of the 5 *DPYD* variant alleles vs. wild-type patients; secondary analysis was conducted of grade 1+ toxicity. Rates of grade 3+ and grade 1+ toxicity in variant carriers vs. wild-type patients were analyzed using a Fisher's exact test to allow for the analysis of groups with counts < 5, using the standard 2-sided $\alpha = 0.05$.

RESULTS

Clinical and genetic data

A cohort of 649 patients who received topical 5-FU treatment at Michigan Medicine between 2012 and 2022 were identified, of whom 201 had genetic data available in MGI and were included in this analysis. These 201 patients were 98% White, 71% men, and the most common indication for topical 5-FU treatment was actinic keratosis (79%; **Table 1**). As expected in a patient cohort in the United States, 7.0% (14/201) of patients carried one of the 5 validated *DPYD* variants leading to a partial DPD deficiency or intermediate metabolizer phenotype (AS = 1.0–1.5).

Occurrence of toxicity and association with *DPYD* genotype

There were no (0%) occurrences of the primary outcome of grade 3+ toxicity; therefore, no statistical analysis could be conducted of the primary end point. There were 22 (11%) occurrences of the secondary outcome of grade 1+ toxicity, all of which were grade 1 or 2 dermatological toxicities. Patients carrying any *DPYD* variant had a nominally higher rate of grade 1+ toxicity than *DPYD* wild-type patients, however, this did not reach statistical significance (21.4% (3/14) vs. 10.2% (19/187), odds ratio = 2.40, 95% confidence interval: 0.10–2.53, $P = 0.19$).

DISCUSSION

Patients who carry diminished activity *DPYD* variants have increased risk of severe toxicity from systemic fluoropyrimidine chemotherapy[11,12,15,16] but whether they have increased risk of toxicity from topical 5-FU is unknown. Prospective trials

indicate that severe, systemic topical 5-FU toxicity is rare,[3,4] but ~6% of the topical dose is absorbed systemically[23] and there is one case report of a patient with complete DPD deficiency who experienced life-threatening systemic toxicity, including stomatitis, bloody diarrhea, vomiting, fever, and chills.[5] Another patient who experienced similar systemic topical 5-FU toxicity tested negative for a null activity (i.e., AS = 0) *DPYD* variant, and no further testing was conducted.[6] Other systemic toxicities, such as neutropenia, angioedema, neurological conditions, and taste abnormalities, have been reported in patients treated with topical 5-FU, most of whom were not tested[7] or did not carry a *DPYD* variant or had normal DPD activity.[8,9] This is the first study, to our knowledge, investigating the risk of topical 5-FU toxicity in a cohort of patients with known *DPYD* genotype or DPD activity. Our results indicate the risk of severe toxicity from topical 5-FU treatment is extremely low, even in patients with partial DPD deficiency.

Table 1 Clinical and genetic information for patients included in the analysis (*n* = 201)

	N (%)
Sex	
Male	143 (71.1%)
Female	58 (28.9%)
Self-reported race	
White	196 (97.5%)
Asian	2 (1.0%)
Black	1 (0.5%)
American Indian/Pacific Islander	1 (0.5%)
Unknown/not reported	1 (0.5%)
Indication	
Actinic keratosis	159 (79.1%)
Verruca	22 (9.14%)
Warts	10 (5.0%)
Other	10 (5.0%)
DPYD genotype	
*DPYD*1/*1* (Wild-type)	187 (93.0%)
*DPYD*1/*2A* (AS = 1.0)	3 (1.5%)
*DPYD*1/*13* (AS = 1.0)	0
*DPYD*1/p.D949V* (AS = 1.5)	1 (0.5%)
*DPYD*1/HapB3* (AS = 1.5)	10 (5.0%)
*DPYD*1/p.Y186C* (AS = 1.5)	0
Total variant carriers	14 (7.0%)
Observed toxicity	
Grade 1+ dermatological toxicity	22 (10.9%)
Grade 3+	0 (0%)

AS, activity score.

Our results do not demonstrate a significant increase in mild, dermatological toxicity in *DPYD* variant carriers receiving topical 5-FU, although this analysis was likely underpowered. Additionally, the estimated effect size (~2.4) is within the range (2×–4×) of the increase in severe toxicity from systemic fluoropyrimidine chemotherapy treatment in *DPYD* variant allele carriers,[13,22] suggesting there may be a similar increase in mild toxicity risk from topical 5-FU. There is limited potential clinical benefit of predicting and avoiding this self-resolving toxicity.[16] Guidance on the use of *DPYD* genotype or DPD phenotype testing prior to topical 5-FU treatment is conflicting (**Table 2**). The DPWG considers *DPYD* genetic testing essential prior to starting fluoropyrimidine treatment regardless of route of administration[18] whereas the European Medicines Agency (EMA) only recommends DPD testing prior to systemic fluoropyrimidine treatment.[19] The US Food and Drug Administration (FDA) does not currently recommend DPD or *DPYD* testing before initiating systemic or topical therapy, although the capecitabine drug label was recently updated to "consider testing."[15,24,25] Based on the lack of severe toxicity and limited potential clinical benefit of avoiding mild toxicity observed in this study, *DPYD* genotype/DPD phenotype testing does not appear to be necessary prior to topical 5-FU treatment.[16]

Table 2 Testing and dosing recommendations by fluoropyrimidine route of administration

	DPYD/DPD testing		Fluoropyrimidine dosing	
	Systemic fluoropyrimidine chemotherapy	Topical 5-fluorouracil	Systemic fluoropyrimidine chemotherapy	Topical 5-FU
DPWG	Testing is essential	Testing is essential	Partial deficiency: reduce doses Complete deficiency: avoid treatment	Complete deficiency: avoid treatment
CPIC	Not applicable[a]	Not applicable[a]	Partial deficiency: reduce doses Complete deficiency: avoid treatment	No statement as to whether dosing recommendations are applicable
EMA	Testing is recommended	Testing recommendation does not apply	Partial deficiency: reduce doses Complete deficiency: avoid treatment	Dosing recommendations are not applicable
FDA[b]	Consider testing (capecitabine[b])	No testing recommendation	Partial deficiency: usual doses Complete deficiency: avoid treatment	Complete deficiency: avoid treatment

5-FU, 5-fluorouracil; CPIC, Clinical Pharmacogenetics Implementation Consortium; DPD, dihydropyrimidine dehydrogenase; DPWG, Dutch Pharmacogenetics Working Group; EMA, European Medicines Agency; FDA, Food and Drug Administration.
[a]The CPIC does not provide testing recommendations. [b]The FDA recently updated the capecitabine drug label to "consider testing." Drug labels for intravenous and topical 5-FU have not been updated and do not recommend testing. Labels for capecitabine, intravenous 5-FU, and topical 5-FU recommend against treatment in patients with complete DPD deficiency but do not recommend dose adjustment in patients with partial deficiency.

There is minimal guidance regarding appropriate dosing of topical 5-FU cream in patients with partial or complete DPD deficiency (**Table 2**). The DPWG and CPIC recommend 50% reductions of systemic fluoropyrimidine chemotherapy doses for patients with partial DPD deficiency (AS = 1.0–1.5) and avoiding fluoropyrimidine chemotherapy treatment in patients with complete DPD deficiency (AS = 0.0–0.5).[17,18] Only the DPWG provides an explicit dosing recommendation for topical 5-FU; the DPWG recommends avoiding topical 5-FU administration for patients with DPD AS = 0.[18] The EMA explicitly states that DPD-guided fluoropyrimidine dosing recommendations do not apply to topical treatments,[19] whereas the CPIC does not specify whether their dosing recommendation should be followed for topical 5-FU treatment.[17] Finally, the FDA recommends avoiding topical 5-FU cream and oral capecitabine treatment in patients with complete DPD deficiency,[2,25] but does not recommend dose adjustment for patients with partial deficiency.[15] These inconsistent recommendations make it challenging for sites that have clinical decision support alerts for patients receiving fluoropyrimidine treatment who carry *DPYD* variants but indicate implementors should be mindful of the administration route when developing and deploying these tools in practice. Our results demonstrate the safety of administering the usual topical 5-FU doses in patients with partial DPD deficiency. It would be prudent to monitor for topical and systemic toxicity in patients with partial DPD deficiency, and perhaps consider switching to the lower strength 2% cream or reducing application frequency if clinically significant toxicity occurred. Unfortunately, due to the absence of patients with complete DPD deficiency in this cohort, their risk of mild or severe toxicity from topical 5-FU remains unknown. Until this information is available, it would be best to avoid topical 5-FU in patients with known DPD deficiency, as recommended by the DPWG and FDA.[2,18]

This retrospective pharmacogenetic association study has several potential limitations that should be considered. Retrospective abstraction of toxicity data from the electronic medical record may have led to some toxicity events not being recorded, as suggested by the comparatively lower rate of mild toxicity in this study (~ 10%) compared with prospective clinical trials (60%–80%).[3,4] This may also be a consequence of collecting toxicity only during the first cycle of topical treatment and not having any means to verify treatment adherence. This is likely true for grades 1–2 toxicity that occurred in patients self-administering treatment at home, although it is unlikely to be a major issue for our primary end point of grade 3+ toxicity that requires medical intervention. Additionally, this study was limited to the 201 patients who met our inclusion criteria and participated in our institutional genetic data repository, 98% of whom were White, precluding adjustment for covariates that may modulate toxicity risk including race. Finally, due to the modestly sized cohort, our study was likely underpowered to detect a statistically significant increase in grades 1–2 toxicity and our cohort did not include any patients with complete DPD deficiency. Additional studies are needed in larger patient cohorts to provide definitive evidence of the increased risks of minor toxicity in patients with partial DPD deficiency and to estimate the risk of severe toxicity from topical 5-FU in the uncommon patients with complete DPD deficiency to inform guidelines recommendations for testing and dosing. An ongoing prospective observational clinical trial of topical 5-FU treatment in patients carrying clinically actionable *DPYD* variants will hopefully provide confirmatory evidence supporting our findings and recommendations for *DPYD* testing and topical 5-FU treatment (https://onderzoekmetmensen.nl/nl/trial/20542).

In conclusion, our study demonstrates the safety of topical 5-FU treatment in *DPYD* variant carriers with partial DPD deficiency, suggesting a lack of potential clinical benefit for pretreatment *DPYD*/DPD testing in these patients. Severe systemic toxicity in a patient with complete DPD deficiency receiving topical 5-FU has been reported[5] previously but the actual risk of this outcome remains unknown. Based on this evidence, and the rarity of complete DPD deficiency (< 0.5%), clinical guidelines should not routinely recommend *DPYD* genotype or DPD activity phenotype testing prior to topical 5-FU treatment. Testing prior to topical 5-FU may be worthwhile in patients with suspected DPD deficiency, perhaps based on previous severe fluoropyrimidine toxicity in the patient or their family member, to determine if the patient has complete DPD deficiency and topical 5-FU treatment should be avoided.

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CONFLICTS OF INTEREST

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AUTHOR CONTRIBUTIONS

J.G., A.L.P., N.L.H., V.S., and D.H. wrote the manuscript. D.L.H. and A.L.P. designed the research. J.G. performed the research. J.G. analyzed the data.

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