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Dihydropyrimidine Dehydrogenase Deficiency Testing for Patients Treated With 5-Fluorouracil and Capecitabine

Key Messages

What Is the Issue?

- Fluoropyrimidines, such as 5-fluorouracil and capecitabine, are drugs used for the treatment of solid tumour cancers. Deficiency in the enzyme dihydropyrimidine dehydrogenase (DPD), which breaks down these drugs, can significantly increase the risk of severe toxicity and death.
- Pretreatment DPD deficiency testing, via genotyping, or phenotyping, followed by dose adjustments is recommended in several countries to reduce adverse drug reactions. However, most evidence is based on genetic variants identified in individuals from European countries such as the Czech Republic, Denmark, France, Ireland, Italy, the Netherlands, Spain, and the UK. This raises uncertainties about the transferability of the safety and effectiveness of these approaches to patients from diverse ethnic origins.
- In Canada, access to DPD deficiency testing is inconsistent and varies widely across provinces and territories.

What Did We Do?

- We conducted a national survey on the current state of DPD deficiency testing and a rapid review to identify and summarize evidence comparing the clinical and cost-effectiveness of DPD deficiency testing and test-guided dose adjustments versus usual care.
- We searched key resources, including journal citation databases, and conducted a focused internet search for relevant evidence published since 2015. One reviewer screened articles for inclusion based on predefined criteria, critically appraised the included studies, and narratively summarized the findings.

What Did We Find?

- The survey results suggest that *DPYD* genotyping is conducted in 5 Canadian provinces, and 2 more are set to start testing later this year. One province indicated that they conduct both *DPYD* genotyping and DPD phenotyping, as required. The cost of testing ranged from CA$50 to CA$500 and was dependent on the testing platform and required turnaround time.
- The evidence suggests that *DPYD* variant carriers are at a higher risk of severe toxicities, hospitalization, and death compared to patients with the wild-type gene and that genotype-guided dose adjustments may improve these clinical outcomes in variant carriers.

Key Messages

- The data for the clinical utility of genotype-guided dosing is based largely on study populations from European countries, decreasing the utility of genotype testing in Canada, where the target population includes numerous ethnic origins. Further research and guideline development is required to support the validity and utility of variants more common in these groups.
- Phenotype testing provides an appealing complementary or alternative test that is independent of ethnic origin; however, evidence supporting its clinical validity and utility is minimal.
- Evidence suggests that *DPYD* testing with subsequent genotype-guide dose adjustments is cost-effective compared to usual care. No evidence was found on the cost-effectiveness of an extended *DPYD* genetic panel or of DPD phenotyping.

What Does This Mean?

- Based on the evidence identified in this report, *DPYD* genotyping may be clinically valid and cost-effective to improve the safety of fluoropyrimidine use in Canada for patients of European descent.
- Clinicians and decision-makers can use the evidence summarized in this review to inform decisions regarding the implementation of DPD deficiency testing.

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Abbreviations

5-FU	5-fluorouracil
CI	confidence interval
CTCAE	Common Terminology Criteria for Adverse Events
DPD	dihydropyrimidine dehydrogenase
FP	fluoropyrimidine
HTA	health technology assessment
ICER	incremental cost-effectiveness ratio
NGS	next-generation sequencing
NRS	nonrandomized study
OR	odds ratio
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
QALY	quality-adjusted life-year
RCT	randomized controlled trial
SR	systematic review
UH_2/U	dihydrouracil to uracil ratio

Key Terminology

Allele: One of 2 or more alternative forms of a gene that arise by mutation and are found at the same place on a chromosome. Every individual has 2 alleles for each gene.

Clinical utility: In this Rapid Review report, this term refers to the probability that pretreatment dihydropyrimidine dehydrogenase testing followed by test-guided dose adjustments reduces fluoropyrimidine-related toxicity or mortality compared to patients who received standards doses.

Clinical validity: In this Rapid Review report, this term refers to the accuracy with which low dihydropyrimidine dehydrogenase activity (identified via either presence of a *DPYD* genetic variant or phenotyping tests for dihydropyrimidine dehydrogenase activity) predicts specific clinical outcomes (e.g., severe toxicity, mortality) following usual care treatment with fluorouracil or capecitabine. This is separate and distinct from analytical validity, which refers to the accuracy and reliability with which a specific test can measure or detect a specific genetic variant. Analytical validity is not considered in this report.

***DPYD* genotyping**: A type of testing to determine whether a genetic variant (genotype) is present in an individual's DNA, specifically their *DPYD* gene. Testing methods include polymerase chain reaction, Sanger sequencing, and next-generation sequencing.

DPD phenotyping: A type of testing to determine the activity level of the dihydropyrimidine dehydrogenase enzyme in an individual's body. Testing methods include measurement of plasma uracil concentrations, dihydrouracil to uracil ratios, and activity in peripheral blood mononuclear cells.

Ethnicity: "A socially defined category or membership of people who may share a nationality, heritage, language, culture, and/or religion."[1]

Ethnic origin: "The ethnic or cultural origins of the person's ancestors. Ancestors may have Indigenous origins, or origins that refer to different countries, or other origins that may not refer to different countries."[2]

Gender: "Gender can refer to the individual and/or social experience of being a man, a woman, or neither. Social norms, expectations and roles related to gender vary across time, space, culture, and individuals."[3]

Health equity: "Equity is the absence of unfair, avoidable or remediable differences among groups of people, whether those groups are defined socially, economically, demographically, or geographically or by other dimensions of inequality (e.g. sex, gender, ethnicity, disability, or sexual orientation). Health is a fundamental human right. Health equity is achieved when everyone can attain their full potential for health and well-being."[4]

PROGRESS-Plus: An acronym used to identify characteristics that stratify health opportunities and outcomes. PROGRESS refers to place of residence, race, ethnicity, culture, language, occupation, gender, sex, religion, education, socioeconomic status, social capital. Plus refers to personal characteristics associated with discrimination (e.g., age, disability), features of relationships (e.g., smoking parents, excluded from school), and time-dependent relationships (e.g., leaving the hospital, respite care, other instances where a person may be temporarily at a disadvantage)[5]

Sex: “The classification of people as male, female, or intersex. Sex is typically assigned at birth and is based on an assessment of one’s reproductive systems, hormones, chromosomes, and other physical characteristics.”[1,3]

Context

Fluoropyrimidines and Fluoropyrimidine-Associated Toxicity

Fluoropyrimidines (FPs), such as 5-fluorouracil (5-FU) and capecitabine, are widely used chemotherapy agents for treating solid tumours, including colorectal, gastric, breast, and head and neck cancers.[6,7] 5-FU is typically administered through IV; whereas, capecitabine is an orally administered prodrug that is converted into 5-FU after absorption. Once inside the cell, these drugs are metabolized into active compounds that disrupt RNA and DNA synthesis, ultimately leading to cell death.[8] While FPs have been shown to be effective in improving overall survival, approximately 30% of patients[9] experience severe (grade ≥ 3)[10] drug-related toxicities. Of note, tegafur is another FP used in the treatment of cancer; however, it will not be discussed in this report as it has not been approved for use in Canada.[11]

5-FU is catabolized in the body by an enzyme called dihydropyrimidine dehydrogenase (DPD). As shown in Figure 1, only 1% to 5% of the original dose of 5-FU mediates the cytotoxic effects on tumour cells, while DPD catabolizes more than 80% of the drug.[12] The remaining 10% is excreted in urine. A deficiency in DPD, caused by mutations in the *DPYD* gene, is found in approximately 2% to 8% of people of European descent.[13,14] This deficiency markedly increases 5-FU levels in the blood, thereby increasing the risk of severe toxicity from FP treatment.

Severe toxicity is defined as adverse events graded as greater than 3 using the Common Terminology Criteria for Adverse Events (CTCAE).[10] FP-related adverse events include hematological (e.g., neutropenia), gastrointestinal (e.g., nausea, diarrhea), cardiovascular (e.g., angina, myocardial infarction), and neurologic toxicities (e.g., hand-foot syndrome). Other outcomes include overall survival, FP-related hospitalization, and FP-related mortality.

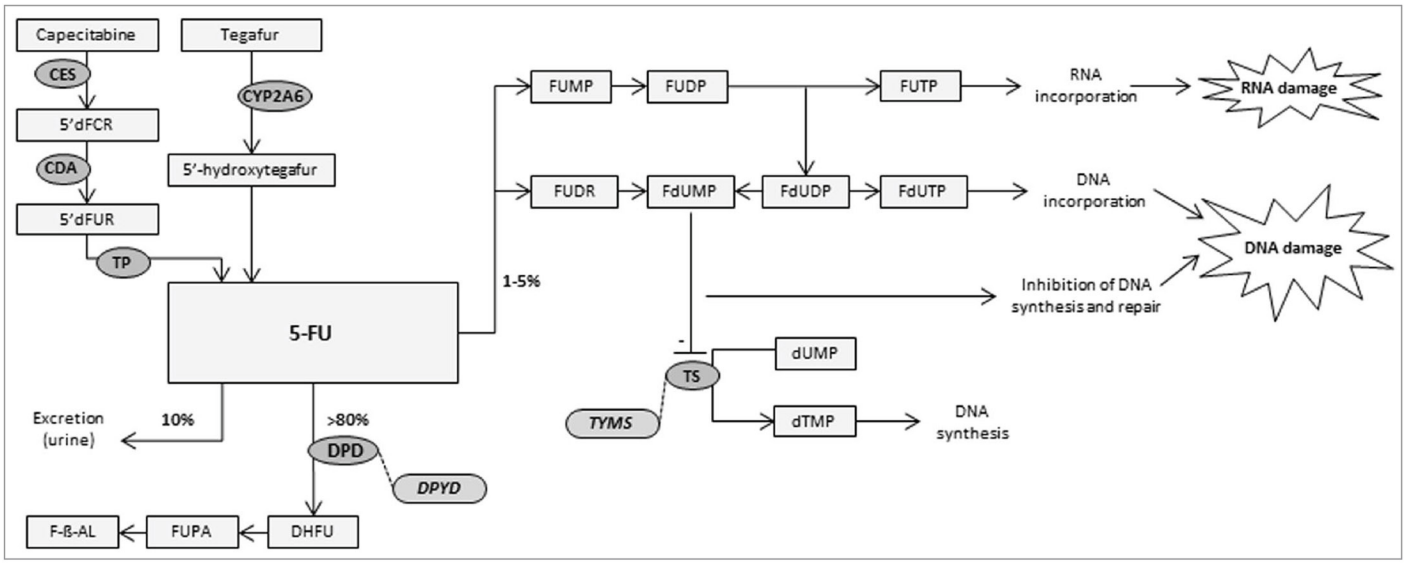
DPD Deficiency Testing: *DPYD* Genotyping Versus DPD Phenotyping

There are 2 types of testing for DPD deficiency: DPD phenotyping and *DPYD* genotyping. *DPYD* genotyping, or genetic testing, focuses on identifying genetic variations within the *DPYD* gene, which encodes DPD; whereas, DPD phenotyping refers to the measurement of DPD activity.[11] In this report, we will use the term DPD deficiency testing when referring to both types of tests.

DPYD Genotyping

DPYD genotyping can be performed using a variety of methods, including real-time polymerase chain reaction (PCR), multiplex PCR, Sanger sequencing, and next-generation sequencing (NGS).[15] PCR is a technique used to amplify specific DNA sequences using synthetic primers to target specific gene segments, followed by repeated cycles of DNA replication to generate millions of copies.[16] PCR alone cannot detect novel mutations — it is limited to confirming known, prespecified variants, and is most effective for identifying common mutations. Sanger sequencing is a method used to determine the nucleotide sequence of a defined region of DNA. While more comprehensive than PCR, it is still limited to targeted regions and known areas of interest.

Figure 1: Fluoropyrimidine Metabolism



5'dFUR = 5'-deoxy-5-fluorouridine; 5'dFCR = 5'-deoxy-5-fluorocytidine; 5-FU = fluorouracil; CES = carboxylesterase; CDA = cytidine deaminase; DHFU = 5,6-dihydrofluorouracil; DPD = dihydropyrimidine dehydrogenase; dTMP = deoxythymidine monophosphate; dUMP = deoxyuridine monophosphate; FdUDP = fluorodeoxyuridine diphosphate; FdUTP = fluorouridine triphosphate; FUDP = fluorouridine diphosphate; FUTP = fluorouridine triphosphate; FUPA = fluoro-beta-ureidopropionate; TP = triphosphate; TS =thymidylate synthase.

NGS is a method of genotyping capable of sequencing entire genes, exomes, or genomes. Similar to PCR or Sanger sequencing, NGS can target specific variants; however, because the targeted region is typically larger, it can detect both known and unknown variants, increasing its breadth, and efficiency. This broader scope makes NGS particularly valuable from an equity perspective, as it allows for the inclusion of diverse genetic backgrounds and rare variants that may be underrepresented in standard testing panels.

To date, more than 50 variants have been identified in the *DPYD* gene with varying levels of evidence regarding their impact on DPD function. The Clinical Pharmacogenetics Implementation Consortium (CPIC) primarily emphasize 4 variants that have been well-characterized and are commonly included in testing panels: *DPYD**2A, *DPYD**13, c.2846A>T, and c.1236G>A;[17] however, a systematic review (SR) published in 2024[18] identified 53 rare or novel variants associated with severe FP-related toxicity. Variants are typically categorized based on their impact on DPD enzyme activity: normal function, decreased function, or no function, and the strength of evidence supporting this claim.

DPD Phenotyping

DPD phenotyping measures the actual enzyme activity, or the metabolic products influenced by DPD function, providing a functional assessment of an individual's ability to metabolize FPs. Three primary methods are commonly used: plasma uracil concentration, the dihydrouracil to uracil (UH_2/U) ratio, and direct measurement of DPD enzyme activity in peripheral blood mononuclear cells (PBMCs).[19] Plasma uracil levels are measured using techniques like high-performance liquid chromatography or liquid chromatography-tandem mass spectrometry. Elevated uracil concentrations suggest reduced DPD activity. The UH_2/U ratio

reflects the balance between uracil and its primary DPD-mediated metabolite; lower ratios indicate impaired metabolism. Measuring DPD activity in PBMCs is considered the gold standard for DPD phenotyping, though this method is technically complex and less commonly available.[17,20]

Each phenotyping approach presents its own challenges, particularly related to preanalytical variables. Uracil and UH_2 levels are highly sensitive to sample handling (e.g., time to processing or improper freezing), patient's compliance to pretest fasting, concurrent medications, and the time of day of the blood draw.[21] PBMC-based testing, while precise, is influenced by circadian rhythms and requires rigorous laboratory infrastructure.[22] Despite these limitations, phenotyping presents a valuable alternative for identifying patients at risk of FP toxicity, particularly in multiethnic populations that may not benefit from the common genetic testing panels.[23] Standardization of testing protocols and interpretation thresholds remains essential for clinical utility and therefore broader clinical implementation. A combined genotype-phenotype approach to DPD deficiency testing is also an option.[23,24]

The clinical utility of DPD deficiency testing lies in the subsequent dose or treatment adjustments of FP drugs. Based on the results of the test, the initial FP dosage can be reduced, or in the case of suspected no function DPD, an alternative treatment can be chosen.[11,17,25]

International and Canadian Context

In April 2020, the European Medicines Agency (EMA) recommended that patients undergo pretreatment testing for DPD deficiency — either through *DPYD* genotyping or phenotyping — to mitigate the risk of severe and potentially fatal toxicities.[26] This was followed by similar recommendations in other European countries including the UK,[27] Germany, Switzerland, and Belgium.[28] Since 2019, France is the only country where DPD testing is mandatory, primarily through phenotyping methods such as plasma uracil measurement.[29,30]

FPs are widely used and effective chemotherapy drugs in Canada. Despite this, no national policies or guidelines on DPD testing exist, and current testing practices vary widely across jurisdictions. This review is important to better understand the current landscape, facilitators, and barriers to DPD testing in Canada to guide the development and implementation of standardized guidelines.

Purpose and Objectives

This report was undertaken to support decision-makers across jurisdictions in Canada by providing a timely update on the status of DPD testing and funding throughout the country, and to summarize relevant evidence on its clinical validity, clinical utility, and cost-effectiveness.

This report also aligns with the CDA-AMC broader efforts to develop a standardized framework to inform decisions about the adoption and implementation of molecular (genetic and genomic biomarker testing) across jurisdictions in Canada.

This report has 3 primary objectives:

- to provide a summary on the status and availability of DPD testing in jurisdictions across Canada
- to identify and summarize available evidence-based guidelines for DPD testing
- to identify, summarize, and critically appraise evidence on the clinical validity, clinical utility, and cost-effectiveness of DPD testing and testing-guided dose adjustments for patients being treated with 5-FU or capecitabine.

Research Questions

- What is the status and availability of genotype and phenotype testing for dihydropyrimidine dehydrogenase deficiency in patients being treated with 5-FU or capecitabine across Canada?
- What are the available evidence-based guidelines on genotype and phenotype testing for dihydropyrimidine dehydrogenase deficiency in patients being treated with 5-FU or capecitabine?
- What is the clinical validity of genotype and phenotype testing for dihydropyrimidine dehydrogenase deficiency in patients being treated with 5-FU or capecitabine?
- What is the clinical utility of genotype and phenotype testing for dihydropyrimidine dehydrogenase deficiency in patients being treated with 5-FU or capecitabine?
- What are the cost implications of genotype and phenotype testing for dihydropyrimidine dehydrogenase deficiency in patients being treated with 5-FU or capecitabine?

Approach

We conducted a survey to inform the status and availability of DPD testing in jurisdictions across Canada, and a Rapid Review of the literature to identify:

- evidence-based guidelines for genotype or phenotype testing and associated dose adjustments
- SRs and primary studies to evaluate the clinical validity of DPD testing, including both *DPYD* genotyping and DPD phenotyping
- SRs and primary studies to evaluate the clinical utility of DPD testing-guided dose adjustments for patients treated with 5-FU and capecitabine
- economic and cost considerations associated with DPD testing for patients treated with 5-FU and capecitabine.

We surveyed representatives from each province and territory responsible for assessment, implementation, or funding decisions regarding DPD testing in March 2025 and April 2025. The survey was hosted on SurveyMonkey[31] and was sent via email, with a reminder email 1 week following the original email. Questions focused on the availability and accessibility of testing, testing methods, and funding details.

An information specialist conducted a customized literature search, balancing comprehensiveness with relevancy, of multiple sources and grey literature on March 4, 2025. One reviewer screened citations and selected studies based on the inclusion criteria presented in and critically appraised included publications using established critical appraisal tools. Appendix 1 presents a detailed description of methods and selection of included studies.

Summary of Survey Findings

Status and Availability of DPD Testing

We received 14 responses regarding the availability of DPD testing from 13 of 13 Canadian jurisdictions — Newfoundland and Labrador, Prince Edward Island, Nova Scotia, New Brunswick, Quebec, Ontario, Manitoba, Saskatchewan, Alberta, British Columbia, Yukon, Northwest Territories, and Nunavut. Respondents provided replies directly via email (for the 3 territories) or via the online survey (13 from Canadian provinces). Details about the availability of DPD testing in Ontario were obtained from public resources. Respondents reported holding positions in medical oncology, genetics, pathology, and pharmacy. A narrative overview of the findings related to the status and availability of DPD testing in Canada is presented in the following section. A list of participating organizations and detailed findings by jurisdiction are available in Appendix 2.

Jurisdictions with DPD Testing

Availability

Five jurisdictions – New Brunswick, Quebec, Ontario, Saskatchewan, and British Columbia - reported the availability of publicly funded DPD testing. One jurisdiction (Alberta) reports that at the time of survey completion, DPD testing is recommended and available with a private payer option only; however, an application for public funding is under way with an estimated availability date of July 2025. Another jurisdiction (Manitoba) reported that while testing was not available at the time of survey completion implementation is under way, with testing set to begin in June 2025. Respondents indicated that testing practices are guided by international and jurisdictional guidelines, safety announcements from regulatory agencies, and established treatment algorithms.

All 5 provinces report conducting genotype testing for the 4 most common tier 1[32] genetic variants:

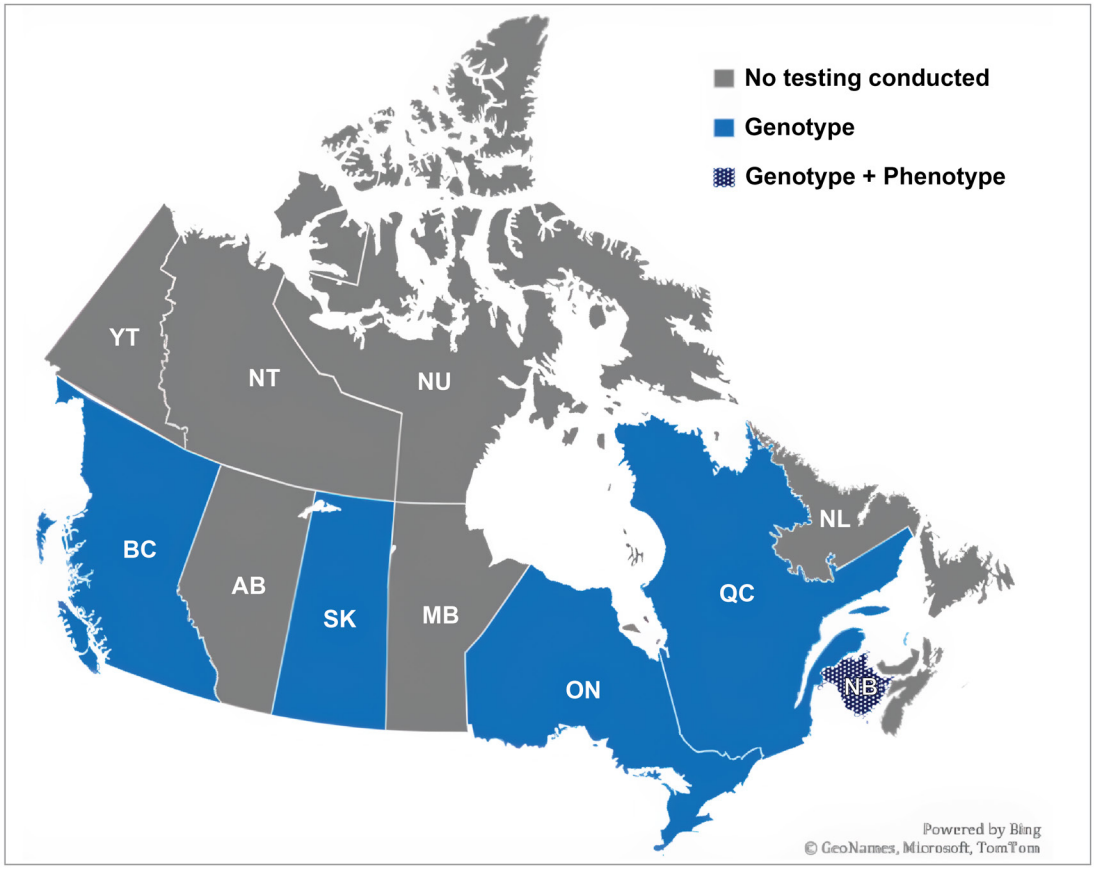
- *DPYD*2A*
- *DPYD*13*
- *DPYD c.2846A>T*
- *DPYD c.1236G>A*.

DPYD c.1236G>A is listed in ON as an optional variant to test. Three provinces reported testing additional variants: HapB3 (*c.1129 to 5923C>G* and c.1236G>A) in Ontario and Saskatchewan, and *c557A>G* and *c2279C>T* in British Columbia. The latter 2 are considered tier 2 variants, which are emerging variants with

less supporting evidence.[32] One province (New Brunswick) noted plans to expand testing to all recognized tier 1 and tier 2[32] variants.

All provinces reported that patient demographic characteristics did not influence the genetic variant tested. One jurisdiction, New Brunswick, also conducts a phenotype test — the UH_2/U ratio via a blood sample. Figure 2 presents the availability of DPD testing across Canada, based on findings from the survey responses.

Figure 2: Availability of DPD Testing Across Canada



NL = Newfoundland and Labrador; PEI = Prince Edward Island; NS = Nova Scotia; NB = New Brunswick; QC = Quebec; ON = Ontario MB = Manitoba; SK = Saskatchewan; AB = Alberta; BC = British Columbia; YT = Yukon; NT = Northwest Territories; NU = Nunavut.

Notes: For Manitoba, the implementation of DPD testing is under way and set to start as soon as June 2025. For Alberta, DPD testing is recommended with a private payer option currently available — publicly funded testing set to start as soon as July 2025.

Survey responses for DPD testing availability were available for 10 provinces and the 3 territories.

Data were derived from the following survey questions: “Do you currently test for dihydropyrimidine dehydrogenase (DPD) deficiency (either via genotype or phenotype testing) in people being treated with fluorouracil and capecitabine in your province or territory?” and “What type of testing do you use?”

Accessibility

In most jurisdictions the same genotype test is used for all patients. While this suggests equality in access to the test, it also suggests minimal consideration for ethnic origin, that might necessitate testing for different

variants. A response from Saskatchewan specified test type may vary based on availability, while a response from New Brunswick noted that patient knowledge or preference can influence testing decisions.

All the provinces reported that DPD testing is generally accessible across the province, although barriers to widespread accessibility were identified as:

- lack of clinician and/or patient awareness
- location of testing centres
- stigma or prejudice based on ethnicity, sex, and/or gender
- turnaround time delays.

Testing Practices

All provinces except 1 reported conducting DPD testing before treatment initiation. A standard order form was used to initiate DPD testing in all but 1 province (Ontario). A response from Saskatchewan indicated that while testing is usually conducted before treatment, it is occasionally conducted after some patients have experienced severe toxicity to FP therapy. Factors that influence when patients are tested were identified as institutional or jurisdictional guidelines, family history of DPD deficiency, and clinician knowledge.

The turnaround times for tests ranged from 5 to 10 days, with most provinces reporting that results were ready a week after testing. For respondents that provided turnaround times for emergency conditions, results were made available within 24 hours to 48 hours.

Resource Implications

DPD testing is publicly reimbursed in 5 provinces. A respondent from British Columbia indicated that all components of the test are publicly funded. Cost estimates per test provided by 5 respondents ranged from CA$50 to CA$500.

Implementation Barriers and Facilitators

Respondents identified several facilitators to the implementation of DPD testing, including:

- number of testing centres (2)
- location of testing centres (2)
- clinician and patient awareness (3)
- clinician and patient educational resources (1)
- reimbursement of test costs (4)
- well-established guidelines (3)
- established billing infrastructure (1)
- electronic medical record system workflow integration (2).

The most reported facilitators of widespread DPD testing were reimbursement of test costs (4 provinces), the presence of well-established guidelines (3 provinces), as well as clinician and patient awareness (3 provinces). Only 1 province identified established billing infrastructure as a facilitator.

Reported barriers to implementation included:

- lack of clinician/ patient awareness of testing (1)
- time constraints related to test turnaround times and treatment schedules (2)
- location of testing sites (2)
- lack of guidelines (1)
- limited lab resources, including lack of technologists and validation plan or platform (1).

The most commonly reported barriers were time constraints and location of testing sites.

Jurisdictions Without DPD Testing

Publicly funded DPD testing was reported unavailable in 5 provinces (Newfoundland and Labrador, Prince Edward Island, Nova Scotia, Manitoba, and Alberta) and the 3 territories. A respondent from Nunavut reported that all oncology patients in the territories are referred to neighbouring provinces where patient assessments (including *DPYD* testing) and treatments are conducted.

A response from Manitoba reported that while testing was not available at the time of survey completion implementation is under way, with testing set to begin in June 2025. A response from Alberta noted that DPD testing is under consideration for 6 of the most common genetic variants (*DPYD**2A, *DPYD**13, c.2846A>T, c.1129 to 5923C>G, c557A > G, c2279C > T), with an application submitted to support public funding. In previous years, DPD testing was not considered as there was no demand from oncologists. The response from Newfoundland and Labrador reported that testing is not available in the province, but if it is requested by a clinician (rarely, and typically in response to greater than anticipated toxicities to FP treatment) tests are sent to the Mayo Clinic. These tests are not funded. Similarly, Prince Edward Island indicated that testing is not available but can be accessed in a neighbouring province.

The barriers to implementation of DPD testing were identified as:

- cost of testing (3)
- lack of guidelines (3)
- limited ability to interpret test results (2)
- time constraints (i.e., test turnaround times and treatment start date) (2)
- electronic medical record workflow and integration (e.g., automatic alerts) (3)
- lack of guidance on test selection (i.e., limited targeted panel of common variants in a population of white people, versus broader panel, versus whole gene sequencing) (1)
- lack of billing infrastructure (2)
- lack of infrastructure and human resources to implement testing (2)
- limited testing availability and capacity volumes (1)
- lack of availability of best test (1)
- perceived undertreating or lack of clinical utility (2)
- lack of clinician and/or patient awareness of testing (1)

- location of testing centres (1).

The most frequently reported barrier was the lack of guidelines (3 responses). The response from Prince Edward Island emphasized a lack of clarity around the clinical utility of testing and the need for national standardization of DPD testing practices. The response from Nova Scotia specified that the potential for undertreating patients was a barrier to implementation.

Summary of Literature Review Evidence

We included 16 publications that met the inclusion criteria of this report. These comprised 1 health technology assessment (HTA), 5 SRs, 5 nonrandomized studies, 1 economic evaluation, and 4 evidence-based guidelines. Appendix 1 presents the PRISMA[33] flow chart of the study selection. A summary of the study characteristics can be found in Appendix 3. Additional references of potential interest that did not meet the inclusion criteria of this review are provided in Appendix 7.

Quality of Evidence

The included publications were critically appraised using established tools. The overall quality of guidelines was high, with 2 guidelines providing a clear link between evidence and recommendations, while 1 provided recommendations based on expert consensus. The quality of evidence assessing clinical validity and clinical utility was variable. While several studies demonstrated methodological strengths, such as a standardized means for measuring outcomes and consistent reporting, most were limited by small sample sizes, historical control groups, and numerous confounding variables. Heterogeneity in study populations and testing methods further limited comparability across studies, so conclusions should be interpreted with caution. Further large observational comparative studies should be conducted to support the findings. The cost studies were appraised based on their alignment with the decision problem, relevance to the setting, and fit for purpose of its main input parameters. A summary of the critical appraisal of the included publications, along with details regarding their strengths and limitations can be found in Appendix 4.

Summary of Findings

Appendix 5 presents additional details regarding the main study findings.

Guidelines Regarding Genotype or Phenotype Testing for DPD Deficiency

Three evidence-based guidelines[32] and 1 consensus-based guideline[32] were included in this review. The consensus document[32] was published in 2024 by the Association for Molecular Pathology (AMP) which is the Pharmacogenomics Working Group of the Clinical Practice Committee. The 3 evidence-based guidelines[11,12,17] were produced by Ontario Health-Cancer Care Ontario (OH-CCO) published in 2023, the Dutch Pharmacogenetics Working Group (DPWG) of The Royal Dutch Pharmacists Association published in 2019, and the CPIC published in 2017, with an update posted on the webpage in 2024. One guideline was developed in Canada,[11] 1 in the Netherlands,[12] and 2 by groups based in the US.[17,32]

AMP[32] provided a minimum set of variants that should be included in clinical genotyping assays and classified them into 2 groups (tier I and tier II). Tier I recommended variants are those that meet the following requirements:

- have an effect on the function of the DPD protein
- are represented commonly in at least 1 population or ancestral group
- have publicly available reference materials
- clinical laboratories can feasibly analyze with standard molecular testing methods.

Tier II variants meet at least 1 of the tier I criteria (but not all) and can be reclassified as tier I if more information becomes available. Although AMP identified variants for each tier, they urge laboratories to consider genetic variations represented in their population to correctly identify patients who may be at risk of developing severe FP toxicity. Specific gene variants identified by AMP for the 2 tiers are presented in Table 1.

Table 1: Tier Classifications of Gene Variants to Test

Tier	Gene variants
1	*c.1905 + 1G>A* *c.1679T>G* *c.1129 to 5923C>G* *c.557A>G* *c.868A>G* *c.2279C>T* *c.2846A>T*
2	*c.299_302del* *c.703C>T* *c.1314T>G* *c.1475C>T* *c.1774C>T* *c.2639G>T*

Three guidelines[11,12,17] provided genotype-based dosing recommendations, classifying patients by their Gene Activity Scores (GAS). This is a standardized system used to quantify DPD phenotype (DPD activity) based on *DPYD* genotype.[34] Because each individual carries 2 copies of a gene (i.e., allele), individuals are assigned a score ranging from 0 to 2, based on the combination of variants that they carry. Carriers of 2 no function or 1 no function and 1 decreased function variant are considered poor metabolizers and given a score of 0.0 or 0.5; carriers of 1 no function or decreased function variant are intermediate metabolizers and are given a score of 1.0 or 1.5; and carriers with 2 fully functional variants are considered normal metabolizers, with a score of 2.

A summary of the recommendations provided by the 3 *DPYD* guideline documents[11,12,17] can be found in Table 2.

Patients With Complete DPD Deficiency (GAS 0 or 0.5)

- DPWG and CPIC recommend avoiding 5-FU or capecitabine.
- In cases where avoiding FP therapy is not possible, the groups recommend starting therapy at a reduced dose and monitoring effects at earliest time points to minimize toxicity.

Patients With Decreased DPD Activity (GAS 1 or 1.5)

- DPWG recommends starting therapy at 50% of the standard dose or to avoid 5-FU and capecitabine.
- CPIC recommends:
  - reducing the starting dose by 50% for patients with GAS of 1
  - reducing the starting dose by 25% to 50% for patients with a GAS of 1.5.

Patients With Fully Functional DPD Activity (GAS 2)

- DPWG and CPIC recommend using the standard dose recommended on the label for 5-FU and capecitabine.

Patients Who Have an Unknown Genotype

- DPWG and CPIC recommend a phenotyping test to determine DPD activity.
- CPIC states that if a phenotyping test is unavailable, a dose reduction of 75% is recommended.

The OH-CCO guideline adapted its recommendations from the CPIC guideline and supplementary materials. However, the group provided recommendations for *DPYD* testing:

- Patients with planned FP-based therapies should be informed about DPD deficiency, risks associated with reduced activity, and available tests to determine functionality.
- They recommend that *DPYD* genotype tests should be involved in the planning of FP-based therapies and screening for clinically relevant *DPYD* should happen before the start of treatment.

In addition, the OH-CCO guideline[11] provided recommendations for implementing *DPYD* testing. These include identifying patients who are candidates for FP-based therapy early so testing can be conducted at the earliest convenience and recommending that *DPYD* testing be a standard part of the prechemotherapy check process with results of the test informing an initial treatment plan.

Clinical Validity of Genotype or Phenotype Testing for DPD Deficiency

Evidence regarding the clinical validity of genotype and phenotype testing for DPD deficiency was available from 1 HTA,[11] 4 SRs,[35-38] and 1 primary study.[9] Two of these SRs[35,36] included a meta-analysis of results. The HTA, published in 2021, was conducted by Ontario Health to assess the validity, utility, and cost-effectiveness of *DPYD* genotyping. The SRs were all published between 2022 and 2024 and aimed to assess the risk of severe toxicity, hospitalization, reduced survival, and death in adult cancer patients (normal versus reduced DPD activity) administered a standard dose of FPs. The primary study, published in 2023, aimed to establish an association between reduced DPD activity determined via PBMCs and adverse events of FP therapy. The 1 HTA and 4 SRs[35-38] included data from a total of 90 unique primary clinical studies; however, there was considerable overlap among the included primary studies. As a result,

the pooled effect estimates and narrative summaries from separate reviews are based on some of the same data (refer to Appendix 6 regarding overlap).

Table 2: Comparison of Genotype-Guided Dosing Recommendations

Test results	Gene activity score	DPWG (2019)	CPIC (2017)
Complete DPD deficiency	0 or 0.5	Avoid 5-FU or capecitabine If there are no other options, start at a reduced dose and start TDM at earliest time point	Avoid 5-FU or capecitabine If there are no other options, start at a reduced dose and start TDM at the earliest time point
Decreased DPD activity	1 or 1.5	Start therapy with 50% of standard dose or avoid 5-FU or capecitabine	For GAS 1: Reduce starting dose by 50% For GAS 1.5: reduce starting dose by 25% to 50%
Fully functional DPD activity	2	Use the standard dose recommended on the label for 5-FU and capecitabine	Use the standard dose recommended on the label for 5-FU and capecitabine
Unknown genotype	May be referred to as PHENO	Carry out a phenotyping test to determine DPD activity	Carry out a phenotyping test to determine DPD activity. If the test is unavailable: reduce dose by 75%

CPIC = Clinical Pharmacogenetics Implementation Consortium; DPD = dihydropyrimidine dehydrogenase; DPWG = Dutch Pharmacogenetics Working Group; FU = fluorouracil; GAS = gene activity score.

Severe (Grade ≥ 3) FP-Related Toxicity

In the SR conducted as part of the HTA,[11] pooled results of 7 studies, that included patients with colorectal, breast, gastrointestinal, esophageal, or head and neck cancer, indicated a higher risk of overall toxicity in *DPYD* variant carriers (any of the 4 main variants) compared to patients with the wild-type gene (risk ratio [RR] = 2.63; 95% confidence interval [CI], 2.15 to 3.96). Similarly, the pooled risk ratio of neutropenia, from 4 included studies was 4.42 (95% CI, 1.59 to 9.18). In 1 included study, 0 of 34 variant carriers experienced hand-foot syndrome compared to 24 of 771 patients with the wild-type gene.

One meta-analysis[36] found an increase in overall toxicity, hematological toxicity, neutropenia, and diarrhea in *DPYD**2A variant carriers compared to patients with wild-type *DPYD* with odds ratios [ORs] of 1.73, 2.37, 1.87, and 1.43, respectively. One meta-analysis[36] found an increase in overall toxicity, hematological toxicity, neutropenia, and diarrhea in *DPYD**2A variant carriers compared to patients with wild-type *DPYD*, with ORs of 1.73, 2.37, 1.87, and 1.43, respectively. No significant difference was found between *DPYD**2A variant carriers and patients with DPYD for gastrointestinal toxicity (OR = 1.22; 95% CI, 0.93 to 1.61).

One SR[37] evaluated toxicity in patients with colorectal cancer who were treated with capecitabine specifically and found 3 studies that reported an increased risk of toxicity in patients who carried the c.1601G>A variant. Two of these studies also found a significant association between the presence of the c.85T>C variant and severe adverse events. Findings on the *DPYD* variant c.496A > G were inconsistent: 1 study reported a significant association with capecitabine toxicity, while 2 others did not.

One SR[38] and 1 nonrandomized study (NRS)[9] reported on severe toxicities and DPD deficiency in patients with various types of cancer, as measured via phenotype methods. Doornhof et al.[9] measured PBMCs, while Paulsen et al[38] included studies that measured plasma uracil concentrations and U/UH_2 or UH_2/U ratios. Paulsen et al[38] narratively summarized 7 observational studies and found that the data regarding the correlation between uracil concentration or the UH_2/U ratio and severe FP-related toxicity is insufficient to draw any reliable conclusions. They suggest the need for adequately powered prospective clinical trials to properly validate the current uracil concentration threshold value proposed by the EMA.[26]

The 1 included NRS[9] found statistically significant associations between DPD deficiency, as measured in PBMCs, and overall, hematological, and gastrointestinal toxicities. A multivariable logistic regression adjusting for age, sex, FP dosage, chemotherapy regimen, kidney and liver function, found that gastrointestinal, cardiovascular and neurologic adverse events were significantly higher in patients with DPD deficiency.

FP-Related Mortality

One meta-analysis[35] found that the presence of *DPYD* variants (*DPYD**2A, *DPYD**13, c.2846A>T, or c.1236G>A) was significantly associated with treatment-related mortality, compared to *DPYD* patients with wild-type, with an OR of 34.86 (95% CI, 13.96 to 87.05). In pooled results across 13 studies, 13 out of 322 variant carriers died from FP-related toxicity, compared to 14 out of 6,952 patients with the wild-type gene. The authors found that the *DPYD**2A variant was the most prevalent among fatalities, followed by *DPYD**13 and c.1129 to 5923C>G and c.1236G>A (HapB3).

One SR[11] narratively summarized 9 observational studies and found that in heterozygous *DPYD* carriers mortality ranged from 0.0% to 100%, and 0.0% to 2.0% in patients with the wild-type gene. Two studies that included only *DPYD**2A carriers found a higher mortality risk in carriers compared to the wild-type gene (RR = 50.00 and 95% CI, 6.21 to 74.53; RR = 52.63 and 95% CI, 10.40 to 120.90).

FP-Related Hospitalization

One SR[11] narratively summarized 5 observational studies reporting rates of hospitalization in *DPYD* variant carriers versus patients with the wild-type gene. Three studies found a higher risk of hospitalization in *DPYD* variant carriers compared to patients with the wild-type gene (RR = 2.26 and 95% CI, 0.69 to 5.14; RR = 4.46 and 95% CI, 3.26 to 5.29; RR = 58.82 and 95% CI, 15.19 to 168.60). The risk ratios of the other 2 studies could not be calculated because they reported frequencies of hospitalizations in variants carriers, but not patients with the wild-type gene.

Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value

The authors of the included HTA[11] calculated the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of *DPYD* genotyping (3 to 4 variants) to detect severe toxicity for 9 observational studies based on data reported within the studies. They found that *DPYD* genotyping had a high specificity for severe toxicity (median of 98.6%), but low sensitivity (median of 8.1%), as many patients with the wild-type gene also experienced severe toxicity. In other words, the test is good at identifying individuals who do not experience severe toxicity (low false positives) but is not as good at identifying those

who do experience severe toxicity (high false negatives). The authors noted that this may be due to the fact that other factors can contribute to severe toxicity including other unmeasured *DPYD* variants and baseline or treatment characteristics (e.g., age, sex, kidney function, cancer type, FP dosage). Previous studies not included in this report, have reported that approximately 30% to 80% of toxicities could be attributable to DPD deficiency.[39] The median PPV and NPV were 61.1% and 84.5%, respectively.

Clinical Utility of Genotype or Phenotype Testing for DPD Deficiency

Evidence regarding the clinical utility of genotype and phenotype testing for DPD deficiency was available from 1 HTA,[11] 2 SRs,[20,38] and 4 NRSs.[40-43] One of the SRs[20] included a meta-analysis of results. The HTA[11] and 2 SRs,[20,38] included data from a total of 67 primary clinical studies, 27 of which were relevant to this research question. There was considerable overlap among the included primary studies of the included HTA and SRs and as a result, the pooled effect estimates and narrative summaries from separate reviews are based on some of the same data (refer to Appendix 6 regarding overlap).

Severe (Grade ≥ 3) FP-Related Toxicity

DPYD-Guided Dose in Variant Carriers Versus Usual Care in Variant Carriers

One study summarized in 2 SRs[38,44] directly compared *DPYD*-guided dose adjustments in variant carriers to usual care in variant carriers. The severe toxicity rates (grade ≥ 3 assessed by the CTCAE[10]) were comparable between the 2 groups (5 out of 22 [22%] and 8 out of 34 [21%], respectively); however, the authors of 1 SR noted that imbalances in the distribution of DPYD variants between groups as well as imprecision in study results reduced the strength of these findings. The NRS by Paulsen et al.[42] found that variant carriers who received reduced doses experienced less severe toxicity compared to those with standard doses (23% of patients and 29% of patients, respectively).

DPYD-Guided Dose Versus Usual Care (No Distinction Between Variant Carriers and Wild-Type Carriers)

One SR with meta-analysis found a statistically significant decrease in both overall toxicity and diarrhea in patients who received *DPYD*-guided dosing versus those who received usual care,[20] suggesting that pharmacogenetic-guided dosing was associated with improved patient outcomes. The NRS by Paulsen et al.[42] found an increased risk of overall grade 3 or higher toxicity in all patients of the *DPYD*-guided group versus a historical control group treated with standard doses (RR = 1.20; 95% CI, 0.92 to 1.57).

DPYD-Guided Dose in Variant Carriers Versus Usual Care in the Wild-Type Gene

Findings regarding risk of severe toxicity in *DPYD*-guided dose reductions in variant carriers versus usual care in patients with the wild-type gene were limited and contradictory. The HTA[44] authors wrote that due to the design of the included studies, they were unable to determine whether reducing the treatment dose results in a risk of severe toxicity that is comparable or lower than that observed in patients with the wild-type gene receiving a standard dose. Paulsen et al[38] summarized 3 studies; 2 of which found higher toxicity rates in variant carriers receiving reduced doses, and 1 of which found higher toxicity rates in patients with the wild-type gene receiving standard doses.

Phenotype-Guided Dose in Patients With Reduced DPD Activity Versus Usual Care in Patients With Normal DPD Activity

UH_2/U ratio: One included NRS found that the incidence of severe toxicity was 11% in patients who received a reduced dose based on their UH_2/U ratio and 13% in patients who received a standard dose.[38]

Plasma Uracil: One study reported similar rates of severe toxicity between patients with reduced DPD activity (U = 16 ng/mL to 150 ng/mL) who received a reduced dose and those with normal DPD activity (U < 16 ng/mL) who received a standard dose (12 out of 27 [44%] versus 43 out of 92 [46%]).[40] However, 26 (28%) of the patients with normal DPD activity received an initial FP dose reduction based on factors other than DPD activity (i.e., fragile baseline condition).

Combined genotype and phenotype dosing: One study reported that 23% (14 out of 60) patients who received reduced doses, based off of a combination of genotype and PBMC levels, experienced severe grade greater than or equal to 3 toxicity, compared to 30% (50 out of 168) of patients with the wild-type gene, and normal PBMC levels.[43]

FP-Related Mortality

FP-related mortality was reported by 1 HTA[44] and 1 NRS.[42] The authors of the HTA[44] noted only 1 FP-related death in a *DPYD* variant carrier (n = 1,103) in the included studies, which occurred "after the patient was wrongly prescribed a standard fluoropyrimidine dose for two cycles" (p. 52).[44] Mortality rates in patients with the wild-type gene across 3 included studies ranged from 0.1% to 0.7%.

The NRS[42] found a decreased risk of FP-related death in variant carriers receiving *DPYD*-guided doses (0 out of 22) versus variant carriers receiving usual care (2 out of 42) (RR = 0.37; 95% CI, 0.02 to 7.46).

FP-Related Hospitalization

Paulsen et al[42] found a decreased risk of FP-related hospitalization in variant carriers receiving *DPYD*-guided doses (0/22) versus variant carriers receiving usual care (8 out of 42) (RR = 0.11; 95% CI, 0.01 to 1.82). The authors of the included HTA[44] note that the point estimates of 4 studies indicated a higher risk of treatment-related hospitalization in variant carriers compared to the wild-type gene, however the confidence intervals also included the possibility of lower risk in *DPYD* carriers.

In a meta-analysis (of 4 studies) conducted by Glewis et al.,[20] it was found that variant carriers had statistically significant more hospitalizations compared to patients with wild-type. However, the authors noted that this could be a result of variation in dose reductions as well as noncompliance to the recommended dose reductions in the included studies. For example, in 1 study, 4 *DPYD* variant carriers received standard FP doses, leading to fatal toxicity in all.

Disease Response

One SR reported no statistically significant difference between variant carriers who received dose adjustments and patients with the wild-type gene who received usual care in terms of complete and partial disease response, based on a meta-analysis of 3 studies (RR = 1.31; 95% CI, 0.93 to 1.85; P = 0.12; I2 =

0%). Similarly, there was no statistically significant difference in stable disease between the same groups, based on meta-analysis of 2 studies (RR = 1.27; 95% CI, 0.66 to 2.44; P = 0.47; I2 = 0%).

Cost Implications of Genotype or Phenotype Testing for DPD Deficiency

Evidence regarding the cost-effectiveness *DPYD* genotyping was available from 1 primary economic evaluation performed as part of the included Ontario Health HTA.[44] The findings from the economic evaluation that was conducted from the perspective of a health care system in Canada suggest that universal pretreatment *DPYD* genotyping (of the following 4 variants: *DPYD*2A, DPYD*13, c.2846A>T, c.1236G>A*) for all patients undergoing treatment with FPs, followed by genotype-guided dose adjustments, is likely cost-effective compared to usual care, at the willingness-to-pay thresholds of $50,000 and $100,000 per quality-adjusted life-year (QALY) gained (91% and 96% probability of being cost-effective, respectively). *DPYD* genotyping remained cost-saving and slightly more effective (resulting in greater QALYs) compared to usual care in all scenarios modelled in sensitivity analyses.

We identified 9 other economic evaluations[45-53] and 1 budget impact analysis, included in the Ontario Health HTA[44] that were excluded from evaluation in this report as they were conducted outside of Canada and therefore have limited transferability to our setting of interest. However, it is worth noting that *DPYD* genotyping was found cost-effective compared to usual care across all studies from different settings. A brief summary of the results of these studies can be found in Appendix 5.

Limitations

Risk of Bias of Included Studies

The authors of the SRs included in this report noted some methodological limitations of the included primary studies affecting the validity and reliability of their results. For example, the comparability of cohorts was limited by numerous confounders in this population including age, sex, body surface area, ethnicity, comorbidities, cancer type, treatment regimen, *DPYD* variant, and kidney and liver function. Because of a lack of clinical equipoise, randomized controlled trials were extremely limited and occurred at the earlier end of the date range. Therefore, the methodological and analytical limitations inherent to both retrospective and prospective cohort studies (e.g., selection bias, missing or incomplete records) were present in the evidence.

Some studies noted discrepancies in *DPYD* testing assays among laboratories[43] and significant variability in uracil measurements due to factors such as participants' food intake and circadian rhythms, and other preanalytical conditions.[38] While in most clinical utility studies, test-guided dose adjustments were made using accepted guidelines,[12,17] exact doses were also dependent on other patient characteristics and clinician preference. Thus, even in the intervention group, doses varied widely.

Evidence Gaps

Evidence regarding the clinical validity and utility of *DPYD* variants beyond the 4 identified by CPIC,[17] as clinically relevant, was limited to 1 guideline[32] and 2 SRs.[36,37] Of note, we did identify 2 large SRs[18,54] in our

literature search that assessed evidence on rare or novel *DPYD* variants; however, they were excluded from the present report as they did not satisfy other search criteria (i.e., they included primarily case reports and case-control studies with limited sample sizes, lacked relevant comparators, and did not consistently report on our outcomes of interest).

Evidence regarding the clinical utility of phenotype-guided dosing was limited to 2 SRs[20,38] and 2 NRS,[40,41] with 2 included testing methods: plasma uracil concentrations and UH_2/U ratio. Of note, our literature search did identify 1 consensus guideline focused on phenotype-guided dose recommendations;[29] however, it was excluded from this report as the recommendations were based on expert consensus rather than a formal SR of evidence.

The assessment of the cost-effectiveness of *DPYD* genotyping and subsequent dose adjustments versus usual care in a public health care payer setting in Canada was limited to 1 economic evaluation.[44] This study only included genotyping for the 4 primary variants. We also found no evidence assessing the cost-effectiveness of DPD phenotyping for reducing FP-related toxicity or mortality. Consequently, no conclusions can be drawn regarding the cost-effectiveness of either extended *DPYD* genetic testing (via NGS or a larger panel of targeted variants) or phenotype testing.

Finally, none of the included studies reported quality of life outcomes specific to DPD testing or test-guided dosing, so the impact of these interventions on patient-reported outcomes is unknown.

Generalizability

We used PROGRESS-Plus criteria[55,56] to guide data extraction and to provide insights into whether the clinical studies conducted to date included diverse patient populations who could be representative of those in Canada. However, the literature we reviewed for this report provided limited information on participant characteristics, often only reporting a few factors such as age, sex, or ethnicity. In cases where participant sex or gender were reported, the authors did not provide any information on how they were defined or measured. Similarly, ethnicity was poorly reported across studies, with some authors reporting patient-reported ethnic groups and others simply reporting the country in which the study was completed. Where it was reported, most studies included participants from European countries, such as the Czech Republic, Denmark, France, Ireland, Italy, the Netherlands, Spain, and the UK. This may limit the generalizability of the evidence to settings in Canada, where patients belong to numerous ethnic groups, including those of African, East Asian, Latin American, Middle Eastern, or South Asian descent.

Relatedly, most studies assessed the 4 *DPYD* variants most prevalent in patients who identified as being of European ancestry, with limited evidence on the validity and utility of other variants. As such, the generalizability of results to diverse population groups is limited.

Some of the phenotype tests included in this report require specific analytical conditions that could be difficult to achieve if sample collection occurs at a location at a great distance from the lab conducting the analysis. Given the geography of Canada and many rural and remote residents, this may not always be achievable.

Conclusions and Implications for Decision- or Policy-Making

This review includes 1 HTA (which included 1 SR and 1 de novo economic evaluation), 4 guideline documents,[11,12,17,32] 5 SRs[20,35-38] (2 with meta-analyses[20,36]), and 5 NRS[9,40-43] regarding the clinical validity, utility, or cost-effectiveness of DPD deficiency testing and test-guided dose adjustments for detecting and preventing severe toxicities, mortality, and hospitalizations.

Summary of Evidence

The survey conducted as part of this report suggests that *DPYD* genotype testing is conducted in 5 jurisdictions in Canada, 1 of which also conducts phenotype testing. Two additional province reported that they are set to begin genotype testing later in 2025. All jurisdictions publicly reimburse the test costs, which ranged from CA$50 to CA$500, depending on testing platform (PCR versus NGS), with NGS costing more. The most frequently reported facilitators in those P/Ts were reimbursement of test costs, the presence of well-established guidelines, and clinician and patient awareness. The most frequently reported barriers in jurisdictions without DPD testing was the lack of well-established guidelines.

The evidence summarized in this report indicates that individuals carrying a *DPYD* variant are likely to be at higher risk of severe toxicity, treatment-related hospitalization, and mortality, compared to patients with the wild-type gene. The evidence also suggests that genotype- or phenotype-guided dosing may reduce severe toxicities and mortality in patients being treated with FPs, without reducing treatment effectiveness (disease response); however, evidence on phenotype-guided dosing is extremely limited. The included studies suggest that FP-related hospitalization may be higher in *DPYD*-guided variant carriers compared to patients with the wild type; however, inconsistency in dose reduction compliance may have affected the results. Large observational comparative studies should be conducted to support these findings. These conclusions may only be applicable to persons with European ancestry, as there is insufficient evidence regarding validity and utility in other groups.

One economic evaluation conducted from a health care perspective in Canada reported that *DPYD* genetic testing for the 4 primary variants (based on data from study populations primarily with European ancestry) with subsequent guided dose adjustments was cost-effective compared to usual care, at the willingness-to-pay thresholds of $50,000 and $100,000 per QALY gained. We did not identify any relevant studies that evaluated the cost-effectiveness of whole genome sequencing or phenotype testing.

Considerations for Future Research

While current evidence on *DPYD* genetic testing supports the clinical validity and utility of a small number of well-characterized variants, questions remain about the relevance of additional, less-studied variants. Future research could focus on assessing the clinical validity for predicting FP-related toxicity, as well as the utility of dose adjustments for these variants. Such studies would provide a more comprehensive understanding of the genetic contributors to DPD deficiency and inform whether expanded testing panels could enhance predictive accuracy and clinical benefit. Further, evidence-based dosing guidelines are also largely limited to 4 *DPYD* variants. As evidence increases regarding other potentially actionable variants, there is a need for

the development and validation of dosing recommendations for these variants. These guidelines should be based on robust clinical outcome data and supported by consensus among pharmacogenomic experts.

Although phenotype testing for DPD deficiency is being used in select jurisdictions, evidence on its clinical utility remains limited. As more data becomes available, future economic evaluations should assess the cost-effectiveness of phenotype testing, both alone and in combination with genotype testing. These analyses would help clarify the value of phenotype-guided dosing strategies and help guide health resource allocation decisions. Similarly, the cost-effectiveness of NGS testing could be assessed.

Finally, given Canada's ethnically diverse population and the equity implications of pharmacogenomic testing, future studies should include detailed reporting of equity-relevant population characteristics including residence, race, ethnicity, culture, language, occupation, gender, sex, religion, education, socioeconomic status, and social capital. This information will be essential to understanding whether findings are generalizable to settings in Canada and whether expanded testing strategies mitigate or inadvertently reinforce existing disparities in cancer treatment outcomes.

Equity Considerations

A lack of diversity in genotype research and *DPYD* variant testing has implications for diverse populations such as Canada's. As described throughout this report, evidence is dominated by 4 *DPYD* variants — *DPYD*2A*, *DPYD*13*, c.2846A>T, and c.1236G>A — identified predominantly in individuals of European ancestry. While these variants have been shown to be clinically valid, relying solely on them may result in missed diagnoses of DPD deficiency in patients from other ethnic origins, who may carry other, less-studied variants. This limits the effectiveness and inclusiveness of genotype-guided dosing strategies and raises concerns about equitable access to safe and effective cancer treatment. Testing panels that include a broader range of variants and DPD phenotype testing, which directly measures enzyme activity regardless of genotype, are compelling options that might allow for a more inclusive and accurate risk assessment, ensuring that the benefits of personalized medicine extend to all patients.

Further, laboratories that conduct DPD testing are typically located in urban centres, thereby limiting equitable access to patients in rural, remote, or underserved communities. This may delay or prevent testing.

Implications for Clinical Practice and Policy-Making

In Canada, implementation of DPD deficiency testing is still in its early stages. In our survey we identified 4 provinces currently offering *DPYD* genotype testing, and 1 conducting both genotype and phenotype testing. While the findings of this report suggest that *DPYD* genetic testing with subsequent dose adjustments may improve clinical outcomes and be cost-effective compared to usual care, there are equity concerns in applying this evidence within the context in Canada. Much of the existing research has focused on 4 *DPYD* variants identified by the CPIC as clinically relevant in populations of European descent. Consequently, there is a risk of disproportionate harms and benefits for groups not of European ancestry, as well as uncertainty around the interpretation of results in these groups. To address these gaps, decision-makers may wish to work with laboratories to consider the feasibility and cost of an extended panel of variants and develop a panel relevant to the patient population present in their jurisdiction or explore whole genome sequencing via

NGS. In the meantime, information could be shared with clinicians regarding the current limitations of *DPYD* genetic testing, and informed consent conversations with patients could outline the insufficiency of evidence to determine the applicability of the test for individuals not of European ancestry.

Phenotype testing may offer a more inclusive alternative, as it is not limited by ancestry. However, current evidence supporting its clinical utility remains limited. Furthermore, we did not find any evidence-based guidelines for phenotype-guided dosing. A combination of genotype and phenotype testing has shown promise in some contexts and may help address clinician concerns about underdosing. Decision-makers may wish to monitor the evolving literature and consider the characteristics of their patient population, to determine which testing strategies to implement to support safe and equitable chemotherapy dosing.

The findings of our survey identified clinician and patient awareness as a facilitator to DPD testing implementation. Jurisdictions may wish to consider a knowledge dissemination plan to increase awareness and access should they introduce testing. Similarly, no clinician or patient-reported outcomes were reported in the included studies. Those who intend to implement DPD testing as a part of routine clinical care may want to consider conducting ongoing monitoring to determine whether any clinician or patient-specific challenges arise. All provinces that reported conducting DPD testing offered public reimbursement, however we did not assess from where jurisdictions allotted funds for this testing (e.g., cancer budget, lab budget, other). Cost of testing will be dependent on the testing method (e.g., PCR, Sanger, NGS) and the expected number of tests, which will be specific to the population and characteristics of each individual province and territory.

A recent Environmental Scan conducted by the CDA-AMC[57] described current assessment frameworks, processes, and guiding principles used to inform the evaluation and implementation of genetic and genomic biomarker testing in cancer care across jurisdictions in Canada. The goal of this report was to support the development of a standardized decision-making framework, in response to the rapid emergence and adoption of precision medicine technologies within Canada. Drawing from a literature review and consultations, the report outlined 3 key categories of assessment criteria when considering the implementation of a genetic biomarker test: evidentiary, implementation, and decision-making. Within these categories, specific criteria included the evaluation of the clinical validity and utility of tests, economic considerations such as cost-effectiveness, barriers and facilitators to implementation, and identification of evidence gaps and future research priorities. All these criteria have been applied in the current report, which may serve as a pilot evidence review when assessing a potential new genetic test. In addition, the survey findings presented here underscore the varied landscape of implementation of genetic and genomic testing, further reinforcing the need for a standardized framework across Canada.

The limitations of the included literature, such as the lack of evidence directly comparing *DPYD* variant carriers with reduced dose to *DPYD* variant carriers with standard doses, the variable quality of primary studies included in identified SRs, the lack of randomized trials to minimize confounding variables, and concerns regarding the generalizability of findings to settings in Canada should be considered when interpreting the conclusions of this report.

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Appendix 1: Detailed Methods and Selection of Included Studies

Please note that this appendix has not been copy-edited.

Literature Search Methods

An information specialist conducted a literature search on key resources including MEDLINE and Embase via OVID, the Cochrane Database of Systematic Reviews, the International HTA Database, the websites of health technology assessment agencies in Canada and major international HTA agencies, as well as a focused internet search. The search approach was customized to retrieve a limited set of results, balancing comprehensiveness with relevance. The search strategy comprised both controlled vocabulary, such as the National Library of Medicine's MeSH (Medical Subject Headings), and keywords. Search concepts were developed based on the elements of the research questions and selection criteria. The main search concepts were dihydropyrimidine dehydrogenase deficiency and testing. Comments, newspaper articles, editorials, conference abstracts, and letters were excluded. Retrieval was limited to the human population. The search was completed on March 4, 2025 and limited to English-language documents published since January 1, 2015.

Selection Criteria and Methods

One reviewer screened citations and selected studies. In the first level of screening, titles and abstracts were reviewed and potentially relevant articles were retrieved and assessed for inclusion. The final selection of full-text articles was based on the inclusion criteria presented in Table 3.

Table 3: Selection Criteria

Criteria	Description
Population	Adult or pediatric patients with planned treatment with fluorouracil or capecitabine, alone or in combination with other therapies
Intervention	**Q1 to Q3:** Pre- or posttreatment *DPYD* genotype testing (on any combination of variants and using any method [e.g., NGS, PCR]); OR pre- or posttreatment phenotype testing for DPD function (using any method [e.g., plasma uracil, dihydrouracil to uracil ratio, peripheral blood mononuclear cells]) **Q4**: Pretreatment *DPYD* testing; or carriers of at least 1 of the *DPYD* variants under assessment *with* subsequent genotype-guided fluoropyrimidine dose reduction; OR pretreatment phenotype testing for DPD function *with* subsequent phenotype-guided fluoropyrimidine dose reduction **Q5**: Pretreatment or reactive DPD testing with subsequent dose adjustments
Comparator	**Q1 to Q2**: NA **Q3**: Wild-type patients (noncarriers of variants of interest) as defined by *DPYD* genotyping, or normal DPD metabolizers based on phenotype test of interest. Reference standard = severe (grade ≥ 3) FP- related toxicity **Q4**: Patients with or without pretreatment *DPYD* genotype or phenotype testing, with no pretreatment pharmacogenetic or pharmacokinetic-guided dose adjustments **Q5**: No DPD testing (usual care)

Criteria	Description
Outcomes	**Q1:** Timing of testing, testing method (genotyping vs. phenotyping), variants tested, testing availability test turnaround time, cost, reimbursement status **Q2:** Recommendations regarding DPD testing and guided treatment adjustments (e.g., timing of testing, testing method, variants to test, dose reductions) **Q3:** testing method, variants tested, specificity, sensitivity, positive predictive value, negative predictive value, toxicity, mortality **Q4**: Safety outcomes: toxicity, mortality, hospitalization; Effectiveness outcomes: progression-free survival, overall survival **Q5:** cost, QALY, ICER
Study designs	**Q1:** NA **Q2:** Evidence-based guidelines **Q3 to Q4:** Health technology assessments, systematic reviews, randomized controlled trials, and nonrandomized studies **Q5:** Economic evaluations

ICER = incremental cost-effectiveness ratio; QALY = quality-adjusted life-year; vs. = versus.

Exclusion Criteria

Articles were excluded if they did not meet the selection criteria outlined in Table 3, they were duplicate publications, were published before 2015, or were not available in English. Systematic reviews in which all relevant studies were captured in other more recent or more comprehensive systematic reviews were excluded. Primary studies retrieved by the search were excluded if they were captured in 1 or more included systematic reviews. Guidelines with unclear methodology or without a formal evidence review were also excluded.

Critical Appraisal of Individual Studies

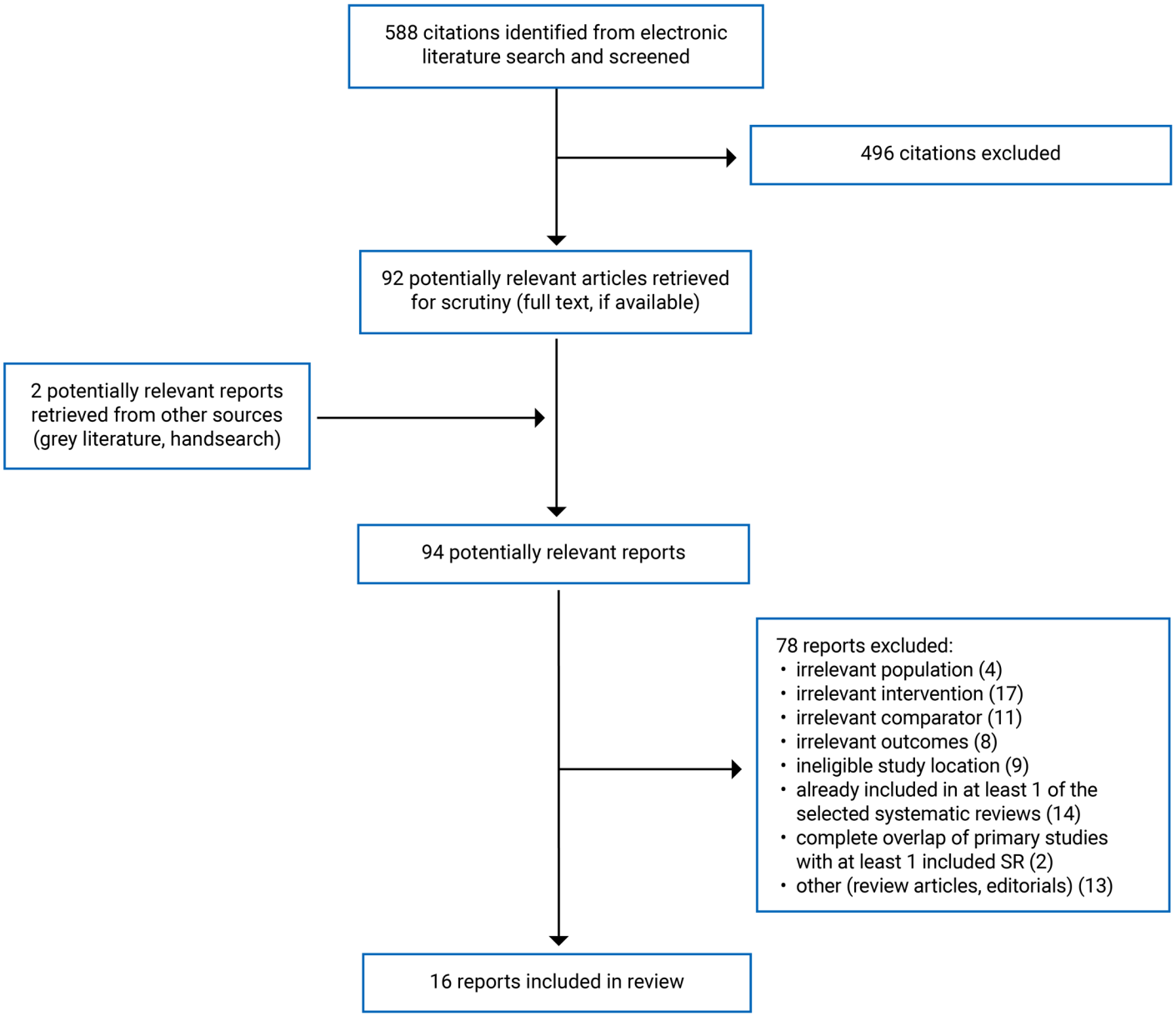
The included publications were critically appraised by 1 reviewer using the following tools as a guide: A MeaSurement Tool to Assess systematic Reviews 2 (AMSTAR 2)[58] for systematic reviews, the Downs and Black checklist[59] for randomized and nonrandomized studies, and the Appraisal of Guidelines for Research and Evaluation (AGREE) II instrument[60] for guidelines. Summary scores were not calculated for the included studies; rather, the strengths and limitations of each included publication were described narratively.

Survey

The invitation to participate in our voluntary survey was sent via email to the representative believed to be responsible for decisions regarding DPD testing and funding in 10 of the 13 Canadian jurisdictions in March and April 2025. Consent was obtained from participants on the first page of the survey, following provision of information regarding the purpose of the survey, time involved, and confidentiality. The survey was hosted by SurveyMonkey, with all appropriate licensing, but respondents also had the option to respond to survey questions directly via email.

The survey consisted of 34 questions more than 13 pages: 1 consent question, 6 demographic questions, 16 questions regarding the status of DPD testing in their jurisdiction, 7 questions assessing barriers and facilitators to implementation, 3 questions on cost and reimbursement, and 1 question requesting any additional information not otherwise captured. The respondent for Nunavut provided information regarding the Northwest Territories and the Yukon. Details regarding the availability of DPD testing for Ontario were pulled from public resources.

Figure 3: Selection of Included Studies



Appendix 2: Survey Findings

Please note that this appendix has not been copy-edited.

Table 4: Information on Survey Respondents and Contacts

Jurisdiction, number of responses	Organization represented by survey respondents
Newfoundland and Labrador (n = 1)	NL Health Services, Cancer Care Program
Prince Edward Island (n = 1)	Health PEI, Cancer Treatment Centre
Nova Scotia (n = 1)	Nova Scotia Health, QEII Health Sciences Centre
New Brunswick (n = 2)	Saint John Regional Hospital (Horizon Health Network) Vitalité Health Network
Quebec (n = 1)	Ministère de la santé et des services sociaux
Manitoba (n = 1)	CancerCare Manitoba
Saskatchewan (n = 3)	Saskatoon Cancer Centre Saskatchewan Cancer Agency University of Saskatchewan
Alberta (n = 2)	Alberta Precision Labs Alberta Health Services
British Columbia (n = 2)	BC Cancer
Nunavut[a] (n = 1)	Department of Health, Government of Nunavut

QEII = Queen Elizabeth II.

[a]Contact provided information on other territories – Northwest Territories and Yukon.

Table 5: Summary of Status and Availability of DPD Testing Across Canada

Jurisdiction, number of responses	DPD Test availability	Test type	Genetic Variants tested	Cost of test (CA$)	TAT
Newfoundland and Labrador (n = 1)	No, tests are sent to Mayo Clinic when requested by a clinician (rare, and typically reactive testing after severe FP toxicity)	Genotype	Mayo clinic tests the 4 common variants and additional ones not specified. *DPYD*2A* *DPYD*13* *DPYD c.2846A>T* *DPYD c.1236G>A*	NA	NA
Prince Edward Island (n = 1)	No, however testing can be accessed via neighbouring province if required	NA	NA	NA	NA
Nova Scotia (n = 1)	No	NA	NA	NA	NA

Jurisdiction, number of responses	DPD Test availability	Test type	Genetic Variants tested	Cost of test (CA$)	TAT
New Brunswick (n = 2)	Yes	Genotype (PCR) and phenotype test (dihydrouracil to uracil ratio)	• *DPYD*2A* • *DPYD*13* • *DPYD c.2846A>T* • *DPYD c.1236G>A*	50 per patient	5 days
Quebec (n = 1)	Yes	Genotype (PCR)	• *DPYD*2A* • *DPYD*13* • *DPYD c.2846A>T* • *DPYD c.1236G>A*	65 (DNA extraction cost = 32 + NAAT analysis cost = 33)	7 to 10 days
Ontario[a] **(n = 1)**	Yes	Genotype	• *DPYD*2A* • *DPYD*13* • *DPYD c.2846A>T* • c.1129 to 5923C>G (required)/ c.1236G>A (optional) (HapB3 when identified together)	NR	NR
Manitoba (n = 1)	No At time of survey testing was in the implementation process and is set to begin June 2025	Genotype (not started yet)	5 variants (specific variants NR)	NR (Funding approved for 100 per case, which does not cover shipping cost)	NR
Saskatchewan (n = 3)	Yes	Genotype (NGS)	• *DPYD*2A* • *DPYD*13* • *DPYD c.2846A>T* • *DPYD c.1236G>A* • HapB3 *(c.1129 to 5923C>G* and *c.1236G>A)* *currently validating full gene sequencing for patients who are negative but still show toxicity	Routine = 300 Urgent = 500	Routine: 5 to 7 days Urgent: 24 to 48 hours
Alberta (n = 2)	No At time of the survey, private payer testing was available and publicly reimbursed testing was in the implementation process with estimated start date of July 2025	Genotype (not started yet)	• *DPYD*2A* • *DPYD*13* • *c.2846A>T* • *c.1129 to 5923C>G* • *c557A > G* • *c2279C > T*	NR	Two weeks

Jurisdiction, number of responses	DPD Test availability	Test type	Genetic Variants tested	Cost of test (CA$)	TAT
British Columbia (n = 2)	Yes	Genotype (PCR)	• *DPYD*2A* • *DPYD*13* • *DPYD c.2846A>T* • *DPYD c.1236G>A* • *c557A > G* • *c2279C > T*	60 plus shipping	Average: 7.5 days Median: 7 days

EMR = electronic medical records; NA = not applicable; NR = not reported; TAT = turnaround time; NAAT = nucleic acid amplification test.
[a]Survey response was pulled from public sources provided by contact.

Table 6: Implementation of DPD Testing across Canada

Jurisdiction, number of responses	Public reimbursement and guidelines followed	Facilitators to implementation and access	Barriers to implementation and access
Newfoundland and Labrador (n = 1)	**Public reimbursement:** NA **Guidelines Followed**: NA	NA	Barriers to implementation: • Cost of testing • Limited understanding of how to interpret test results • Time constraints (e.g., test TAT, treatment start date) • Cost of test development and validation • EMR workflow and integration (e.g., automatic alerts) • Lack of guidelines and guidance about the best test to conduct i.e., limited targeted panel of common variants in Caucasian population • Billing infrastructure • Lack of infrastructure and human resources to implement testing
Prince Edward Island (n = 1)	**Public Reimbursement:** NA **Guidelines Followed:** NA	NA	Barriers to implementation: • Lack of clinician and/or patient awareness of testing • Limited understanding of how to interpret test results • Time constraints (e.g., test TAT, treatment start date) • EMR workflow and integration (e.g., automatic alerts) • Perceived lack of utility (e.g., low variant prevalence, lack of guidelines regarding genotype-guided dose adjustments)

Jurisdiction, number of responses	Public reimbursement and guidelines followed	Facilitators to implementation and access	Barriers to implementation and access
Nova Scotia **(n = 1)**	**Public reimbursement:** NA **Guidelines Followed**: NA	NA	Barriers to implementation: • Undertreating of patients after testing is done • Best test is not available • Potential undertreating of toxicity • Direct *serum* levels of DPD are not available
New Brunswick **(n = 2)**	**Public reimbursement:** Yes **Guidelines Followed:** *DPYD* pharmacogenomic testing recommendations by the AMP	• Clinician and patient awareness • Reimbursement of the cost of tests	Barriers to implementation: • Location of testing sites (e.g., urban or rural area) Barriers to accessibility: • Stigma or prejudice based on ethnicity, sex, gender, age, or other
Quebec **(n = 1)**	**Public reimbursement:** Yes **Guidelines Followed:** Provincial clinical tools, *INESSS: Fluoropyrimidine-Based Treatments: Best Strategies to Reduce the Risk of Severe Toxicities Caused by Dihydropyrimidine Dehydrogenase Deficiency*, are available to ensure therapy is safe and beneficial. Standardized prescriptions and patient advice are developed provincially and used by HCPs	NR	NR
Ontario[a] **(n = 1)**	**Public Reimbursement:** Yes **Guidelines Followed:** OH-CCO guidelines	• Number of testing centres • Location of testing centres • Well-established guidelines • Reimbursement of test costs	NR
Manitoba **(n = 1)**	**Public Reimbursement:** Yes (coverage for $100 once implemented) **Guidelines Followed:** NR	NR	Barriers to implementation: • Location of testing centres (urban vs. rural) • EMR system workflow integration (e.g., automatic alerts) • Billing infrastructure • Lack of infrastructure and human resources to implement testing Barriers to access DPD testing: • Location of testing centres

Jurisdiction, number of responses	Public reimbursement and guidelines followed	Facilitators to implementation and access	Barriers to implementation and access
Saskatchewan **(n = 3)**	**Public Reimbursement:** Yes **Guidelines Followed:** OH-CCO guidelines, US FDA safety announcements, and EMA recommendations	• Reimbursement of the cost of tests • Number of testing centres • Clinician and patient awareness • Well-established guidelines • EMR system workflow integration (e.g., automatic alerts)	Barriers to implementation: • Location of testing centres (urban vs. rural) • Lack of guidelines • Lack of technologists and validation plan or platforms in the labs • Time constraints (e.g., test TAT, treatment start date) Barriers to access DPD testing: • Delays in receiving results
Alberta **(n = 2)**	**Public Reimbursement**: No (funding application under way, anticipated July 2025 start date) **Guidelines Followed:** *Fluoropyrimidine Treatment in Patients with Dihydropyrimidine Dehydrogenase (DPD) Deficiency* (CancerCare Alberta Clinical Practice Guideline, adapted from OH-CCO guidelines)	NR	• Cost of testing • Lack of guidelines
British Columbia **(n = 2)**	**Public Reimbursement:** Yes **Guidelines Followed:** Jurisdictional guidelines based on the CPIC genotype-guided based dosing	• Location of testing (e.g., urban and rural) • Clinician and patient educational resources • Reimbursement of test costs • Clinician and patient awareness • Well- established guidelines • Established billing infrastructure • EMR system workflow integrations (e.g., automatic alerts)	• Lack of clinician and/or patient awareness of testing • Time constraints (e.g., test TAT, treatment start date)

AMP = Association for Molecular Pathology; CCO = Cancer Care Ontario; CPIC = Clinical Pharmacogenetics Implementation Consortium; DPD = dihydropyrimidine dehydrogenase; EMA = European Medicine Agency; EMR = electronic medical records; *FDA* = NA = not applicable; NR = not reported OH = Ontario Health; TAT = turnaround time.

[a]Survey response was pulled from public sources provided by contact.

Appendix 3: Characteristics of Included Publications

Please note that this appendix has not been copy-edited.

Summary of Study Characteristics

Summaries of study characteristics are organized by research question. Additional details regarding the characteristics of included publications are provided in Table 7 to Table 10.

Included Studies for Question 2: Guidelines on Genotype or Phenotype Testing for DPD Deficiency

Three evidence-based guidelines[11,12,17] and 1 consensus-based guideline[32] were included in this review. The consensus document[32] was published in 2024 by the AMP which is the Pharmacogenomics Working Group of the Clinical Practice Committee. The 3 evidence-based guidelines[11,12,17] were produced by OH-CCO published in 2023, the Dutch Pharmacogenetics Working Group (DPWG) of The Royal Dutch Pharmacists Association published in 2019, and the CPIC published in 2017, and an update posted on the webpage in 2024. One guideline was developed in Canada,[11] 1 in the Netherlands,[12] and 2 by groups based in the US.[17,32] One guideline[32] was developed through joint consensus among subject matter experts from multiple professional organizations including the CDC, CPIC, and DPWG. Three guidelines[11,12,17] conducted a systematic literature review to create the evidence base for recommendations. Recommendations were developed by evidence and expert consensus.[11,17] The OH guideline[11] was additionally reviewed by external experts and peer consultations. The DPWG[12] used a 5-point rating scale (0 to 4) created by Swen et al.[61] to assess the strength of evidence, while CPIC[17] used a scale modified from Valdes et al.[62] (weak, moderate, high). The CPIC group determined the strength of recommendations, while OH and DPWG did not report a formal method for rating the strength of recommendations. The consensus-based guideline by the AMP was a technical testing standard intended to provide laboratory guidance for pharmacogenomic testing of *DPYD* variants. Three guidelines focused on patients who are candidates for systemic treatment with FPs (5-FU or capecitabine) to provide genotype-guided dosing recommendations to minimize the risk of severe toxicity. The DPWG also considered recommendations for cutaneous administration of 5-FU or capecitabine. The OH group additionally provided recommendations on *DPYD* testing procedures. Intended users were clinicians,[11,17] physicians,[11,12] pharmacists,[12] or laboratory technicians[32] involved in the care of patients with cancer.

Included Studies for Question 3: Clinical Validity of Genotype or Phenotype Testing for DPD Deficiency

We identified 1 HTA,[44] 4 SRs,[35-38,44] and 1 primary study[9] to address this research question. Two of the SRs[35,36] included a meta-analysis of results. The HTA[44] and SRs[35-38,44] included data from a total of 90 unique primary clinical studies; however, there was considerable overlap among the included primary studies. As a result, the pooled effect estimates and narrative summaries from separate reviews are based on some of the same data, although not all reviews reported the same outcomes. A citation matrix illustrating the degree of primary study overlap is presented in Appendix 6.

The authors of the SR conducted as part of the HTA by Ontario Health[44] searched for systematic reviews and HTAs published from database inception to February 2020, to use as a source of primary studies published until their literature search dates. They then performed a search for primary clinical studies published from January 2018 (earliest search end date for included SRs and HTAs) to February 2020. In total, the authors included 29 primary clinical studies (25 relevant to this research question; clinical validity).

In the 2 included SRs with meta-analyses[35,36] study design was limited to RCTs or cohort studies. The SR by de Moraes et al[35] included 9 RCTs, 11 retrospective cohort, and 16 prospective cohort studies published up to June 18, 2024, while the SR by Kim et al[36] included 11 RCTs published up to October 18, 2021.

The SR by Cura et al[37] searched for studies of any design published up to December 30, 2022 that evaluated association between mutations in genes involved in the breakdown of capecitabine with toxicity or treatment effectiveness. They identified 12 studies in total, 6 of which were relevant to the present review. Finally, Paulsen et al[38] conducted 2 separate literature searches, both for any human clinical trials published up to June 10, 2021. The first search for measure of participants *DPYD* genotype, and the second for measurement of participants' DPD phenotype in plasma (uracil and/or dihydrouracil). They found 10 genotyping studies and 7 phenotyping studies relevant to this research question.

The primary study included[9] in this question was conducted in the Netherlands and published in 2023.

The HTA,[44] 2 of the SRs[36,37,44] and the NRS[9] provided information on the age, sex, and ethnicity of participants from the included primary studies; however, the authors did not report how sex was defined or measured. In most cases, ethnicity was poorly reported within the primary studies. None of the included SRs provided participant information for other PROGRESS-Plus criteria,[55,56] such as place of residence, race, culture, language, occupation, religion, education, socioeconomic status, or social capital.

Four of the SRs[35-37,44] focused solely on *DPYD* genotyping to test DPD activity, while 1 SR[38] included 12 studies focused on *DPYD* genotyping and 9 studies on DPD phenotyping (via plasma U concentrations and UH_2/U ratios). The NRS[9] evaluated DPD activity measured in PBMCs.

Outcomes assessed across the HTA, 4 SRs[35-38,44] and 1 NRS[9] to address research question 3 included:

- severe (grade ≥ 3) toxicity (overall and by category: cardiovascular, gastrointestinal, neurologic, and hematological)
- FP-related mortality
- FP-related hospitalization
- sensitivity, specificity, PPV, and NPV of *DPYD* genotyping to predict severe FP-related toxicity.

Included Studies for Question 4: Clinical Utility of Genotype or Phenotype Testing for DPD Deficiency

We identified 1 HTA,[44] 2 SRs[20,38] and 4 primary studies[40-43] to address this research question. One of the SRs[20] included a meta-analysis of results. The HTA[44] and SRs[20,38] included data from a total of 67 primary

clinical studies, however only 27 were relevant to the present question. There was considerable overlap among the included primary studies and as a result, the pooled effect estimates and narrative summaries from separate reviews are based on some of the same data, although not all reviews reported the same outcomes. A citation matrix illustrating the degree of primary study overlap is presented in Appendix 6.

The authors of the HTA by Ontario Health[44] published in 2021, searched for SRs and HTAs published from database inception to February 2020, to use as a source of primary studies published until their literature search dates. They then performed a search for primary clinical studies published from January 2018 (earliest search end date for included SRs and HTAs) to February 2020. In total, the authors included 29 primary clinical studies (6 relevant to this relevant to this research question).

In the 1 included SR with a meta-analysis,[20] published in 2022, included study designs was limited to RCTs or cohort studies. It included 8 prospective studies, 6 retrospective studies, and 3 combined retrospective and prospective studies published up to December 7, 2020. The SR by Paulsen et al.,[38] published in 2022, conducted 2 separate literature searches, both including any human clinical trials published up to June 10, 2021. The first search looked for studies assessing participants' *DPYD* genotype, and the second for studies assessing participants' DPD phenotype (as measured by plasma uracil and/or dihydrouracil concentrations). The authors included 21 primary studies in total, 5 of which were relevant to this research question.

All 4 included NRSs[40-43] were published between 2022 and 2024, and were conducted in hospital settings in Spain,[40] the Netherlands,[43] Denmark,[42] and France.[41] Two are retrospective cohort studies,[40,43] 1 retrospective, before-and-after study with a propensity score analysis,[41] and 1 prospective cohort, before-and-after study with a historical control group.[42]

All included studies for this research question[20,38,40-44] provided information on the age and sex of participants; however, the authors did not report how sex was defined or measured. The HTA[44] and 1 SR}[20] reported on the ethnicity of study participants. None of the included studies provided participant information for other PROGRESS-Plus criteria,[55,56] such as place of residence, race, culture, language, occupation, religion, education, socioeconomic status, or social capital.

The HTA,[44] 1 SR,[20] and 1 NRS[42] assessed genotype-guided dosing only. 1 SR[38] included studies assessing either genotype or phenotype-based dosing. Two of the included NRSs[40,41] evaluated phenotype-guided dosing based on plasma uracil concentrations, and 1 NRS[43] assessed a combined genotype and phenotype dosing method. In that study, DPD activity levels were measured in PBMCs.

Outcomes assessed across the 1 HTA, 2 SRs and 4 NRS to address research question 4 included:

- severe (grade ≥ 3) toxicity (overall toxicity and specific categories or types: cardiovascular, gastrointestinal, neurologic, and hematological), assessed using the CTCAE[10]
- FP-related mortality
- FP-related hospitalization
- disease response.

Included Studies for Question 5: Cost Implications of Genotype or Phenotype Testing for DPD Deficiency

We identified 1 economic evaluation, conducted as part of an Ontario HTA,[44] to address this research question. The study assessed the cost-effectiveness of *DPYD* genotyping followed by genotype-guided dosing versus usual care. We found no relevant evidence regarding the cost-effectiveness of phenotype testing for DPD deficiency versus usual care; therefore, no summary can be provided. Of note, we found 9 additional economic evaluations[45-53] and 1 budget impact analysis[44] assessing *DPYD* genotyping, which were excluded from this report due to their settings (i.e., they were not conducted from a Canadian health care perspective). A brief summary of the results of these excluded economic evaluations can be found in Appendix 5.

The included economic evaluation[44] conducted a probabilistic cost-utility analysis and a cost-effectiveness analysis, using a decision-tree model with a 6-month time horizon, from the perspective of the Ontario Ministry of Health, in Ontario, Canada. Outcomes for the probabilistic cost-utility analysis were costs and QALYs, and for the cost-effectiveness analysis were the proportion of patients with severe FP-related toxicities and the number of severe toxicities.

Clinical model inputs (e.g., patient characteristics, *DPYD* variant prevalence, probabilities of severe toxicity) were drawn from various sources of published literature, pooled prevalences from the meta-analysis conducted as part of the HTA[44] to inform clinical validity, clinical expert opinion, various sources of published literature and assumptions where required. Cost inputs were drawn from the Canadian Institute of Health Information (CIHI), the Ontario Health Insurance Program (OHIP), Ontario Drug Benefit (ODB) program, the pan-Canadian Oncology Drug Review (pCODR), laboratory expert opinion, and various sources of published literature. Costs were inflated to 2020 CA$.

The patient population was based on the characteristics of patients who received FPs in Ontario from 2014 to 2019, including different types of cancer (e.g., colorectal, breast, gastrointestinal, other), and receiving either 5-FU, capecitabine, or a combination regimen. Age and sex of the patient population was reported however, the authors did not report how sex was defined or measured. The evaluation did not provide information for other PROGRESS-Plus criteria,[55,56] such as place of residence, race, ethnicity, culture, language, occupation, religion, education, socioeconomic status, or social capital. The intervention in this study was pretreatment *DPYD* genotyping for the 4 primary variants (*DPYD**2A, *DPYD**13, c.2846A>T, c.1236G>A) in all patients with planned FP treatment, followed by genotype-guided dose adjustments made according to the 2017 CPIC guidelines,[17] compared to no testing and standard doses.

Table 7: Characteristics of Included Guidelines

Intended users, target population	Intervention and practice considered	Major outcomes considered	Evidence collection, selection, and synthesis	Evidence quality assessment	Recommendation development and evaluation	Guideline validation
AMP (2024)[32]						
Intended users: Clinical laboratories and assay manufacturers who develop, validate, and/or offer *DPYD* pharmacogenomic testing Target population: NA	Standardized clinical testing for selected *DPYD* genetic variants in clinical laboratories	NA	NR	NR	*DPYD* variants were reviewed into 2 tiers by subject matter experts from multiple professional organizations.	NR
Ontario Health/CCO (2023)[11]						
Intended users: Health care providers involved in the care of cancer patients who have planned systemic treatment with FPs (medical oncologists, nurses, pharmacists) Target population: Cancer patients who are candidates for systemic treatment with FPs (5-FU or capecitabine)	*DPYD* testing and genotype-guided dosing	Toxicity and treatment effectiveness	Not stated specifically for this guidance but a systematic review and standard meta-analytic methods are used for synthesis.	NR	Not stated specifically for this guidance but the recommendations on basis of synthesized evidence and an informal consensus of working group members on suitability for practice in Ontario, and expert opinion and consultation.	Not stated specifically for this guidance but internal review for methodological rigour, external review, targeted peer review (clinical and methodological quality and relevance of recommendations), and professional consultation (feedback, quality and relevance check).
DPWG (2019)[12]						
Intended users: Physicians and pharmacists Target population: Patients being treated or with planned treatment	Genotype-guided dosing recommendations	Toxicity	Systematic literature review and relevant literature was summarized by 1 of the authors.[a]	Quality was assessed on a 5-point scale ranging from 0 (lowest- data on file) to 4 (highest-	Recommendations were based on evidence.	NR

Intended users, target population	Intervention and practice considered	Major outcomes considered	Evidence collection, selection, and synthesis	Evidence quality assessment	Recommendation development and evaluation	Guideline validation
with FPs (5-FU and capecitabine)				well performed controlled studies or meta-analysis)		
CPIC (2017)[17]						
Intended users: Clinicians Target population: Patients being treated with FPs (5-FU, capecitabine, and tegafur) for which for which genotype data are available	Genotype-guided dosing recommendations	Toxicity and treatment efficacy	Systematic literature review. Publications were reviewed and included in evidence tables.	Evidence was graded using a scale modified from Valdes et al.[62] (high, moderate, and weak)	Recommendations reflect expert consensus based on clinical evidence. Strength of recommendations are based on weighting ethe evidence from a combination of preclinical functional and clinical data and some existing disease-specific consensus guidelines (Strong, moderate, optional, and no recommendation).	Not stated specifically but generally CPIC reports an extensive pre-and postsubmission review and approval process.

5- FU = 5-fluorouracil; AMP = Association for Molecular Pathology; CCO = Cancer Care Ontario; CPIC = Clinical Pharmacogenetics Implementation Consortium; DPWG = Dutch Pharmacogenetics Working Group; OH = Ontario Health; NR = not reported.

[a]Evidence was given a clinical impact score on a 7-point scale ranging from AA# (positive effect) to F (highest negative effect).

Table 8: Characteristics of Included HTA and Systematic Reviews

Study citation, country, funding source	Study designs and numbers of primary studies included	Variants evaluated or type of phenotype test	Population characteristics	Ethnicity groups Reported	Intervention and comparator(s)	Relevant outcomes
HTAs						
Ontario Health (2021) Canada	**Study design:** A SR of clinical and economic	**Variants tested:** • *PYD**2A • *PYD**13	People with planned cancer treatment with 5-fluorouracil	Ethnicity of patients was reported in 13 studies, NR in 17 studies.	**Clinical Validity** **Intervention:** Carriers of at least 1 of the	**Clinical outcomes:** • Overall severe toxicity • Severe gastrointestinal

Study citation, country, funding source	Study designs and numbers of primary studies included	Variants evaluated or type of phenotype test	Population characteristics	Ethnicity groups Reported	Intervention and comparator(s)	Relevant outcomes
Funding: Ontario Health is funded by the government of Ontario, Canada	evidence, including SRs published from database inception to February 2020 and primary studies published between January 2018 and February 2020. The HTA also included a de novo cost-utility analysis and budget impact analysis, which are described in Appendix 5. **Number of included studies:** Four SRs and 3 HTAs. 29 primary clinical studies were included (25 relevant to validity, 6 relevant to utility, (6 RCTs, 23 cohort studies).	• c.2846A>T • c.1236G>A	or capecitabine (monotherapy or in combination regiments) **Cancer type:** Colorectal cancer affected all patients in 12 studies and 35% to 85% of patients in 9 studies. Also included were breast, gastrointestinal, esophageal, and head and neck cancers. **Age:** Mean age of included clinical validity studies was 47 to 67 years. Mean age of included clinical utility studies was 58 to 65 years. **Sex:** 42% to 73% of patients in included clinical validity studies were male. 35% to 59% of patients in included clinical utility studies were male.	In included clinical validity studies 67% to 100% of patients were Caucasian. In included clinical utility studies, 98% to 100% were Caucasian. # of studies reporting the follow ethnic groups: • Caucasian: 13 • Black/African American: 3 • Asian: 4 • Afro-Caribbean: 1 • African: — • 'Other': 8	*DPYD* variants under assessment (*DPYD**2A, *DPYD**13, c.2846A>T, c.1236G>A), as defined by *DPYD* genotyping **Comparator:** wild-type patients **Clinical Utility** **Intervention:** *DPYD* genotyping of the variants under assessment (*DPYD**2A, *DPYD**13, c.2846A>T, c.1236G>A) before the start of treatment, or carriers of at least 1 of the *DPYD* variants under assessment, followed by genotype-guided FP dose reduction **Comparator:** patients with no testing; patients with phenotype tests for DPD function before the start of treatment; or wild-type patients or *DPYD* carriers without a genotype--	toxicity • Severe hematological toxicity • Severe dermatological toxicity • Overall survival • Progression-free survival **Cost outcomes:** • Costs • QALYs • ICER

Study citation, country, funding source	Study designs and numbers of primary studies included	Variants evaluated or type of phenotype test	Population characteristics	Ethnicity groups Reported	Intervention and comparator(s)	Relevant outcomes
					guided FP dose reduction	
SRs						
Cura et al. (2023)[37] Spain **Funding source:** the Instituto de Salud Carlos III	12 studies in total; 6 studies (RCTs and cohort studies) relevant to the present review	**Variants evaluated:** • c.IVS14 + 1G > A • 1679T > G • c.2846A>T • c.1236G>A • c.2194G > A • c.85T > C • c.1601G > A • c.1627A > G • c.496A > G • c.1906 to 14763G > A • c.1906 to 19696G > T • c.775A > G • g.97539400G > A • g.97523004G > A • 1896T > C • c.680 + 2545T > C	Colorectal cancer patients treated with capecitabine-based regimens (monotherapy or in combination with other antineoplastic agents)	**European ancestry:** 5 studies **Asian ancestry:** 1 study	**Intervention:** *DPYD* genotyping, FP treatment in *DPYD* variant carriers **Comparator**: FP treatment in patients with the wild-type gene.	**Outcomes:** FP-related severe toxicity (graded using the CTCAE); overall and grouped by type: GI, cardiovascular, asthenia, cutaneous, respiratory
Kim et al. (2022)[36] Korea **Funding source:** National Research Foundation funded by the Korean government and	**Study designs:** SR and meta-analysis of studies published up to October 2021. **Number of**	**Variants evaluated:** • c.2194G > A (rs1801160)	Cancer patients receiving FP-based regimens	**European ancestry:** 6 studies (1x Czech Republic, 1x Netherlands, 1x Spain, 1x UK and Ireland, 1x Italy, 1x 'multiple sites in EU')	**Intervention:** Genotyping of the rs1801160 *DPYD* variant, followed by treatment with standard dose of FPs **Comparator:** Patients	**Outcomes:** FP-related severe toxicity (graded using the CTCAE); overall and grouped by type: gastrointestinal, hematological, neutropenia, and diarrhea.

Study citation, country, funding source	Study designs and numbers of primary studies included	Variants evaluated or type of phenotype test	Population characteristics	Ethnicity groups Reported	Intervention and comparator(s)	Relevant outcomes
Gyeongsang National University.	**primary studies**: 6 studies total.				with wild-type gene treated with standard dose of FPs	
Paulsen et al. (2022)[38] Denmark **Funding source:** Danish Cancer Society and the Region of Southern Denmark	**Study design:** An SR of both genotyping and phenotyping studies (2 separate lit searches) published up to June 2021. **Number of included studies:** 21 total (12 genotyping studies [10 clinical validity; 2 clinical utility] and 9 phenotyping studies [7 clinical validity; 2 clinical utility).	**Variants evaluated:** • *DPYD**2A • *DPYD**13 • c.2846A>T • c.1236G>A **Phenotype tests evaluated:** • Plasma uracil concentration [U] • Dihydrouracil to Uracil ratio (UH_2/U)	Cancer patients receiving systemic 5-FU, capecitabine or tegafur **Age:** NR	NR	**Intervention:** *DPYD* genotyping and treatment with systemic 5-FU, capecitabine or tegafur, in *DPYD* variant carriers or DPD phenotyping and treatment with systemic 5-FU, capecitabine or tegafur in low DPD activity patients **Comparator:** *DPYD* genotyping and treatment with systemic 5-FU, capecitabine or tegafur, in patients with the wild-type gene, or DPD phenotyping and treatment with systemic 5-FU, capecitabine or tegafur in normal DPD activity patients	**Outcomes:** Incidence of severe (grade ≥ 3) toxicity (graded using the CTCAE)
De Moraes et al. (2024)[35]	**Study design:** SR and meta-analysis of clinical studies published up to	**Variants evaluated:** • *DPYD**2A	Solid tumour (nonhematologic) cancer patients receiving standard dose	**Ethnicity groups:** NR **Study locations:** percentage of studies conducted in:	**Intervention:** *DPYD* genotyping followed by FP treatment in *DPYD* variant carriers	• Risk of treatment-related mortality

Study citation, country, funding source	Study designs and numbers of primary studies included	Variants evaluated or type of phenotype test	Population characteristics	Ethnicity groups Reported	Intervention and comparator(s)	Relevant outcomes
Brazil **Funding:** None	June 2024. **Number of included studies:** 36 total. Nine RCTs, 11 retrospective cohorts, and 16 prospective cohorts.	• *DPYD**13 • c.2846A>T • c.1236G>A	of FP chemotherapy. Total of 16,005 patients. **Cancer type:** Most (86%) of the studies focused on colorectal cancer. **Age:** NR **Sex**: 47% male, 36% female, 17% sex NR.	Europe: 78.38% Asia: 18.92% Americas: 2.7% Oceania: 2.7%	**Comparator**: *DPYD* genotyping followed by FP treatment in patients with the wild-type gene.	
Glewis et al. (2022)[20] Australia **Funding**: none	**Study design:** SR with meta-analysis of publications up to December 2020. **Number of included studies:** 17 (retrospective and prospective cohorts).	**Variants evaluated:** • *DPYD**2A (15 studies) • *DPYD**13 • c.2846A>T • c.1236G>A **Phenotype test:** UH_2/U ratio (1 study) Combination of genotyping and/or phenotyping (1 study)	Patients 18 years or older with a diagnosis of solid cancer and treated with capecitabine or 5-FU based chemotherapy regimen (monotherapy or combination therapy).	Nine studies reported on ethnicity: the majority included “Caucasians” [from original source] while 2 studies reported on a population from India	**Intervention:** Pharmacogenetic-guided dosing (PGD) for FPs (genotype/phenotype or combination testing	• Grade 3/4 overall toxicity • Grade 3/4 diarrhea • Overall treatment response • Hospitalizations • Overall survival • Progression-free survival

CI = confidence interval; GI = gastrointestinal; CTCAE = Common Terminology Criteria for Adverse Events; NA = not applicable; NR = not reported.; OR = odds ratio.

Table 9: Characteristics of Included Primary Clinical Studies

Study citation, country, funding source	Study design	Variants tested or phenotype test	Population characteristics	Intervention and comparator(s)	Clinical outcomes, length of follow-up
Studies Included for Question 3 (Clinical Validity)					
Doornhof et al. (2023)[9] Netherlands **Funding source:** NR	Retrospective Cohort of patients treated between January 2017 and January 2021 at a single hospital in the Netherlands.	**Phenotype Test:** DPD activity levels in PBMCs (nmol/mg/hour).	**Inclusion criteria:** Patients older than the age of 18 treated with FP therapy (5-FU or capecitabine) **Excluded**: patients without pretreatment DPD phenotype measurement. **Number of participants:** 481 **Age, median (*IQR*):** 66 (58 to 73) years. **Sex, male (%):** 47.2% **Mean BSA (m²):** 1.89 ± 0.21 **Mean DPD (*sd*):** 9.7 (2.85) nmol/mg/h **Ethnicity groups:** NR **Other PROGRESS-Plus criteria**: NR[a]	**Intervention:** FP treatment in patients with minor or moderate DPD deficiency, as measured in PBMCs (between 50 and 70%, or < 50% of the population average of 9.6 nmol/mg/hour, respectively) *with* dose corrections based on CPIC guidelines. **Comparator:** FP treatment in patients with normal DPD activity, as measured in PBMCs (> 70% of the population average of 9.6 nmol/mg/hour)	**Outcomes:** FP-related adverse events (grade 1 to 2 and grade ≥ 3) overall and by group: cardiovascular, hematological, gastrointestinal, neurologic, dermatological, other. **Follow-up:** NR
Studies Included for Question 4 (Clinical Utility)					
Ockeloen et al. (2023)[43] Netherlands **Funding source:** None	Retrospective cohort of patients treated between January 2014 and December 2019 at a single academic hospital in the Netherlands.	**Variants evaluated:** • *DPYD**2A, • *DPYD**13, • c.2846A>T, • c.1236G>A (**Lab method:** Sanger sequencing) **Phenotype Test:** DPD enzyme activity assay using ex vivo peripheral blood mononuclear cells (PBMCs).	**Inclusion criteria:** All patients older than 18 years diagnosed with cancer that were treated with FPs (5-FU or capecitabine). **Number of participants:** 228 **Age (years), mean (sd):** 62.6 (10.4) **Male sex, n (%):** 131 (57.5%) **Ethnicity groups: "**The patients in this study were of different	**Intervention:** *DPYD* genotyping and DPD phenotyping via PBMCs, followed by initial dose reductions guided by the DPWG[12] guidelines. **Comparator:** *DPYD* genotyping and DPD phenotyping via PBMCs, followed by initial dose reductions guided by the DPWG[12] guidelines	**Outcomes:** Initial dose reduction Overall grade ≥ 3 toxicity

Study citation, country, funding source	Study design	Variants tested or phenotype test	Population characteristics	Intervention and comparator(s)	Clinical outcomes, length of follow-up
		(**Lab method:** Ultra high-performance liquid chromatography mass spectrometry)	ethnic backgrounds, although the majority was Caucasian" (p.6)[43] **Other PROGRESS-Plus criteria**: NR[a]		
Paulsen et al. (2023)[42] Denmark **Funding source:** Region of Southern Denmark and the Danish Cancer Society	Prospective analysis of cancer patients with a historic group as controls, at a single hospital in Denmark. Patients in the intervention group were enrolled between September 2020 and December 2021. The control group was treated with their first dose of FP between June 2017 and June 2020, at the same hospital as the intervention group.	**Variants evaluated:** • *DPYD**2A, • *DPYD**13, • c.2846A>T, • c.1236G>A/HapB3 (real-time PCR and LAMP Human DPD deficiency kit) **Phenotype Test:** Plasma concentration of uracil [U] (Liquid chromatography-tandem mass spectrometry method)	**Inclusion criteria:** Patients planned for their first systemic treatment with 5-FU, capecitabine, or tegafur, regardless of the tumour type. **Number of participants:** 722 **Age (years), mean (sd):** 66.7 (9.4) **Male sex, n (%):** 456 (63%) **Ethnicity groups:** NR **Other PROGRESS-Plus criteria**: NR[a]	**Intervention:** Pretreatment *DPYD* genotyping followed by initial FP genotype-guided dose reductions. Post hoc DPD phenotyping via plasma uracil concentrations were also conducted. **Comparator:** Standard dosing of FP treatment, with *post hoc DPYD* genotyping	**Outcomes:** • Overall grade ≥ 3 FP-TOX • FP-related hospitalizations • FP-related death • Discontinuation of FP due to toxicity **Time of assessment:** After the first 3 cycles of FP treatment
Tejedor-Tejeda et al. (2023)[40] Spain **Funding source:** Unfunded	Retrospective cohort of patients treated between September 2020 and April 2021 at a single hospital in Spain.	**Phenotype Test:** Plasmatic Uracil (ng/mL) High-performance liquid chromatography system.	**Inclusion criteria:** Patients diagnosed with gastrointestinal tumours and planned FP-related treatment (5-FU or capecitabine) **Number of participants:** 119 **Age (years), mean:** 64 **Male sex (%):** 47.2% **Ethnicity groups:** NR **Other PROGRESS-Plus criteria**: NR[a]	**Intervention:** FP treatment in patients with DPD deficiency, as measured by plasma uracil concentrations *with* dose adjustments (guidance for dose adjustments is unclear but appears to be based on a combination of clinician experience, baseline patient characteristics, and uracil measurements). **Comparator:** FP treatment in patients with no DPD	**Outcomes:** FP-related adverse events (total and separated by CTCAE grade: 1, 2, 3, and 4) **Follow-up:** NR

Study citation, country, funding source	Study design	Variants tested or phenotype test	Population characteristics	Intervention and comparator(s)	Clinical outcomes, length of follow-up
				deficiency, as measured by plasma uracil concentrations *with* dose adjustments (guidance for dose adjustments is unclear but appears to be based on a combination of baseline patient characteristics and clinician experience).	
Laures et al. (2022)[41] Netherlands **Funding source:** NR	Retrospective cohort of patients treated between 2017 and 2019 at 3 oncology centres in Frances.	**Phenotype Test:** Plasmatic Uracil (ng/mL) Ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method.	**Inclusion criteria:** All patients older than 18 years of age treated with 5-FU and who had an available pretherapeutic uracil concentration measurement. **Number of participants:** 292 [198 with DPD deficiency screening study group, 94 in reference group] **Age (years), mean:** 64 **Male sex (%):** 57.9% **Ethnicity groups:** NR **Other PROGRESS-Plus criteria**: NR[a]	**Intervention:** FP treatment in patients who underwent DPD deficiency screening (plasma uracil concentrations *with* dose adjustments as required (based on **Comparator:** FP treatment in patients who did not undergo DPD deficiency screening and therefore were treated with standard doses of FP.	**Outcomes:** Median toxicity severity score (the following CTCAE grade 3/4 toxicities were included in the calculation of the score: anemia, neutropenia, thrombocytopenia, nausea, vomiting, mucositis, diarrhea, alopecia, and hand-foot syndrome) **Follow-up:** Four treatment cycles (i.e., 8 weeks)

NA = not applicable; NR = not reported.; IQR = interquartile range; BSA = body surface area; DPD = dihydropyrimidine dehydrogenase; sd = standard deviation; PMBCs = peripheral blood mononuclear cells; CPIC = clinical pharmacogenetics implementation consortium; AE = adverse event, CTCAE = common terminology criteria for adverse events.

[a]The main PROGRESS-Plus criteria include place of residence, race, ethnicity, culture, language, occupation, gender, sex, religion, education, socioeconomic status, and social capital, personal characteristics associated with discrimination (e.g., age, disability), features of relationships, and time-dependent relationships.[55,56]

Table 10: Characteristics of Included Economic Evaluation

Study citation country, funding source	Type of analysis, time horizon, perspective	Population characteristics	Intervention and comparator(s)	Approach	Source of clinical, cost, and utility data used in analysis	Main assumptions
Ontario Health (2021)[44] Canada **Funding:** Ontario Health is funded by the government of Ontario, Canada	**Analysis**: probabilistic cost-utility analysis and cost-effectiveness analysis **Time horizon**: 6 months **Perspective**: Ontario Ministry of Health.	Any adults with planned cancer treatment with fluorouracil or capecitabine (monotherapy or in combination) **Age**: 63.6 years **Sex:** 49.2% male;50.8% female **Cancer type:** 40% colorectal 22% breast 10% upper gastrointestinal 28% other (e.g., pancreatic, prostrate, skin, lymphoid)	**Intervention:** Universal pretreatment *DPYD* testing for 4 variants (*DPYD*2A, DPYD*13, c.2846A>T, c.1236G>A*), followed by guided FP dosing for variant carriers following the CPIC guidelines. **Comparator**: Usual care (no testing and standard dosing).	Decision-tree model.	The clinical model was based on the treatment pathways in Ontario, clinical guidelines, and published economic analyses. Clinical inputs and model state transition probabilities (e.g., prevalence of DPYD phenotypes, probabilities of overall severe toxicity, hospitalization, death) were drawn from various sources of published literature, pooled prevalences from a meta-analysis conducted as part of this HTA, clinical expert opinion, and assumptions where required. Health-utility inputs were drawn from various sources of published literature, with weighted averages calculated by the authors for baseline utility values and disutility values associated with severe toxicity. Cost inputs were sourced from the Ontario Health Insurance Program (OHIP) Schedule of benefits A441, the Schedule of Benefits	• Most patients would be heterozygous *DPYD* variant carriers. • All *DPYD* poor metabolizers treated with standard-dose FPs would experience severe toxicities. • *DPYD* poor metabolizers treated with an alternative regimen would have similar risks of overall severe toxicity as *DPYD* in patients with the wild-type gene. • To capture the potential QALY loss due to rare but fatal toxicities, death due to severe FP-related toxicity was assumed to occur after the first or second cycle of chemotherapy. • Treatment-related hospitalization would occur only in patients with severe toxicities. • Approximately 1% to 2% of *DPYD* tests

Study citation country, funding source	Type of analysis, time horizon, perspective	Population characteristics	Intervention and comparator(s)	Approach	Source of clinical, cost, and utility data used in analysis	Main assumptions
					for Laboratory Services in Ontario, the Ontario Drug Benefit Formulary, Ontario Health (Cancer Care Ontario) dosing guidelines, consultation with laboratory experts, the Canadian Institute for Health Information (CIHI), and various sources of literature.	would fail and need to be rerun. • *DPYD* genotype-guided dosing and treatment adjustments would follow the 2017 CPIC guidelines.

NR = not reported. CPIC = Clinical Pharmacogenetics Implementation Consortium; NHS = National Health Service; QALY = quality-adjusted life-years.

Appendix 4: Critical Appraisal of Included Publications

Please note that this appendix has not been copy-edited.

Summary of Critical Appraisal

Critical appraisal summaries are organized by study design. Table 11 to Table 15 present additional details regarding the strengths and limitations of the included publications.

Evidence-Based Guidelines

The overall scope and purpose were clearly outlined in all guidelines,[11,12,17,32] including their objectives and target population, although the health questions being addressed in the guidelines were not explicitly stated or clearly defined in any of the documents. All guidelines reported details regarding their guideline development group and target users.[11,12,17,32] Two guidelines specified that a multidisciplinary group of professionals was involved in formulating the recommendations.[12,17]

A systematic literature review was used by 3 guideline development groups[11,12,17] however, only 2 groups[12,17] provided details about the criteria, search strategies, and strengths and limitations of evidence. Systematic review details of the OH-CCO guideline[11] were provided in a HTA published by Ontario Health.[44] One guideline group relied on consensus between subject matter experts but details on how the evidence base was created were scarce.[32] Two guidelines described the methods used to formulate recommendations, expert consultation and consensus, though neither described how expert consensus was achieved.[11,17] A clear explicit link between the recommendations and evidence was provided in 2 guidelines with a table showing the rationale and strength of evidence.[11,17]

Only 1 guideline included details of an external review process with the names and occupation of each external reviewer provided.[11] None of the groups provided details about procedures for updating the guideline, although 1 guideline was updated.[17] Recommendations in each of the guidelines were specific, clear, and unambiguous with key recommendations easy to identify.

Two guidelines[11,17] described the facilitators and barriers of applying the recommendations, as well as implementation tools and workflow that intended users could apply. One guideline provided guidelines for implementing *DPYD* testing.[11] None of the guidelines reported potential resource implications for applying the recommendations. Two guidelines[12,17] provided a funding statement but only 1[12] specified that the funding bodies were not involved in the development of guideline. Conflicts of interest were declared in all 4 documents,[11,12,17,32] but one[11] did not include a formal statement.

Systematic Reviews

The authors of all 6 SRs,[20,35-38,44] including the SR from the HTA,[44] clearly defined their objectives and eligibility criteria, conducted comprehensive literature searches across multiple databases, and provided details on key search terms and search dates. They also included flow charts illustrating the study selection

along with their reasons for excluding studies. These methodological strengths increase the reproducibility of the SRs. The review methods for 3 of the SRs[20,35,44] were established before conducting the reviews (i.e., they were documented in registered protocols), reducing the risk of reporting bias. In 5 SRs,[20,35-38] study selection was performed by either 2 or 3 authors independently.

The quality of the included primary studies was assessed using transparent and satisfactory techniques in 5 SRs,[20,35-37,44] and publication bias was assessed by the authors of 4 SRs[20,35,36,44] using funnel plots and/or Egger's and Begg's regression tests. In all cases, the authors suggested that the risk for publication bias was low. Three of the SRs[20,35,44] reported the characteristics of included studies in sufficient detail (e.g., study design, number of participants, study location) and used appropriate methods for the statistical combination of results and assessing statistical heterogeneity (e.g., the I2 statistic).

As for methodological limitations, the authors of 5 SRs[20,35-38] did not report conducting a grey literature search, increasing the risk of missing relevant studies that are not published commercially and that may be inaccessible via bibliographic databases (i.e., nonindexed studies). Three SRs[36-38] did not report that methods were established a priori. None of the SRs reported the sources of funding for the included primary studies, potentially influencing conflicts of interest within the studies. All the SRs,[20,35-38,44] limited included studies to those published in English or did not specify which languages were eligible for inclusion, potentially introducing language bias and omitting relevant data from non-English studies. In all 6 SRs[20,35-38,44] it was unclear if data extraction and critical appraisal were conducted by a single reviewer or multiple reviewers, creating a risk for inaccuracies in these processes.

The generalizability of findings from all 6 SRs[20,35-38,44] to settings in Canada was unclear because of limited reporting on the characteristics of primary study participants (e.g., across PROGRESS-Plus criteria[55,56]). Finally, the authors of 1 SR[11] did not state their potential conflicts of interest, and the sources of funding for 3 SRs[35,36,44] was unclear.

Nonrandomized Cohort Studies

The authors of all 5 nonrandomized cohort studies (NRS)[9,40-43] provided clear descriptions of their study objectives, outcomes, interventions, comparators, participant eligibility criteria, and main findings. Principal confounders were listed and compared between groups in 4 of the studies.[9,41-43] Additional methodological strengths were that the measurement of outcomes, including FP-related toxicity, were standardized using the CTCAE.[10] However, 1 study[40] only reported on the CTCAE grade of AE, and not on the specific type of AE. The authors of 4 NRSs[9,41-43] reported estimates of uncertainty (e.g., confidence intervals) and P values. All 5 studies[9,40-43] recruited patients treated in large hospitals, with care providers and treatment pathways representative of the target population. For 1 NRS,[42] the proportion of patients who were asked to participate in the study and those who agreed was not stated, potentially introducing selection bias and differences between the study sample and source population. In 1 NRS,[43] the sampling method was not well described so we were unable to determine whether the study sample was representative of the source population. The authors of all 5 NRSs[9,40-43] declared that they had no potential conflicts of interest related to this work

and reported their sources of funding, which were unrelated to industry and considered unlikely to have influenced the study's findings.

Several factors affected the internal and external validity of the included NRSs. In all 5 NRSs,[9,40-43] subjects were not blinded to the intervention they received, potentially introducing performance or detection bias. In none of the studies was there any indication that the outcome assessors (i.e., those grading the AEs experienced by participants) were blinded to the intervention, which could lead to bias in their severity grading. However, all studies reported standardized grading using a validated tool[10] which could help minimize this bias. Additionally, no mention was made in any of the studies regarding missing data or incomplete records. Missing confounders can introduce selection bias and missing outcomes could result in attrition bias or systematic bias if the missing data patterns are related to the incidence of AEs.

In 2 studies[41,42] different intervention groups were recruited over different periods of time (pre- and post-implementation of standardized DPD testing at the study centres), potentially introducing other variables (e.g., referral patterns, staff changes, treatment guidelines) that might have confounded the incidence of severe toxicities. Due to the nature of the intervention in the included studies (FP dose reductions based on DPD activity), compliance with the intervention was not always reliable. In 1 NRS,[9] some patients were missing phenotype data and therefore received doses based on other factors. In another study[43] some low DPD activity patients did not receive the recommended dose reduction because testing was performed after the start of treatment.

Although the authors reported some relevant baseline participant characteristics (e.g., age, sex, body surface area, comorbidities), many important characteristics that stratify health opportunities and outcomes were not reported, such as race, ethnicity, culture, language, place of residence, socioeconomic status, and other PROGRESS-Plus criteria.[55,56] As a result, it remains unclear whether the findings of these NRSs[9,40-43] can be generalized to settings in Canada. Further, the 2 studies that involved *DPYD* genotyping[42,43] only evaluated the 4 variants most relevant in populations of European descent, limiting the generalizability of results to more ethnically diverse populations that might carry other *DPYD* variants affecting DPD activity.

Economic Evaluations

The authors of the economic evaluation[44] clearly stated their research question, objectives, the economic importance of the research question, the interventions compared, and rationale for conducting the analysis from the perspective of the Ontario Ministry of Health using a 6-month horizon. They provided detailed information on the sources of the effectiveness estimates, utility values, and treatment costs. The authors recorded the currency and price data used and the methods for adjusting prices for inflation, described their approach to sensitivity analyses, reported incremental analyses, provided an answer to the study question, and summarized the findings with conclusions accompanied by appropriate caveats.

The primary strength of the economic evaluation[44] is its concordance with the decision problem of interest to this report. Given that it was conducted from a health care perspective in Canada, it is more generalizable to other settings in Canada. The model-based evaluation allowed for the comparison of broad patient

populations, including numerous cancer types, and different chemotherapy regimens (5-FU, capecitabine, and alternative treatments for *DPYD* poor metabolizers). Similarly, the choice of a decision-tree model was justified due to the acute nature of chemotherapy treatment and its associated adverse events, which typically resolve within a few months.[62] The intervention (*DPYD* genotyping followed by genotype-guided dosing) is conducted already in some provinces across Canada and so is of direct interest. The dose adjustments were guided by an evidence-based guideline.[17]

The primary limitation of the economic evaluation is the uncertain generalizability of results among broad ethnic groups. The studies from which the clinical input data on genotype-guided dosing utility were sourced, generally did not report any PROGRESS-Plus criteria,[55,56] except age and sex. Further, the evaluation focused on 4 primary variants that are much less common in other ethnic groups other than those of European descent. Therefore, *DPYD* variant prevalences and probability data for severe toxicities, hospitalizations, and mortality may have limited generalizability beyond the ethnic groups represented in the studies; a critical limitation given the diverse population of Canada. Conclusions about cost-effectiveness may be different if the intervention included testing for a broader panel of variants that better represented this population. Similarly, health-utility inputs were not drawn from sources in Canada and therefore may not accurately reflect preferences across Canada.

Finally, the study[44] only looked at the impact of *DPYD* genotyping, without any analysis of phenotype testing for DPD activity. Therefore, no evidence is available regarding the cost-effectiveness of phenotype testing.

Table 11: Strengths and Limitations of Guidelines Using AGREE II Tool[60]

Item	AMP (2024)[32]	OH-CCO (2023)[11]	DPWG (2019)[12]	CPIC (2017)[17]
Domain 1: Scope and purpose				
1. The overall objective(s) of the guideline is (are) specifically described.	Yes	Yes	Yes	Yes
2. The health question(s) covered by the guideline is (are) specifically described.	NA	Partial Yes	Partial Yes	Partial Yes
3. The population (patients, public, and so forth) to whom the guideline is meant to apply is specifically described.	NA	Yes	Partial Yes	Yes
Domain 2: Consultations				
4. The guideline development group includes individuals from all relevant professional groups.	Yes	Partial Yes	Yes	Partial Yes
5. The views and preferences of the target population (patients, public, and so forth) have been sought.	No	No	No	No
6. The target users of the guideline are clearly defined.	Yes	Yes	Yes	Partial Yes

Item	AMP (2024)[32]	OH-CCO (2023)[11]	DPWG (2019)[12]	CPIC (2017)[17]
Domain 3: Rigour of development				
7. Systematic methods were used to search for evidence.	No	Yes, from the HTA conducted by OH	Yes	Yes
8. The criteria for selecting the evidence are clearly described.	NA	No	Yes	Partial Yes
9. The strengths and limitations of the body of evidence are clearly described.	NA	No	Partial Yes	Yes
10. The methods for formulating the recommendations are clearly described.	Partial Yes	Partial Yes	No	Partial Yes
11. The health benefits, side effects, and risks have been considered in formulating the recommendations.	NA	Partial Yes	No	Yes
12. There is an explicit link between the recommendations and the supporting evidence.	NA	Partial Yes	Yes	Yes
13. The guideline has been externally reviewed by experts before its publication.	NR	Yes	No	No
14. A procedure for updating the guideline is provided.	NR	Partial Yes	No, but they provide statement that says guidelines are regularly updated	No, but guideline was updated in 2024
Domain 4: Clarity of presentation				
15. The recommendations are specific and unambiguous.	Yes	Yes	Yes	Yes
16. The different options for management of the condition or health issue are clearly presented.	NA	Partial Yes	Yes	Yes
17. Key recommendations are easily identifiable.	Yes	Yes	Yes	Yes
Domain 5: Applicability				
18. The guideline describes facilitators and barriers to its application.	No, but the technical feasibility for laboratories to use the standard testing methods were considered when classifying variants	Yes	No	Partial Yes
19. The guideline provides advice and/or tools on how the recommendations can be put into practice.	NA	Yes	No	Yes

Item	AMP (2024)[32]	OH-CCO (2023)[11]	DPWG (2019)[12]	CPIC (2017)[17]
20. The potential resource implications of applying the recommendations have been considered.	NR	No	No	No, there is clear statement at beginning that cost-effectiveness is beyond scope of guideline
21. The guideline presents monitoring and/or auditing criteria.	NA	No	No	No
Domain 6: Editorial independence				
22. The views of the funding body have not influenced the content of the guideline.	NR	Partial Yes	Yes	Yes
23. Competing interests of guideline development group members have been recorded and addressed.	Yes	No	Yes	Yes

AGREE II = Appraisal of Guidelines for Research and Evaluation II; AMP = Association for Molecular Pathology; CCO = Cancer Care Ontario; CPIC = Clinical Pharmacogenetics Implementation Consortium; DPWG = Dutch Pharmacogenetics Working Group; HTA = health technology assessment; NA = not applicable; NR = not reported; OH = Ontario Health.

Note: Categories Yes, Partial Yes, and No were determined using the Likert Scale on the AGREE-II tool. Yes (Scores 5 to 7); Partial Yes (Scores 3 to 4); No (Scores 1 to 2).

Table 12: Strengths and Limitations of HTAs and SRs Using AMSTAR 2[58]

Strengths	Limitations
De Moraes et al. (2024)[35]	
• The research question and inclusion criteria provided details on the population, intervention, comparator, and outcomes of interest. • The authors registered an *a prior* protocol within the PROSPERO database that included details on the search strategy, inclusion/exclusion criteria, risk of bias assessment, analysis plan, and discussion of heterogeneity. • The authors searched 4 databases, and the literature search was conducted within 24 months of publication. • Two reviewers independently performed study selection and assessment of risk of bias of included studies. • The authors provided a list of excluded studies with exclusion reasons. • The authors justified combining the data for a meta-analysis and used an appropriate weighted technique to combine study results. • The authors discussed that most of the included studies had a low risk of bias (score 8 to 9 on NOS). • The authors found that their main outcome showed low heterogeneity (I2 = 2%). • The authors investigated potential publication bias, with visual inspection of contour-enhanced funnel plots and using Egger's regression asymmetry and Begg's rank correlation tests. • The authors reported no competing interests.	• The authors did not provide an explanation for their selection of study designs. • The authors did not justify the exclusion of publications in languages other than English. • The authors did not report searching the bibliographies of included studies, study registries, grey literature, or consulting content experts in the field for other potentially relevant articles. • The authors did not report whether data extraction was completed independently by 2 reviewers. • The authors did not report the sources of funding for individual studies included in the review.

Strengths	Limitations
Cura et al. (2023)[37]	
• The population, intervention, and outcomes of interest were clearly stated. • The authors searched 2 databases and provided their search strategy (supplementary table). • The literature search was conducted within 24 months of publication. • Two authors independently selected studies and performed data extraction. • The characteristics of included studies are provided. • The authors provide a good discussion regarding heterogeneity of results in the review. • The authors reported no competing interests and described their funding sources.	• The inclusion criteria did not describe the comparator. • There was no statement regarding a priori review methods or an established protocol. • No explanation was provided for included study designs. • The authors did not justify restrictions based on language. • There was no report of searching reference lists, grey literature, or trial registries for potentially relevant studies. • Excluded studies with justification was not provided. • Funding sources for individual studies included was not provided.
Glewis et al. (2022)[20]	
• The population, intervention, comparators, and outcomes of interest were clearly stated. • The review methods were established before conducting the review (The review was registered in PROSPERO, [CRD42020223768]). • The systematic search included multiple databases, and the search was conducted within 24 months of completion of the review. • Key search terms and the full search strategy were provided. • Three authors independently performed study selection. • The authors described the included studies in detail. • The authors used a satisfactory technique for assessing the risk of bias from patient selection bias and cohort comparability in individual studies. • The authors assessed the potential impact of RoB in individual studies on the results of the meta-analysis and provided a discussion of the likely impact on results. • The authors used appropriate methods to test for heterogeneity and publication bias in the results. • Review authors stated no competing interests and no funding received for this work.	• The authors did not report searching reference lists of included studies, a targeted grey literature search, or consultation with content experts to identify potentially relevant articles. • Authors did not justify restricting publications to those published in English or explain their selection of eligible study designs. • The authors did not report performing data extraction in duplicate. • The authors did not provide a list of excluded studies with justification for exclusion. • Authors did not assess RoB from confounding or dose-response gradient.
Kim et al. (2022)[36]	
• Includes details on population, intervention, outcomes, and controls. • Authors searched 3 databases (PubMed, Web of Science, and Embase), provided search strategy, and provided publication restriction categories. • Two independent reviewers performed search and screening. • The study authors clearly defined basic study characteristics (PICO), although they did not include research designs. • Authors justified the meta-analysis data and performed	• Authors did not justify their choice of included study designs • There was no explicit statement regarding a priori methods or protocol • Did not discuss searching reference lists, grey lit, trial registries, experts, and so forth • No statement provided on number of authors that completed data extraction • Number of studies excluded and reasons provided, however list of specific studies not provided

Strengths	Limitations
and reported a weighted analysis, and reported the heterogeneity score (I2 = 30%). • There was no heterogeneity between the included studies (I2 = 30%). • Authors carried out an adequate investigation of publication bias (Egger's test and Begg's test) and discussed the likely impact on results. • The authors reported no competing interests.	
Paulsen et al. (2022)[38]	
• The research question and inclusion criteria provided details on the population, intervention, comparators, and outcomes of interest. • The authors searched 2 databases and provided a detailed search strategy. • The authors discussed searching the citations of the included studies and through study trial registries. • Three independent reviewers selected studies, with a consensus procedure described. • A full list of excluded studies was provided as an appendix, with specific exclusion reasons provided. • The authors reported the study design, intervention, comparators, and outcomes for all included studies. • The authors discussed the heterogeneity of included studies, and limitations of certain studies were discussed. • Authors discussed that the wide range in results among included studies was indicative of heterogeneity in study populations and treatment regimens, and that a pooled analysis was not relevant. • Most authors had no conflicts of interest. One author described their funding sources.	• The follow-up time frame for included was not provided. • The authors did not explain decisions for specific publication restrictions. • The authors did not state that they had a priori methods or an established protocol before completing the review. • The authors did not provide an explanation for the type of study designs included in the review. • The authors did not report searching the grey literature or consulting experts for potentially relevant articles. • The authors did not report how many authors performed the data extraction. • Detail on the population characteristics of the included studies was not provided. • The authors did not perform any formal assessment of quality or risk of bias in the included studies.
Ontario Health HTA (2021)[44]	
• The population, intervention, comparators, and outcomes of interest were clearly stated. • The review methods were established before conducting the review (The HTA was registered in PROSPERO, the international prospective register of systematic reviews). • The systematic search included multiple (5) databases. • Key search terms and the full search strategy were provided. • Database autoalerts in MEDLINE and Embase were created for the duration of the assessment period. A targeted grey literature search was conducted, and content experts were consulted for further relevant articles. • A list of excluded but potentially relevant studies was provided, with justification for exclusion provided. • The authors described the included studies in detail. • The authors used a satisfactory technique for assessing the risk of bias in individual studies (Newcastle-Ottawa scale and	• The authors did not explain their selection of eligible study designs • Authors did not justify restricting publications to those published in English • Only 1 author performed study selection and data extraction • Sources of funding for the individual studies included in the HTA were not reported • Review authors did not state their potential conflicts of interest or funding sources

Strengths	Limitations
the ROBIS tool). • The review authors used appropriate weighted methods for meta-analysis when appropriate and in the absence of heterogeneity. • Only low risk of bias RCTs were included. • The authors carried out an adequate investigation of publication bias and discussed its likely impact on the results.	

AMSTAR 2 = A MeaSurement Tool to Assess Systematic Reviews 2; NA = not applicable; NR = not reported; ROBIS = Risk of Bias in Systematic Reviews; PROSPERO = international prospective register of systematic reviews.

Table 13: Strengths and Limitations of Clinical Studies Using the Downs and Black Checklist[59]

Strengths	Limitations
Tejedor-Tejeda (2024)[40]	
Reporting • Authors clearly described the aim of the study, main outcomes, participant inclusion criteria, interventions, comparator, and main findings • Participant characteristics (e.g., age, sex) were reported • Adverse events were reported **External Validity** • The study was conducted at a large hospital in Spain, with treatment and settings that appear to be representative of the care that most patients receive. • As a retrospective study, no informed consent was required so all consecutive patients treated at the study centre over an 8-month period were included and therefore should be representative of the entire population. **Internal Validity – bias** • Compliance with the intervention was reliable • Outcome measures were valid **Internal Validity – confounding** • Participants in different intervention groups were recruited from the same population, and over the same period **Other** • The authors declared no conflicts of interest and no sources of funding that may have influenced the findings of the study.	**Reporting** • Exclusion criteria are not described • For all outcomes, the authors reported simple outcome data, but did not report the odds ratios between the groups, 95% CIs, or actual P values • AEs were reported based on CTCAE grade, but no details regarding the type of AEs were provided. • Authors did not report whether there was any missing data or incomplete records. **External Validity** • There was limited reporting of the characteristics of study participants (e.g., across PROGRESS-Plus criteria[55,56]) **Internal Validity – Bias** • Patients were not randomized to different study groups (retrospective cohort study) • Any difference in follow-up lengths between patients was not reported **Internal Validity – confounding** • Factors besides uracil levels that could affect the incidence of adverse events were not reported **Power** • The authors did not conduct a sample size calculation
Doornhof (2023)[9]	
Reporting • Authors clearly described the aim of the study, main outcomes, participant eligibility criteria, interventions, comparator, and main findings • Participant characteristics (e.g., age, sex, BSA) were reported • For all outcomes, the authors reported simple outcome data, the odds ratios between the groups, 95% CIs, and actual P	**External Validity** • There was limited reporting of the characteristics of study participants (e.g., across PROGRESS-Plus criteria[55,56]) **Internal Validity – bias** • Patients were not randomized to different study groups (retrospective cohort study) • Any difference in follow-up lengths between patients was not

Strengths	Limitations
values • No participants were lost to follow-up • Adverse events were reported **External Validity** • The study was conducted at a large hospital in the Netherlands, with treatment and settings that appear to be representative of the care that most patients receive. • As a retrospective study, no informed consent was required so all consecutive patients treated at the study centre over a four-year period were included and therefore should be representative of the entire population. **Internal Validity – bias** • Authors conducted appropriate statistical analyses and univariate and multivariate log regressions. • Compliance with the intervention was reliable. • Outcome measures were valid. **Internal Validity – confounding** • Participants in different intervention groups were recruited from the same population, and over the same period • Factors besides DPD phenotype that could affect the incidence of adverse events (e.g., dosage, hepatic function, kidney function, chemotherapy regimen, relevant patient characteristics) were corrected for using log regression **Other** • The authors declared no conflicts of interest	reported **Power** • The authors did not conduct a sample size calculation **Other** • The authors did not disclose any sources of funding that may have influenced the findings of the study
Ockeloen et al. (2023)[43]	
Reporting • Authors clearly described the aim of the study, main outcomes, participant inclusion criteria, interventions, comparator, and main findings • Participant characteristics (e.g., age, sex, BSA) were reported • For all outcomes, the authors reported simple outcome data, or actual P values • Adverse events were reported **External Validity** • The study was conducted at a single academic hospital in the Netherlands, with treatment and settings that reflect common practice for these patients. **Internal Validity – bias** • Outcome measures were valid **Internal Validity – confounding** • Participants in different intervention groups were recruited from the same population over the same period of time. • The distribution of known confounders (i.e., age, sex, BSA, tumour and treatment type) in the different groups was described and analyzed. No difference was found between groups.	**Reporting** • Exclusion criteria are not described • Authors did not report whether there was any missing data or incomplete records **External Validity** • There was limited reporting of the characteristics of study participants (e.g., across PROGRESS-Plus criteria[55,56]) • The intervention only involved *DPYD* genotyping for the 4 variants most common in populations of European ancestry, and are less prevalent in other ethnic groups. **Internal Validity – bias** • Patients were not randomized to different study groups (retrospective cohort study) • Any attempts to blind those measuring the outcomes of the intervention were not reported • Compliance with the intervention was not always reliable, as patients with a *DPYD* variant or with low DPD activity did not always receive the recommended dose reduction according to guidelines, because sometime testing was performed after the start of treatment.

Strengths	Limitations
Other • The authors declared no conflicts of interest and no sources of funding that may have influenced the findings of the study.	**Internal Validity – confounding** • Not all *DPYD*$^{variant\_no}$-DPD^{normal_activity} patients were included in the study so there may be some selection bias. However, the included patients were randomly selected. **Power** • The authors did not conduct a sample size calculation
Paulsen et al. (2023)[42]	
Reporting • Authors clearly described the aim of the study, main outcomes, participant inclusion and exclusion criteria, interventions, comparator, and main findings • Participant characteristics (e.g., age, sex) were reported • For all outcomes, the authors reported simple outcome data, relative risk between groups, 95% CIs, and actual P values • Adverse events were reported **External Validity** • The study was conducted at 1 hospital in Denmark, with treatment and settings that appear to be representative of the care that most patients receive. • The intervention under study was implemented as a new standard of care at the study centre and thus the staff, setting, and treatment, of the participants were representative of the treatment the majority of patients receive. • The patients in the different groups were recruited from the same population over the same period of time. **Internal Validity – bias** • Outcome measures were valid (adverse events were graded according to CTCAE v5.0) • The time period between the intervention and outcome assessment was the same for the intervention and control groups (after the first 3 treatment cycles) • Authors conducted appropriate statistical analyses. **Internal Validity – confounding** • Participants in different study groups were recruited from the same hospital. • The distribution of known confounders (i.e., age, sex, BSA, tumour and treatment type) in the different groups was described and analyzed. No difference was found between groups. **Other** • The authors declared no conflicts of interest and no sources of funding that may have influenced the findings of the study.	**Reporting** • Authors did not report the number of patients lost to follow-up. **External Validity** • There was limited reporting of the characteristics of study participants (e.g., across PROGRESS-Plus criteria[55,56]). • The intervention only involved DPYD genotyping for the 4 variants most common in populations of European ancestry, limiting generalizability to other ethnic groups. • The proportion of the patients who were asked to participate in the study and those who agreed was not stated. No comparison between the study sample and source population was provided. **Internal Validity – bias** • Patients were not randomized to different study groups • Any attempts to blind those measuring the outcomes of the intervention were not reported • Compliance with the intervention was reliable, with all *DPYD* variant carriers receiving the correct dose reductions. **Internal Validity – confounding** • The intervention group and control group were recruited over different periods of time (before-and-after the implement of standardized *DPYD* genotyping) • Confounders were compared between groups however significance of differences was not calculated, and no adjustments were made in the analyses to account for any differences. **Power** • The authors conducted a sample size calculation based on reported frequencies of grade 3 or higher toxicities, and minor allele frequencies in the population. However, the sample size was not reached and therefore the study did not have sufficient power to detect clinically relevant difference.
Laures et al. (2022)[41]	
Reporting • Authors clearly described the aim of the study, main outcomes, participant inclusion criteria, interventions, comparator, and	**Reporting** • Exclusion criteria are not described • Authors did not report whether there was any missing data

Strengths	Limitations
main findings • Participant characteristics (e.g., age, sex, height, weight, BSA) were reported • For all outcomes, the authors reported simple outcome data, means with standard deviations, medians with min-max range, or actual P values • A list of principal confounders was provided and compared between groups (age, gender, BMI, creatinine clearance, performance status, tumour type and stage chemotherapy, use of biotherapy and use of radiotherapy) • Adverse events were reported **External Validity** • The study was conducted at 1 tertiary oncology centre and 2 secondary centres in France, with treatment and settings that appear to be representative of the care that most patients receive. • As a retrospective study, no informed consent was required so all consecutive patients treated at the study centre over a 14-month period were included and therefore should be representative of the entire population. **Internal Validity – bias** • Compliance with the intervention was reliable • Outcome measures were valid **Internal Validity – confounding** • Participants in different intervention groups were recruited from the same population **Other** • The authors declared no conflicts of interest that may have influenced the findings of the study. • The authors declared no funding received for this work.	or incomplete records. **External Validity** • There was limited reporting of the characteristics of study participants (e.g., across PROGRESS-Plus criteria[55,56]) **Internal Validity – bias** • Patients were not randomized to different study groups (retrospective cohort study) • Any difference in follow-up lengths between patients was not reported **Internal Validity – confounding** • Participants in different intervention groups were recruited over different periods of time **Power** • The authors did not conduct a sample size calculation

NA = not applicable; NR = not reported.; BSA = body surface area; CTCAE = Common Terminology Criteria for Adverse Events.

Table 14: Strengths and Limitations of Economic Evaluations

Item Appraisal criteria	Strengths	Limitations
Ontario HTA (2021)[44]		
Decision problem: Does the scope of the economic evaluation align with the decision problem of interest regarding target population(s), intervention(s), comparator(s), outcome(s), time horizon, perspective, setting, and model?	• The time horizon is stated (6 months). Justification for the time horizon is provided (based on the assumption that chemotherapy efficacy would be similar between *DPYD* carriers who received a reduced dose and wild-type patients. While this has been shown in some studies, the evidence is comparing 2 different populations, and is not conclusive) • The structure of the decision-tree model was	• Details of the subjects from whom the valuations were obtained (various published literature sources) were not provided. • The study only looks at the impact of *DPYD* genotyping of the 4 main variants. It does not include an analysis of the cost-effectiveness of extended *DPYD* genotyping (with more variants) or phenotyping tests.

Item Appraisal criteria	Strengths	Limitations
	clearly described, and appropriate given the natural history of the disease and treatment in question.	
PROGRESS-Plus criteria[55,56]	• The authors report on age and sex of participants in included studies.	• Population data did not include any other PROGRESS-Plus criteria such as ethnicity, race, or socioeconomic status. • Studies used for clinical inputs did not report on PROGRESS-Plus criteria.
Clinical inputs: natural history of the disease, clinical effectiveness, safety and harms, health utilities and disutilities	• The source study of *DPYD* variant prevalences and probability of severe toxicity inputs contained all 3 groups of interest (*DPYD* wild-type gene with standard dose; *DPYD* variant carrier with reduced dose; *DPYD* variant carrier with standard dose). • All events relevant for the health system are included in the model, and are consistent with other economic evaluations. • Assumptions pertaining to the model structure are described and justified. • Overall health-utility values are used (no treatment-specific utilities). • Treatment decisions and dose adjustments in the model were based off an appropriate source (CPIC guidelines). • Baseline health utility and disutility associated with severe toxicity were weighted averages based on the 3 most common cancer types and toxicity types, respectively.	• The main source of severe toxicity probability estimates was an observational study with a small number of *DPYD* carriers (due to low frequency in the population), and an overrepresentation of the variants with expected weakest effects on DPD. • Health utilities used were not from sources across Canada and therefore may not accurately reflect preferences in Canada. • The model did not factor in potential nonadherence to genotype-guided treatment recommendations. • Probabilities of severe toxicity and hospitalization in *DPYD* poor metabolizers on an alternate chemo regiment were based on assumption.
Cost Inputs: unit costs and resource use	• Sources of treatment cost inputs are provided • Quantities of resource use, including testing resources and sample, days in hospital, and drug doses, are reported separately from their unit costs • Methods for the estimation of quantities and unit costs are described • Currency and price data were recorded (2020 CA$) • No discounting rate was applied and it is justified (short time horizon).	• Costs related to infrastructure (e.g., testing equipment), overhead, licensing, accreditation, or personnel training were not considered • Testing costs vary greatly based on number of samples per run, and thus would depend on availability and demand for testing in other provinces.
Reporting quality	• The report adheres to reporting guidelines:[63] ○ The authors clearly state the research question and its economic importance, and the form, perspective, intervention and comparators, and primary outcome measures for the evaluation.	

Item Appraisal criteria	Strengths	Limitations
	○ Details of the design and results of the study used for effectiveness estimates were provided. ○ Details of the methods of synthesis were given for pooled prevalence estimates or calculated means. ○ Methods to value health states and benefits were stated. ○ The methods for currency conversions and price adjustments were given. ○ Details of statistical tests and confidence intervals were given. ○ The approach to the sensitivity analysis was given. ○ The choice of variables and their ranges for the sensitivity analysis were justified (ranges were based on mean and standard errors). ○ Major outcomes from both the reference case analysis and the scenario analyses were presented in disaggregated and aggregated form. ○ The answer to the study question was given. ○ Conclusions follow from the data reported ○ The conclusions were accompanied by appropriate caveats. **Other** ● Sources of funding were disclosed (Ontario Health is funded by the Ontario government)	

CA$ = Canadian dollars; CHEERS = Consolidated Health Economic Evaluation Reporting Standard; DPD = dihydropyrimidine dehydrogenase.

Appendix 5: Literature Review Findings

Please note that this appendix has not been copy-edited.

Table 15: Summary of *DPYD* Testing Recommendations From Ontario Health, Cancer Care Ontario

Recommendations	Evidence supporting recommendations	Quality of evidence and strength of recommendations
"Patients with planned fluoropyrimidine-based therapies should be informed about DPD deficiency, available tests to detect deficiency, and the potential risks associated with fluoropyrimidine treatment if a deficiency is detected. It is important to note that with universal access to DPD testing, the risks should be minimal."	1 HTA, 2 evidence-based guidelines based on SR and expert consensus, 1 SR with MA, 2 narrative reviews, 1 NRS (retrospective case series) and product monographs to establish the prevalence and risks associated with DPD deficiency for patients with planned FP-based therapy.	NR
"Prospective *DPYD* genotyping should be included in the planning of fluoropyrimidine-based therapies."	3 NRS (retrospective chart review, 2 prospective cohort designs) that showed that prospective genotyping reduced the incidence of toxicity and treatment induced mortality	NR
"Prior to initiating fluoropyrimidine-based therapies, patients should be screened for clinically relevant *DPYD* variants: c.1905+1G>A, c.2846A>T, c.1679T>G, and c.1236G>A."	1 HTA, 1 NRS (prospective cohort design), and 1 EE showing that upfront testing minimizes toxicity and reduces costs for health care systems associated with treatment side effects.	NR
"Initial dose adjustments for fluoropyrimidine treatments[a] should be made according to the *DPYD* genotype identified, as part of an informed discussion with patients based on consideration of risks and benefits. During subsequent cycles, the dose should be re-adjusted according to the patient's tolerance to minimize toxicity and to optimize the treatment's effectiveness."	1 evidence-based guideline based on SR: 1 HTA, and 1 prospective clinical trial showing that individualized genotype-guided dosing reduces the risk of toxicity for patients.	NR

DPD = dihydropyrimidine dehydrogenase; EE = economic evaluation; HTA = health technology assessment; MA = meta-analysis; NR = not reported; NRS = nonrandomized study; SR = systematic review.

[a]Genotype-guided dosing recommendations for patients are adapted from the 2017 CPIC Guidelines and Supplementary Tables[17] which are subject to updates and modifications. Refer to Table 16.

Table 16: Summary of Genotype-Guided Dosing Recommendations in Included Guidelines

Genotype and phenotype of patient	Recommendations	Evidence supporting recommendations	Quality of evidence and strength of recommendations
DPWG (2019)[12]			
Carrier of 2 variants associated with fully	**5-FU/capecitabine** **Systemic:** "Avoid FU and capecitabine."	Relevant studies included NRS, SR with MA, narrative reviews, case reports, and	NR

Genotype and phenotype of patient	Recommendations	Evidence supporting recommendations	Quality of evidence and strength of recommendations
dysfunctional DPD activity GAS: 0	"If it is not possible to avoid FU and capecitabine: determine the residual DPD activity **in** mononuclear cells from peripheral blood and adjust the initial dose accordingly." **Cutaneous:** "Avoid FU"	in vitro studies showing the *DPYD* gene variants and possible effects on FP-based toxicity	
Carrier of 2 variants associated with reduced functionality of DPD activity ***or*** Carrier of 1 variant associated with reduced functionality of DPD activity and 1 variant associated with fully dysfunctional DPD activity GAS: PHENO[b]	**5-FU/capecitabine:** "Determine the residual DPD activity in mononuclear cells from peripheral blood and adjust the initial dose based on phenotype and genotype or avoid FU and capecitabine**."**	Relevant studies included NRS, SR with MA, narrative reviews, case reports, and in vitro studies showing the *DPYD* gene variants and possible effects on FP-based toxicity.	NR
Carrier of 1 variant associated with fully dysfunctional DPD activity GAS: 1	**5-FU/capecitabine:** "Start with 50% of the standard dose or avoid FU and capecitabine."	Relevant studies included NRS, SR with MA, narrative reviews, case reports, and in vitro studies showing the *DPYD* gene variants and possible effects on FP-based toxicity.	NR
Carrier of 1 variant associated with reduced functionality of DPD activity GAS: 1.5	**5-FU/capecitabine:** "Start with 50% of the standard dose or avoid FU and capecitabine."	Relevant studies included NRS, SR with MA, narrative reviews, case reports, and in vitro studies showing the *DPYD* gene variants and possible effects on FP-based toxicity.	NR
Carrier of no variants associated with either reduced functionality or fully dysfunctional DPD activity GAS: 2	Patients should receive a standard dose for 5-FU, capecitabine, and tegafur	Relevant studies included NRS, SR with MA, narrative reviews, case reports, and in vitro studies showing the *DPYD* gene variants and possible effects on FP-based toxicity	NR
CPIC (2017)[17]			
Patients carrying 2 no function alleles ***or*** an individual carrying 1 no function plus 1 decreased function allele	**For people with GAS 0.5:** Strongly recommended to avoid use of 5-FU-containing regimens. If no FP-free regimens are a suitable therapeutic option,	Relevant references were specified for each gene variant with references that support the major findings in the guideline Supplementary Materials. Study types	Strength of recommendation: strong.

Genotype and phenotype of patient	Recommendations	Evidence supporting recommendations	Quality of evidence and strength of recommendations
Complete DPD deficiency[a] GAS: 0 and 0.5	5-FU administered at a strongly reduced dose combined with early TDM (at the earliest time point) may be considered for patients. To estimate starting dose, a phenotyping test should be considered if available. If no phenotyping test is available, it is estimated that a dose reduction of at least 75% would be required. **For people with GAS 0:** Avoid use of 5-FU or 5-FU prodrug-based regimens.	included in vitro, ex vivo, and clinical.	
An individual carrying 1 normal function allele with 1 no function allele ***or*** 1 decreased function allele, ***OR*** an individual carrying 2 decreased function alleles. Decreased DPD activity[a] GAS: 1 or 1.5	"Reduce starting dose based on activity score followed by titration of dose based on toxicity or therapeutic drug monitoring (if available - increase the dose in patients experiencing no or clinically tolerable toxicity in the first two cycles to maintain efficacy or decrease the dose in patients who do not tolerate the starting dose to minimize toxicities" **For patients with GAS 1**: reduce starting dose by 50% **For patients with a GAS 1.5**: reduce starting dose by 25% to 50%.	Relevant references were specified for each gene variant with references that support the major findings in the guideline Supplementary Materials. Study types included in vitro ex vivo, and clinical.	Strength of recommendations for **GAS 1:** Strong and for **GAS 1.5:** moderate.
Patients carrying 2 normal functioning alleles Normal DPD activity and not at risk of severe FP toxicity GAS: 2	"Use label recommended dosage and administration."	Relevant references were specified for each gene variant with references that support the major findings in the guideline Supplementary Materials. Study types included in vitro ex vivo, and clinical.	Strength of recommendation: Strong.

CPIC = Clinical Pharmacogenetics Implementation Consortium; DPD = dihydropyrimidine dehydrogenase; DPWG = Dutch Pharmacogenetics Working Group; FU = fluorouracil; GAS = gene activity score; MA = meta-analysis; NRS = nonrandomized study; NR = not reported; SR = systematic review; TDM = therapeutic data monitoring.

[a]Patients are at risk of severe or even fatal drug toxicity when treated with FP drugs.

[b]DPD enzyme activity cannot be predicted correctly, an additional phenotyping test is required to determine the DPD enzyme activity.

Table 17: Clinical Validity Findings by Outcome — Severe (Grade ≥ 3) Toxicity

Citation	Details (e.g., evidence source, number of participants, variants evaluated)	Intervention (variant carrier or DPD deficient pt)	Comparator (wild-type or normal DPD activity pt)	Difference between groups
Genotyping				
***DPYD* variant carrier vs. the wild-type gene**				
Kim et al. (2022)[36]	Six observational studies, n = 6,119, variants evaluated: c.2194G > A only.	The c.2194G > A (rs1801160) variant is associated with an elevated risk of FP-related toxicity and is a good candidate for DPD deficiency screening before treatment with FPs.		
Overall toxicity	Six observational studies, n = 5,331	291/546 (53.3%)	1837/4785 (38.4%)	OR = 1.72, 95% CI 1.44 to 2.07, P < 0.001, I2 = 30%
Gastrointestinal toxicity	Three observational studies, n = 3,915	70/407 (17.2%)	480/3508 (13.7%)	OR = 1.22, 95% CI, 0.93 to 1.61, P = 0.15, I2 = 0%
Hematological toxicity	Three observational studies, n = 2,278	149/284 (52.5%)	683/1994 (34.3%)	OR = 2.37, 95% CI, 1.48 to 3.81, P = 0.0003, I2 = 59%
Neutropenia	Three observational studies, n = 3,919	152/411 (36.9%)	782/3508 (22.3%)	OR = 1.87, 95% CI, 1.49 to 2.34, P < 0.00001, I2 = 63%
Diarrhea	Three observational studies, n = 4,121	98/404 (24.3%)	748/3717 (20.1%)	OR = 1.43, 95% CI, 1.12 to 1.83, P = 0.004, I2 = 9%
Paulsen et al. (2022)[38]	Twelve observational studies, n = 8,328, variants evaluated: *DPYD**2A, *DPYD**13, c.2846A>T, c.1236G>A.	• *DPYD* variant carriers are at a higher risk of overall severe (grade ≥ 3) toxicity when treated with standard doses of FPs than patients with the wild-type gene. • Prevalence of severe (grade ≥ 3) toxicity in variant carriers treated with a standard dose ranged from 14% to 89%, and in patients with the wild-type gene with a standard dose ranged from 10% to 49%. • There is great heterogeneity in patient populations, treatment regimens, and reported toxicities among included studies. Therefore, a pooled analysis was not relevant.		
Ontario Health (2021)[44]				
Overall toxicity	Carriers of any of the 4 variants (*2A, *13, c.2846A>T, c.1236G>A), 7 observational studies, n = NR	NR	NR	RR = 2.63, 95% CI, 2.15 to 3.96
		• Six of 7 studies indicated a higher risk in *DPYD* carriers treated with a standard FP dose compared to patients with the wild-type gene. The point estimate of the RR in the 7th study was consistent with an increased risk in *DPYD* carriers, but the CI included the possibility of a lower risk. • This analysis included heterozygous carriers only and did not include homozygous or compound heterozygous carriers. • Heterozygous carriers of any of the 4 variants under assessment may have a higher risk of severe toxicity when treated with a standard FP dose, compared to patients with the wild-type gene treated with a standard dose.		

Citation	Details (e.g., evidence source, number of participants, variants evaluated)	Intervention (variant carrier or DPD deficient pt)	Comparator (wild-type or normal DPD activity pt)	Difference between groups
	*DPYD**2A carriers only, 16 observational studies, n = NR.	The RR from 15 of 16 studies indicated a higher risk in *DPYD**2A carriers compared to patients with the wild-type gene; however, in 8 studies the CIs also included the possibility of a lower risk in *DPYD**2A carriers.		
	*DPYD**13 carriers only, 7 observational studies, n = NR.	• The RR in 5 studies ranged from 1.01 to 4.64, but the CIs in 2 studies included the possibility of lower risk in *DPYD**13 carriers. • RRs could not be calculated for 2 studies in which *DPYD**13 carriers did not experience severe toxicity.		
	c.2846A>T carriers only, 13 observational studies, n = NR.	• The RR from 12 of 13 studies ranged from 1.02 to 21.64, indicating a higher risk in c.2846A>T carriers vs. patients with the wild-type gene; however, the CIs of 4 studies also included the possibility of no difference between groups or a lower risk in c.2846A>T carriers. • The RR could not be calculated for 1 study in which c.2846A>T carriers did not experience severe toxicity.		
	c.1236G>A carriers only, 6 observational studies, n = NR.	• One study reported a higher risk of overall severe toxicity in carriers vs. wild-type patients (RR 5.12, 95% CI, 2.54 to 9.87) • The point estimates of the RR were closer to 1 and the results were imprecise in 5 studies: 1.25 (0.91 to 1.61), 1.82 (0.47 to 5.21), 1.09 (0.91 to 1.19), 0.83 (0.37 to 1.63), and 1.10 (0.67 to 1.62).		
Neutropenia	Carriers of any of the 4 variants (*2A, *13, c.2846A>T, c.1236G>A), 2 observational studies, n = NR	NR	NR	RR = 4.42; 95% CI, 1.59 to 9.18
	*DPYD**2A carriers only, 9 observational studies, n = NR.	Point estimates of RRs indicated a higher risk in *DPYD**2A carriers compared to patients with the wild-type gene in all studies; however, the CIs of 3 studies also included the possibility of a lower risk in *DPYD**2A carriers.		
	*DPYD**13 carriers only, 2 observational studies, n = NR.	• In 1 study, 1 (25.0%) *DPYD**13 carrier who received a standard FP dose and 561 (36.4%) patients with the wild-type gene also treated with a standard dose developed severe neutropenia (RR 0.73, 95% CI, 0.02 to 2.10). • In another study, no *DPYD**13 carriers had severe neutropenia, but 8 (1.4%) wild-type patients did (P = 1.00).		
	c.2846A>T carriers only, 5 observational studies, n = NR.	In 4 of 5 studies, the RRs indicated a higher risk of neutropenia in variant carriers; however, the CIs of 2 studies also included the possibility of a lower risk in carriers.		
	c.1236G>A carriers only, 1 observational study, n = NR	17 (22.1%)	184 (9.8%)	RR = 2.26; 95% CI, 1.38 to 3.40
Diarrhea	Carriers of any of the 4 variants (*2A, *13, c.2846A>T, c.1236G>A), 2 observational studies, n = NR	RRs from 2 studies suggested a higher risk of diarrhea in carriers of any of the 4 variants compared to the wild-type gene (RR = 2.35, 95% CI, 0.94 to 4.81; RR = 6.09, 95% CI, 2.37 to 12.66)		

Citation	Details (e.g., evidence source, number of participants, variants evaluated)	Intervention (variant carrier or DPD deficient pt)	Comparator (wild-type or normal DPD activity pt)	Difference between groups
	*DPYD**2A carriers only, 11 observational studies, n = NR	RRs from 9 studies indicated an increased risk in *DPYD**2A carriers compared to patients with the wild-type gene; however, CIs of 3 studies also included the possibility of a lower risk in *DPYD**2A carriers		
	*DPYD**13 carriers only, 3 observational studies, n = NR	2 (50%)	190 (12.3%)	RR 4.07, 95% CI, 0.62 to 7.71
		1 (100%)	18 (22%)	RR 4.55, 95% CI, 1.72 to 6.32
		0 (0%)	34 (5.8%)	P = 1.00
	c.2846A>T carriers only, 6 observational studies, n = NR	In all 6 studies, RRs indicated a higher risk in c.2846A>T carriers compared to the wild-type gene; however, the CIs of 2 studies also included the possibility of a lower risk in carriers		
	c.1236G>A carriers only, 2 observational studies, n = NR	11 (14.3%)	234 (12.5%)	RR = 1.14; 95% CI, 0.61 to 1.92
		14 (50%)	125 (23.1%)	RR = 2.16; 95% CI, 1.35 to 3.34
Hand-foot syndrome[a]	Carriers of any of the 4 variants (*2A, *13, c.2846A>T, c.1236G>A), 1 observational study, n = NR	0/34 (0%)	24/771 (3.1%)	P = 0.62
	*DPYD**2A carriers only, 3 observational studies, n = NR.	• In 1 study, severe HFS occurred in 3 (42.9%) *DPYD**2A carriers treated with a standard FP dose and 242 (43.2%) wild-type patients treated with a standard dose (RR = 0.99; 95% CI, 0.25 to 1.82). • In another study, 1 carrier developed severe HFS (100.0%) compared to 5 (4.8%) patients with the wild-type gene (RR = 20.83; 95% CI, 5.55 to 35.60). • In a third study, none of the patients, *DPYD**2A carriers or wild-type, experienced severe HFS.		
	c.2846A>T carriers only, 1 observational study, n = NR	4 (50%)	241 (43.1%)	RR = 1.16; 95% CI, 0.40 to 1.91
	c.1236G>A carriers only, 1 observational study, n = NR	26 (92.9%)	459 (85.0%)	RR = 1.09; 95% CI, 0.91 to 1.95
Cura et al. (2023)[37]	6 observational studies, n = 1,853, variants evaluated: any	• This review did not identify any studies reporting significant associations between the 4 primary *DPYD* variants (*2A, *13, c.2846A>T, or c.1129 to 5923C>G and c.1236G>A (HapB3)) and capecitabine-related toxicity. • The authors report a noticeable increase in research examining *DPYD* variants beyond the 4 currently recognized as clinically relevant. • Three studies reported an increased risk of toxicity in c.1601G > A (rs1801160) variant carriers treated with capecitabine compared to patients with wild-type patients. Two of these studies also found a significant association between the c.85T > C (rs2297595) variant and severe adverse events.		

Citation	Details (e.g., evidence source, number of participants, variants evaluated)	Intervention (variant carrier or DPD deficient pt)	Comparator (wild-type or normal DPD activity pt)	Difference between groups
		• Findings on the c.496A > G (rs1801265) variant were inconsistent: 1 study reported a significant association with capecitabine toxicity, while 2 others did not. • One study identified a significantly higher risk of severe toxicity in the c.483 + 18G > A (rs17376848) variant carriers, but this finding was not supported by 2 other studies with larger sample sizes.		
Phenotyping				
Reduced DPD activity ([U] > 16ng/mL) vs. normal DPD activity ([U] < 16 ng/mL)				
Paulsen et al. (2022)[38]	Seven observational studies, n = 2,818, phenotype tests: plasma [U], and U/UH_2 or UH_2/U ratios	• Data regarding the correlation between U concentration or UH_2/U ratio and severe FP-related toxicity is contradictory. • One study found that U concentration was superior to UH_2/U in predicting toxicity, while another found that median pretreatment U levels were comparable in patients with and without grade ≥ 3 toxicity. • DPD phenotyping provides an approach to identify patients with rare *DPYD* variants (that might not be tested for in genotyping), as well as other possible causes of low DPD activity. However, the measurement is highly sensitive to preanalytical conditions. • "Evidence supporting the current threshold values of [U] proposed by EMA is sparse. There is a clear need for proper validation in adequately powered prospective clinical trials." (p. 338).[38,]		
DPD activity < 70% (as measured in PBMCs) vs. Normal DPD activity				
Doornhof et al. (2023)[9]	Retrospective cohort; 481 participants			
	Cardiovascular toxicity	NR	NR	OR = 2.090; 95% CI, 1.067 to 4.092; P = 0.032
	Gastrointestinal toxicity	NR	NR	OR = 2.917; 95% CI, 1.459 to 5.832; P = 0.002
	Neurologic toxicity	NR	NR	OR = 2.249; 95% CI, 1.135 to 4.459; P = 0.020
	Grade ≥ 3 hematological toxicity	NR	NR	OR = 0.939; 95% CI, 0.276 to 3.189; P = 0.919
	Grade ≥ 3 other toxicities	NR	NR	OR = 3.166; 95% CI, 1.244 to 8.057; P = 0.016
DPD activity < 50% (as measured in PBMCs) vs. Normal DPD activity				
Doornhof et al. (2023)[9]	Retrospective cohort; 481 participants			
	Cardiovascular toxicity	NR	NR	OR = 1.320; 95% CI, 0.390 to 4.463; P = 0.655
	Gastrointestinal toxicity	NR	NR	OR = 1.623; 95% CI, 0.516 to 5.099; P = 0.407

Citation	Details (e.g., evidence source, number of participants, variants evaluated)	Intervention (variant carrier or DPD deficient pt)	Comparator (wild-type or normal DPD activity pt)	Difference between groups
	Neurologic toxicity	NR	NR	OR = 1.383; 95% CI, 0.362 to 5.282; P = 0.636
	Grade ≥ 3 hematological toxicity	NR	NR	OR = 5.252; 95% CI, 1.124 to 24.543; P = 0.035
	Grade ≥ 3 other toxicities	NR	NR	OR = 2.223; 95% CI, 0.412 to 11.982; P = 0.353

CI = confidence interval; EMA = European Medicines Agency; HFS = hand and foot syndrome; NA = not applicable; NR = not reported; OR = odds ratio; RR = risk ratio; U = uracil; U/UH_2 = uracil to dihydrouracil.

[a]Also known as palmar-plantar erythrodysesthesia.

Table 18: Clinical Validity Findings by Outcome — Sensitivity, Specificity, PPV, NPV of *DPYD* Genotyping (3 to 4 variants) to Detect Severe Toxicity

Citation	Details	Sensitivity (%), median (min-max)	Specificity (%), median (min-max)	PPV (%), median (min-max)	NPV (%), median (min-max)
Ontario Health (2021)[44]	9 observational studies[a], n = NR	8.1 (3.5 to 21.6)	98.6 (95.0 to 100.0)	61.1 (13.0 to 100.0)	84.5 (50.5 to 91.5)
		• *DPYD* genotyping has a high clinical specificity (to detect severe toxicity), but a low clinical sensitivity, as many patients with the wild-type gene also experienced severe toxicity. • Other factors may contribute to severe toxicity including other unmeasured *DPYD* variants, and baseline characteristics (e.g., age, sex, cancer type).			

CI = confidence interval; NA = not applicable; NR = not reported; NPV = negative predictive value; PPV = positive predictive value.

Note: Severe FP-related toxicity was used as the reference standard to calculate sensitivity, specificity, PPV, and NPV (i.e., if toxicity occurred in a *DPYD* variant carrier, this was considered a true positive; if toxicity occurred in a patient with the wild-type gene, this was considered a false-negative).

[a]1 study calculated and reported sensitivity, specificity, PPV, and NPV values. For the remaining 8 studies, outcomes were calculated by the Ontario Health (2021)[44] authors for each included study based on data presented within the study. Results are summarized here using median and min-max.

Table 19: Clinical Validity Findings by Outcome — Other Patient-Related Outcomes

Citation	Evidence source, number of participants	Intervention (*DPYD* variant carrier)	Comparator (wild-type)	Difference between groups
FP-Related Mortality				
De Moraes et al. (2024)[35]	4 RCTs, 9 observational studies, n = 7,274, variants evaluated: *DPYD**2A, *DPYD**13, c.2846A>T, HapB3 (c.1236G>A and c.1129 to 5923C>G)	13/322 (4.0%)	14/6952 (0.2%)	OR = 34.86; 95% CI, 13.96 to 87.05; P < 0.000001; I2 = 2%
		the *DPYD**2A variant was the most prevalent among fatalities, followed by *DPYD**13 and c.1129 to 5923C>G and c.1236G>A (HapB3).		

Citation	Evidence source, number of participants	Intervention (*DPYD* variant carrier)	Comparator (wild-type)	Difference between groups
Ontario Health (2021)[44]	9 observational studies, n = NR, carriers of any of the 4 variants: *DPYD**2A, *DPYD**13, c.2846A>T, c.1236G>A	• Two studies that included *DPYD**2A carriers found a higher risk in carriers compared to patients with the wild-type gene (RR = 50.00; 95% CI, 6.21 to 74.53 and RR = 52.63; 95% CI, 10.40 to 120.90). • One study reported a death in the only homozygous carrier identified but did not report on deaths in patients with the wild-type gene. • Three of the 9 studies did not report on mortality in the patients with the wild-type gene.		
FP-Related Hospitalization				
Ontario Health (2021)[44]	5 observational studies, n = NR, carriers of any of the 4 variants: *DPYD**2A, *DPYD**13, c.2846A>T, c.1236G>A	• Three studies found a higher risk of hospitalization in *DPYD* variant carriers compared to patients with the wild-type gene. ◦ Lunenburg 2018, n = 805, RR = 2.26 (95% CI, 0.69 to 5.14) ◦ Loganayagam 2013, n = 430, RR = 4.46 (95% CI, 3.26 to 5.29) ◦ Boisdron-Celle 2007, n = 243, RR = 58.82 (95% CI, 15.19 to 168.60) • Two studies reported frequency of hospitalizations in variants carriers but not patients with the wild-type gene.		

CI = confidence interval; NR = not reported; OR = odds ratio.

Table 20: Clinical Utility Findings by Outcome — Severe (Grade ≥ 3) Toxicity

Citation	Evidence source, number of participants	Intervention	Comparator	Difference between groups
GENOTYPING				
***DPYD*-guided dose in variant carriers vs. usual care in variant carriers**				
Paulsen et al. (2022)[38]	One prospective/retrospective cohort, n = 828, variants evaluated: *DPYD**2A, *DPYD**13, c.2846A>T, c.1236G>A.	Only 1 study compared *DPYD* variants carriers who received a standard dose to *DPYD* variant carriers who received a reduced dose. Toxicity rates in each group were 21% (8/34) and 22% (5/22), respectively.		
Ontario Health (2021)[44]	**Variants evaluated:** • *DPYD**2A • *DPYD**13 • c.2846A>T • c.1236G>A.	Only 1 study directly compared *DPYD* carriers treated with a reduced FP dose to *DPYD* carriers treated with a standard dose. However, due to imprecision in the study results and imbalances in the distribution of *DPYD* variants between the groups, it remains uncertain whether genotype-guided dose reduction in heterozygous carriers effectively reduces the risk of severe toxicity or toxicity-related hospitalization.		
Paulsen et al. (2023)[42]	Prospective cohort vs. historical control, n = 722	5/22 (23%)	12/42 (29%)	RR = 0.80; 95% CI, 0.32 to 1.97
***DPYD*-guided dose (all patients) vs. usual care (all patients)**				
Glewis et al. (2022)[20]	17 observational studies	• "This systematic review and meta-analysis support the hypothesis that PGD improves patient outcomes in terms of grade 3/4 toxicity and in particular overall toxicity and diarrhea without impacting on overall response and treatment-related death. Future research is needed to focus on other ethnic populations, with consistency of dose reduction in the *DPYD* variant allele carrier and reporting outcomes adjusted based on confounders." (p.135)[20]		

Citation	Evidence source, number of participants	Intervention	Comparator	Difference between groups
		• Overall, the results support the hypothesis that PGD improves patient outcomes compared to non-PGD, (reduced grade ≥ 3 overall toxicity and grade ≥ 3 diarrhea) without impacting treatment response or treatment-related death.		
Overall toxicity	5 observational studies, n = 4,271, PGD vs. non-PGD	871/4091 (21.3%)	121/180 (67.2%)	RR = 0.32, 95% CI, 0.27 to 0.39, P < 0.00001, I2 = 32%
Diarrhea	6 observational studies, n = 2,163, PGD vs. non-PGD	67/1611 (4.2%)	43/552 (7.8%)	RR = 0.38; 95% CI, 0.24 to 0.61; P < 0.0001; I2 = 0%
Paulsen et al. (2023)[42]	Prospective cohort vs. historical control, n = 722	63/230 (27%)	112/492 (23%)	RR = 1.20; 95% CI, 0.92 to 1.57
***DPYD*-guided dose in variant carriers vs. usual care in pts with the wild-type gene**				
Paulsen et al. (2022)[38]	Two observational studies, n = 2,538, variants evaluated: *DPYD**2A, *DPYD**13, c.2846A>T, c.1236G>A.	• One study found that *DPYD* variants carriers that receive reduced FP (22%) doses did not have comparable toxicity rates to patients with the wild-type gene who received standard doses (14%). • Another study found that overall toxicity in *DPYD* variant carriers who received a reduced dose was still higher (39%) than patients the wild-type gene who received a standard dose (23%). • The third study found that the incidence of overall grade ≥ 3 toxicity was 21.1% in patients with the wild-type gene (n = 1,347) and 13% (n = 47) in variant carriers who received a dose reduction. However, post-hoc genotyping revealed that 41 of the original wild-type patients were carriers of the HapB3 variant. This groups had an overall grade ≥ 3 toxicity frequency of 24%.		
Ontario Health (2021)[44]	Six observational studies, n = NR, variants evaluated: *DPYD**2A, *DPYD**13, c.2846A>T, c.1236G>A.	Due to the design of the included studies, the authors were unable to determine whether reducing the treatment dose in *DPYD* variant carriers results in a risk of severe toxicity that is comparable to or lower than that observed in patients with the wild-type gene who are receiving a standard dose.		
Phenotyping				
UH_2/U ratio				
Paulsen et al. (2022)[38]	One prospective study, n = 218	One study found that the incidence of severe toxicity was 13% in patients who received a standard dose and 11% in patients who received a reduced dose based on their UH_2/U ratio.		
DPD-guided dose (U = 16 to 150ng/mL) vs. standard dose in normal DPD activity (U < 16ng/mL) pts.				
Tejedor-Tejeda et al. (2024)[40]	Retrospective cohort, n = 119[a]	12/27 (44%)	43/92 (46%)[b]	NR
Uracil-based dosing **DPD-guided dose (all patients) vs. usual care (all patients)**				

Citation	Evidence source, number of participants	Intervention	Comparator	Difference between groups
Laures et al. (2022)[41]	Retrospective cohort vs. historical controls, n = 292			
	Treatment Cycle 1	5.6%	8.5%	NR
	Treatment Cycle 2	4.2%	9.8%	NR
	Treatment Cycle 3	4.3%	9.8%	NR
	Treatment Cycle 4	3.4%	4.4%	NR
Combination genotyping and phenotyping guided dosing				
Paulsen et al. (2022)[38]	One prospective study, n = 1,116, phenotype tests: [U] and [UH_2] measurements, variants evaluated: *DPYD**2A, *DPYD**13, c.2846A>T.	• One study used a multiparametric approach including genotyping, [U], and [UH_2] measurements to determine initial dose reductions. The incidence of grade ≥ 3 toxicity was 10.8% (n = 718) in the reduced dose patients and 17.6% (n = 398) in the standard dose patients. • "The widely used cut-off value for [U]-based phenotyping appears insufficiently substantiated. There is still an unmet need for prospective evidence connecting pre-treatment testing, dose adjustments and clinical outcomes." (p. 340).[38]		
Genotype and phenotype-guided (PBMCs) dose vs. standard dose				
Ockeloen et al. (2023)[43]	Retrospective cohort, n = 228			
	DPYD$^{variant\_no}$-DPD^{normal_activity}	7/34 (21%)	45/148 (30%)	NR
	DPYD$^{variant\_yes}$-DPD^{normal_activity}	2/10 (20%)	2/6 (33%)	NR
	DPYD$^{variant\_no}$-DPD^{low_activity}	3/11 (27%)	2/13 (15%)	NR
	DPYD$^{variant\_yes}$-DPD^{low_activity}	2/5 (40%)	1/1 (100%)	NR
	DPYD$^{variant\_yes}$	4/15 (27%)	3/7 (43%)	NR
	DPD$^{low\_activity}$	5/16 (31%)	3/14 (21%)	NR
	All patients	14/60 (23%)	50/168 (30%)	NR

NA = not applicable; NR = not reported.; PGD = pharmacogenetic-guided dosing; RR = relative risk.

[a]These results refer to the incidence of toxicity of any grade (1 to 4).

[b]Twenty-six (28%) of these patients received an initial FP dose reduction based on factors other than DPD activity (i.e., fragile baseline condition).

Table 21: Clinical Utility Findings by Outcome — Other Patient-Related Outcomes

Citation	Evidence source, number of participants	Intervention	Comparator	Difference between groups
FP-related mortality				
***DPYD*-guided dose in variant carriers vs. usual care in variant carriers**				
Paulsen et al. (2023)[42]	Prospective cohort vs. historical control, n = 722	0/22 (0%)	2/42 (4.8%)	RR = 0.37, 95% CI, 0.02 to 7.46
***DPYD*-guided dose cohort (all patients) vs. usual care (all patients)**				
Paulsen et al. (2023)[42]	Prospective cohort vs. historical control. n = 722	1/230 (0.4%)	6/492 (1.2%)	RR = 0.36, 95% CI, 0.04 to 2.94

Citation	Evidence source, number of participants	Intervention	Comparator	Difference between groups
Ontario Health (2021)[44]	—	• "The only fluoropyrimidine-related death reported in *DPYD* carriers who were treated with a reduced dose occurred after the patient had been wrongly prescribed a standard fluoropyrimidine dose for two cycles. Therefore, we could not determine what the outcome would have been if the patient had received a reduced dose. In wild-type patients, one study reported 2 (0.1%) deaths, a second study reported three (0.3%) deaths, and a third study reported 10 (0.7%) deaths" (p.52)[44] • No homozygous or compound heterozygous *DPYD* carriers were included in the study populations, likely due to their very low prevalence. These individuals typically have markedly reduced or absent DPD activity and are therefore at high risk for severe FP-related toxicity, which highlights the importance of *DPYD* genotyping for identifying these high-risk patients.		
FP-related hospitalizations				
***DPYD*-guided dose in variant carriers vs. usual care in variant carriers**				
Paulsen et al. (2023)[42]	Prospective cohort vs. historical control, n = 722	0/22 (0%)	8/42 (19%)	RR = 0.11; 95% CI, 0.01 to 1.82
***DPYD*-guided dose cohort (all patients) vs. usual care (all patients)**				
Paulsen et al. (2023)[42]	Prospective cohort vs. historical control, n = 722	23/230 (10%)	40/492 (8.1%)	RR = 1.23; 95% CI, 0.75 to 2.00
Ontario Health (2021)[44]	—	Hospital length of stay was shorter among *DPYD* carriers who received a reduced dose compared to those treated with a standard dose, but the evidence was uncertain.		
***DPYD*-guided dose in variant carriers vs. usual care in the wild-type gene**				
Glewis et al. (2022)[20]	4 observational studies, n = 3,727	28/158 (17.7%)	399/3569 (11.2%)	RR = 1.49, 95% CI, 1.05 to 2.12, P = 0.03, I^2 = 0%.
Ontario Health (2021)[44]	—	• The point estimates of 4 studies indicated a higher risk of treatment-related hospitalization; however, the confidence intervals also included the possibility of lower risk in *DPYD* carriers • Based on 3 studies, the median number of days in hospital ranged from 4 to 6.5 in *DPYD* carriers and 5 to 13 in patients with the wild-type gene.		
Complete and partial disease response				
***DPYD*-guided dose in variant carriers vs. usual care in the wild-type gene**				
Glewis et al. (2022)[20]	3 observational studies, n = 351	28/70 (40.0%)	106/281 (37.7%)	RR = 1.31, 95% CI, 0.93 to 1.85, P = 0.12, I2 = 0%.
Stable disease				
***DPYD*-guided dose in variant carriers vs. usual care in the wild-type gene**				
Glewis et al. (2022)[20]	2 observational studies, n = 277	8/33 (24.2%)	26/244 (10.7%)	RR = 1.27, 95% CI, 0.66 to 2.44, P = 0.47, I2 = 0%.

NA = not applicable; NR = not reported; RR = risk ratio, CI = confidence interval.

Table 22: Summary of Findings of Included Economic Evaluations

Main study findings	Authors' conclusion
Ontario Health HTA (2021)[44]	
Reference Case Analysis Results The authors conducted a probabilistic analysis to capture parameter uncertainty. When possible, they specified distributions around input parameters using the mean and standard error. A total of 5,000 simulations were run and calculated the expected values of costs and outcomes for each strategy. The average total cost for the *DPYD* genotyping strategy was estimated as $1,920.82 (95% CrI: $1,308.71 to $2,743.56) and $2,065.70 (95% CrI: $1,340.67 to $3,060.75) for usual care. Difference, per patient, between *DPYD* Genotyping and Usual Care (No testing), Mean (95% CrI): • Total costs: - $144.88 (−$543.10 to $101.91) • Proportion of patients with severe toxicities: −0.22% (−1.63% to 1.37%) • Number of severe toxicities: −0.02 (−0.05 to 0.02) • QALYs: 0.0011 (0.0003 to 0.0023) • ICER ($/QALY): Dominant (less costly and more effective) At the willingness-to-pay values of $50,000 and $100,000 per QALY, *DPYD* genotyping is highly likely to be cost-effective (91% and 96% probability, respectively). **Scenario Analyses Results** The authors examined additional structural and parameter uncertainty by conducting several scenario analyses. The modelled inputs included changes to the prevalence of *DPYD* intermediate and poor metabolizers, the source of effectiveness and resource use estimates, the probability of treatment-related hospitalization, days of hospitalization, impact of severe toxicities on quality of life, alternative chemotherapy for poor metabolizers, cost of an extra physician visits, and cost of *DPYD* genotyping test. *DPYD* genotyping remained cost-saving and slightly more effective (greater QALYs) in all scenarios.	*"DPYD* genotyping may be slightly more effective and less costly compared to usual care (no testing) because fewer patients would have severe fluoropyrimidine-related toxicity. At the commonly used willingness-to-pay values of $50,000 and $100,000 per QALY gained, *DPYD* genotyping is likely cost-effective compared to usual care (91% and 96% probability, respectively)." (p. 88)[44]

CrI = Credible interval; ICER = incremental cost-effectiveness ratio; QALY = quality-adjusted life-year.

Table 23: Summary of Excluded Economic Evaluations

Study citation, setting, year cost	Population	Analytic technique, study design, perspective, time horizon	Intervention and comparators	Outcomes	Summary of outcomes
Ontario Health (2021)[44] Ontario, Canada **2020 CA$**	adults who had planned FP-based anticancer treatment	• Budget Impact Analysis • Reference case analysis and sensitivity analyses • Ontario Ministry of Health perspective • 5-year time horizon	**Intervention**: Pretreatment *DPYD* testing **Comparator:** Usual care (No *DPYD* testing and standard dose of FPs)	Costs	We estimated that publicly funding pretreatment *DPYD* genotype testing may be cost-saving (a total of $714,963 saved over the next 5 years, provided that the implementation, service delivery, and program coordination costs do not exceed our estimated amounts). The cost of testing would be about $834,527 over the next 5 years.
Koleva-Kolarova et al. (2023)[45] UK **2020/21 GBP**	FP-based chemotherapy in women with metastatic breast cancer	• Cost-utility analysis • Decision-tree model followed by Markov model • Public health care payer perspective • Lifetime horizon (cycle length of 2 months)	**Intervention:** Pretreatment 'ToxNav' panel (that includes 18 *DPYD* genes and 1 other ENOSF1) followed by test-guided dose adjustments **Comparator:** No testing and standard dosing/standard of care	Costs QALY ICER	ToxNav was dominant over standard of care, producing 0.19 additional quality-adjusted life-years and savings of £78,000 per patient over a lifetime. The probabilistic sensitivity analysis showed □97% probability of the ToxNav strategy to be dominant.
Fragoulakis et al. (2023)[46] Italy **EUR (Year NR)**	Patients receiving capecitabine, 5-FU, or irinotecan for diagnosed colorectal cancer	• Trial-based model (GLM and imputation of missing data) • Health care payer perspective • Time horizon not specified	**Intervention:** Prospective genotyping (for 4 main *DPYD* variants + 3 variants in UGT1A1, followed by test-guided dosing (using DPWG guidelines) **Comparator**: No testing (standard doses)	Costs QALY	The total cost of the study arm was estimated at €380 (approximately US$416; 95% CI, 195 to 596) compared to €565 (approximately US$655; 95% CI, 340 to 724) of control arm. The mean survival in study arm was estimated at 1.58 (+ 0.25) LYs vs. 1.50 (

Study citation, setting, year cost	Population	Analytic technique, study design, perspective, time horizon	Intervention and comparators	Outcomes	Summary of outcomes
					+ 0.26) (Log Rank test, X2 = 4.219, df = 1, P value = 0.04). No statistically significant difference was found in QALYs. ICER was estimated at €13,418 (approximately US$14,695) per QALY, while the acceptability curve indicated that when the willingness-to-pay was under €5,000 (approximately US$5,476), the probability of PGx being cost-effective overcame 70%.
Brooks et al. (2022)[48] US **2020 US$**	Patients with stage III colon cancer and planned treatment using fluorouracil or capecitabine	• Cost-effectiveness analysis • Markov model • Health care payer perspective • 5-year time horizon	**Intervention:** Upfront *DPYD* testing for 4 main variants with subsequent dose adjustments **Comparator**: No DPD testing and standard dose	costs/QALY Proportion of people experiencing severe toxicity (grade ≥ 3) • Hospitalization costs • Cost of test • Six-month interval Probability of death Following adjuvant chemotherapy for stage III colon cancer	Pretreatment *DPYD* genotyping was cost-effective in 96% of iterations.
Deenen et al. (2016)[49] Netherlands **2014** £	Cancer patients intended to undergo FP treatment	• Cost-minimization analysis • Decision-tree model • Health care payer perspective • Time horizon not specified	**Intervention:** Pretreatment *DPYD* genotyping for 1 variant (*DPYD**2A) **Comparator:** Usual care (no testing)	Health: Proportion of people experiencing severe toxicity (grade ≥ 3) Cost: Total cost per patient	Pretreatment *DPYD* genotyping was slightly more effective and less costly.

Study citation, setting, year cost	Population	Analytic technique, study design, perspective, time horizon	Intervention and comparators	Outcomes	Summary of outcomes
Henricks et al. (2019)[50] Netherlands **2019** £	Cancer patients intended to undergo FP treatment	• Cost-minimization analysis • Trial-based model • Decision analytical model • Health care payer perspective • Time horizon not specified	**Intervention**: Pretreatment *DPYD* genotyping for 4 variants (*DPYD**2A, c.2846A>T, c.1679T>G, and c.1236G>A) **Comparator:** Usual care (no testing)	Health: Proportion of people experiencing severe toxicity (grade ≥ 3) Cost: Total cost per patient	Upfront *DPYD*-guided dose individualization, improving patient safety, is cost-saving, or cost-neutral, but is not expected to yield additional costs.
Fragoulakis et al. (2019)[47] Italy **2018** £	Cancer patients treated with FP-based chemotherapy	Trial-based model (GLM) Third-party payer (sickness funds) Time horizon not specified	**Intervention:** Retrospective *DPYD* testing for the 4 main variants (no guided dosing). *DPYD* extensive metabolizers (i.e., wild-type gene) [Group A] **Comparator:** *DPYD* intermediate or poor metabolizers (i.e., variant carriers) [Group B]	Clinical benefit expressed as quality-adjusted life-years (QALYs) per genotype group, direct costs	Findings suggest that *DPYD*-guided FPs treatment represent a cost-saving choice for individuals having cancer in the Italian health care setting.
Murphy et al. (2018)[51] Ireland **2012 €**	Patients commencing FP chemotherapy for colorectal cancer	Cost-benefit analysis Decision-tree model Private hospital payer perspective Time horizon not specified	**Intervention:** Prospective *DPYD* testing for 4 variants (c.1905 + 1G > A, c.2846A>T, c.1679T>G, and c.1601G > A) 2A, 4, 13, 2846A > T) with NO dosing adjustments (assumed reduction in AEs for those with variants) **Comparator:** Reactive *DPYD* testing	Costs associated with the index admission only	Toxicity costs for *DPYD* carriers totalled €232,061, vs. €23,718 for upfront cohort testing, with a benefit of approximately €120,000.
Cortejoso et al. (2016)[52] Spain **€ (year NR)**	Cancer patients treated with FPs	Cost-effectiveness analysis Trial-based model Health care payer perspective Time Horizon not specified	**Intervention:** *DPYD* genotyping for 3 variants (*2A, *13, and 2846A > T) **Comparator:** Cost of treating severe FP-induced neutropenia	Costs of *DPYD* genotyping vs. costs of treating severe Neutropenia Cases of neutropenia	We demonstrated that real-time *DPYD* genotyping using TaqMan probes is cost-effective in all FP-based treatments.

Study citation, setting, year cost	Population	Analytic technique, study design, perspective, time horizon	Intervention and comparators	Outcomes	Summary of outcomes
				prevented/1000 patients treated	
Abushanab et al. (2025)[53] Qatar **2023 to 2024 £**	Patients with local or metastatic breast cancer undergoing FP-based treatments	Cost-utility analysis Two stage decision analysis (6 months horizon) + Markov model (lifetime horizon) Public health care payer perspective	**Intervention:** *DPYD* genetic testing and personalized dosing of capecitabine/5-fluorouracil (5-FU) dosing (which variants tested was not specified) **Comparator:** SOC (No testing and standard doses)	Short-term: cost/ success (survival without grade 3/4 toxicity at 6 months) Long-term: cost/QALY	*DPYD* genetic testing for breast cancer is cost-saving and cost-effective.

CA$ = Canadian Dollar; £ = euro; £= British Pound; GLM = generalized linear model; NR = not reported; US$ = US Dollar; QAR = Qatari Riyal; QALY = quality-adjusted life-year; SOC = standard of care.

Appendix 6: Overlap Between Included Systematic Reviews

Please note that this appendix has not been copy-edited.

Table 24: Overlap in Relevant Primary Studies Between Included Systematic Reviews

Primary study citation	Cura et al. (2023)	Kim et al. (2022)	Ontario Health HTA (2021)	De Moraes et al. (2024)	Glewis et al. (2022)	Paulsen et al. (2022)
Rosmarin D, et al. Gut. 2015;64(1):111 to 120.	Yes	No	No	No	No	No
Falvella FS, et al. Br. J. Clin. Pharmacol. 2015;80(3):581 to 588.	Yes	No	No	Yes	No	No
Pellicer M, et al. Pharmacol.Res. 2017;120:133 to 137	Yes	Yes	No	No	No	No
Pellicer M, et al. Pharmacogenomics. 2017;18(3):1215 to 1223.	Yes	No	No	No	No	No
Varma A, et al. Asian Pac J Cancer Prev. 2019;20(10):3093 to 3100.	Yes	No	No	No	No	No
Puerta-Garcia E, et al. Surg Oncol. 2020;35:388 to 398.	Yes	No	No	No	No	No
Deenen MJ, et al. Clin Cancer Res. 2011;17(11):3455 to 68	No	Yes	Yes	Yes	No	Yes
Lee AM, et al. J Natl Cancer Inst. 2014;106(12):1 to 12.	No	No	Yes	Yes	No	No
Rosmarin D, et al. J Clin Oncol. 2014; 32:1031 to 39.	No	No	No	Yes	No	No
Froehlich TK, et al. Int J Cancer. 2015;136(3):730 to 39.	No	No	Yes	Yes	No	Yes
Jennings BA, PLoS One. 2013;8(10):e78053.	No	No	Yes	Yes	No	No
Loganayagam A, et al. Br J Cancer. 2013;108(12):2505 to 15.	No	No	Yes	Yes	No	No
Morel A, et al. Mol Cancer Therap. 2006;5(11):2895 to 904.	No	No	No	Yes	No	No
Meulendijks D, et al. Int J Cancer. 2015;138(1):245 to 53.	No	No	Yes	No	No	No
Kleibl Z, et al. Neoplasma. 2009;56:303 to 316.	No	Yes	No	No	No	No
Boige V, et al. JAMA Oncol. 2016;2(5):655 to 662.	No	Yes	Yes	Yes	No	Yes
Madi A, et al. Eur J Cancer. 2018;102:31 to 39.	No	Yes	No	No	No	No

Primary study citation	Cura et al. (2023)	Kim et al. (2022)	Ontario Health HTA (2021)	De Moraes et al. (2024)	Glewis et al. (2022)	Paulsen et al. (2022)
Iachetta F, et al. Br J Cancer. 2019;120(8):834 to 39.	No	Yes	Yes	No	No	No
Henricks LM, et al. Int J Cancer. 2019;144(9):2347 to 54.	No	No	Yes	No	Yes	No
Kleinjan JP, et al. Anticancer Drugs. 2019;30(4):410 to 5.	No	No	Yes	No	Yes	No
Lee AM, et al. Pharmacogenet Genomics. 2016;26(3):133 to 7.	No	No	Yes	No	No	No
Lunenberg C, et al. Eur J Cancer. 2018;104:210 to 8.	No	No	Yes	No	Yes	Yes
Toffoli G, et al. Int J Cancer. 2015;137(12):2971 to 80.	No	No	Yes	Yes	No	No
Stavraka C, et al. Breast Cancer Res Treat. 2019;175(2):511 to 7.	No	No	Yes	No	Yes	No
Meulendijks D, et al. Br J Cancer. 2017;116(11):1415 to 24.	No	No	Yes	No	No	Yes
Maharjan AS, et al. Clin Colorectal Cancer. 2019;18(3):e280-e6.	No	No	Yes	No	No	No
Nahid NA, et al. Cancer Chemother Pharmacol. 2018;81(1):119 to 29.	No	No	Yes	Yes	No	No
Cremolini C, et al. Oncotarget. 2018;9(8):7859 to 66.	No	No	Yes	Yes	No	No
Etienne-Grimaldi MC, et al. PLoS ONE. 2017;12(5):e0175998.	No	No	Yes	Yes	No	Yes
Cellier P, et al. BMC cancer. 2011;11:98.	No	No	Yes	No	No	No
Braun MS, et al. J Clin Oncol. 2009;27(33):5519 to 28.	No	No	Yes	Yes	No	No
Schwab M, et al. J Clin Oncol. 2008;26(13):2131 to 8.	No	No	Yes	Yes	No	No
Sulzyc-Bielicka V, et al. Pharmacol Rep. 2008;60(2):238 to 42.	No	No	Yes	No	No	No
Boisdron-Celle M, et al. Cancer Lett. 2007;249(2):271 to 82.	No	No	Yes	No	No	Yes
Largillier R, et al. Clin Cancer Res. 2006;12(18):5496 to 502.	No	No	Yes	Yes	No	No
Salgueiro N, et al. Genet Med. 2004;6(2):102 to 7.	No	No	Yes	Yes	No	No

Primary study citation	Cura et al. (2023)	Kim et al. (2022)	Ontario Health HTA (2021)	De Moraes et al. (2024)	Glewis et al. (2022)	Paulsen et al. (2022)
Wigle TJ, et al. Clin Transl Sci. 2021;14(4):1338 to 48.	No	No	Yes	No	No	Yes
Henricks LM, et al. 2018;19(11):1459 to 67.	No	No	Yes	No	No	No
Fernandez MA, et al. Eur J Hosp Pharm. 2019;26:A229 to 30.	No	No	No	Yes	No	No
Amirfallah A, et al. J Pers Med. 2018;8(4):13.	No	No	No	Yes	No	No
Boige V, et al. J Clin Oncol. 2010;28:2556 to 64.	No	No	No	Yes	No	No
Boisdron-Celle M, et al. Semin Oncol. 2017;44(1):13 to 23.	No	No	No	Yes	Yes	Yes
Botticelli A, et al. Anticancer Drugs. 2017;28(5):551 to 6.	No	No	No	Yes	No	No
Cai X, et al. Eur Rev Med Pharmacol Sci. 2014;18(8):1247 to 58.	No	No	No	Yes	No	No
Cerić T, et al. Bosn J Basic Med Sci. 2010;10(2):133 to 9.	No	No	No	Yes	No	No
Detailleur S, et al. Ann Gastroenterol. 2021;34(1):68 to 72.	No	No	No	Yes	No	No
Dhawan D, et al. Indian J Med Res. 2013;137(1):125 to 9.	No	No	No	Yes	No	No
Gross E, et al. PLoS ONE. 2008;3(12):e4003.	No	No	No	Yes	No	No
Joerger M, et al. 2015;75(4):763 to 72.	No	No	No	Yes	No	No
Kristensen MH, et al. J Int Med Res. 2010;38(3):870 to 83.	No	No	No	Yes	No	No
Ruzzo A, et al. Br J Cancer. 2017;117(9):1269 to 77.	No	No	No	Yes	No	Yes
Salgado J, et al. 2007;17(2):325 to 8.	No	No	No	Yes	No	No
Toffoli G, et al. Clin Pharmacol Ther. 2019;105(4):994 to 1002.	No	No	No	Yes	No	No
Vivaldi C, et al. Pharmacogenomics J. 2021;21(2):233 to 42.	No	No	No	Yes	No	No
Ohnuma S, et al. Ann Oncol. 2015;26:ix8.	No	No	No	Yes	No	No

Primary study citation	Cura et al. (2023)	Kim et al. (2022)	Ontario Health HTA (2021)	De Moraes et al. (2024)	Glewis et al. (2022)	Paulsen et al. (2022)
Ghoche A. Ann Oncol. 2023;34:S88.	No	No	No	Yes	No	No
Negarandeh R. et al. BMC Cancer. 2020;20:1 to 7.	No	No	No	Yes	No	No
Van Kuilenberg AB, et al. Clin Pharmacokinetics. 2012;51:163 to 74.	No	No	No	No	Yes	No
Deenen MJ, et al. J Clin Oncol. 2016;34(3):227 to 34.	No	No	No	No	Yes	No
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Patil V, et al. South Asian J Cancer. 2016;5(04):182 to 5.	No	No	No	No	Yes	No
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Yang CJ, et al. Cancer Chemother Pharmacol. 2011;67:49 to 56.	No	No	No	No	Yes	No
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Launay M, et al. Clin Cancer Drugs. 2017;4(2):122 to 8.	No	No	No	No	Yes	Yes
Lunenburg CA, et al. Pharmacogenomics. 2016;17(7):721 to 9.	No	No	No	No	Yes	No
Magnani E, et al. Intern Emerg Med. 2013;8:417 to 23.	No	No	No	No	Yes	No
Henricks LM, et al. Lancet Oncol. 2018;19(11):1459 to 67.	No	No	No	No	No	Yes
de With M, et al. Clin Pharma Ther. 2022;112(1):62 to 68.	No	No	No	No	No	Yes
Ciccolini J, et al. Ther Drug Monit. 2006;28(5):678 to 85.	No	No	No	No	No	Yes
Capitain O, et al. Dose-Response. 2020;18(3): 155932582095136.	No	No	No	No	No	Yes
Shakeel F, et al. Pharmacogenomics et al. 2021;22(3):145 to 55.	No	No	No	No	No	Yes
Meulendijks D, et al. Int J Cancer. 2016;138(11):2752 to 61.	No	No	No	No	No	Yes

Primary study citation	Cura et al. (2023)	Kim et al. (2022)	Ontario Health HTA (2021)	De Moraes et al. (2024)	Glewis et al. (2022)	Paulsen et al. (2022)
Del Re M, et al. Pharmacogenomics. 2019;19(6):556 to 563.	No	No	No	No	No	Yes

Appendix 7: References of Potential Interest

Please note that this appendix has not been copy-edited.

Previous CDA-AMC Reports

Vu T, Spry C. *Raltitrexed in Patients With Dihydropyrimidine Dehydrogenase Deficiency*. Canadian Agency for Drugs and Technologies in Health (CADTH); 2022. Accessed May 14, 2025. https://www.canjhealthtechnol.ca/index.php/cjht/article/view/RC1463

Guidelines and Recommendations

Beumer JH, Chu E, Allegra C, et al. Therapeutic Drug Monitoring in Oncology: International Association of Therapeutic Drug Monitoring and Clinical Toxicology Recommendations for 5-Fluorouracil Therapy. *Clin Pharmacol Ther*. 2019;105(3):598-613. doi:10.1002/cpt.1124 PubMed

Review Articles

Hertz DL. Assessment of the clinical utility of pretreatment *DPYD* testing for patients receiving fluoropyrimidine chemotherapy. *J Clin Oncol*. 2022;40(33):3882-3892. doi:10.1200/JCO.22.00037 PubMed

White C, Scott R, Paul C, et al. Ethnic diversity of DPD activity and the *DPYD* gene: review of the literature. *Pharmacogenomics Pers Med.* 2021;14:1603-1617. doi:10.2147/PGPM.S337147. PubMed

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