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Impact of Clinical and Pharmacogenetic Variables on the Risk of Fluoropyrimidine-Related Adverse Events within Ontario

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Abstract

Severe life-threatening adverse events are a major limitation of fluoropyrimidine chemotherapy, these events are disastrous for patients and costly to the healthcare system. Identifying patients at high risk for developing adverse events would allow for preventive dose reduction, improving patient outcomes and reducing healthcare cost. This thesis uses data pulled from provincial databases to quantify the adverse event burden of fluoropyrimidines in Ontario. We found that 31% of Ontario patients treated with fluoropyrimidine systemic chemotherapy developed a severe adverse event that requires acute medical care. Patients that required acute medical therapy were significantly costlier to the public system averaging an increased cost of $16,754 (CAD). Therefore, there is a substantial potential to reduce cost to the health care system and pay for preventive screening strategies. A known risk factor for fluoropyrimidine adverse events is Dihydropyrimidine dehydrogenase (DPD, gene *DPYD*) deficiency. We demonstrate that screening for DPD deficiency through targeted genetic testing and genotype-guided dosing reduces the risk of adverse events in genetically deficient patients. Unfortunately, 31% of wildtype patients still experience a severe adverse event, requiring further research into predictors of adverse event risk. Following up on genetic variants, we tested for a recently described intrageneric *DPYD* deletion through a nested case-control study. We found the deletion associated with toxicity, however the variant was too rare to decide on the overall relevancy to clinical testing at this time. Next, we looked beyond genetics and tested the impact of plasma folate level on adverse event risk. Despite previous literature suggesting an association we found that plasma folate levels were not predictive of fluoropyrimidine adverse event risk. Therefore, there remains a significant number of unexplained fluoropyrimidine-related adverse events. In conclusion, while folate level did not show a predictive value, pharmacogenetic testing was found to be feasible and effective at reducing adverse events. To this end, we worked with Health Quality Ontario to translate these findings, pretreatment *DPYD* testing is now recommended to be a publicly funded test in Ontario.

Keywords

Fluoropyrimidines, Adverse Events, Pharmacogenetics, Dihydropyrimidine Dehydrogenase, Chemotherapy, Health Economics

Summary for Lay Audience

Fluoropyrimidines are a class of widely used chemotherapy drugs; however, they cause significant side effects which can be lethal and occur in up to one in three patients. This thesis discusses the use of fluoropyrimidines in Ontario and investigates methods to reduce the number of severe side effects experienced by patients. A known risk factor for fluoropyrimidine side effects is deficiency of the key enzyme that degrades the drug; patients with the deficiency are exposed to higher drug levels and more side effects. In chapter 2 we found targeted screening for this genetic deficiency is an effective way to protect some patients from experiencing toxic outcomes. Chapter 3 tested for the presence of a newly described deficiency causing variant. We found that a patient carrying the novel variant experienced severe side-effects and required a reduced dose of fluoropyrimidines. However, the variant was rare and the overall utility at the provincial level remains uncertain. Chapter 4 investigated whether the environmental risk factor of dietary folate intake could improve the prediction of fluoropyrimidine side effect risk. We measured plasma folate levels in a sample of genetically competent patients and tested for an association between plasma folate level and fluoropyrimidine side effects. We found that plasma folate level had no impact on the risk for severe side effects, but did notice that other clinical characteristics such as the patients' sex were predictive of their risk. Finally, Chapter 5 places the previous work into the larger context by directly assessing the health care burden of fluoropyrimidine use in Ontario. We found that 31% of fluoropyrimidine treated patients require emergency medical care due to severe side effects. Patients that were forced to receive fluoropyrimidine-related emergency medical treatment were significantly costlier to the health care system than those patients that did not. Combining the results of chapters 2 and 5 allowed for a convincing argument for knowledge translation at the policy level. Our work has now been used to support recommendations that Ontario publicly fund genetic testing prior to fluoropyrimidine chemotherapy. This is a significant advancement for the field and patient safety.

Co-Authorship Statement

Chapter 2 contains data adapted from the published work Theodore J. Wigle, *et al*. "Impact of pretreatment dihydropyrimidine dehydrogenase genotype-guided fluoropyrimidine dosing on chemotherapy associated adverse events." Clinical and Translational Science (2021). As first author TJW performed data collection, designed data analysis, completed data analysis and wrote the manuscript. BJP, WAT, RML, JL, SN, VP, DK, JM, VS, SS, YC, SW, EW, UIS and RBK assisted in data collection. BJP, SM, VP, DK, SS, YC, UIS and RBK reviewed data analysis. WAT, UIS and RBK initiated the trial. RBK Supervised all aspects of the work. Finally, all authors were asked to review and comment on the manuscript.

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List of Abbreviations

5-MTHF	5-Methyltetrahydrofolate
5-FDHU	5-Fluorodihydrouracil
5-FU	5-Fluorouracil
5,10-MTHF	5,10-Methylenetetrahydrofolate
ADR	Adverse Drug Reaction
ADME	Absorption Distribution Metabolism Elimination
AE	Adverse Event
AS	Activity Score
CNV	Copy-Number Variation
CPIC	Clinical Pharmacogenetics Implementation Consortium
CTCAE	Common Terminology Criteria for Adverse Events
DCP	Dataset Creation Plan
DHF	Dihydrofolate
DNA	Deoxyribonucleic acid
DPD	Dihydropyrimidine Dehydrogenase
DPYD	Dihydropyrimidine Dehydrogenase Gene
EHR	Electronic Health Record
EMA	European Medicines Agency
FBAL	α-fluoro-β-alanine
FDA	Food and Drug Administration
FdUMP	Fluorodeoxyuridine monophosphate
FdUTP	Fluorodeoxyuridine triphosphate
FUMP	Fluorouridine monophosphate
FUPA	α-fluoro-β-ureidopropionic acid

FUTP	Fluorouridine triphosphate
FUR	Fluorouridine
FdUR	Fluorodeoxyuridine
HPLC	High Pressure Liquid Chromatography
IQR	Interquartile Range
MAF	Minor Allele Frequency
mRNA	Messenger Ribonucleic Acid
MS/MS	Tandem Mass Spectrometry
MTHFR	Methylenetetrahydrofolate Reductase
MTX	Methotrexate
pABG	p-aminobenzoyl glutamic acid
PD	Pharmacodynamics
PK	Pharmacokinetics
RA	Rheumatoid Arthritis
RNA	Ribonucleic Acid
SD	Standard Deviation
SNV	Single Nucleotide Variation
THF	Tetrahydrofolate
TS	Thymidylate Synthase
TYMS	Thymidylate Synthase Gene

Chapter 1

1 Introduction

1.1 Introduction

In medicine an ideal therapy can be applied to all individuals with a similar condition and return all members of the population to a healthy state without consequence. To this end an ideal drug could be given at a constant dose and would perfectly heal all members of the population without side-effects, regardless of differences between the individuals. In reality no therapy is without risk, and extremely few are perfectly successful in treating the underlying pathology. Balancing the therapeutic benefit and deleterious consequences of a therapy requires defining the factors that influence these outcomes; for drugs this is the study of pharmacology. Pharmacology incorporates both what the body does to the drug, pharmacokinetics and what the drug does to the body, pharmacodynamics[1]. Understanding these principles can lead to the improvement of the therapeutic index: the ratio of patients benefiting from a therapy to patients experiencing adverse outcomes. This thesis describes basic principles of pharmacology and then directly relates them to the specific case of fluoropyrimidine-chemotherapy. Ultimately, the data presented here describe the benefit of prospective pharmacogenetic intervention and the work required to implement this process in the province of Ontario.

1.2 Brief Review of Pharmacology

Understanding the degree of inter-person variability and how this impacts the outcomes for different drugs allows for the safe and effective use of pharmacotherapies[2]. Pharmacokinetics describes the transit of the drug into the body, delivery to the target site, and the processes that alter it along the way. The classic delineation of pharmacokinetics is absorption, distribution, metabolism, and excretion (ADME). Inter-patient differences in any of these processes can dramatically affect the outcome that the patient experiences. Broadly speaking, absorption is the route of entry from the external environment into the body (e.g. oral, intravenous, etc.). The route of administration has a major impact on the absorption of drugs and part of this effect is driven by the molecular mechanisms for facilitating its uptake. Commonly, the absorption of drugs is determined by chemical properties such as hydrophobicity, which allows compounds to more easily cross cell membranes and be absorbed. But it is not uncommon for drugs to be actively transported through the intestinal barrier by uptake transport proteins. Once the drug has

entered the circulation, it must move to the site of action through the process of distribution. Accessing the site of action may require crossing additional barriers such as the blood-brain barrier or the cell membrane of the target cell. Of note, these transitions can also be facilitated by host transport proteins. Excretion can be an active process through excreting the compounds into waste products such as feces or urine, or elimination can be a passive process such as filtering metabolites into the urine. The metabolism of xenobiotics occurs throughout the process from administration to elimination. Altering the structure of the drug changes the chemical properties and thereby can change the ability of the drug to be absorbed, distributed or eliminated. As well, altering the structure of the drug often impacts its ability to interact with its target and further links the pharmacokinetics and pharmacodynamics. Many metabolic processes rely upon the function of enzymes and these enzymes can be distributed through the body or located in specialized tissues such as the liver. Understanding the metabolic processes that a drug is subjected to, where they occur, and how they modify the potency of the agent is an important component of pharmacology. These processes define pharmacokinetics and disturbances to any of the ADME principles can significantly change the outcome of a therapy.

The counterpart to pharmacokinetics is pharmacodynamics, which details how drugs exert their effects on the biologic systems. A common mechanism of action is for drugs to interfere with function of enzymes through acting as inhibitors of these catalysts. Inhibiting enzymes can directly interfere with cellular metabolism and prevent the efficient transmission of cellular signals. In addition to enzyme, inhibition drugs can be designed to directly modulate the cellular signaling. Through this process drugs can be designed to block the signaling of an overactive system or stimulate a hypoactive system. This mechanism is about regulating the endogenous process using the existing cellular machinery. Finally, in certain pathologies it can be beneficial to circumvent the homeostatic processes and mechanically damage the cell infrastructure. For example, Oxaliplatin is a platinum derivate drug used in the treatment of cancer and directly interferes with the structure of the deoxyribonucleic acid (DNA) strands. These platinum-based drugs cause the physical cross linking of DNA which prevents replication and leads to breaks in the DNA. Cellular machinery can attempt to either export the drug or

repair the damage but is not the direct target for the mechanism of action.[3] As with pharmacokinetics, pharmacodynamics is subject to variation, which has implications for the efficacy and toxicity of drugs.

Given the complexity of pharmacologic systems, it is unsurprising that patients exhibit a wide variability in drug response. Generally, there are three inter-related sources of variability in pharmacology, genetics, environment, and subject physiology (Fig. 1.1). Genetics define the innate architecture of the receiving organism. The drug transporters, metabolism enzymes and target proteins involved in drug response are all subject to variation at the genetic level. The relationship between genetic variation and the phenotypes of drug response is the study of pharmacogenomics, and for individual drug-gene interactions this is termed pharmacogenetics. Another source of variation is the environmental exposure of the individual. Environmental factors such as diet, lifestyle, and concomitant medication use can prime or stress a system. These two sources of variation are not siloed, there are important interactions. Genetic variation can prime individuals to be susceptible to environmental stimuli and environmental exposure can imprint on the genetics through epigenetic modifications. This complex network can be unique to each drug-patient interaction, defining these parameters and how they impact the drug response allows for the better prediction of patient outcomes and thereby more effective therapy.

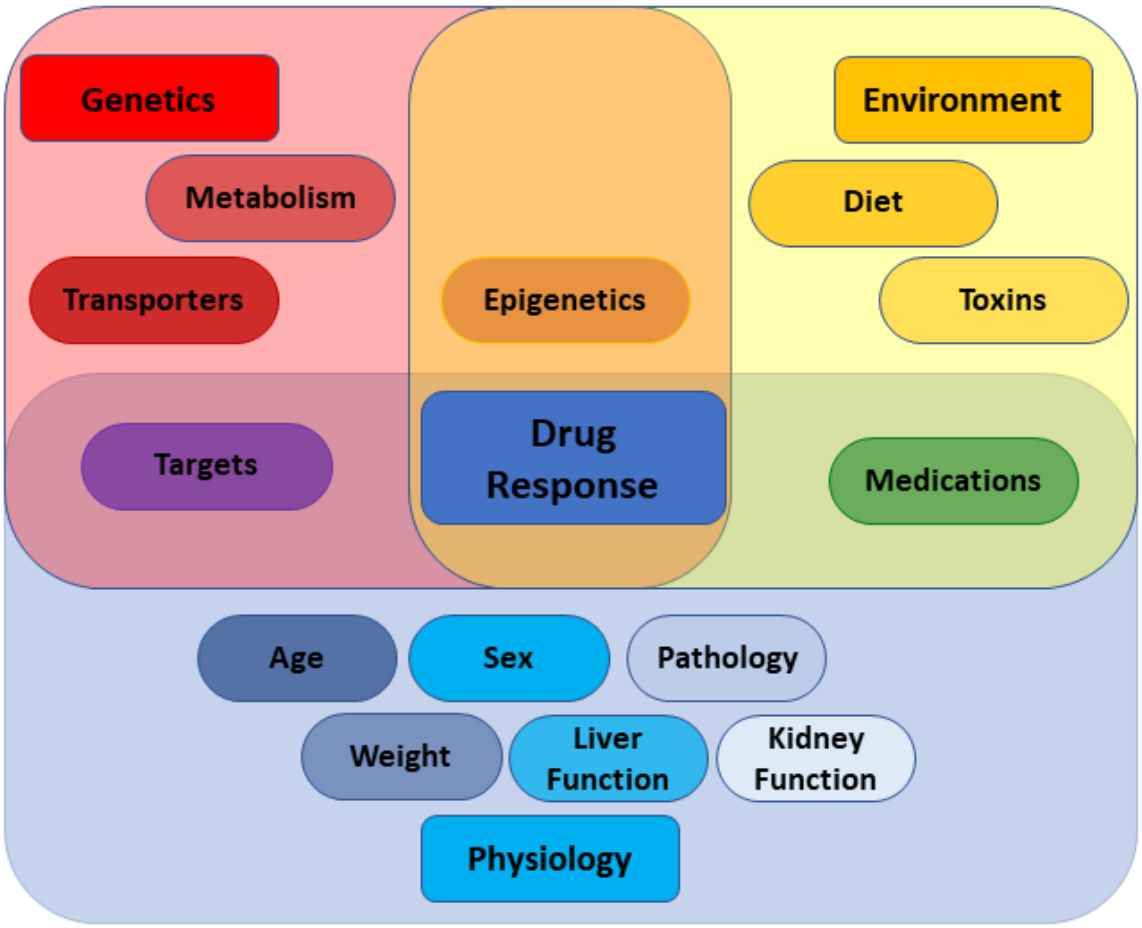

Figure 1.1 Sources of variability in drug response

Variability in drug response can occur from intrinsic and extrinsic factors. The genetic makeup of the individual can determine their ability to uptake and metabolize the drug of interest. Genetics is not an isolated factor as environmental pressures can impact on genetics through epigenetic modifications. As well the pathophysiology of the disease can lead to genetic variants that further alter the drug response. The general physiology of the patient can determine their overall health and metabolic state. The physiology is the phenotypic outcome of their genetic and environmental outcome. Some comorbid conditions may require medications that can lead to drug-drug interactions and impair the drug response. Inputs from the environment including dietary nutrients and toxins can modify both the metabolism and mechanism of action of the drug. Overall, many factors influence drug response and exist in an interconnected network leading to a high potential variability.

1.3 Genetic Variation and Drug Response

Pharmacogenomics links genetic variation and drug response. DNA is considered an extremely stable form of information storage. However, due to the scale of the human genome and the complexity of cellular replication, genetic variation is common[4]. At a large-scale genomic variation is known as structural variation, this involves the deletion, duplication, translocation or inversion of extended portions of genetic code[5]. Normally, diploid individuals carry two alleles of each gene, one copy inherited from each parent, structural variation results in the loss or gain of these alleles. Without compensatory regulatory changes, structural variation leads to changes in the gene dosage and consequent transcription level of the gene of interest[6]. For pharmacogenes involved in the metabolism of a drug, this means that patients that carry a deletion would consequently have 50% of the enzymatic activity, conversely the gain of a copy would result in roughly 150% of enzyme activity. The cytochrome p450 (CYP) superfamily enzymes are significant players in drug metabolism as they catalyze the most frequent metabolic process in drug metabolism, the oxidation of xenobiotics during first pass metabolism[7]. Cytochrome P450 2D6 (CYP2D6) is a member of this superfamily and is known to metabolize one fifth of commonly prescribed medications[8]. *CYP2D6* is a highly polymorphic gene and one form of this polymorphism is whole gene duplication and deletion. The variation of CYP2D6 causes a range of phenotypes from poor metabolizers with less than two functional copies to ultra-rapid metabolizers with more than two functional copies[9]. These phenotypes result in an increased variability of the pharmacokinetics of CYP2D6 substrates by altering the metabolism and thereby the systemic exposure of these agents. Clinical guidelines are currently available for 26 CYP2D6 substrate drugs including selective serotonin reuptake inhibitors[10]. This *CYP2D6* example demonstrates the effect of structural variants on metabolism but structural variation in not limited to one aspect of pharmacokinetics. The pharmacogenetic effects of structural variation can affect proteins throughout pharmacokinetic and pharmacodynamic spectrums from uptake transport proteins to the pharmacodynamic targets. Smaller structural variants can occur within genes such as duplication or deletion of individual exons, referred to as exon-level copy number variations (CNVs)[11]. Interpreting the effect of CNVs can be more difficult than predicting

the phenotype of whole gene duplication and deletions. The phenotypic outcome of a small CNV depends on the functional impact on that allele. At the nucleic acid level, an intra-gene CNV may result in errors of transcription or processing of the ribonucleic acid (RNA) product from pre-RNA to mature messenger RNA (mRNA). At the translational level, changing the variant coding sequence can have deleterious effects on the formation of the functional protein product[12]. The outcome of intra-gene deletions can be as significant as whole gene deletions when they prevent the production of functional protein products. Combined, these forms of structural variants contribute significantly to pharmacogenomics, additional variation at different scales serve to both complement and compound the effects of these structural variants.

While the importance of genetic structural variants is evident, single nucleotide variants (SNVs) account for more than 99.9% of variants in the human genome[13]. Despite their small size, SNVs have broad implications and can occur throughout the genome in both coding and non-coding regions. The impacts of SNVs in a pharmacogenetic study rely upon the ability of the SNV to generate a functional change in the gene product. Synonymous SNVs can impact pharmacogenetics through altering regulatory elements or through interfering with RNA processing[14]. Non-synonymous SNVs occur within the coding sequence and have direct impact on the pharmacogenes protein products[15]. The most severe non-synonymous SNV introduces a premature stop codon. Truncated proteins are the greatest divergence from normal and the most likely to have a significant effect on function. Alternatively, non-synonymous SNVs change an individual codon leading to an alternative residue at that position in the protein structure. If the residue substituted is similar to that dictated by the original gene and is in a region of little importance for the structure or function of the protein these substitutions can be well-tolerated. However, when the location and similarity of the substituted residue differs from the original residue the predicted outcome on the protein function can change significantly, single residue substitutions can completely obliterate protein function. In pharmacogenetics, this introduces a range of phenotypic consequences for genetic variants. Therefore, after identifying variants within pharmacogenes it is essential to functionally characterize these variants to determine how they impact the overall pharmacology for the drug of interest.

1.4 In addition to structural variation 469 missense and/or possible loss of function, SNVs have been found in the *CYP2D6* gene[4,16]. While many of these variants are very rare, present in less than 0.1% of the population, the remaining SNVs produce a range of phenotypic consequences. This has led to the development of a CYP2D6 activity score system to define the impact of these various genetic alleles[17]. The CYP2D6 activity score is an important example in pharmacogenetics as it incorporates both structural variation and SNVs into the score. For example, duplication of a functional allele increases the overall enzymatic potential, but duplication of a defective allele does not. Thanks to the understanding of CYP2D6 variation, clinical guidelines are now in place to aid in treating these patients when genotype information is known[10,18]. Although not yet a clinical standard, applying *CYP2D6* pharmacogenetics in the dosing of selective serotonin reuptake inhibitors (SSRIs) is now being explored. Given CYP2D6 activity regulates the systemic exposure of SSRIs, applying *CYP2D6* pharmacogenetics may help determine an effective dose in this difficult to manage drug class.

Additional Factors Affecting Drug Response

While pharmacogenomics defines a portion of the variability in drug response, it does not act in a vacuum and many other factors influence drug response. Intrinsic characteristics such as age, sex, and body composition are common factors that affect interpatient variability of drug response[2]. Metabolism changes throughout life from newborns to the elderly and age serves as a marker of the ability of individuals to properly metabolize and respond to pharmacotherapy[19,20]. As with other biologic processes, drug response can vary by sex based upon both hormonal differences and differences in the regulation of metabolic enzymes[21]. Multiple factors including diet and lifestyle impact the body composition of patients, variability in body composition impacts multiple pharmacokinetic and pharmacodynamic properties[22]. Diet bridges the gap between

intrinsic factors and external influences. For example, diet impacts the pharmacodynamic properties of drugs through the influence of nutritional co-factors. Such an interaction plays an important role during methotrexate (MTX) therapy for rheumatoid arthritis (RA). Low-dose MTX is used as an immunosuppressant that reduces inflammation in RA, however MTX is an antifolate that directly inhibits multiple enzymes of the folate cycle[23]. The inhibition of the folate cycle is not the primary mechanism of action in treating RA, but leads to dose limiting toxicities that result in discontinuation of the drug[24]. Therefore, clinicians have developed a protocol to supplement low-dose MTX therapy with folic acid as a supportive therapy. This prevents the off-target folate deficiency while preserving the immunosuppressant properties reducing the inflammation in RA patietns[25]. This interaction introduces the concept of drug-drug interactions, in this case the folate supplementation does not directly affect either the MTX pharmacokinetics or it's disease specific mechanism of action.

Drug-drug interactions represent a significant source of inter-patient variability and occur when one compound causes changes to the pharmacokinetic or pharmacodynamic properties of a co-administered compound. The CYP superfamily of enzymes are particularly important in drug-drug interactions as CYP enzymes are sensitive to both induction and inhibition by many compounds. In addition to the genetic variations previously discussed, CYP2D6 serves as the fulcrum of multiple drug-drug interactions such as the relationship between metoprolol (CYP2D6 substrate) and the SSRIs paroxetine and fluoxetine (CYP2D6 inhibitors). The inhibition of CYP2D6 by SSRIs results in reduced metabolism of metoprolol, leading to increased adverse drug reactions[26]. This unintended cross reaction between the two drugs increases the interpatient variability, such scenarios can be managed by selecting alternative therapies to avoid the interaction. The example here also demonstrates the variability that can be created by layering multiple factors into a single system. At a genetic level, CYP2D6 harbors SNVs whose phenotypic effect can be masked or amplified by larger structural variations. Once the gene dosage is understood, the variability continues to be altered by interaction with drugs that further modify the enzymatic activity. This demonstration of the interconnection of the sources of variability is conceptually helpful but has yet to incorporate a final major source of variation, the impact of comorbid conditions.

Comorbid conditions alter the physiology of the patient and can have significant impact on the pharmacokinetic properties leading to interpatient variability in drug response. Hepatic and renal comorbidities represent the important players in interpatient variability[27]. The liver is a major site of metabolism for both nutrients and xenobiotics, hepatic pathologies such as nonalcoholic fatty liver disease (NAFLD) alter the activity of drug metabolizing enzymes[28]. Variation in the metabolic efficiency of the liver leads to variation across the other pharmacokinetic parameters including absorption distribution and excretion. For example, when NAFLD progresses to end stage non-alcoholic steatohepatitis (NASH), the liver function is significantly impaired and this leads to clinically significant changes in the pharmacokinetics of many commonly administered drugs[29]. The liver also plays a role in drug excretion through the biliary excretion of some compounds, in comparison the urinary system is a primary route of excretion with a substrate specific role in metabolism. Impaired renal function commonly reduces the clearance of many xenobiotics, increasing both the peak and average exposure to the drug[30]. The phenotypic consequences depend on the pharmacodynamic properties of the drug, including the therapeutic concentration and the tolerance for increased exposure. Interpreting these changes within the clinical context of the individual patient allows for a personalized approach to patient care to compensate for the variability. While general comorbidities such as hepatic and renal diseases impact the pharmacology of xenobiotics, the pathology being treated can also influence the pharmacology.

1.5 Cancer and Pharmacotherapy Considerations

Cancer is a common and complex pathology with diverse capabilities that create special considerations during pharmacotherapy. Cancer is an evolving disease defined by the ability of a subset of cells to acquire hallmark characteristics that allow them to escape their regulated life cycle[31,32]. These hallmark characteristics provide a survival benefit to cancer cells allowing the cancer to outcompete its neighbouring somatic tissue and grow indefinitely, without regard for the health of the host organism. In general, there are three strategies to treat cancers namely surgery, radiation, and pharmacotherapy. Surgery and radiation are effective at treating localized bulky disease but in many cases, pharmacotherapy is essential[33]. Since the hallmarks of cancer represent the essential skill

set required by cancers to survive, therapies are most effective when they impair these processes[34]. Modern pharmacotherapies include biologic agents and targeted small molecule inhibitors, which are designed to directly impair a single metabolic process that is specifically deregulated in cancer but not in normal tissues[34,35]. These treatments are highly effective, however, the precision that gives them their efficacy is also their downfall[36,37]. Targeted therapies that effect a single process are limited in their application to cancers that are reliant on that pathway. The diversity of cancers between disease sites, between individuals and between microenvironments is in part due to the ability to exploit multiple pathways in a context dependent manner. This explains how the targeted nature of modern pharmacotherapies can limit their utility. The second major limitation of targeted therapies is the rapid and substantial development of resistance amongst cancer cells. The underlying genomic instability of cancer results in genetic heterogeneity amongst cancer cells of an individual patient[32,38]. Conceptually, resistance to targeted therapy follows the principals of natural selection. Targeted therapies are a strong selective pressure which quickly reduce the ability of susceptible cells to continue their line allowing cells that carry a resistance mechanism to outcompete and become the dominant cell type of the cancer[39]. Due to these limitations of targeted therapy, alternative less targeted pharmacotherapies still retain a significant role in treating cancers in the modern era.

Chemotherapy drugs beyond targeted therapies more broadly affect fundamental requirements of cell viability rather than being specific to deregulated pathways of cancer. An example class of chemotherapies are antimetabolites[40]. Antimetabolites mimic endogenous substrates involved in DNA synthesis, but are effectively non-functional, interfering with the ability of cells to replicate[41]. Cancer cells are especially susceptible to these poisons as cancer cells limit the regulation of cell cycle in order to increase the replicative rate. However, certain normal tissues such as gastrointestinal epithelium and hematopoietic cells are physiologically rapidly replicating and can suffer similar cytotoxicity to cancer cells[42]. This emphasizes the importance of understanding the factors that impact the therapeutic index of chemotherapies. Classic chemotherapy drugs are not impervious to resistance and in addition to the specific resistance mechanisms seen for targeted therapies classic chemotherapy drugs are susceptible to pharmacokinetic

variations[43]. Cancer as a physiologic state has a significant impact on the pharmacology of the drugs used to treat the disease. Firstly, extensive cancerous disease can compromise organ systems such as hepatic metabolism and renal clearance. Impairment of these organ systems alter the pharmacokinetics of the therapies administered systemically. Secondly, aggressive treatment of cancer can lead to nutritional deficiency which can limit necessary co-factors from a pharmacodynamic perspective. Finally, the cancer itself can act as a unique tissue with specific absorption, metabolism and excretion[43]. The complexity of these tumours is driven by the genomic instability, which enables the other hallmark traits and resistance mechanisms. The genomic diversity of cancer cells can give tumours unique pharmacokinetic properties compared to the host tissue. For example, tumours can increase the expression of efflux transporters, this effectively provides resistance to the chemotherapy as drug molecules cannot be retained in the target cells[44]. This diversity of tumours increases the difficulty of predicting the pharmacology of chemotherapies. The potential for differences in effect between the tumour and the healthy tissue is yet another factor to consider when determining the therapeutic index and utility of a drug.

1.6 Fluoropyrimidine Chemotherapies

The fluoropyrimidine class of chemotherapies are antimetabolites commonly used in the treatment of solid tumour malignancies[45]. As antimetabolites, they interfere with DNA synthesis and stability impacting multiple hallmarks of cancer. Fluoropyrimidines serve as core components in over 50% of modern chemotherapy regimens including the primary regimens for gastrointestinal tract tumours[46]. For example, in the treatment of colorectal adenocarcinoma, fluoropyrimidines are used throughout treatment from neoadjuvant radiosensitizers, to adjuvant or palliative systemic therapies. In Canada, colorectal cancer is the third most common cancer by incidence and the second most common cause of cancer mortality[47]. These trends continue globally such that an estimated 1.14 million patients were diagnosed with colorectal cancer in 2020[48]. While screening efforts are improving the early detection of colorectal cancers, the majority of patients will require some form of fluoropyrimidine chemotherapy[49]. The broad utility of fluoropyrimidine chemotherapy beyond colorectal cancer includes both head and neck

cancers, and advanced breast cancer, resulting in millions of patients worldwide treated with fluoropyrimidines annually[46]. The major limitation of fluoropyrimidine chemotherapy is the narrow therapeutic index of these regimens, as approximately 30% of patients experience a severe adverse drug reaction during fluoropyrimidine chemotherapy[50]. Fluoropyrimidine-related adverse events (AEs) commonly include diarrhea, mucositis, neutropenia, and hand-foot syndrome[46]. The use of fluoropyrimidine chemotherapy is an excellent example of how understanding the pharmacology of a drug allows for clinical intervention.

The anti-tumour properties of fluoropyrimidines were originally described in 1957 by Charles Heidelberger and colleagues at the University of Wisconsin[51]. Fluoropyrimidines were developed following earlier work that noted tumours preferentially consumed more uracil than comparable normal tissue[52,53]. Knowing that substituting a hydrogen atom for a fluorine atom in a chemical structure can dramatically change its biologic activity, Heidelberger collaborated with Robert Duchinsky of Hoffman-La Roche Inc. to synthesize a variety of fluorinated pyrimidines[51]. Amongst the candidate molecules, 5-fluorouracil (5-FU) was found to be the lead compound and is still a mainstay clinically in modern chemotherapy regimens. Additionally, capecitabine has been developed as an orally administered prodrug of 5-FU[54,55] (Fig. 1.2). Following first pass metabolism, capecitabine metabolites are actively transported in target tissues where they are converted intracellularly to 5-FU. The general pharmacokinetics of capecitabine are now defined, however, the link between the pharmacokinetic parameters and the pharmacodynamic effect is not as clear as for the parent compound 5-FU[56]. However, since the end product is the conversion of capecitabine to 5-FU, the same downstream mechanisms of action and metabolic processes apply for both drugs[57].

In the original report describing fluorinated pyrimidines as a class, 5-FU demonstrated broad anti-tumor efficacy against four different solid tumour models in both rats and mice. As well, in this pioneering work Heidelberger *et al*. identified two potential mechanisms of action. It was foundthat 5-FU was directly incorporated into the nucleic acids, and that thymidine synthesis was inhibited[51]. Heidelberger led a series of studies over the next decade that progressively demonstrated multiple metabolic pathways and

proposed mechanisms of action. First, it was demonstrated that 5-FU is subjected to the same catabolic pathway as endogenous uracil[58], with the degradation of 5-FU occurring primarily in the liver[58,59]. Conversely, Heidelberger's group demonstrated that the anabolic formation of fluorinated nucleotides was responsible for the anti-tumor activity of 5-FU[60,61], but it took many additional studies to elucidate the relevance of each pathway. The anabolic metabolism is such that once 5-FU has entered the cell it can be converted from a nucleobase to a nucleoside with either a ribose or deoxyribose sugar forming fluorouridine (FUR) or fluorodeoxyuridine (FdUR) respectively. Kinases phosphorylate these nucleosides to the nucleotides fluorouridine monophosphate (FUMP) and fluorodeoxyuridine monophosphate (FdUMP) respectively. Alternatively, 5-FU can be directly converted into FdUMP through the action of the orotate phosphoribosyltransferase. Both FUMP and FdUMP can be further phosphorylated to their di- or triphosphate states and then incorporated into their respective nucleic acids[62,63] (Fig. 1.3). The formation of these fluorinated nucleotides represents a lethal synthesis as the false nucleotides contribute to the anti-tumour properties of fluoropyrimidines.

Heidelberger *et al*. originally noted two potential mechanisms of action, the incorporation of fluorinated nucleotides into nucleic acids and inhibition of thymidine synthesis[51]. In subsequent studies, Heidelberger concluded that the incorporation of fluorinated nucleotides into nucleic acid was not the key mechanism of action[64]. However, further research has now demonstrated that the mechanism of action is context dependent. Fluorouridine triphosphate (FUTP) incorporation during RNA synthesis leads to failure of preRNA processing[65], irregular tRNA modification[66], and incomplete polyadenylation of mRNA[67]. When fluorodeoxyuridine triphosphate (FdUTP) is mistakenly incorporated into DNA, cells enter repeated cycles of attempted excision repair, which ultimately destabilizes the DNA[68]. These mechanisms of action help explain some of the variability in antitumor effects noted by Heidelberger during the early studies[69]. Additionally, Heidelberger's lab correctly identified another major mechanism of action, which was the interference with thymidine synthesis[51]. In follow-up studies, it was demonstrated that 5-FU prevents the methylation of uridine during thymidine synthesis[60]. This led to the discovery that FdUMP inhibits the enzyme Thymidylate Synthetase (TS, gene name

TYMS), effectively inhibiting the sole pathway for *de novo* synthesis of thymidine[69,70]. The reaction catalyzed by TS requires 5,10-mthylenetetrahydrofolate (5,10-MTHF) to serve as a methyl donor. Subsequently it was found that FdUMP is an irreversible inhibitor of TS that forms a covalent bond within the catalytic site, however this bond cannot be formed in the absence of 5,10-MTHF[71,72]. It is believed that TS inhibition is the primary mechanism of action of fluoropyrimidine chemotherapeutics[73].

The basic understanding of 5-FU metabolism and mechanisms of action continued to be unraveled across decades following the initial discovery. Yet the drugs were rapidly advanced to clinical trials in humans, the first preliminary report of human experiments was published just one-year after the initial description of the class[74]. This early maximum tolerated dose finding study described many key aspects of the fluoropyrimidine pharmacokinetics which have proved true in follow-up studies. . First, toxic effects were short lived following withdrawal of the drug, implying rapid clearance. Second, antitumor effects were seen only in patients with severe toxicity, implying a narrow therapeutic index. Third, beneficial outcomes were only seen in solid tumours, implying this subset of cancers should be the target population[74]. The full elucidation of fluoropyrimidine clinical pharmacokinetics was hindered by the lack of sensitivity of available analytic techniques. Heidelberger's lab had limited early success demonstrating significant variation in the absorption of fluoropyrimidines based on the route of administration. Orally administered 5-FU is slowly absorbed and much more variable than direct I.V. infusion[75]. In this investigation, there was significant variation in the degradation of fluoropyrimidines and intriguingly a significantly reduced degradation in a patient with severe liver cirrhosis[76]. A companion study demonstrated that even with I.V. administration only 1% of the administered 5-FU underwent the lethal synthesis required for anti-tumour effect[76]. The pharmacokinetic variability was further substantiated demonstrating that the degradation was saturable resulting in dose dependent variation in kinetics[75], ultimately resulting in therapeutic differences between regimens based on dosing schedule[77]. It has subsequently been demonstrated that both the therapeutic efficacy and toxicity profile of 5-FU are dependent on the systemic exposure attained[78-81]. Clinical pharmacokinetic modelling has demonstrated that both age and sex are significant predictors of fluoropyrimidine toxicity[82-92]. The positive correlation between

increased age and risk of fluoropyrimidine toxicity has been suggested to relate to general physiologic changes in hepatic function and a decreased metabolism[46,91]. The sex related differences are also attributed to underlying differences in metabolism[93,94]. Despite the mounting evidence of the importance of 5-FU, systemic exposure the full mechanism of 5-FU metabolism was not well understood during the first 30 years of use.

In 1987, Heggie *et al*. were the first to accurately describe the clinical pharmacokinetics of 5-FU, specifically the importance of catabolic metabolism[95]. Heidelberger's group had originally proposed that the key transition was first from FdUR and FUR back to 5-FU by nucleoside phosphorylase[96]. Heggie *et al.* used an improved high-pressure liquid chromatography (HPLC) method to simultaneously isolate 5-FU and the individual catabolites from plasma, bile, and urine samples. As proposed by Heidelberger's earlier work, 5-FU catabolism mirrors uracil catabolism[51,58]. First, 5-FU is converted to 5-fluorodihydrouracil (5-FDHU), which is then hydrolyzed to α-fluoro-β-ureidopropionic acid (FUPA), which is cleaved to α-fluoro-β-alanine (FBAL)[59]. Heidelberger had concluded that FUPA was likely the major catabolite[58,59]. However, the assays employed in these early studies was not capable of detecting 5-FDHU or FBAL at physiologically relevant times and concentrations. This shortcoming in available techniques combined with the previous assumption that nucleoside phosphorylase activity was the key to fluoropyrimidine inactivation obscured the importance of the catabolic pathway. Heggie *et al.* were able to demonstrate the rapid conversion of 5-FU to 5-FDHU, with an elimination half-life of 12.9 minutes for unchanged 5-FU. The elimination half-life of 5-FDHU was 61.9 minutes and in contrast to Chaudhuri *et al.*[58], Heggie *et al*. determined FUPA to be a transient metabolite with a prolonged half-life of 238 minutes[95]. Ultimately, over 80% of administered 5-FU is subjected to the catabolic metabolism and excreted in the urine as FBAL[95]. This study provided new insight into the importance of the pyrimidine catabolic pathway in the metabolism and excretion of 5-FU.
Coincidentally two years prior to Heggie *et al*.'s publication, a case report was presented on severe 5-FU toxicity in a patient with familial pyrimidinemia and pyrimidinuria[97]. Familial pyrimidinemia results from the inability to metabolize endogenous pyrimidines such as uracil. In the context of 5-FU clinical pharmacokinetics, this was the first link between an inborn error of metabolism and clinical toxicity.

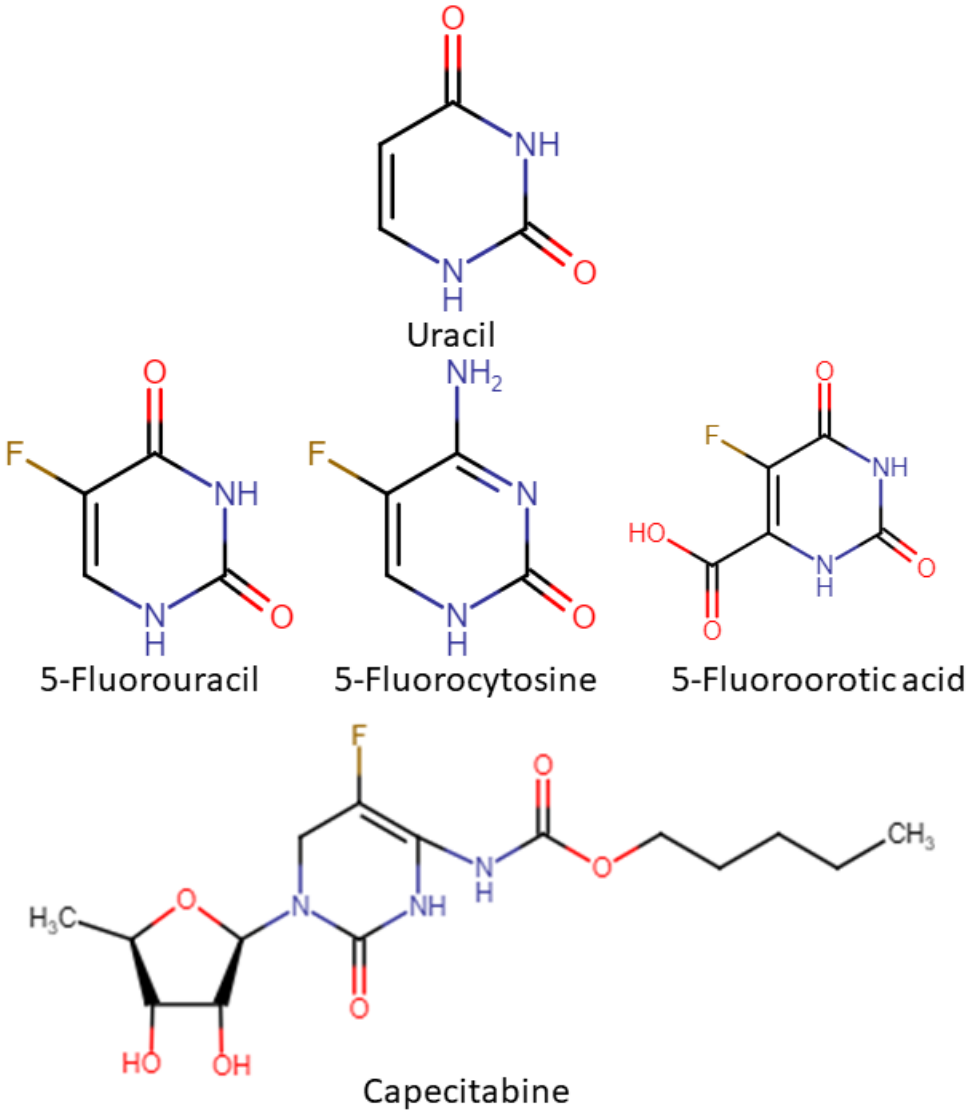

Figure 1.2 Chemical structure of uracil and fluorinated derivatives.

Demonstrating the structural similarities between fluoropyrimidines and uracil. The excessive utilization of uracil by tumours drove the development of the fluoropyrimidine class. The first fluoropyrimidines ever produced were 5-fluorouracil, 5-fluorocytosine and a derivative of fluorouracil 5-flurorоorotic acid. 5-fluorouracil proved to be the lead compound for drug development and clinical use. Capecitabine is a pre-prodrug form of fluorouracil that require enzymatic conversion during first-pass metabolism, and at the target site.

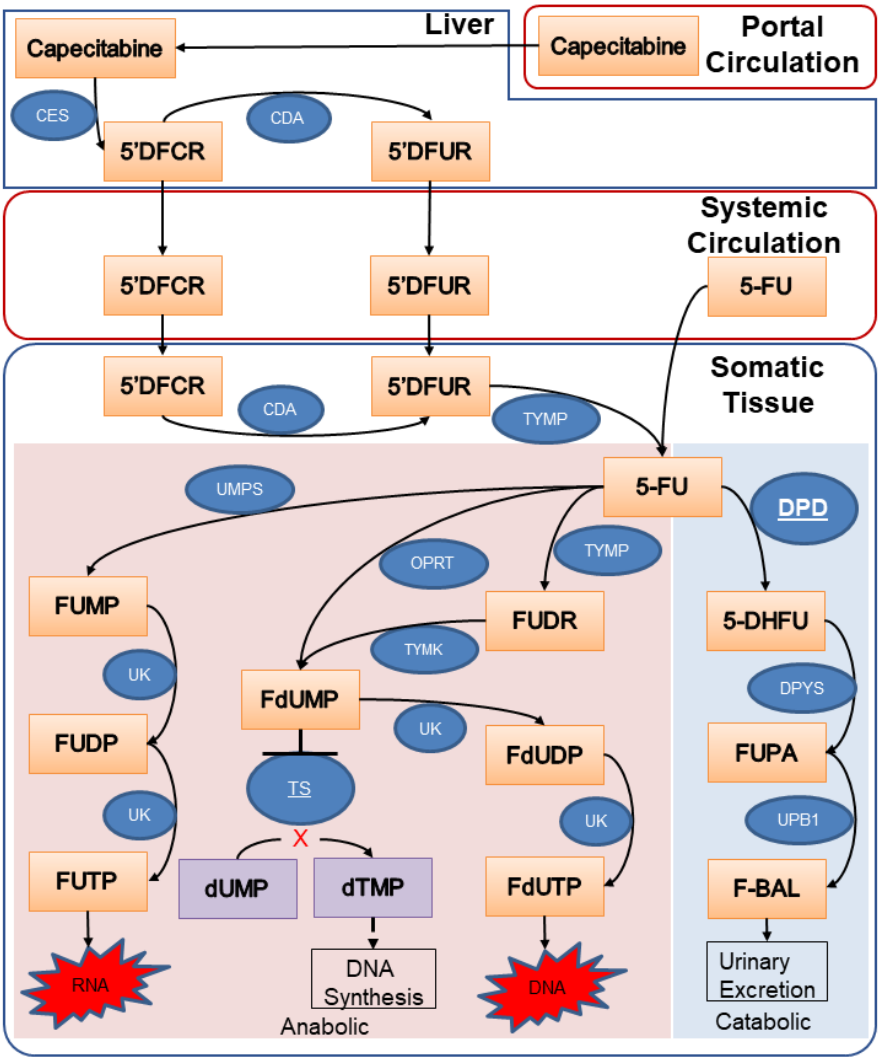

Figure 1.3 Metabolism of clinically relevant fluoropyrimidines

Capecitabine is orally ingested and subjected to first pass metabolism in the liver. 5'DFUR is the primary capecitabine metabolite in systemic circulation, whereas 5-FU is administered intravenously into systemic circulation. The circulating capecitabine metabolites are converted to 5-FU intracellularly at the site of action. Over 80% of intracellular 5-FU is subjected to catabolic metabolism (blue shading) and the metabolites are excreted in the urine. Dihydropyrimidine dehydrogenase (DPD) is the rate limiting enzyme in the catabolic cascade and determines the elimination efficiency. Less than 1% of 5-FU undergoes lethal synthesis to fluorinated nucleotides through anabolic metabolism (orange shading). FdUMP acts as a thymidylate synthase (TS) inhibitor impairing DNA synthesis. Alternative mechanisms of action include direct incorporation of fluorinated nucleotides into RNA or DNA causing disfunction. Enzymes (blue ovals): carboxylesterase (CES), cytidine deaminase (CDA), thymidine phosphorylase (TYMP), uridine monophosphate synthase (UMPS), orotate phosphoribosyltransferase (OPRT), thymidine kinase (TYMK), uridine kinase (UK), dihydropyrimidinase (DPYS), and Beta-ureidopropionase (UPB1).

1.7 Dihydropyrimidine Dehydrogenase

Shortly following the first case report Diasio *et al*.[98] published a second case report of severe 5-FU induced toxicity in a patient with familial pyrimidinemia and a symptomology remarkably similar to what Tuchman *et al*. had reported[97]. However, Diasio *et al.* were able to experimentally identify the specific enzymatic deficiency and open a field of research that continues to grow today. Dihydropyrimidine dehydrogenase (DPD, gene *DPYD*) catalyzes the first and rate limiting reaction in the uracil catabolic pathway[99,100] and DPD has an equal affinity for both uracil and 5-FU[101]. Diasio *et al*. demonstrated that the proband in their case report had a familial DPD deficiency that was inherited in an autosomal recessive fashion[98]. A series of reports in the 1990s reaffirmed this association between familial DPD deficiency and 5-FU toxicity[102-104], and severe toxic outcomes in DPD deficient patients continue to be reported in modern treatment regimens[105]. Given this association between DPD deficiency and fluoropyrimidine induced toxicity, many studies have attempted to quantify the variability of DPD activity as a marker of 5-FU pharmacokinetics. DPD is a ubiquitously expressed enzyme, important sites for its metabolism of fluoropyrimidines include hepatocytes, peripheral blood mononuclear cells, and mucosal cells of the intestine[106,107]. The high DPD expression in the intestinal lining and liver result in extensive first-pass metabolism limiting the bioavailability of oral 5-FU[108]. At the population level, DPD enzyme activity demonstrates significant interpatient variability of between 6 to18-fold based on the population and tissue sample analyzed[93,109-114]. Complete DPD deficiency has also been identified as an autosomal recessive disorder with variable penetrance[115], this adds to the complexity of assessing DPD activity at a population level. At the intra-individual level, DPD activity displays significant variability. DPD activity and expression in peripheral sites such as PBMCs has poor correlation with expression more centrally such as in hepatocytes[112,116]. The tissue specific variation has also been demonstrated within related tissues. DPD enzymatic activity demonstrated significant intra-patient variability when assessed in normal colonic mucosa, inflamed mucosa and cancerous tissue from the same patient[117]. Overlaying the variability within and between tissue types, DPD expression and activity follow diurnal rhytms[111,118-125]. This cyclical variation in DPD activity led to the proposal of timed dose regimens, however, the interpatient variability in the cyclical

activity limited the therapeutic benefit of these complex applications[122,126,127]. The extensive variability of DPD activity helps to explain why attempts to directly correlate DPD enzymatic activity and fluoropyrimidine clearance showed weak associations[128,129]. Therefore, despite the crucial role of DPD activity in fluoropyrimidine catabolism, the direct assessment of DPD activity is of little clinical utility. In parallel to assessing DPD activity as a phenotypic marker for 5-FU pharmacokinetics, many investigators have interrogated *DPYD* as a pharmacogenetic source of variability in 5-FU pharmacokinetics.

1.8 Pharmacogenetic influence of *DPYD*

The association between DPD deficiency and 5-FU induced toxicity started the search for the human *DPYD* gene and gaining an understanding of the potential pharmacogenetic influence. The human *DPYD* gene was cloned and characterized in the mid 1990s[130-132].This rapidly led to the first discovery of a SNV implicated in familial DPD deficiency[133,134]. This variant *DPYD* c.1905+1G>A (rs3918290, formerly *2A) is an intronic splice site variant that leads to the skipping of exon 14 during pre-mRNA processing. The missing 165 nucleotides in the mature mRNA result in a complete loss of function for this variant[135]. However, it was quickly realized that DPD deficiency could not be linked to a single defect[136,137]. Currently there are 482 missense and/or loss of function *DPYD* SNVs recorded in the Genome Aggregation Database[4,138]. Determining the clinical relevancy of these many mutations requires two considerations, the phenotypic impact of each variant and the prevalence in the population. In the early discovery of *DPYD* variants, the phenotypic effect was measured directly in patients by assessing the DPD activity of PBMCs[133,134]. Direct assessment of DPD activity in patients carrying *DPYD* variants is limited by the known sources of variation such as circadian rhythm and sex differences noted above[119]. With the advance of *in vitro* methodologies, variants can now be transiently expressed in model systems and the DPD phenotype assessed directly with limited external confounders[139,140]. This improves the ability to predict the translation of novel genetic variants in terms of DPD activity[141]. The magnitude of phenotypic change caused by a *DPYD* variant is significant but the clinical relevance of variants also considers the prevalence within a population. In order to determine the prevalence of *DPYD* variants in clinically relevant populations, many

retrospective genotype association studies have been completed using samples of patients from fluoropyrimidine based clinical trials[142-148]. The benefit of measuring the allele frequency of variants in these trials is that this process also allows for tests of association between the genotypes and clinical outcomes such as toxicities. Through meta-analyses of these genetic association studies, a short list of four clinically relevant variants has been determined[147,149-151]. Due to the principle of gene dosage discussed earlier, carrying a detrimental genetic variant means that regardless of the physiologic variation of DPD activity the maximal response is blunted in these patients. Understanding the properties of these four variants allows for the potential implementation of *DPYD* genotype-guided fluoropyrimidine dosing.

The first clinically relevant *DPYD* variant is also the first variant to have been identified, *DPYD* c.1905+1G>A[152]. As noted above, this variant results in a complete loss of DPD enzymatic activity[139]. The allele frequency of variants varies by the genetic background of the population being discussed. The clinical trials used for the determination of genotype-phenotype association were composed of >95% Caucasians of European descent. Therefore, it is common practice in the literature to report the minor allele frequency (MAF) amongst Europeans as the clinically relevant value. The MAF of *DPYD* c.1905+1G>A is 0.79%, meaning approximately 1.6% of patients are carriers[152]. The second clinically relevant variant is *DPYD* c.1679T>G (rs55886062, formerly *13)[152]. *DPYD* c.1679T>G is a nonsynonymous missense variant resulting in the substitution of isoleucine to serine at residue 560 of DPD (p.I560S), this substitution places a hydrophilic residue in a well-conserved hydrophobic region of the protein stucture[153]. The resulting instability produces a complete lack of DPD enzymatic activity[139,140]. The MAF of *DPYD* c.1679T>G is significantly lower than the other clinically relevant variants at 0.06%[152]. The third clinically relevant variant is *DPYD* c.2846A>T (rs67376798). This missense variant results in the substitution of aspartic acid to valine at residue 949 of DPD (p.D949V), this mutation results in a conformational change interfering with cofactor binding[153]. *DPYD* c.2846A>T results in a less efficient enzyme conformation with up to 60% reduced DPD activity[139,140], and is present at a MAF of 0.37%. Both *DPYD* c.1679T>G and *DPYD* c.2846A>T were first identified through exon-sequencing of *DPYD* in clinically DPD deficient patients[136,153]. The fourth

clinically relevant variant was also identified in a sample of DPD deficient patients, however, it was identified by haplotype mapping[154]. The fourth clinically relevant variant is an intronic variant, *DPYD* c.1129-5923C>G (rs75017182, part of Haplotype-B3). This intronic variant reduces the pre-mRNA splicing efficiency translating to approximately a 35% reduction in DPD enzymatic activity[155]. As a member of *DPYD* haplotype-B3, *DPYD* c.1129-5923C>G is in complete linkage disequilibrium with *DPYD* c.1236G>A and *DPYD* c.1236G>A is commonly used as the genotype tested variant[156]. *DPYD* c.1236G>A is the most common of the four clinically relevant variants with a MAF of 2.4%[152]. All four clinically relevant variants have been demonstrated to increase the risk of severe fluoropyrimidine-related AEs[147,149-151]. Combined, the four clinically relevant variants are present in 7% of populations of European descent. A pharmacogenetic intervention to prospectively reduce the fluoropyrimidine-dosing in these patients is one strategy for improving the therapeutic index. However, given that nearly a third of patients experience fluoropyrimidine-related AEs, other factors must affect the susceptibility for fluoropyrimidine-related AEs. Extensive studies of the *DPYD* gene have found additional variants beyond SNVs, including CNVs and epigenetic changes in the promoter region[157-159]. However, to date these alternate genetic changes have been either too rare or failed to prove significantly predictive of enzyme activity to be considered clinically relevant[160]. Additional factors that impact the pharmacology of fluoropyrimidines still need to be investigated to improve the prediction of patients at increased risk for fluoropyrimidine-related adverse events.

1.9 Folate and Fluoropyrimidine Pharmacodynamics

A portion of the adverse events unexplained by *DPYD* pharmacogenetics might be explained by additional sources of variation such as interindividual variation in endogenous co-factors. As discussed in Section 1.6 Fluoropyrimidine Chemotherapies, a central mechanism of action for fluoropyrimidine chemotherapy is the inhibition of TS by FdUMP. Importantly, FdUMP only efficiently inhibits TS in the presence of an excess of 5,10-MTHF[71,72], this inextricably links fluoropyrimidine therapy to folate metabolites and the folate cycle (Fig. 1.4). The importance of this association has been demonstrated through *in vivo* animal models and clinically through the use of adjuvants[161]. The

Houghton lab were early leaders in this region, they demonstrated that the cytotoxicity of 5-FU did not directly correlate with intracellular concentration of the active metabolite FdUMP[162]. It was then demonstrated that despite a low conversion rate from 5-FU to FdUMP of 1%, the intracellular FdUMP concentration was sufficient to saturate all TS binding sites. Despite an excess of FdUMP, they noted that only a maximal of 50% of TS binding sites were actually occupied[163]. Knowing that efficient FdUMP-TS interaction requires an excess of 5,10-MTHF, Houghton *et al*. tested supplementing 5-FU with folic acid derivatives in a murine model of colorectal cancer xenografts. This demonstrated that folinic acid, a formyl derivative of folic acid, improved the inhibition of TS *in vivo*[164]. The addition of bolus folinic acid (leucovorin) to 5-FU based chemotherapy was then demonstrated to improve the clinical response rate in colorectal cancer[165]. In comparison, when the combination of capecitabine and folinic acid was explored the toxicity profile made this combination untenable[54,108,166]. The differences in pharmacologic effect between the two drug-drug interactions has not been fully elucidated but folinic acid is now included in modern regimens as adjuvant for 5-FU and not for capecitabine.

Folinic acid represents a purposefully exogenous drug to exploit the relationship between folate and fluoropyrimidines but there remains a question over the reaction between fluoropyrimidines and endogenous folate species. The folate cycle is a crucial endogenous process, and dietary folate is essential for homeostatic processes. The folate cycle is the inter-conversion of different folate species to allow for their cyclical use as co-factors in various biochemical processes such as DNA synthesis. Folate refers to a group of compounds related by a core chemical structure which differ by their redox state and functional groups (Fig. 1.5). Folates are composed of three subunits a pterin ring, a para-aminobenzoic acid and a glutamate tail. The pterin ring is the site of oxidation and folate transits through three oxidation states in the folate cycle. Fully oxidized is folic acid, a partially reduced structure known as dihydrofolate and finally fully reduced tetrahydrofolate. The fully reduced THF forms the backbone for further modification by functional groups at the C5 and C10 positions. The pABA linker joins the pterin ring to the glutamate tail; in plasma the glutamate tail of folates are one unit long but intracellularly the glutamate tail is extended through polyglutamation, which aids in the

retention of the molecule at the target site. As essential vitamins folates must be supplemented from dietary sources, as such there exists inter-individual variation in the folate status of individuals. Given the importance of 5,10-MTHF in fluoropyrimidine mechanism of action, this has led to the inquiry of the role of folate status for the pharmacodynamic impact of fluoropyrimidine chemotherapy. For example, in murine tumor xenograft models, mice and rats fed folate deficient diets were resistant to 5-FU chemotherapy[167,168]. However, the effect was small and in at least one model could be reversed by the concomitant administration of folinic acid[168]. Translating these pre-clinical findings into clinical relevance has proved challenging. Despite five studies attempting to correlate plasma folate levels and fluoropyrimidine pharmacodynamics the results have been inconclusive[169-173]. The standing hypothesis in the clinical literature is that patients with high endogenous plasma levels of folates are primed for increased TS inhibition by fluoropyrimidines. The clinical outcome of this increased interaction is either increased efficacy or increased toxicity. A positive association between plasma folate and 5-FU toxicity was found by Sharma *et al.*[169] but two reports failed to replicate these findings[170,173]. Additionally, amongst capecitabine treated patients an association between plasma folate and toxicity was found in two studies[171,172], however, these studies used a modified outcome measure that complicates interpreting the relevance of these findings in the overall adverse event literature. Therefore, the relevance of plasma folate level on fluoropyrimidine pharmacodynamics remains to be elucidated.

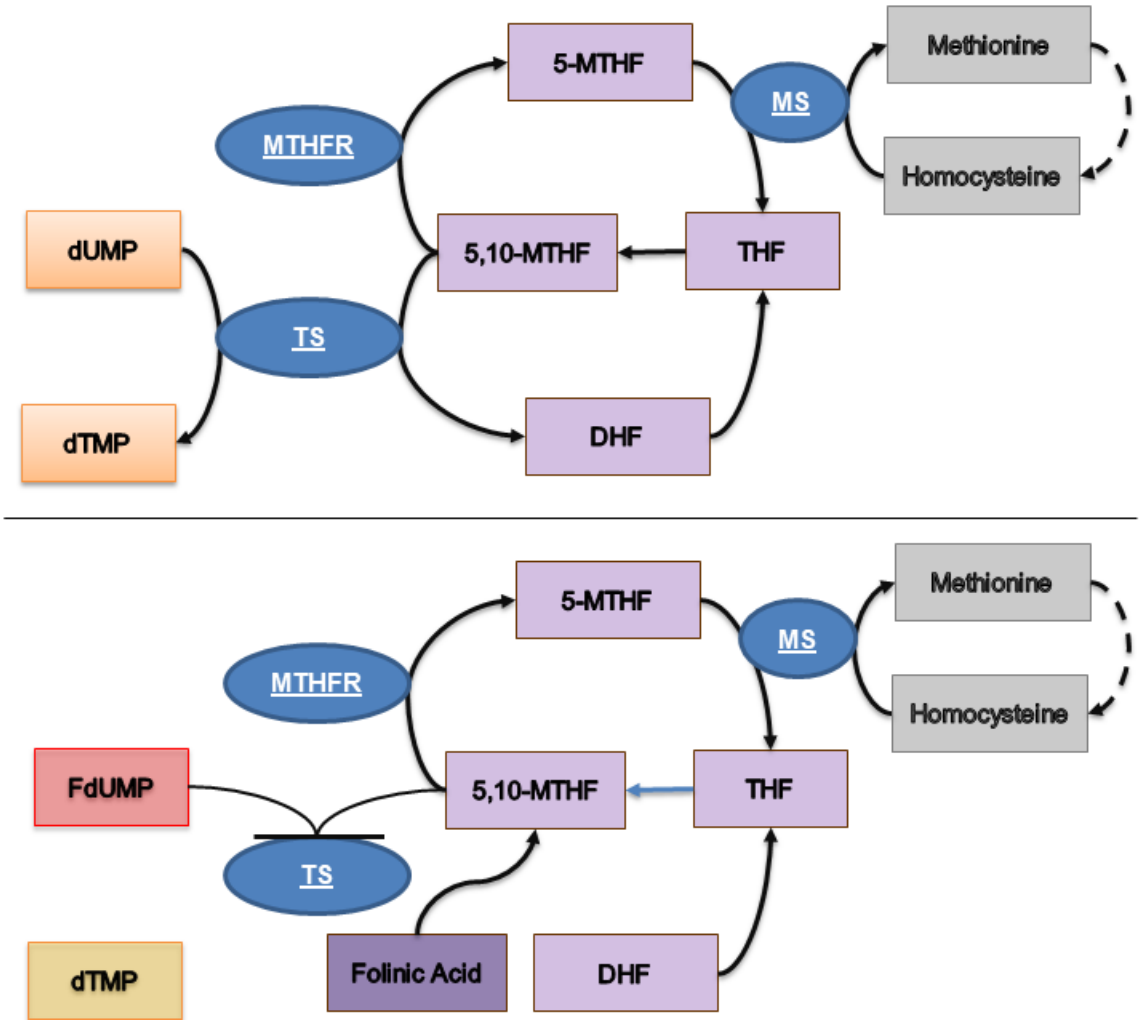

Figure 1.4 Interaction between folate cycle and fluoropyrimidines

Thymidylate synthetase uses 5,10-methylenetetrahydrofolate (5,10-MTHF) as the methyl donor for *de novo* synthesis of thymidine. 5,10-MTHF is an intermediate in the folate cycle, which includes tetrahydrofolate (THF), dihydrofolate (DHF) and 5-methyltetrahydrofolate (5-MTHF). Fluoropyrimidines act as irreversible inhibitors of TS when in the presence of 5,10-MTHF. In order to ensure an efficient inhibition of TS clinically an excess of 5,10-MTHF is insured through the coadministration of folinic acid.

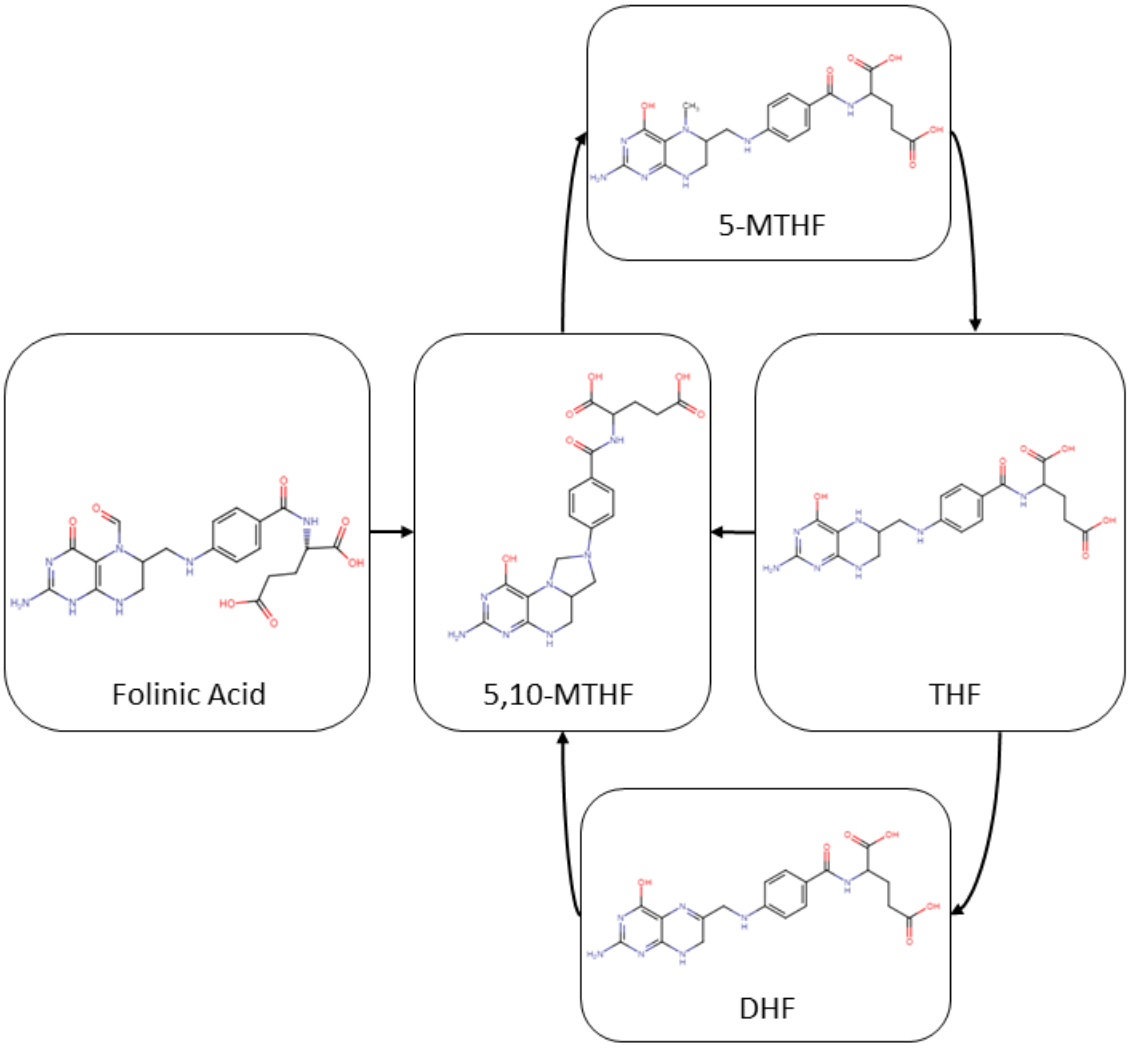

Figure 1.5 Structures of clinically relevant folate species

Folates are a structurally related group of compounds with a pterin ring, p-aminobenzoyl acid and glutamate tail. The different folate species differ by oxidation of the pterin ring. Folinic acid is an exogenous form of folate administered as an adjuvant during fluoropyrimidine chemotherapy. Folinic acid is a formyl derivative of folate that rapidly converts to 5,10-MTHF *in vivo*. Abbreviations: 5,10-methylenetetrahydorfolate (5,10-MTHF), tetrahydrofolate (THF), dihydrofolate (DHF) and 5-methyltetrahydrofolate (5-MTHF).

1.10 Rationale

The narrow therapeutic index of fluoropyrimidine chemotherapy can lead to excessive toxicity and discontinuation of therapy. We now have a significant understanding of the pharmacology of fluoropyrimidines, however, translating this knowledge base into clinical practice has been limited to date. Significant changes to regimen structure have evolved over time such that modern regimens focus more on sustained fluoropyrimidine exposure. For example, capecitabine is a "take home" medication given daily for extended courses while 5-FU is given as a combination bolus and continuous I.V. infusion. But the dosing of these regimens is still based on body composition and variability in exposure is common. Dose modifications are left at the discretion of the treating oncologists using clinical gestalt or reflexively in response to toxicity[174]. A major shortcoming has been acting upon the established relationship between DPD deficiency and fluoropyrimidine AEs. Expert groups have attempted to provide guidance for implementation, however, these guidelines are not considered practice setting in their own right and require further validation and support from national agencies[152,175,176]. In North America, both the Food and Drug Administration and Health Canada have included warnings of the risk DPD deficiency on fluoropyrimidine packaging[177-180]. However, neither agency has recommended any form of DPD deficiency screening. In contrast, significant implementation of DPD deficiency screening has occurred in Europe.

Two Dutch studies have demonstrated the efficacy of *DPYD* genotype-guided dosing using clinically relevant variants[181,182]. Further, these trials demonstrated that genotype-guided dosing was cost effective in the Dutch Health Care System[182,183]. As well, DPD deficiency screening through phenotypic testing has become a standard practice in France[184,185]. As a result of the growing support, the French Medicines Agency requested a review of DPD deficiency screening by the European Medicines Agency (EMA). In April 2020, the EMA released their recommendations in favor of screening for DPD deficiency through either phenotypic or genotypic strategies[186]. Additional European nations have subsequently published DPD deficiency screening guidelines[187,188]. Despite these significant advancements in Europe, translating these findings to North American practice has lagged. One limitation of the translational relevance of these European

findings is the suggestion that there are geographical differences in toxicity profiles. Haller *et al*. found that patients in capecitabine-based clinical trials in the United States, experienced more severe fluoropyrimidines-related AEs than comparable patients in European and Asian trials[189]. As well, due to the variation in healthcare systems across the world, defining the economic potential of an intervention requires context specific definitions. Therefore, regional data is required to support the local implementation of *DPYD* genotype-guided fluoropyrimidine dosing. Despite the demonstrated benefits of DPD deficiency screening in European populations, there remains a significant burden of unexplained fluoropyrimidine-related AEs. A dose nomogram that incorporates *DPYD* genetic variation, age and sex has been proposed but did not reach implementation[92]. Additional factors such as baseline plasma folate level may prove to be a valuable variable in predicting patients at increased risk of fluoropyrimidine-related AEs. An improved model for assessing fluoropyrimidine-related AEs has significant potential for improving patient care (Fig. 1.6). Therefore, the goal of this thesis is to improve the prediction and prevention of fluoropyrimidine-related adverse events in Ontario.

1.11 Hypothesis

Fluoropyrimidine-related adverse events represent a significant burden to the Ontario Healthcare System. Clinical pharmacology variables including genetics and endogenous substrates will allow for the reduction of adverse events.

1.12 Aims

1. To study the efficacy of preemptive *DPYD* genotype-guided dosing for reducing fluoropyrimidine-related adverse events.
2. To define the burden of fluoropyrimidine-related adverse events in Ontario.
3. To study the impact of plasma folate level on the risk of fluoropyrimidine-related AEs

This work presented in this thesis achieved these aims, providing a better understanding of fluoropyrimidine use in the province of Ontario. This work sets the stage for further projects to continually improve patient outcomes during fluoropyrimidine therapy.

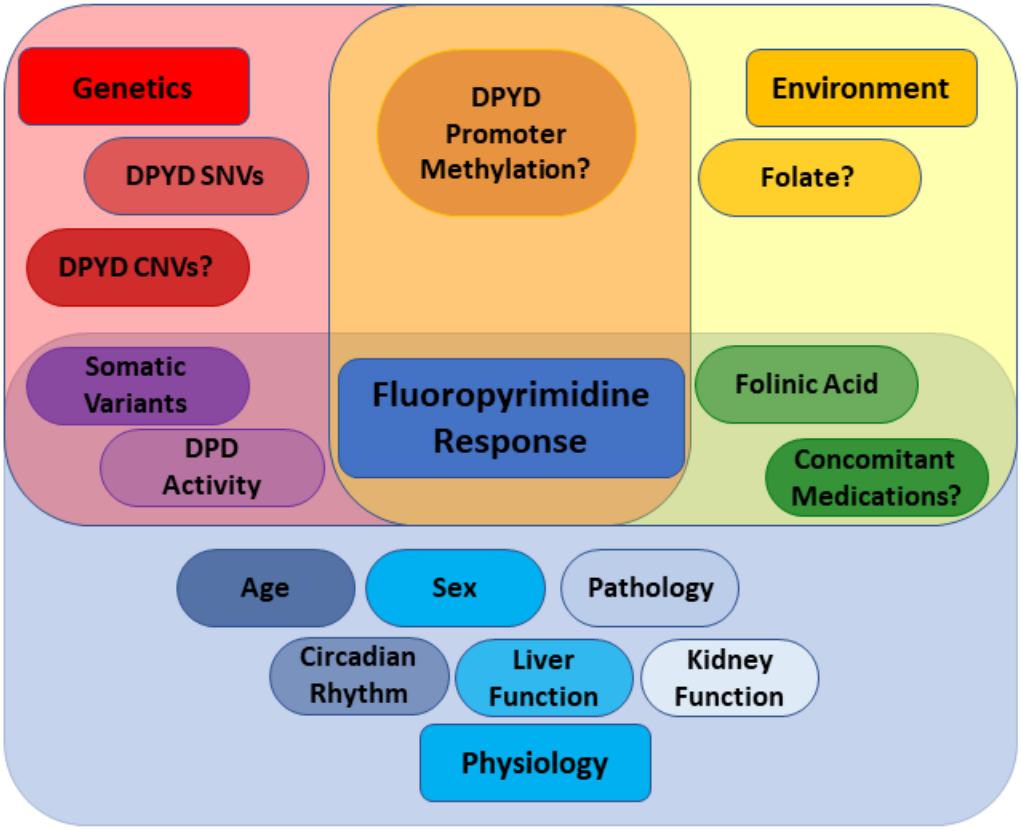

Figure 1.6 Sources of variability in fluoropyrimidine response

Fluoropyrimidine pharmacology is highly variable with known and proposed sources of variation. The pharmacogenetics of fluoropyrimidine response are driven by variation in dihydropyridine dehydrogenase (*DPYD*). But the pharmacogenetics of *DPYD* are not in isolation there are proposed epigenetic influences on promoter methylation and variability in the enzyme activity that cannot be directly determined from known genetic variants. The degree of variability in fluoropyrimidine response demonstrates that additional factors beyond *DPYD* variation are at play. Environmental factors such as dietary co-factors and folate status may play a role. As well the general health condition of the patient is known to influence response with age, sex, liver and kidney function all having previously associated with significant differences in fluoropyrimidine response.

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Chapter 2

2 Impact of pre-treatment dihydropyrimidine dehydrogenase (*DPYD*) genotype-guided fluoropyrimidine dosing on chemotherapy associated adverse events.[1]

[1] This chapter represents published work by Wigle, Theodore J., *et al*. "Impact of pretreatment dihydropyrimidine dehydrogenase genotype-guided fluoropyrimidine dosing on chemotherapy associated adverse events." Clinical and Translational Science (2021). DOI: https://doi.org/10.1111/cts.12981.

2.1 Introduction

5-fluorouracil (5-FU) and capecitabine are fluoropyrimidines used in the treatment of solid tumours[1-4]. Unfortunately, approximately 30% of patients experience severe fluoropyrimidine-related toxicity[5,6]. Dihydropyrimidine dehydrogenase (DPD, gene name *DPYD*) is the rate-limiting enzyme in fluoropyrimidine catabolism[7]. DPD deficiency increases the risk of fluoropyrimidine-related toxicity[8], and there are heritable *DPYD* variants associated with decreased enzyme function and thereby DPD deficiency[9]. Meta-analyses have narrowed the list of clinically relevant genetic variants allowing the implementation of targeted genotype-guided dosing[10-12].

In 2013, the Clinical Pharmacogenetics Implementation Consortium (CPIC®) published a consensus guideline detailing fluoropyrimidine dosing recommendations for three *DPYD* variants associated with reduced enzymatic activity: *DPYD* c.1905+1G>A (*2A, rs39818290), *DPYD* c.2846A>T (rs67376798), and *DPYD* c.1679T>G (*13, rs55886062)[13]. For heterozygous carriers of an individual variant, a 50% dose reduction was recommended, while avoidance of fluoropyrimidines therapies was recommended for homozygous or compound heterozygous variant carriers. A fourth intronic variant, *DPYD* c.1129-5923C>G (rs75017182, in linkage disequilibrium with *DPYD* c.1236G>A (rs56038477)) was added to the guideline in 2017[9]. These recommendations were also refined based on enzymatic activity scores (AS)[14]. The AS of each patient is the sum of the individual alleles where each allele is assigned a score of 0-1 based on functional characterization. The AS of clinically relevant alleles are 0 for *DPYD* c.1905+1G>A or *DPYD* c.1679T>G and 0.5 for *DPYD* c.2846A>T or *DPYD* c.1129-5923C>G. A 25-50% dose reduction was recommended for intermediate metabolizers with an AS of 1.5 and a 50% dose reduction was recommended for an AS of 1 and avoidance for an AS of 0-0.5. In 2018, results from Henricks *et al*.[15] lead to further updates of the CPIC guidelines to recommend a 50% dose reduction for AS of either 1-1.5[16]. In addition, following a well-tolerated initial dose reduction CPIC encourages cautious dose escalation and with concurrent therapeutic drug monitoring if available. Of note, the CPIC guidelines provide

reference for patients with available genotype data and do not comment on the necessity of pre-emptively determining the *DPYD* genotype.

In addition to CPIC guidelines for response to known *DPYD* variants, Dutch and French initiatives have published guidelines that explicitly recommend DPD deficiency screening prior to fluoropyrimidine therapy[17,18]. Despite these recommendations, adoption of pre-treatment *DPYD* genotyping in Canada has been limited and currently is widely accessible only in Quebec. Given the abundance of data linking complete DPD deficiency to severe toxicity, a randomized controlled trial of pre-treatment DPD deficiency screening was considered to be inappropriate for our centre. The only two-arm comparative study of DPD deficiency screening was terminated prematurely due to the fluoropyrimidine-related death of a DPD deficient patient in the control arm[19]. However, two prospective *DPYD* single arm genotype-guided studies were completed in the Netherlands, the first examined the impact of one variant (*DPYD* c.1905+1G>A)[20], and the second assessed four variants (*DPYD* c.1905+1G>A, *DPYD* c.2846A>T, *DPYD* c.1679T>G and *DPYD* c.1236G>A)[15]. These studies demonstrated that genotype-guided dosing reduces the risk of adverse events (AEs) for *DPYD* variant carriers in a European population compared to the historical rate in *DPYD* variant carriers receiving the standard of care.

In contrast, the impact of pre-treatment *DPYD* genotype-guided fluoropyrimidine dosing in a North American setting is unpublished. There is an important distinction between results from a European population and the potential results in a North American population. Work by Haller *et al*. has identified regional variation in fluoropyrimidine-related AEs between the United States and Europe[21]. Therefore, there is a need for regional data to support regional implementation. Here, we conducted a study to determine the impact of pre-treatment *DPYD* genotype-guided dosing on patient safety at a tertiary care centre in London, Ontario Canada. We hypothesized that *DPYD* variant carriers who received genotype-guided dosing would have no greater risk of fluoropyrimidine-related AEs as compared to non-carriers.

2.2 Patients and Methods

2.2.1 Study Sample

We conducted a single-centre retrospective study of patients referred to the Personalized Medicine clinic at London Health Sciences Centre ((LHSC), London, Ontario) for *DPYD* genotype testing between December 1st, 2013 and November 30th, 2019. The study was approved by the Institutional Review Board at Western University and all patients provided written informed consent. Of 1,945 patients referred for testing, 1,845 were tested prior to fluoropyrimidine treatment. At initiation the study was based on the 2013 CPIC guideline[13]; however, in response to the 2017 update[9] the *DPYD* c.1236G>A variant was added to the testing panel in May 2018. Consequently, forty-one *DPYD* c.1236G>A carriers identified retrospectively from enrollment between December 1st, 2013 and May 1st 2018, were removed from the study as they did not receive appropriate genotype-guided dosing. Two compound heterozygous carriers were identified amongst the genotype-guided patients and the treating oncologists were advised to select an alternative therapy. 1,394 patients who initiated treatment through LHSC prior to December 1st, 2019 were included in the genotype-guided study (Fig. 2.1, baseline characteristics summarized in Table 2.1). Prior chemotherapy, radiation therapy, concurrent antineoplastic therapies, and other concomitant medications were allowed. Baseline characteristics for patients lost to follow-up are shown in Table 2.2.

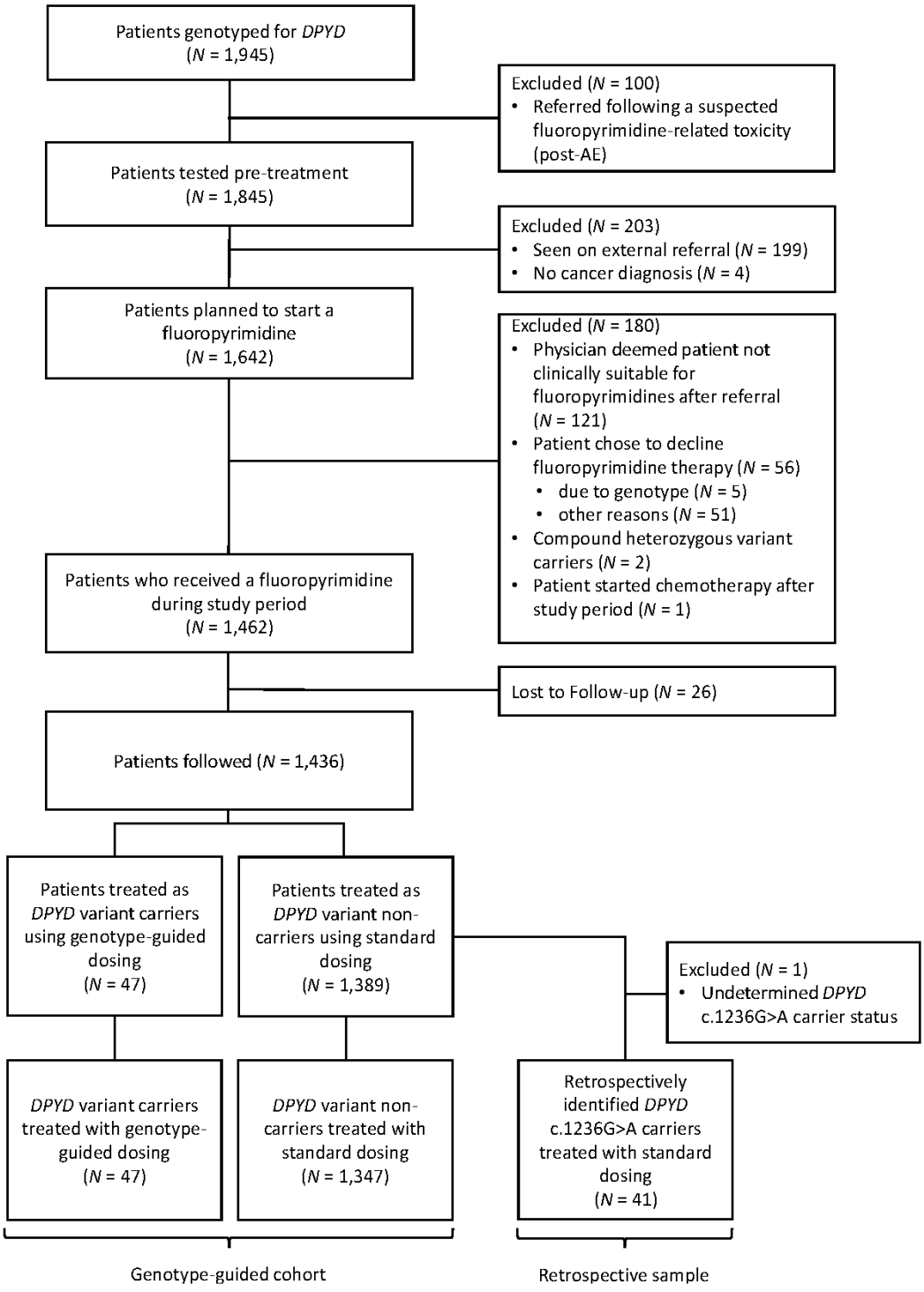

Figure 2.1 CONSORT Diagram

Flow chart of patient inclusion and exclusion from initial referral to the final cohorts.

Table 2.1: Baseline Characteristics of Study Population

	Genotype-guided Cohort		Retrospective sample
Characteristic	**Non-Carrier (*N* = 1,347)**	**Carrier (*N* = 47)**	**c.1236G>A Carrier (*N*=41)**
Sex, *N* (%)			
Female	605 (44.9)	25 (53)	13 (31)
Male	742 (55.1)	22 (47)	28 (68)
Race, *N* (%)			
White	1,267(94)	45 (96)	40 (98)
Other [a]	32 (2.4)	1 (2)	1 (2)
Unknown [b]	48 (3.6)	1 (2)	0 (0)
Age, mean (SD), y	64 (12)	62 (13)	66 (10.4)
Body Surface Area, mean (SD), m^2	1.9 (0.3)	1.9 (0.2)	1.94 (0.27)
Tumour Site, *N* (%)			
Colorectal	779 (57.8)	25 (53)	21 (51)
Gastric & Esophagus	189 (14.0)	7 (15)	5 (12)
Pancreas	106 (7.9)	6 (13)	2 (5)
Breast	89 (6.6)	3 (6)	1 (2)
Anus	48 (3.6)	1 (2)	3 (7)
Head & Neck	27 (2.0)	2 (4)	3 (7)
Other [c]	109 (8.1)	3 (6)	6 (15)
Regimen, *N* (%)			
Capecitabine with Radiation	277 (20.6)	11 (23)	11 (27)
Capecitabine Monotherapy [d]	229 (17.0)	7 (15)	8 (20)
Capecitabine with Oxaliplatin	130 (9.7)	2 (4)	2 (5)
Capecitabine with Other Agents [e]	68 (5.0)	3 (6)	1 (2)
FOLFOX [d]	228 (16.9)	8 (17)	9 (22)
FOLFIRI/FOLFIRINOX	135 (10.0)	8 (17)	0 (0)
5-FU with Cisplatin/Carboplatin	128 (9.5)	4 (9)	3 (7)
5-FU with Other Agents [f]	152 (11.3)	4 (9)	7 (17)
DPYD Genotype, *N* (%)			
Wild-type	1347 (100)	0 (0)	0 (0)
c.2846A>T heterozygous	0 (0)	19 (40)	0 (0)
c.1905+1G>A heterozygous	0 (0)	9 (19)	0 (0)
c.1679T>G heterozygous	0 (0)	1 (2)	0 (0)
c.1236G>A heterozygous	0 (0)	18 (38)	41 (100)

[a] Other Race includes <1% each of Black, Asian, and First Nations individuals.
[b] Due to self-declaration of Race not all patients provided this information and it remains unknown.
[c] Other included appendix and small bowel, genitourinary, hepatobiliary, and primary site unknown.
[d] Including with and without biologic agents.
[e] Including gemcitabine, lapatinib, temozolomide, docetaxel, epirubicin and mitomycin + radiation.
[f] Including the Degramount, FEC-D, and FLOT regimens, in addition to mitomycin + radiation.

Table 2.2: Baseline Characteristics of Patients Lost to Follow-Up

Characteristic	Lost to follow-up (n=26)
Sex, No. (%)	
Female	8 (31)
Male	18 (69)
Race, No. (%)	
White	26 (100)
Age at chemotherapy initiation, mean (SD), y	67 (10)
Body Surface Area, mean (SD), m^2	2.0 (0.2)
Tumour Site, No. (%)	
Colorectal	9 (35)
Gastric & Esophagus	4 (15)
Pancreas	1 (6)
Other [a]	12 (46)
Regimen, No. (%)	
Capecitabine Monotherapy [b]	1 (6)
Capecitabine with Platinum Agents [c]	1 (6)
5-FU with Platinum Agents + Radiation	3 (17)
5-FU with Platinum and Other Agents + Radiation	2 (11)
5-FU with Other Agents + Radiation	1 (6)
Unknown	18 (69)
DPYD Genotype, No. (%)	
Wild-type	25 (96)
c.1236G>A heterozygous	1 (4)

[a] Other included appendix and small bowel, genitourinary, hepatobiliary, and primary site unknown.
[b] Including with and without biologic agents.
[c] Including gemcitabine, lapatinib, temozolomide, docetaxel, epirubicin and mitomycin + radiation.

2.2.2 DPYD genotype testing and dosing recommendations

Whole blood samples were collected from each patient and DNA was extracted using the MagNA Pure Compact Instrument (Roche). DNA was assessed on a ViiA 7 real-time PCR system (Thermo Fisher Scientific) using TaqMan® allelic discrimination assays (Thermo Fisher Scientific) for *DPYD* c.1905+1G>A (assay ID: C__30633851_20), *DPYD* c.2846A>T (assay ID: C__27530948_10), *DPYD* c.1679T>G (assay ID: C__11985548_10), and *DPYD* c.1236G>A (assay ID: C__25596099_30). Variant *DPYD* c.1236G>A is known to be in strong linkage disequilibrium with *DPYD* c.1129-5923C>G and was used as a proxy for genotyping which is in alignment with the CPIC guidelines.

Results and dosing recommendations were provided to the referring physicians within the patients' electronic health records (EHRs). Recommendations were as follows: for non-carriers, dose as per standard of care; for simple heterozygous carriers, apply a 50% initial dose reduction and consider attempting dose escalation in subsequent cycles pending patient tolerance. A 25-50% initial dose reduction was recommended for heterozygous carriers of *DPYD* c.1236G>A upon its addition to the testing panel, with the same additional recommendation to attempt dose escalation based on patient tolerability. Avoidance of fluoropyrimidines in homozygous or compound heterozygous variant carriers was recommended throughout the study, recommendations are summarized in Table 2.3. Final treatment decisions were at the discretion of the treating oncologist.

Table 2.3: Genotype-Guided Dose Recommendations

Variant Status	*DPYD* Genotype	AS [a]	Recommendation
Non-Carrier	-/-	2	Standard Dosing
Simple Heterozygous Carriers	-/c.1236G>A [b]	1.5	25-50% Dose Reduction
	-/c.2846G>A	1.5	50% Dose Reduction
	-/c.1905+1G>A	1	
	-/c.1679T>G	1	
Compound Heterozygous Carriers	c.1236G>A/c.2846A>T [c]	1	Avoid Fluoropyrimidines
	c.1236G>A/c.1905+1G>A	0.5	
	c.1236G>A/c.1679T>G	0.5	
	c.2846A>T/c.1905+1G>A	0.5	
	c.2846A>T/c.1679T>G	0.5	
	c.1905+1G>A/c.1679T>G	0	
Homozygous Carriers	c.1236G>A/c.1236G>A [d]	1	
	c.2846A>T/c.2846A>T [d]	1	
	c.1905+1G>A/c.1905+1G>A	0	
	c.1679T>G/c.1679T>G	0	

[a] The predicted AS, assuming non tested variants are functional with an AS of 1 per allele.
[b] *DPYD* c.1236G>A was added to the testing panel in 2018 as a proxy for Haplotype-B3 and the causative variant *DPYD* c.1129-5923C>G.
[c] Despite an AS of 1 we recommend avoiding fluoropyrimidines in c.1236G>A/c.2846A>T patients, however no patients with this genotype were detected.
[d] Despite an AS of 1 we recommend avoiding fluoropyrimidines in c.1236G>A/c.1236G>A or c.2846A>T/c.2846A>T patients, however no patients with this genotype were detected.
Abbreviations and Symbols: Activity Score (AS); Negative for tested variants (-)

2.2.3 Data Collection

Treatment data including regimen, dose and radiation use were collected from LHSC pharmacy records. Clinical variables and toxicity data were obtained by standardized review of the patients' EHRs by trained study personnel, each record was reviewed independently by two study members. Toxicity data were recorded from clinic notes, admission records, discharge summaries and emergency room reports. Severe AEs included grade ≥3 toxicities according to the National Cancer Institutes' Common Terminology Criteria for Adverse Events (CTCAE) v.5.0[22]. Only those AEs determined to be possibly, probably or definitely related to the fluoropyrimidine components were included in the outcome, following the standard definitions proposed in the NIH protocol template for phase II/III trials[23]. Based on these principles the definitions require the AE to occur within 30 days of fluoropyrimidine administration, be pharmacologically plausible, and not be attributable to another component of the regimen. The effect of removing and reinstating the fluoropyrimidine were also considered when these challenges occurred. Based on the literature the major toxicity categories considered were gastrointestinal (including primarily: diarrhea, oral mucositis and nausea/vomiting), myelosuppression (primarily neutropenia/febrile neutropenia, as well as thrombocytopenia, and unexplained anemia), cardiac (sudden onset cardiac toxicity during fluoropyrimidine administration), and Palmar-Plantar erythrodysesthesia (Hand-Foot Syndrome (HFS)). The remaining AEs in which fluoropyrimidines were likely contributors were grouped under the other heading. The AEs reported by the initial reviewers and attribution of causality was reviewed by a medical oncology fellow under the supervision of a practicing medical oncologist. Conflicts in the records were reviewed by the initial coders and the reviewing medical oncologist. Patients were followed for their entire treatment period and until toxicity resolved.

2.2.4 Outcomes

The primary outcome was severe (grade ≥3, CTCAE v.5.0) fluoropyrimidine-related AEs. We included a secondary outcome of early fluoropyrimidine-related AEs during the first two cycles of treatment. Secondary outcomes further included fluoropyrimidine-

related AEs by toxicity category, proportion of patients discontinuing fluoropyrimidines due to fluoropyrimidine-related AEs and fluoropyrimidine-related deaths.

2.2.5 Statistical Methods

The primary outcome was compared between *DPYD* variant carriers and non-carriers using a Chi-squared test. Other dichotomous outcomes were compared using Chi-squared or Fisher's Exact test as appropriate. Fisher's Exact tests were used when cell values in contingency tables were $\leq$5. A test of non-inferiority between AEs in the variant carriers and non-carriers was performed using a two-one sided test of equivalence. The smallest effect size of interest (SESOI) was determined using the lower bound for the 95% CI of the risk for the *DPYD* c.1236G>A variant carriers in the literature multiplied by the event rate in non-carriers in this study. The *DPYD* c.1236G>A variant demonstrated the lowest increased risk and using this value to set the SESOI was considered a conservative approach. Unadjusted relative risk was used to show the risk of grade $\geq$3 AE in our genotype-guided study and within the literature. Unadjusted relative risks are reported due to the low number of events amongst variant carriers, and for consistency with previous genotype-guided studies. A multivariable logistic regression determined the adjusted odds ratios, adjusted for age, sex, regimen and initial intensity. A Wilcoxon-Mann-Whitney test was used to compare the number of cycles administered between variant carriers and non-carriers. Descriptive statistics are shown using number (percentage), mean (SD), and median (interquartile range (IQR)) as applicable. Reported P-values are for two-sided tests, with $P<0.05$ considered significant. All analyses were performed using R (version 4.0.2, R Foundation Inc.; http://cran.r-project.org/). In addition, the package "tidyverse" was used for data processing and both "epiR" and "TOSTER" were used for analysis (Appendix A).

2.3 Results

2.3.1 Study Population

Among the 1,394 patients provided genotype-guided dosing the mean (SD) age was 64 (12) years and 764 (54.8%) were male. The most common primary tumour site was colorectal (804, 57.7%). Overall fluoropyrimidine use was distributed between capecitabine (727, 52.2%) and 5-FU (667, 47.8%). Forty-seven patients (3.4%) were heterozygous carriers for one of *DPYD* c.2846A>T (19, 1.4%), c.1236G>A (18, 1.3%), *DPYD* c.1905+1G>A (9, 0.6%), or *DPYD* c.1679T>G (1, <0.1%). The retrospectively identified *DPYD* c.1236G>A carriers did not appear to differ from the primary study, with the most common primary tumour site being colorectal (21, 51%), and an approximately equal use of capecitabine (23, 56%), and 5-FU (18, 44%). However, the retrospective sample contained more males (28/41, 68%) than females. The baseline characteristics are summarized in Table 2.1.

2.3.2 Physician Compliance with Dose Recommendations

We confirmed that variant carriers were treated according to the dose recommendations provided to the treating oncologist. The mean initial dose intensity was 52% (18) of ideal for variant carriers and 88% (14) for non-carriers (Table 2.4). Variant carriers received a median (IQR) of 6 (2-7) cycles of fluoropyrimidine treatment, and non-carriers received a median of 4 (2-6) cycles. We also assessed the mean dose intensity throughout the treatment period and found that variant carriers received a mean dose intensity over the total treatment period of 55% (15) while mean intensity for non-carriers was 84.2% (14.7).

Table 2.4: Severe Fluoropyrimidine-related Adverse Events During Full Follow-up

	Genotype-guided Cohort							Retrospective Sample
	Non-Carrier (*N* = 1,347)	Carrier (*N* = 47)	*P*-value [a]	c.1905+1G>A (*N* = 9)	c.2846A>T (*N* = 19)	c.1679T>G (*N* = 1)	c.1236G>A (*N* = 18)	c.1236 G>A (*N* = 41)
Initial dose intensity, mean (SD)	87.4 (15.2)	52 (18)	NA	47 (16)	47 (21)	43 (N/A)	59 (13)	85 (17)
Dose intensity, mean (SD)	84.2 (14.7)	55 (13)	NA	46 (8)	55 (15)	50 (N/A)	59 (12)	85 (17)
Treatment Cycles, Median (IQR)	4 (2-6)	6 (2-7)	0.201	6 (2-8)	6 (4-8)	6 (N/A)	4 (2-6)	2 (2-4)
Total Severe AEs [b] (All Cycles), *N* (%)	***N* (%)**			***N* (%)**				***N* (%)**
Global [c]	418 (31.0)	11 (23)	0.265	3 (33)	5 (26)	0 (0)	3 (17)	14 (34)
Gastrointestinal	167 (12.4)	6 (12)	0.940	2 (22)	2 (11)	0 (0)	2 (11)	7 (17)
Myelosuppression	157 (11.7)	6 (12)	0.816	2 (22)	2 (11)	0 (0)	2 (11)	2 (5)
Cardiac	33 (2.4)	0 (0)	0.625	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
HFS	35 (2.6)	1 (2)	>0.99	0 (0)	1 (5)	0 (0)	0 (0)	2 (5)
Other [d]	113 (8.4)	2 (4)	0.425	1 (11)	1 (5)	0 (0)	0 (0)	5 (12)
AE-related Death [e]	10 (0.7)	0 (0)	>0.99	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Discontinued Treatment [f]	232 (17.2)	10 (21)	0.437	2 (22)	3 (16)	0 (0)	5 (28)	7 (17)

[a] *P*-value for treatment cycles was calculated based on Wilcoxon-Mann-Whitney test. *P*-values for Fluoropyrimidine-related AEs calculated using the following tests: 'Global', 'Gastrointestinal', 'Myelosuppression', and 'Discontinued Treatment' utilized Chi-squared tests; 'Cardiac', 'HFS', 'Other', and 'AE-related Death' utilized Fisher's Exact Test.

[b] Grade ≥3 by Common Terminology Criteria for Adverse Events v.5.0.

[c] Global includes all fluoropyrimidine-related AEs grade ≥3 and fluoropyrimidine-related deaths. This does not include discontinuation.

[d] Other grade ≥3 AEs included: fatigue, infections, neurotoxicities, and lab abnormalities.

[e] At least one fluoropyrimidine-related AE contributed significantly to death.

[f] Patients discontinuing treatment with fluoropyrimidines due to a fluoropyrimidine-related AE of any grade.

Abbreviations: AEs, adverse events; HFS, hand-foot syndrome; IQR, interquartile range; NA, not applicable.

2.3.3 Toxicity Outcomes

There were no significant differences in the primary or secondary toxicity outcomes between genotype-guided variant carriers and non-carriers. We observed that 23% (11/47) of variant carriers, and 31.0% (418/1,347) of non-carriers experienced severe fluoropyrimidine-related AEs during their total treatment periods (P = 0.265) (Table 2.4). We next examined severe fluoropyrimidine-related AEs that occurred during the early cycles (1-2) of fluoropyrimidine treatment. We found that 13% (6/47) of genotype-guided variant carriers compared to 21.4% (284/1,347) of genotype-guided non-carriers experienced an early fluoropyrimidine-related AE (P = 0.167) (Table 2.5). Secondary analyses of the major AE categories, proportion discontinuing fluoropyrimidines due to AEs and fluoropyrimidine-related deaths, during the total treatment period or the first two cycles, did not show any significant differences between genotype-guided variant carriers and non-carriers. Additionally, we performed non-inferiority testing comparing the risk for global severe fluoropyrimidine-related AEs between carriers and non-carriers both during the total treatment and limited to the early cycles (Fig. 2.2). In both early and total treatment periods the confidence intervals included no difference but did not cross the non-inferiority margin. Therefore, we conclude that genotype guided variant carriers do not experience increased risk of fluoropyrimidine-related adverse events compared to non-carriers receiving the standard of care dosing practices. We determined the unadjusted relative risk (RR) of grade $\geq$3 fluoropyrimidine-related AEs in our genotype-guided variant carriers to allow for comparison to literature values (Table 2.6)[11,15]. We report unadjusted RR values due to the small number of genotype-guided variant carriers in our cohort and the literature. We obtained historical values for RR of fluoropyrimidine-related AEs without genotype-guidance from a meta-analysis by Meulendijks et al[11]. In our cohort, genotype-guided variant carriers were not at a significantly elevated risk for severe fluoropyrimidine-related AEs compared to non-carriers. Indeed, with the recommended 50% dose reduction the RR was 1.08 (95% CI: 0.43-2.74) for c.1905+1G>A carriers, and 0.85 (95% CI: 0.40-1.82) for c.2846A>T carriers. With the recommended 25-50% dose reduction recommendations the RR for

genotype-guided c.1236G>A carriers was 0.54 (95% CI: 0.19-1.52). Finally, the single c.1679T>G carrier in our genotype-guided cohort was treated with a 50% dose reduction and did not suffer any fluoropyrimidine-related AEs during treatment[11]. Additionally, we performed a secondary calculation of multivariable logistic regression adjusting for age, sex, regimen and initial intensity of therapy. Due to the small sample size of variant carriers there is significant potential for the introduction of bias during adjustment for multiple variables. However, we note that there were no significant differences in the adjusted odds ratio compared to the unadjusted RR predictions (Table 2.7).

Table 2.5: Severe Fluoropyrimidine-related Adverse Events During Cycles 1 & 2

	Genotype-guided Cohort							Retrospective Sample
	Non-Carrier (*N* = 1,347)	Carrier (*N* = 47)	*P*-value [a]	c.1905+1G>A (*N* = 9)	c.2846A>T (*N* = 19)	c.1679T>G (*N* = 1)	c.1236G>A (*N* = 18)	c.1236 G>A (*N* = 41)
	N (%)			*N* (%)				*N* (%)
Early Severe AEs [b] (Cycles 1-2), *N* (%)								
Global [c]	284 (21.1)	6 (13)	0.167	2 (22)	3 (16)	0 (0)	1 (5)	10 (24)
Gastrointestinal	131 (9.7)	3 (6)	0.616	1 (11)	1 (5)	0 (0)	1 (5)	5 (12)
Myelosuppression	102 (7.6)	5 (11)	0.401	2 (22)	2 (11)	0 (0)	1 (5)	1 (2)
Cardiac	26 (1.9)	0 (0)	>0.99	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
HFS	13 (1.0)	0 (0)	>0.99	0 (0)	0 (0)	0 (0)	0 (0)	2 (5)
Other [d]	68 (5.0)	2 (4)	>0.99	1 (11)	1 (5)	0 (0)	0 (0)	3 (7)
AE-related Death [e]	8 (0.6)	0 (0)	>0.99	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Discontinued Treatment [f]	137 (10.2)	5 (11)	0.808	2 (22)	1 (5)	0 (0)	2 (11)	4 (10)

[a] *P*-values for Fluoropyrimidine-related AEs calculated using the following tests: 'Global' utilized Chi-squared test; 'Gastrointestinal', 'Myelosuppression', 'Cardiac', 'HFS', 'Other', 'AE-related Death', and 'Discontinued Treatment' utilized Fisher's Exact Test.

[b] Grade ≥3 by Common Terminology Criteria for Adverse Events v.5.0.

[c] Global includes all fluoropyrimidine-related AEs grade ≥3 and fluoropyrimidine-related deaths. This does not include discontinuation.

[d] Other AEs included: fatigue, infections, neurotoxicities, and lab abnormalities.

[e] At least one fluoropyrimidine-related AE contributed significantly to death.

[f] Patients discontinuing treatment with fluoropyrimidines due to a fluoropyrimidine-related AE of any grade.

Abbreviations: AEs, adverse events; HFS, hand-foot syndrome; IQR, interquartile range.

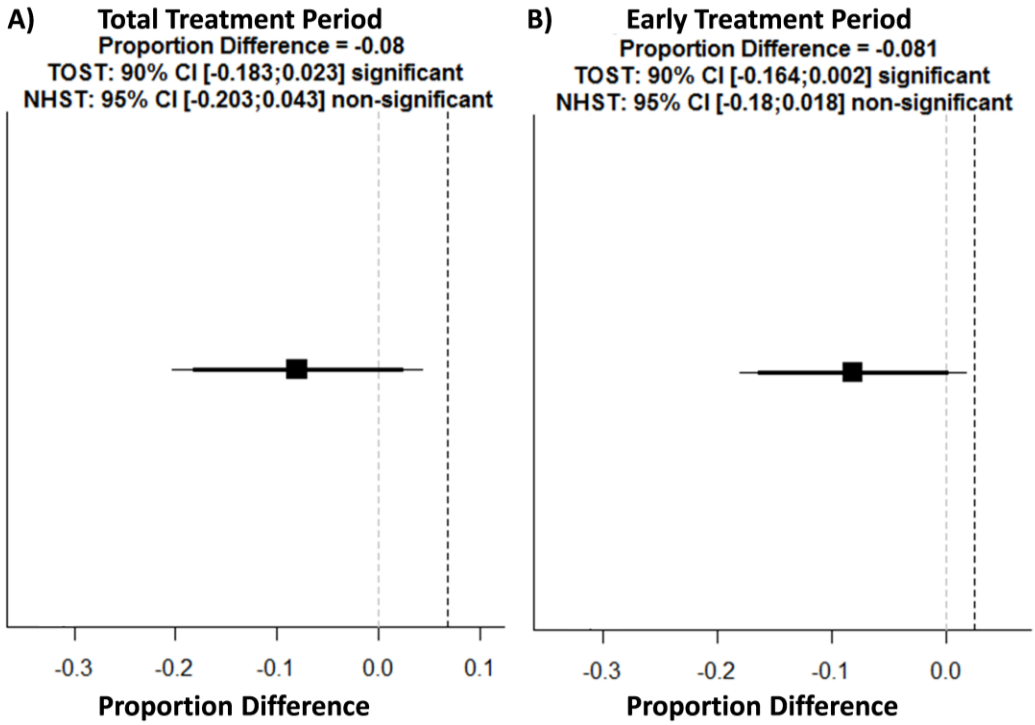

Figure 2.2 Noninferiority Testing for Total and Early Treatment Periods

Plotting results of non-inferiority comparison for global severe fluoropyrimidine-related AEs between genotype-guided variant carriers and non-carriers. Difference is variant carriers minus non-carriers. The genotype guided variant carriers do not experience an increased risk of severe fluoropyrimidine-related AEs in full follow-up (A), the inferiority bound is 6.82%. As well, the genotype-guided variant carriers do not experience an increased risk of severe fluoropyrimidine-related AEs during cycles 1 & 2 (B), the inferiority bound is 2.52%. Abbreviations: AEs, Adverse Events.

Table 2.6: Relative Risk of Severe Fluoropyrimidine-related Adverse Events

DPYD Variant	Genotype-guided Dosing Current Cohort [a] Unadjusted RR (95% CI) [d]	Patients Treated Without Genotype-guided Dosing [b] Unadjusted RR (95% CI) [d]	Genotype-guided Dosing Literature Cohort [c] Unadjusted RR (95% CI) [d]
c.1905+1G>A	1.08 (0.43-2.74)	2.87 (2.14-3.86)	1.31 (0.63-2.72)
c.2846A>T	0.85 (0.40-1.82)	3.11 (2.25-4.28)	2.00 (1.19-3.34)
c.1679T>G	NA [e]	4.30 (2.10-8.80)	NA [e]
c.1236G>A	0.54 (0.19-1.52)	1.72 (1.22-2.42)	1.69 (1.18-2.42)

[a] Our genotype-guided cohort: 50% dose reduction recommended for carriers of c.1905+1G>A, c.2846A>T, and c.1679T>G; 25% -50% dose reduction for carriers of c.1236G>A.
[b] Meulendijks *et al.* historical cohort derived from a meta-analysis[11]: standard of care dosing with adjustment due to tolerability resulting in the assumption that given no genotype was known the dose intensity was equivalent between *DPYD* variant carriers and non-carriers.
[c] Henricks *et al.* genotype guided cohort[15]: 50% dose reduction recommended for carriers of c.1905+1G>A or c.1679 T>G; 25% dose reduction for carriers of c.2856A>T or c.1236G>A. Followed by dose escalation pending patient tolerance.
[d] Unadjusted relative risks with 95% confidence intervals are discussed due to small sample size of variant carriers in genotype-guided cohorts. Risks are calculated compared to non-carriers of the individual variant of interest.
[e] Only one c.1679T>G carrier was detected in each genotype-guided cohort. In both cohorts, the carrier was treated with 50% dose reduction and did not suffer a fluoropyrimidine-related adverse event.
Abbreviations: CI, confidence interval; NA, not applicable; RR, relative risk.

Table 2.7: Adjusted Odds Ratio of Severe Fluoropyrimidine-related Adverse Events

DPYD Variant	Model 1 (Unadjusted) [a] OR (95% CI)	Model 2 (Age & Sex) [b] OR (95% CI)	Model 3 (Age, Sex, Regimen, Initial Intensity) [c] OR (95% CI)
c.1905+1G>A	1.12 (0.24-4.26)	1.07 (0.23-4.14)	1.80 (0.36-7.17)
c.2846A>T	0.80 (0.26-2.11)	0.81 (0.26-2.14)	0.78 (0.24-2.12)
c.1236G>A	0.45 (0.10-1.36)	0.43 (0.10-1.30)	0.43 (0.10-1.33)
c.1679T>G	NA [d]	NA [d]	NA [d]

[a] Model 1: OR for severe global fluoropyrimidine-related adverse events unadjusted for cofounders.
[b] Model 2: OR for severe global fluoropyrimidine-related adverse events adjusted for age and sex.
[c] Model 3: OR for severe global fluoropyrimidine-related adverse events adjusted for age, sex, regimen, and initial intensity.
[d] There was a sole c.1679T>G carrier in this study, they did not experience a severe adverse event.
Abbreviations: Odds Ratio (OR); Confidence Interval (CI); Not Applicable (NA).

2.3.4 Retrospectively Identified *DPYD* c.1236G>A carriers

DPYD c.1236G>A carriers identified retrospectively in May 2018 (N = 41) were removed from the genotype-guided cohort as they were treated as *DPYD* variant non-carriers. We predicted that these *DPYD* c.1236G>A carriers would experience an increased risk of fluoropyrimidine-related AEs given they were treated with standard dosing. However, *DPYD* c.1236G>A carriers treated with standard dosing did not experience an elevated toxicity profile (Table 2.4). In brief, 34% (14/41) of the retrospectively identified *DPYD* c.1236G>A carriers experienced a severe fluoropyrimidine-related AE during the total treatment period and 24% (10/41) experienced an early severe fluoropyrimidine-related AE (Table 2.5). In summary, compared to the genotype-guided cohort the unadjusted relative risk of global severe fluoropyrimidine-related adverse events was 1.09 (0.71-1.68).

2.4 Discussion

We report the impact of pre-treatment *DPYD* genotype-guided dosing on fluoropyrimidine-related AEs in a Canadian hospital assessed through retrospective follow-up of the Personalized Medicine Clinic. We show that when treated with genotype-guided dosing for *DPYD* c.1905+1G>A, *DPYD* c.1679T>G, *DPYD* c.2846A>T, or *DPYD* c.1236G>A, the proportion of variant carrying patients who experienced severe fluoropyrimidine-related AEs was not statistically different from non-carriers. We found that a 50% dose reduction for *DPYD* c.1905+1G>A and *DPYD* c.2846A>T carriers ameliorated the severe fluoropyrimidine-related AE risk compared to the historical RR for carriers treated with full dose (Table 2.6). Previously, Henricks *et al.* reported that a 25% initial dose reduction in carriers of *DPYD* c.2846A>T did not eliminate the elevated risk of severe fluoropyrimidine-related AEs[15]. Together these findings suggest that an initial 50% dose reduction is an appropriate dosing strategy for carriers of *DPYD* c.1905+1G>A and *DPYD* c.2846A>T, consistent with the current CPIC guidelines[9].

The Personalized Medicine Clinic attempted to provide *DPYD* genotype-guided dosing in alignment with the best available evidence. Indeed, the genotyping for *DPYD* c.1236G>A as a proxy for variant *DPYD* c.1129-5923C>G starting in 2018 reflects the latest CPIC guideline recommendations that note the association of *DPYD* c.1129-5923C>G with severe fluoropyrimidine-related AEs[11,24]. In order to account for this in this analysis we carried out retrospective genotyping for *DPYD* c.1236G>A for patients who had been enrolled prior to inclusion of this variant as part of the *DPYD* test panel. We hypothesized that our patients who were *DPYD* c.1236G>A carriers treated with standard dosing would exhibit an increased risk of fluoropyrimidine-related AEs in alignment with previous meta-analysis data as cited in the CPIC guidelines[11,14]. However, the retrospectively identified *DPYD* c.1236G>A carriers in our study did not demonstrate an increased risk. In the meta-analysis by Meulendijks *et al.* which demonstrated an increased risk associated with *DPYD* c.1236G>A the included studies consisted of only European populations (N = 4,261)[11]. Subsequently to the meta-analysis's publication, a large association study of American colorectal cancer patients (N = 1,953) demonstrated no

significant association between *DPYD* c.1129-5923C>G and fluoropyrimidine-related AEs (RR 1.27; 95%CI: 0.97-1.67) in their population[25]. In the American study they also confirmed that the proxy variant *DPYD* c.1236G>A was in complete LD with the causal variant *DPYD* c.1129-5923C>G. However, the American study did demonstrate a trend towards an association for the primary outcome and a statistically significant association in a secondary outcome linking the *DPYD* c.1129-5923C>G variant with severe neutropenia[25]. We propose the difference between the findings of Lee *et al.* and Meulendijks *et al.* and the proposed regional difference in overall fluoropyrimidine AE risk between American and European populations are related[11,21,25]. We suggest the lack of significant association in our *DPYD* c.1236G>A carriers may reflect an underlying difference in the baseline risk threshold between Europeans and North Americans. However, this difference was not proven and may be due to the limited sample size of retrospective *DPYD* c.1236G>A carriers in this study. CPIC currently supports a 50% dose reduction for *DPYD* c.1236G>A carriers, followed by dose escalation if the patient tolerates the reduced dose. More evidence is needed to elucidate the extent of the potential regional effect on carriers of this variant. In the mean time we continue to support the CPIC recommendations for *DPYD* c.1236G>A carriers. Given the uncertainty, therapeutic drug monitoring may be useful to limit AEs during dose escalations[26].

2.4.1 Limitations

The first major limitation of our study is the experimental design. A robust two-arm comparative study directly comparing genotype-guided dosing to standard of care therapy would have provided stronger evidence to support these findings. However, a two-arm comparative study was deemed inappropriate given the existing body of evidence associating *DPYD* variation and fluoropyrimidine-related AEs prior to initiating the program at the Personalized Medicine Clinic. The retrospective collection of AE outcomes also limits the design. However as listed in the methods sections, systems were in place to limit the bias of this data collection and the pragmatic nature was necessary given limitations of the clinic at the time of study initiation. As well our study design lacks disease progression or survival outcomes. However, it has previously been shown

that carriers of *DPYD* c.1905+1G>A treated with a 50% starting dose reduction achieved the same fluoropyrimidine exposure as non-carriers treated with standard dosing[20]. As well, a retrospective survival analysis showed no difference in survival outcomes between variant carriers receiving genotype-guided dosing and non-carriers receiving standard dosing[27]. These data suggest that the *DPYD* variant carriers treated with a dose reduction achieve the same systemic exposure and therapeutic outcomes. The four variants tested in this study have been validated in studies predominated by Caucasians of European descent, as was our study population. Additional *DPYD* variants may play an important role in other patient populations (e.g. *DPYD* c.557A>G in people of African descent[28]). Further research in other patient populations is needed to validate the utility of *DPYD* genotype-guided dosing in more diverse populations. Finally, this study employed only *DPYD* genotype testing as a pre-treatment screening method for DPD deficiency, we did not assess other methods of detecting DPD deficiency in this patient population.

2.4.2 Conclusion

Health Canada and the Food and Drug Administration include warnings that DPD deficient patients are at an increased risk of severe AEs on fluoropyrimidine product labels[1-4]. However, to date neither agency has recommended any pre-treatment screening methods despite consensus guidelines from expert groups in Europe[17,18]. In March of 2019 the French Medicines Agency triggered a formal review of pre-emptive DPD deficiency screening by the European Medicines Agency (EMA), and in April 2020 the EMA issued a recommendation for DPD deficiency testing prior to initiation of fluoropyrimidines. Our data support equivalent efforts to study and implement DPD deficiency screening through DPYD genotype testing be undertaken within North America.

2.5 Chapter 2 References

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Chapter 3

3 Case-Control study of Fluoropyrimidine-related Adverse Events and Dihydropyridine Dehydrogenase Exon 4 Deletion in Colorectal Cancer

3.1 Introduction

Chapter 2 demonstrated the clinical utility of *DPYD* genotype-guided fluoropyrimidine dosing. However, when testing targeted *DPYD* genotype-guided dosing approximately a third of *DPYD* wild-type patients experienced a severe fluoropyrimidine-related adverse event (AE). Given that *DPYD* is known to be highly polymorphic investigating additional and newly described genetic variants is key to ensuring the safety of fluoropyrimidine chemotherapy. Chapter 3 describes a nested case-control study screening for the presence of a recently discovered *DPYD* exon 4 deletion in the patient population reported in Chapter 2.

Historical estimates state that approximately one third of patients treated with fluoropyrimidine based chemotherapies experience severe adverse drug reactions[1,2]. This significant detriment to patients also represents a large burden on the healthcare system. Pharmacogenetics can play a role in limiting the risk of fluoropyrimidine-related AEs. Dihydropyrimidine dehydrogenase (DPD, E.C. 1.3.1.2, gene name *DPYD*) is the rate-limiting enzyme of fluoropyrimidine catabolism[3]. DPD deficiency results in reduced clearance of fluoropyrimidines and corresponding increased systemic exposure[4]. Increased systemic exposure is known to result in an increased risk for severe fluoropyrimidine-related AEs. As demonstrated in Chapter 1[5] and in the literature[6,7], proactively dose reducing patients carrying a decreased function *DPYD* allele is an effective strategy to protect those patients at greatest risk for fluoropyrimidine-related AEs. The Dutch Pharmacogenetics Working Group, the Swiss Group of Pharmacogenomics and Personalised Therapy and the European Medicines Agency recommend *DPYD* genotyping as an appropriate companion diagnostic in the prescribing of fluoropyrimidines[8-10]. Despite the growing support for *DPYD* genotyping the sensitivity of this method is low given the relative scarcity of currently accepted clinically actionable variants.

In populations of European descent, the combined carrier frequency of the four clinically actionable *DPYD* variants (c.1905+1G>A, c.2846A>T, c.1679T>G, and c.1236G>A) is approximately 7%[11]. Therefore, given standard of care dosing results in approximately one third of patients experiencing an AE, if genotype-guided dose reduction perfectly

protected variant carriers from severe fluoropyrimidine-related AEs there would be approximately one-fifth of patients experiencing AEs. These AEs that are unexplained by the current clinically relevant *DPYD* variants remain an important target for intervention. Henricks *et al.* found that when tested for the four clinically relevant variants 23% of *DPYD* "wild-type" patients still experienced a severe fluoropyrimidine-related AE[7]. As well, in Chapter 2 it was demonstrated that 31% of *DPYD* wild-type patients experienced a severe fluoropyrimidine-related AE[5]. Both of these genotype-guided studies conclude that *DPYD* genotype-guided fluoropyrimidine dosing is beneficial to patients and support its implementation but highlight avenues for further investigation.

The *DPYD* locus is large spanning over 840 kilobases in genomic length representing 3,078 bp of coding sequence broken into 23 exons. The four clinically relevant variants represent a small minority of the SNVs within this region that are estimated to have both a prevalence and phenotypic consequence that warrants upfront testing in populations of European descent[11]. Yet SNVs are not the sole source of genetic variation within the *DPYD* locus, additional variation such as whole gene copy number variation (CNV) and exonic deletions have also been reported[12-14]. To this end, Saarenheimo *et al.* recently published a prospective study in which *DPYD* variation was interrogated by targeted exon sequencing and multiple ligation dependent probe amplification (MLPA) prior to fluoropyrimidine chemotherapy in an effort to better identify patients with a genetic basis for DPD deficiency in a sample of 167 Finnish patients[15]. Combining Sanger sequencing with MLPA allows for the simultaneous detection of SNVs, and CNVs. Saarenheimo *et al.* found 9 (5.4%) carriers of known clinically relevant *DPYD* variants, and an additional 4 (2.4%) carriers of a previously undescribed *DPYD* exon 4 deletion. Saarenheimo *et al.* were unable to characterize the break point of this novel deletion due to the large flanking introns encompassing ~106 kb. However, they did confirm DPD deficiency in patients carrying the *DPYD* exon 4 deletion through assessment of DPD activity in peripheral blood mononuclear cells. The prevalence of this deletion in the Finnish population and the resulting clear DPD deficiency makes this deletion a promising candidate for a clinically relevant variant. To screen for the presence of this *DPYD* exon 4 deletion in our Canadian population we conducted a retrospective nested case-control study.

3.2 Patients and Methods

3.2.1 Patients

Patients for this study were selected from the previously published population characterized in chapter 2[15]. Briefly patients were originally recruited for a single-centre study of genotype-guided dosing at London Health Sciences Centre in London, Ontario, Canada between December 1, 2013 and November 30, 2019. The study was approved by the Institutional Review Board at Western University and all patients provided written informed consent. The sample size for this analysis was defined *a priori* to be 250 patients with a 1 case :1 control matching. Patients were considered to be cases if they experienced at least one severe fluoropyrimidine-related AE during their full follow-up period, and conversely control patients experienced no severe fluoropyrimidine-related AEs. Exclusion criteria included carrying a clinically relevant *DPYD* variant (*DPYD* c.1905+1G>A, *DPYD* c.1679 T>G, *DPYD* c.2846 A>T, and *DPYD* c.1236G>A), primary tumour site other than colon or rectum, or lacking sufficient DNA in the Personalized Medicine bio-bank for reanalysis. AE case and control patients were then matched by age, sex and treatment regimen.

3.2.2 Data Collection

Details on the generation of the patient level dataset have been discussed in chapter 2[5]. In brief, patients were seen at the Personalized Medicine Clinic for *DPYD* genotype-guided fluoropyrimidine dosing in alignment with the CPIC guidelines[11,16]. The patients were then pragmatically followed through their electronic health records including pharmacy dispensing records, clinic notes, and emergency room reports. All AEs were recorded and graded according to the National Cancer Institutes' Common Terminology Criteria for Adverse Events version 5.0[17], severe AEs are those events grade$\geq$3. Only those events considered severe and related to fluoropyrimidine therapy were included.

3.2.3 Detection of DPYD Exon 4 Deletion

We utilized TaqMan® copy number variation assay to quantify the presence of a *DPYD* exon 4 deletion. We utilized a FAM labeled probe against *DPYD* exon 4 (Thermo Fisher

Scientific, Cat: 4400291), and a Vic labeled probe against the control gene RNAse P (Thermo Fisher Scientific, Cat: 4316844). Patients were tested on 96 well plates in batches of 30 with healthy volunteer DNA samples used as cross refence between plates. Relative quantification (RQ) was determined according to manufacturer instructions, a RQ of approximately 1 is interpreted as diploid, a 50% reduction in RQ is a predicted haploid deletion.

3.3 Results & Discussion

3.3.1 Patients

Of 1,394 patients reported in the primary study there were 429 cases and 965 control patients to select from in this follow-up investigation. After excluding known *DPYD* variant carriers ($Case_n$: 11; $Control_n$: 36) and patients with primary disease sites other than colorectal cancer ($Case_n$: 178; $Control_n$: 390) we could choose a well-matched sample from the remaining 240 case and 538 control patients (Fig. 3.1). The *a priori* sample size was 250 patients matched 1 case: 1 control. The planned matching intentionally inflates the proportion of patients experiencing severe fluoropyrimidine-related AEs from 31% in the primary study to 50% in this investigation. This inflation was intended to increase the chances of detecting *DPYD* exon 4 deletion carriers in our population. The case and control groups were matched for sex, age and drug regimen with no large differences between groups in the baseline characteristics, summarized in Table 3.1.

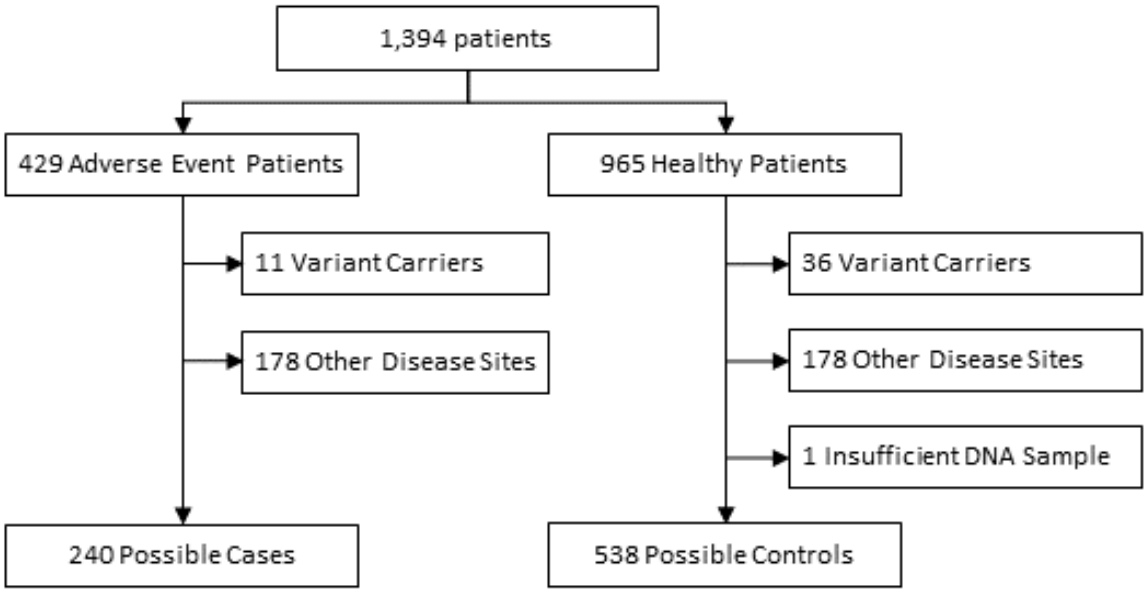

Figure 3.1: Patient Flow Diagram for Selecting Case-Control Sample

Patients for the nested case control were selected from the genotype-guided population described in chapter 2. Only patient's wildtype for the genotyped *DPYD* variants and with a primary tumour of the colon or rectum were eligible. 150 eligible cases were selected at random, control patients were matched to cases by age, sex and regimen.

Table 3.1 Baseline Characteristics

Characteristic	Case	Control
Total (N)	**125**	**125**
Sex (N)		
Female	63	63
Male	62	62
Age in years, mean (SD)	65.0 (10.4)	65.6 (9.9)
Race [a]		
White	124	114
Black	0	2
Asian	1	1
Unknown	0	8
Treatment characteristics		
BSA (m^2), mean (SD) [b]	1.9 (0.2)	1.9 (0.3)
Initial Dose Intensity, mean (SD) [c]	91 (14)	87 (14)
Average Dose Intensity, mean (SD)	81 (15)	84 (13)
Treatment Cycles, median (IQR) [d]	6 (3-7)	6 (3-8)
Regimens		
Capecitabine with radiation	14	13
Capecitabine monotherapy	35	35
Capecitabine with oxaliplatin	26	26
FOLFOX [e]	31	31
FOLFIRI/FOLFIRINOX [e]	13	14
5-FU with radiation	6	6

[a] Race was self-declared by participants based upon their grandparents, in some cases the patients were unable to provide the information.

[b] Body Surface Area.

[c] Dose intensity reflects the percentage of ideal dose for each patient given their regimen and body surface area.

[d] The number of treatment cycles attempted in each patient, some cycles were ended prematurely due to adverse events

[e] Includes patients with and without additional biologic therapy

3.3.2 Adverse Events

The adverse drug reactions seen in the case patients are representative of the major fluoropyrimidine-related AE toxicity categories. Each patient had at least one event therefore there are more events than patients in the case sample. The largest category was gastrointestinal events accounting for 44.5% of AEs, amongst which diarrhea was the most common AE in the sample. The next major category was myelosuppression, with severe neutropenia being the most common AE in this category. Finally, there were 25 cases of hand-foot syndrome (HFS, also known as palmar-plantar erythrodysesthesia). There were a small number of events that were determined to be fluoropyrimidine-related but did not fall under the classic toxicity categories. The most common AEs in the other category were infections, both respiratory and infective colitis, with the most severe outcome leading to fulminant sepsis. Less common AEs in the other category were severe fatigue and acute kidney injury including the sequelae of severe lab abnormalities. Finally, 5 patients that died due to fluoropyrimidine-related AEs were included in the study. The rate of fluoropyrimidine-related death in the primary study was only 0.6%[5], 2% in this small sample is an over representation. The over representation of death was intended to enrich the patient population to discover if the novel *DPYD* exon 4 deletion was present in any patients outside the Finnish population. The AEs included in the cases group are summarized in Table 3.2.

Table 3.2 Fluoropyrimidine-related Adverse Events

Category	No.
No. of patients	125
No. of Adverse Events	157
Gastrointestinal	
Diarrhea	47
Colitis	11
Mucositis [a]	6
Nausea/Vomiting [b]	4
Myelosuppression	
Neutropenia	31
Febrile Neutropenia	11
Anemia	2
HFS [c]	25
Other [d]	15
Death	5
[a] Includes Mucositis Oral and Esophagitis	
[b] Includes either nausea or vomiting	
[c] Hand-Foot Syndrome (defined as palmar-plantar erythrodysesthesia syndrome)	
[d] Includes: hypokalemia, acute kidney injury, infection, fatigue and a maculopapular-rash	

3.3.3 Exon 4 Deletion

We identified one patient with a haploid *DPYD* exon 4 deletion, representing an overall Minor Allele Frequency (MAF) of 0.2% or 0.4% amongst patients that experienced a fluoropyrimidine-related AE (Fig. 3.2). This patient was a 78-year old female with stage IV colorectal adenocarcinoma. This patient was receiving palliative intent capecitabine monotherapy initiated at 100% ideal dose intensity. They experienced grade 2 oral mucositis during the first cycle of therapy, and was given analgesia for the discomfort. The capecitabine dose was reduced by 35%, however by the end of the second cycle they had developed grade 3 diarrhea and grade 2 HFS in addition to persistent grade 2 oral mucositis. The patient was provided supportive therapy through IV fluid resuscitation and analgesia, the capecitabine therapy was held for three weeks to allow the patient to recover. Following the resolution of these AEs capecitabine monotherapy was reinitiated with an additional 30% reduction (now at 35% ideal dose). The patient was able to continue on for an additional 9 cycles of capecitabine monotherapy before discontinuing due to a change in the goals of care.

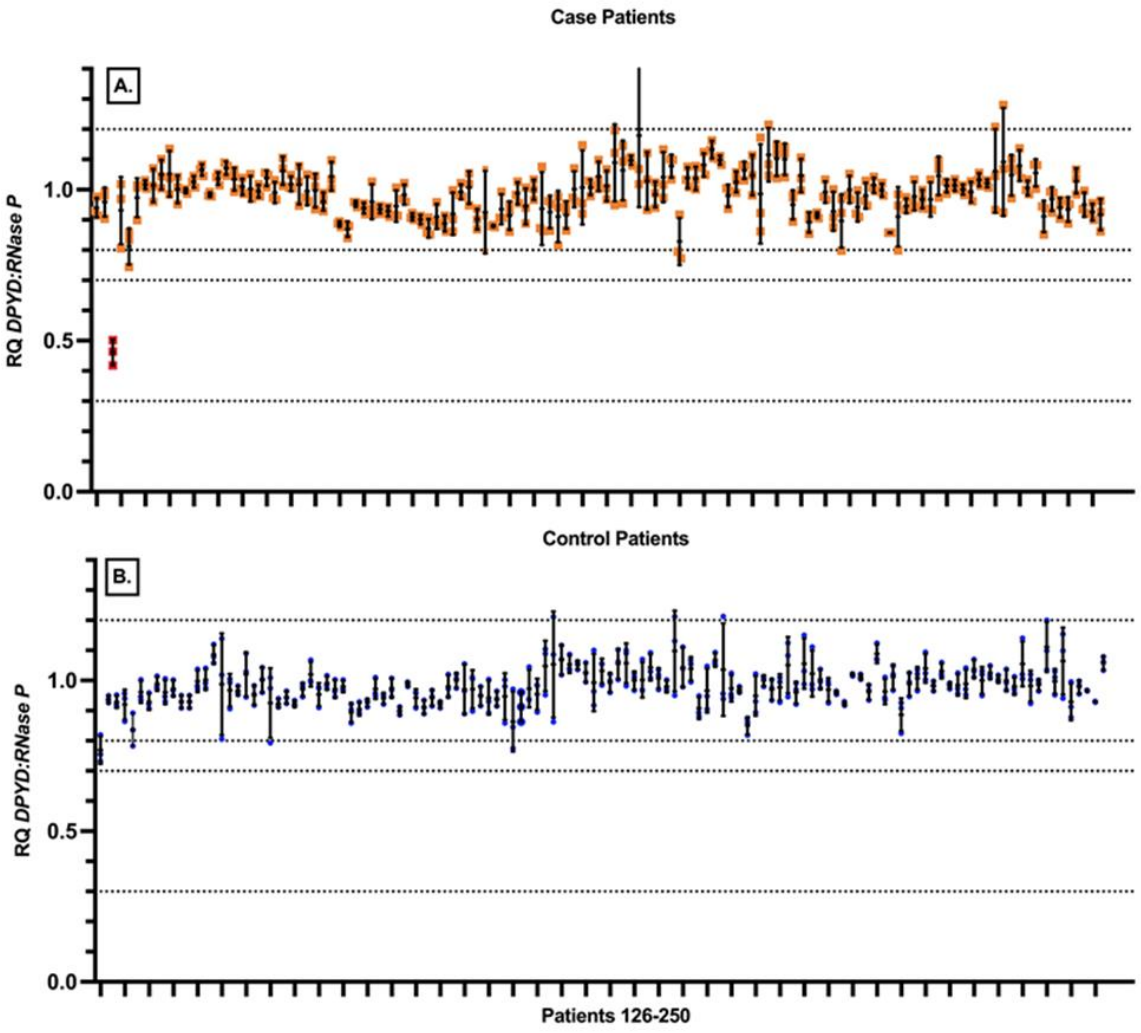

Figure 3.2. *DPYD* Exon 4 Copy Number Variation

Plotting *DPYD* exon 4 amplification relative to *RNaseP* as relative quantification (RQ) values for case (A.) and control (B.) patients. A RQ value of 1 suggests a diploid copy number, while a ratio of 0.5 implies a haploid deletion. There is a single patient with a predicted deletion of *DPYD* exon 4 amongst the case patients highlighted in red. Points are replicates, bars are mean ± standard deviation.

3.3.4 Discussion

A retrospective nested case-control study was conducted to test for an association between a recently identified *DPYD* exon 4 deletion and fluoropyrimidine-related AEs. We identified a single patient carrying a haploid deletion (MAF 0.2%), this patient experienced a severe fluoropyrimidine-related AE during therapy. The scarcity of deletion carriers identified in this enriched design implies the true MAF in our population is likely much lower than that reported in the Finnish study (1.2%). This may limit the applicability of pretreatment screening for this deletion for patients not of Finnish descent. However, it is crucial to note that amongst the *DPYD* variants currently considered clinically actionable, *DPYD* c.1679T>G has a MAF of ~0.03%. This acknowledges there are two components in determining the clinical actionability of genetic variants, with both the prevalence and phenotypic effect being crucial aspects. While no direct *in vitro* characterization has been completed for this novel *DPYD* exon 4 deletion there are two significant pieces of evidence to suggest that deletion of exon 4 results in a complete loss of DPD enzymatic activity. First, Saarenheimo *et al*. assessed the DPD activity in PBMCs for three *DPYD* exon 4 deletion carriers, and demonstrated significantly decreased DPD activity. Second, van Kuilenburg *et al*. have previously demonstrated that a splice site variant which skips exon 4 results in a frame shift and consequent premature stop codon producing a non-functional DPD protein[14]. These two findings suggest that *DPYD* exon 4 deletion would result in a patient with DPD deficiency. Therefore, we suggest that while the novel *DPYD* exon 4 deletion true MAF outside of Finland is likely <0.2% the variant should still be considered as a future candidate for clinical testing. Elucidating the clinical relevance of the *DPYD* exon 4 deletion requires large scale determination of the MAF, and confirmatory associations studies as have been completed for the four current clinically actionable *DPYD* variants.

3.3.5 Conclusion

The deletion of *DPYD* exon 4 can be identified in Canadian patients. However, the low observed frequency of this variant requires further characterization before a recommendation of its clinical utility can be confidently made.

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Chapter 4

4 Investigating the association between plasma folate status and fluoropyrimidine-related adverse events

4.1 Introduction

Chapters 2 and 3 investigated the role of *DPYD* genetic variation in fluoropyrimidine-related AEs. It is clear that *DPYD* genotype-guided dosing is an effective method to prevent a subset of fluoropyrimidine-related AEs in Ontario[1]. However due to the rarity of clinically relevant genetic variants compared to the overall burden of fluoropyrimidine-related AEs, *DPYD* genotyping alone cannot protect the majority of at-risk individuals prior to treatment with fluoropyrimidines. Studies that test the influence of additional clinical and genetic covariables are needed to improve our ability to identify at-risk patients. Chapter 4 reports on the association between total plasma folate and fluoropyrimidine-related AEs.

Fluoropyrimidine chemotherapeutics such as 5-fluorouracil (5-FU) and capecitabine act as anti-metabolites through interfering with nucleotide synthesis and incorporation of fluorinated nucleotides into DNA and RNA molecules. Both capecitabine and 5-FU are metabolized to 5-fluorodeoxyuridine monophosphate (FdUMP), which is an irreversible thymidylate synthase (TS) inhibitor[2,3]. TS uses the co-factor 5,10-methylenetetrahydrofolate (5,10-MTHF) to catalyze the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP). When TS is inhibited by fluoropyrimidine metabolites, the imbalance in dUMP and dTMP impairs DNA synthesis, destabilizes DNA and leads to cell death[4]. For effective inhibition of TS, FdUMP must interact with TS in the presence of the co-factor 5,10-MTHF[2,3]. During the clinical development of 5-FU it was suggested that the necessity of the 5,10-MTHF co-factor was limiting the efficacy of 5-FU as a single agent[5-8]. This hypothesis led to the testing of folinic acid as an adjuvant to 5-FU chemotherapy; folinic acid is an exogenous formylated folic acid that is readily converted to 5,10-MTHF *in vivo*[9]. Folinic acid has proven to improve the therapeutic index of 5-FU[10,11]. In contrast, when folinic acid is used as an adjuvant to capecitabine the toxicity profile is significantly worsened and this application failed in early phase clinical trials[12,13].

The ternary complex of TS, 5,10-MTHF and FdUMP link the efficacy of fluoropyrimidines to the availability of components in the folate cycle. This suggests that the folate status of individuals may impact the efficacy of fluoropyrimidine

chemotherapy. For example, in an animal model of colorectal cancer tumours, folate deficient mice were resistant to 5-FU, however this effect was reversed by the concomitant administration of folinic acid[14]. The clinical relevance of plasma folate status during fluoropyrimidine-chemotherapy has yet to be resolved and the limited number of studies in this area have produced conflicting results[15-19]. Sharma *et al.* originally reported a significant association between serum folate level and severe fluoropyrimidine-related AEs in a secondary safety analysis of 81 patients treated with capecitabine monotherapy[19]. While Yap *et al.*[17]and Chan *et al.*[16] purported to have replicated the association in capecitabine treated patients both these articles used a modified definition of severe adverse event lowering the grade of severity from 3 to 2, confusing the utility of this data. Amongst patients treated with 5-FU and folinic acid Alvarez-Cabellos *et al.* failed to detect a significant association[18], but Yan *et al.*[15] reported a positive association using a compound outcome that included grade $\geq$3 toxicity and/or discontinuing therapy. The controversy surrounding these findings and limited sample sizes of the published works warrants further interrogation of the association between folate levels and fluoropyrimidine-related AEs. Chapter 2 reported on genotype-guided fluoropyrimidine dosing completed at the Personalized Medicine Clinic in London Ontario. During this project patients were requested to donate plasma to the Personalized Medicine biobank for future research. Given the large biobank that was accumulated over six-years of enrollment, this resource represented the largest sample available for testing the association between plasma folate status and the risk of fluoropyrimidine-related AEs. However, measuring folate status in archival plasma samples requires special consideration due to the instability of folate during sample collection, processing and storage.

Folate species are sensitive to oxidation in blood samples which ultimately leads to cleavage of the bond between the pterin ring and p-aminobenzoic acid subunits[20]. Compounding this issue folate remains unstable despite storage down to -20^{o}C[21-24]. Fortunately, Hannisdal *et al.* have developed a method for determining the plasma folate level through the quantitative conversion of folate and its degradation products to a core component namely p-aminobenzoyl glutamate (N-(4-Aminobenzoyl)-L-glutamic acid (pABG))[25]. This validated high pressure liquid chromatography tandem mass

spectrometry (HPLC-MS/MS) method allows for the analytical recovery of folate as pABG equivalents from multiple storage conditions[26,27]. Using an adapted version of this method allowed interrogation of the total folate status of patients seen for *DPYD* genotype-guided fluoropyrimidine dosing using archival plasma samples. In addition to total plasma folate we interrogated genetic variation within methylenetetrahydrofolate reductase (MTHFR, gene *MTHFR*). MTHFR catalyzes the reduction of 5,10-MTHF to 5-methyltetrahydrofolate (5-MTHF) reducing the pool of the essential co-factor 5,10-MTHF. Due to this interaction it has been hypothesized that decreased MTHFR activity may be associated with increased fluoropyrimidine-related AEs[28]. There are two common *MTHFR* variants that result in decreased enzymatic activity *MTHFR* c.1298A>C (rs1801131) and *MTHFR* c.677C>T (rs1801133) which have been the primary targets of past association studies[28]. However previous analyses have not accounted for the underlying total plasma folate levels and *MTHFR* variant status together. Therefore, this chapter reports on the retrospective analysis of total plasma folate status in conjunction with *MTHFR* variants amongst *DPYD* wild-type patients treated with fluoropyrimidine chemotherapy. The standing hypothesis in the clinical literature is that patients with high endogenous plasma folate levels are primed for increased inhibition of TS by fluoropyrimidines. The predicted clinical outcome of the increased TS inhibition is an increase toxicity profile amongst patients with high endogenous folate levels.

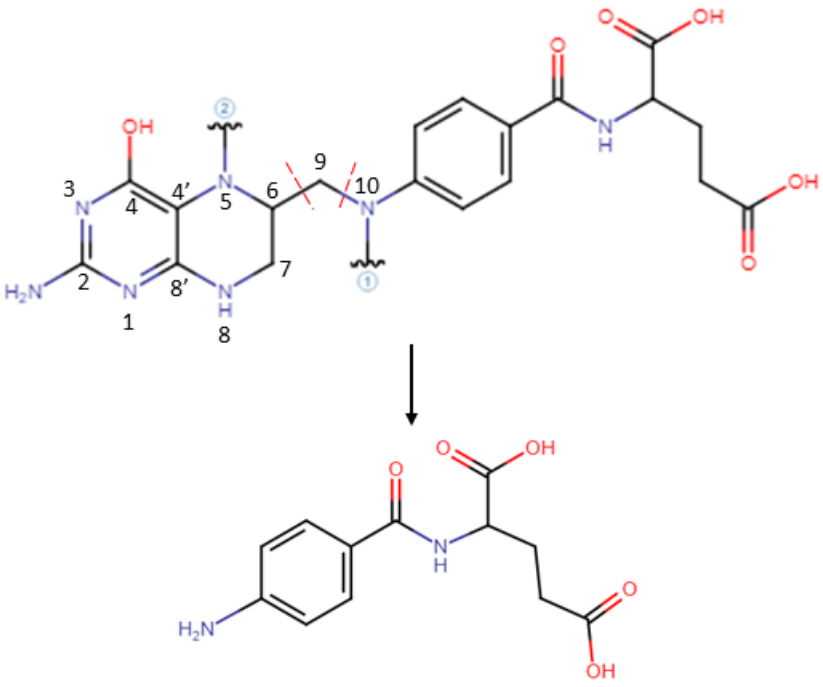


Figure 4.1 Structures of generic mono-glutamated folate backbone and pABG

The folate backbone shared between the different species differs based upon the bond structure between atoms 5-6, 7-8 and based upon the R groups at position 1 and 2. The bonds between 5-6 and 7-8 are sensitive to oxidation during sample collection, and worse yet the 6-9 and 9-10 bonds are sensitive to oxidative cleavage during sample collection. These degradations make it impossible to accurately determine the concentration of different folate species if samples are not carefully collected and stored immediately. However, given the shared backbone, chemically driving the lysis of the 9-10 bond allows for the collection and measurement of the remaining portion of the backbone, giving a relative folate equivalent. In plasma folates are mono-glutamated and therefore the fragments are measured as p-aminobenzoyl glutamate (pABG).

4.2 Patients and Methods

4.2.1 Patients

Patients for this study were selected from the previously published population characterized in chapter 2[1]. Patients were originally recruited for a single-centre study of genotype-guided dosing at London Health Sciences Centre in London, Ontario, Canada between December 1st, 2013 and November 30th, 2019. The study was approved by the Institutional Review Board at Western University and all patients provided written informed consent. Pre-treatment whole blood samples were drawn in K_2EDTA vacutainer tubes, 400 µL of whole blood was used for genomic DNA extraction using the MagNA Pure Compact Instrument (Roche) platform. Remaining whole blood was separated by centrifugation at 5,000 x g for 10 minutes at 4 ^{o}C and up to 1 mL of plasma was retained and stored at -80 ^{o}C. Selection of the sample for retrospective assessment of total plasma folate status was conducted amongst *DPYD* wild-type patients (i.e. exclude carriers of *DPYD* c.1905+1G>A, *DPYD* c.1679 T>G, *DPYD* c.2846 A>T, or *DPYD* c.1236G>A). Patients with gastrointestinal primary tumours were genotyped as part of an unpublished candidate gene study using a custom Open Array TaqMan ® genotype panel (Thermo Fisher Scientific). Patients not genotyped on the Open Array panel or failing to have genotype calls for either *MTHFR* variants of interest were excluded from the study. Lastly, patients with insufficient archival plasma samples (<100 µL) were excluded.

4.2.2 Adverse Event Outcomes

Details on the generation of the patient level dataset have been discussed in Section 2.2.3 Data Collection[1]. Briefly, patients referred to the Personalized Medicine Clinic prior to fluoropyrimidine chemotherapy were provided *DPYD* genotype-guided dosing in alignment with the contemporary CPIC guidelines[29,30]. The patients were then pragmatically followed through their electronic health records including pharmacy dispensing records, clinic notes, and emergency room reports. All AEs were recorded and graded according to the National Cancer Institutes' Common Terminology Criteria for Adverse Events version 5.0[31], severe AEs are those events grade ≥3. Only those events considered severe and related to fluoropyrimidine therapy were included. The primary

outcome in this study was any severe fluoropyrimidine-related AE during the full follow-up period (Total AEs). The secondary outcome in this study was having a severe fluoropyrimidine-related AE during the first two cycles of chemotherapy (Early AEs).

4.2.3 Chemicals

pABG (cat: A593770), and tamoxifen (cat: D786540) standards were obtained from Toronto Research Chemicals. Reagents used in the quantitative conversion of endogenous folate species to pABG were all ACS Reagent grade including perchloric acid (cat: 77230), potassium hydroxide (cat: 221473-500G), potassium bicarbonate (cat: 237205-100G), potassium permanganate (cat: 223468-25G), hydrogen peroxide (cat: 216763-100ML), and acetic acid (cat: 695092-5000ML-GL), purchased from Sigma-Aldrich. All solvents were HPLC-grade including acetonitrile (cat: A996-4), methanol (cat: A546-4) and water (cat: W6-4) purchased from Fisher Chemical. pABG stocks were prepared in 20mM phosphate buffer with 1% (m/v) ascorbic acid (Sigma cat: A02778-25G) and 10%(v/v) acetonitrile in water then adjusted to pH 7.2. The stock solutions of pABG were aliquoted and stored at -80 ^{o}C. Tamoxifen stocks were prepared in acetonitrile and stored at -20 ^{o}C.

4.2.4 Conversion of Endogenous Folate to pABG

This assay was adapted from the work of Hannisdal *et al*. to use tamoxifen as internal calibrator and manual pipetting[25]. Hannisdal *et al*. used a carbon (^{13}C) labeled internal standard during their assay development, we were unable to attain a ^{13}C pABG standard. Attempts were made to use a deuterated pABG internal standard, however due to the conditions to the ionization the deuterated standard was susceptible to proton exchange with the solvent and the result was a decay in the internal standard signal. Therefore, tamoxifen was used as an alternative, this calibrator does not define the retention time but the signal was stable and account for inter-sample variation. First 100 µL of plasma was mixed with 15 µL of 100 µM tamoxifen in acetonitrile. Next, the plasma was deproteinized by adding 33 µL of 1.6 M perchloric acid. Samples were gently mixed by hand and the protein precipitate was pelleted by centrifugation at 5,000 x g for 5 minutes. Next, 90 µL of the supernatant was transferred to a fresh tube and mixed with 34µL of a

solution containing 1.44 M KOH and 1.2 M potassium bicarbonate. Potassium perchlorate was allowed to precipitate for 15 minutes at room temperature before centrifugation at 10,000 x g for 5 minutes. 80 µL of the supernatant was transferred to a new tube and treated with 7 µL of 0.13 M potassium permanganate. After 20 minutes standing at room temperature, 10 µL of hydrogen peroxide (30%(v/v)) was added causing the rapid release of gas. The solution was then mixed by vortex and spent 20 minutes at room temperature before centrifugation at 10,000 x g for 5 minutes. Then 80 µL of the supernatant was pipetted to a new tube with 15 µL of 3 M perchloric acid. This final mixture was allowed to stand for 30 minutes at 4 ^{o}C before centrifugation at 12,500 x g for 10 minutes. The final transfer of 80µL of the supernatant was loaded into pre-cooled sample vials for storage at 4 ^{o}C. Prepared samples were stored at 4 ^{o}C for up to 24 hours prior to initiating the HPLC-MS/MS procedure, total run length of each HPLC-MS/MS batch was up to 24 hours.

4.2.5 HPLC-MS/MS

Processed plasma samples were placed in a 4^{o}C sample tray. The ZORBAX Stable Bond C8 reversed-phase column (150mm x 4.6 mm inner diameter; particle size, 3.5µm, Agilent Technologies) was conditioned for 30 minutes with 520mM acetic acid in water with a 0.5mL/min flow rate prior to each run. The mobile phases were 520mM acetic acid in water (Solvent A) and 100% methanol (Solvent B). 60µL of the sample was injected through a 100 µL loading loop and the chromatography gradient was performed as follows: 0–0.2 minutes, 100% A at 0.75 mL/min; 1.0 minute, 60% A at 0.75mL/min; 2.99 minutes, 30% A at 0.75mL/min; 3.0–4.0 minutes, 30% A at 0.5mL/min; 4.01 minutes, 30% A at 0.75 mL/min; 4.20 minutes, 5% A at 0.75 mL/min; 7.20 minutes, 5% A at 0.75 mL/min; 7.4 minutes, 100% A at 0.75 mL/min and finally 11 minutes, 100% A at 0.75 mL/min . All gradient steps were linear. The column effluent was directed to waste for the first 3 minutes and loaded into the mass spectrometer between 3.01-11 minutes. The electrospray ionization source conditions included a temperature of 400 °C, voltage of 3,500 V, sheath gas pressure of 50 psi and auxiliary gas pressure of 10 psi. The analytes were detected by selective-reaction monitoring in the positive-ion mode of the TSQ-Vantage mass spectrometer (Thermo Fisher Scientific). pABG was monitored

through the transition of 267.1→120.1 m/z with a collision energy of 17 eV eluting at a retention time of 3.65 minutes, while tamoxifen was monitored through the 372.0→72.0 m/z transition with a collision energy of 24 eV eluting at 6.52 minutes. The standard curve used to quantify pABG consisted of 6 concentrations ranging from 5-100 nM, with each curve measured with three intra-run quality control (Qc) samples and three inter-run Qc samples. The standard curves were prepared in triplicate with pABG spiked into pooled plasma from healthy volunteers, the background pABG was measured in triplicate and subtracted from the remaining points of the curve.

4.2.6 Statistical Analysis

Descriptive statistics are presented as mean (standard deviation), count (percentage), and median (inter-quartile range) as appropriate. Before performing multivariate logistic regression, individual covariates were compared against the primary and secondary outcomes. As well prior to multivariate modeling assessment of collinearity between pABG concentrations and baseline covariates was completed. Continuous variables (age, BSA) were visualized by scatter plots and tested using a simple linear regression, while categorical variables (sex, drug, genotypes) were plotted by variable state and tested using unpaired student's t-test or one-way ANOVA as applicable. Multivariate logistic regression was performed with the base model including pABG, age and sex for face validity, the drug variable was added due to the results of univariate analysis. Model performance was compared using receiver-operator curves and predicted vs observed probability curves. The primary statistical analyses were performed GraphPad Prism version 9.0.0 for Mac OS (GraphPad Software), and post-hoc power analysis was completed in G*Power version 3.1.9.7[32].

4.3 Results

4.3.1 Patients

From the 1,394 patients treated with *DPYD* genotype-guided dosing in Chapter 2[1], 8 patients declined to be included in the Personalized Medicine biobank and the 47 *DPYD* variant carriers were excluded. A total of 639 patients were assessed on the Open Array genotype panel, 14 patients failed to amplify for *MTHFR* rs1801131 and 2 additional patients failed to amplify for *MTHFR* rs1801133. An additional 48 patients had insufficient plasma samples for analysis of pABG (Fig. 4.2). The final sample of 575 patients had a mean age of 65 (12) years and 94.8% of the patients were white. As race was a self-declared variable during study enrollment it is unknown for 6.1% of patients, and Black and Asian individuals compose less than 1% each of the sample. The vast majority of patients had colorectal (89.6%) or gastroesophageal (9.7%) tumours, while pancreatic and anal primary tumours made up less than 1% each of the sample. This patient population differs in the representation of disease sites compared to the total population presented in section 2.3.1 Study Population. The genotype panel was targeted towards patients with gastrointestinal tumours, therefore no head and neck or breast cancer patients were included in the current study. The limited number of disease sites subsequently reduces the number of treatment regimens used in this sample population. Reducing potential confounding regimens theoretically improves the power to detect the true association between pABG and AEs. A sample size of 575 patients is powered to detect 1.25 odds increase in AE risk for each SD above the mean pABG concentration. The median pABG concentration was 15.3 nM (IQR: 9.5-25.2), and the mean pABG concentration was 20.0 nM (19.9). A summary of the adverse events in this patient sample are noted in Table 4.2. During complete follow-up 35.6% of patients experienced a severe fluoropyrimidine-related AE (Total AEs). Within the first and second cycles of chemotherapy 23.6% of patients experienced a severe fluoropyrimidine-related AE (Early AEs). Both the proportions of Total and Early AEs are slightly greater than seen in the total patient population, however this was an insignificant difference. The distribution of AEs across the toxicity categories is similar to those reported for the *DPYD* wild-type patients discussed in section 2.3.3 Toxicity Outcomes.

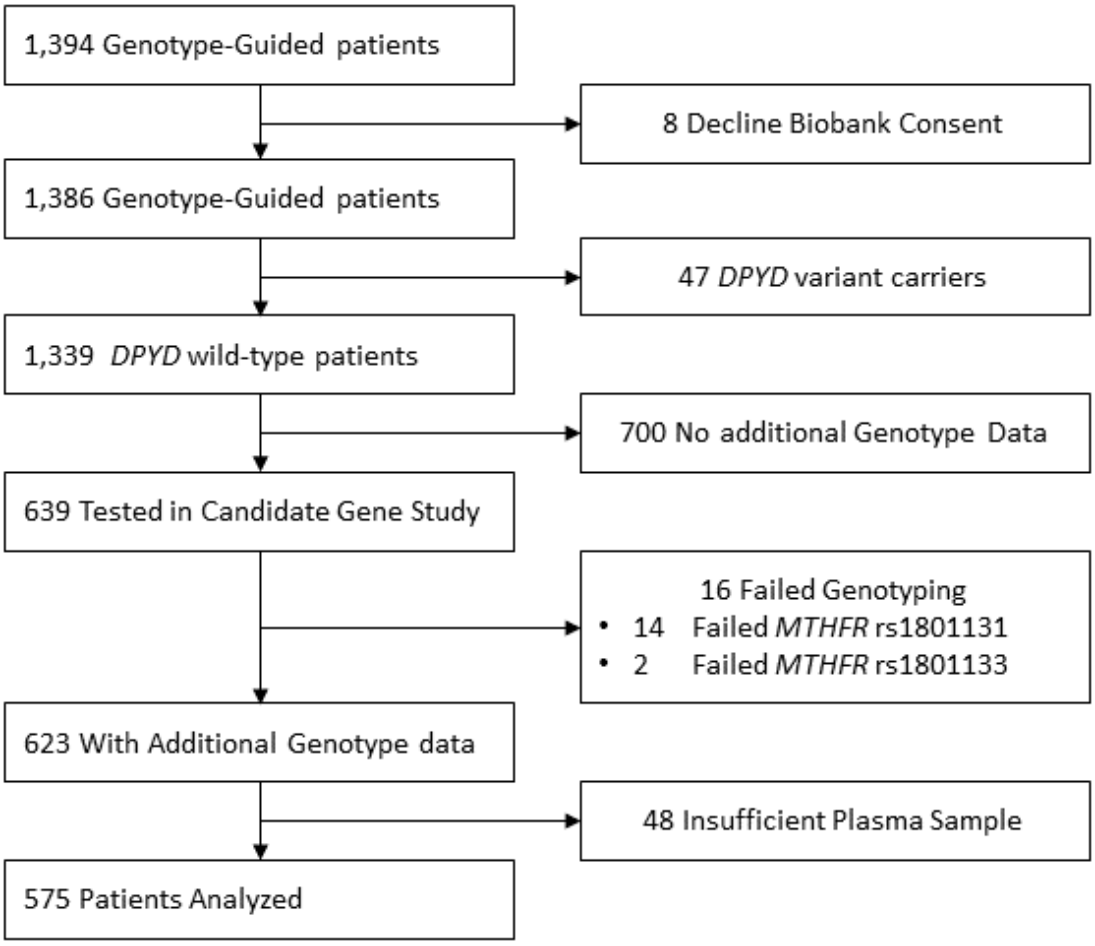


Figure 4.2 CONSORT Diagram

Flowchart of patient inclusion and exclusion. The patient sample was selected from the final cohort reported in Chapter 2.

Table 4.1 Baseline Characteristics

pABG, median (IQR)	15.3	(9.5-25.2)
Sex	**N**	**%**
Female	241	43.1
Male	318	56.9
Age in years, mean (SD)	65	(12)
Race	**N**	**%**
White	530	94.8
Other[a]	11	2.0
Unknown	34	6.1
Tumour Site	**N**	**%**
Colorectal	516	92.3
Gastroesophageal	55	9.8
Other[b]	4	<1%
Stage	**N**	**%**
I	18	3.2
II	97	17.4
III	297	53.1
IV	163	29.2
Treatment characteristics	**N**	**%**
BSA (m2), mean (SD)	1.9	(0.2)
Initial Dose Intensity, mean (SD)	89	(15)
Average Dose Intensity, mean (SD)	85	(14)
Regimens	**N**	**%**
Capecitabine with radiation	154	27.5
Capecitabine monotherapy	114	20.4
Capecitabine with oxaliplatin	69	12.3
FOLFOX	150	26.8
FOLFIRI/FOLFIRINOX	28	5.0
5-FU with other agents[c]	60	10.7
***MTFR* rs1801131**	**N**	**%**
T/T	275	47.8
T/G	243	42.3
G/G	57	9.9
***MTFR* rs1801133**	**N**	**%**
G/G	255	44.3
G/A	262	45.6
A/A	58	10.1
[a] Other race includes <1% each of Black, Asian and/or First Nations individuals		
[b] Other sites include <1% each of pancreatic and anal tumours		
[c] Other agents included Cisplatin/Carboplatin, mitomycin, and deGramont regimen		

Table 4.2 Summary of Fluoropyrimidine-related Adverse Events

Complete Follow-up (Total AE)	**N**	**%**
Global[a]	205	35.6
Gastrointestinal	73	12.6
Myelosuppression	77	13.3
Cardiac	20	3.4
Hand-Foot Syndrome	19	3.3
Other[b]	57	9.9
Death	4	0.6
Cycles 1 & 2 (Early AE)	**N**	**%**
Global[a]	136	23.6
Gastrointestinal	62	10.7
Myelosuppression	48	8.3
Cardiac	16	2.7
Hand-Foot Syndrome	6	1.0
Other[b]	30	5.2
Death	4	0.6
[a] Global includes at least 1 fluoropyrimidine-related adverse event from any toxicity category.		
[b] Other includes fluoropyrimidine-related adverse events such as infections, fatigue, neurotoxicity, acute kidney injury and the sequelae there-of.		

4.3.2 Univariate Association with Adverse Events

Each baseline characteristic and treatment characteristic were compared individually against the outcomes of both Total AEs (Fig. 4.3) and Early AEs (Fig.4.4) prior to developing the multivariate logistic regression model. Plasma pABG concentration was a poor predictor of both Total AEs (Tjur's R^2 0.001) and Early AEs (Tjur's R^2 0.001). In an unbiased assessment of multivariate regression this would lead to plasma pABG concentration being excluded from the model. However due to the intent of the study to test for the association between pABG and fluoropyrimidine-related AEs it was retained for the multivariate modeling. An increase in age has previously been reported to correlate with an increased risk for severe fluoropyrimidine-related AEs[33,34]. Yet, in this sample of patients, age poorly predicted both the Total AE (Tjur's R^2 0.001) and Early AE (Tjur's R^2 0.002) outcomes. Despite being a poorly predictive factor in the univariate analysis age was selected for inclusion in the multivariate analysis for face validity of the model given the previously published reports. The treatment characteristics of body surface area (BSA) and average dose intensity during treatment also failed to discriminate between healthy patients and those experiencing a fluoropyrimidine-related AE for both the Total AE and Early AE outcomes. Without additional support from the literature or being driven by the hypothesis of the study these variables were not appropriate for inclusion in the multivariate modeling. In contrast both sex and which drug (capecitabine or 5-FU) the patient's regimen utilized, were predictive of fluoropyrimidine-related AEs in univariate analyses. Treatment regimen was simplified to the drug variable because there were too few events in each regimen to reliably incorporate regimen into multivariate modeling in the future. In this univariate analysis it is clear that patients treated with capecitabine had a lower risk of experiencing a fluoropyrimidine-related AE for both the Total AE ($p<0.001$) and Early AE ($p<0.001$) outcomes. As well sex was predictive of the fluoropyrimidine-related AEs with women at increased risk for Total AEs ($p=0.004$), but the association was not significant for the Early AE outcome ($p=0.131$). Neither *MTHFR* rs1801131 or *MTHFR* rs1801133 were individually predictive for fluoropyrimidine-related AEs in either Total AE or Early AE outcomes.

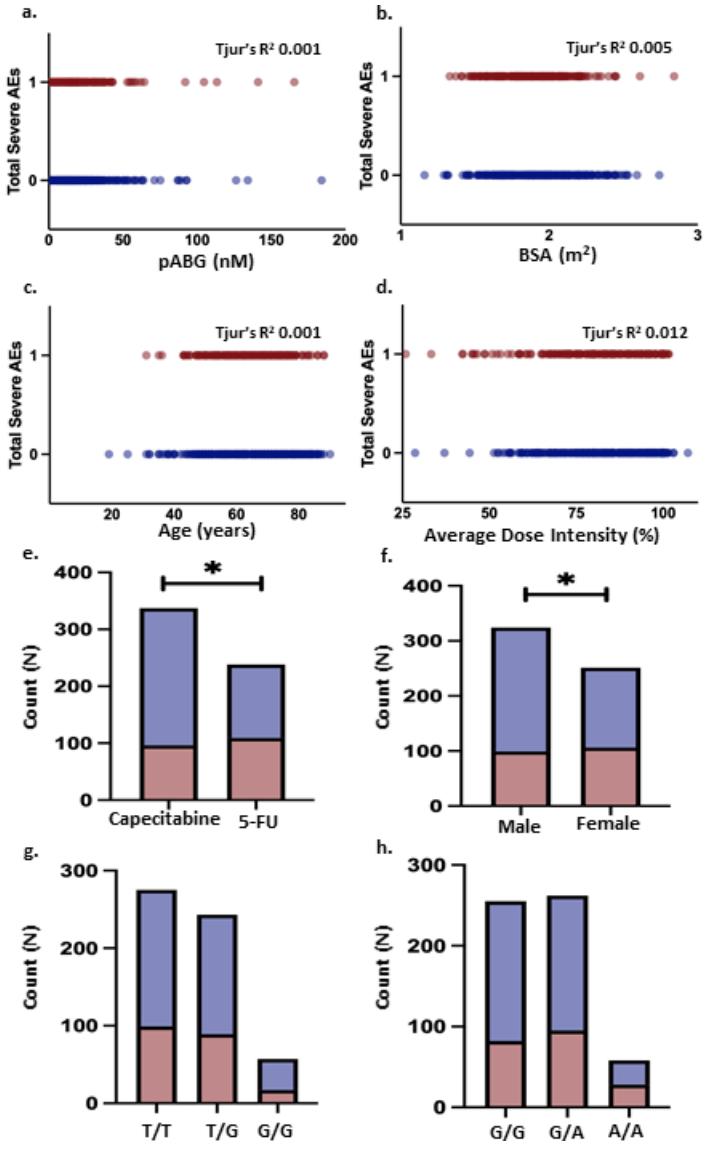


Figure 4.3 Univariate Analysis of Total Adverse Events

Continuous characteristics including pABG concentration (a.), body surface area (BSA) (b.), age (c.), and average dose intensity (d.) were assessed by logistic regression. None of the continuous characteristics had a significant association with the Total AE outcome. Categorical characteristics including drug (e.), sex (f.), *MTHFR* rs1801131 (g.), and *MTHFR* rs1801133 (h.) were assessed by χ^2 tests. Patients treated with 5-FU were at a significantly increased risk compared to capecitabine, and females were at a significantly increased risk compared to males. In contrast neither *MTHFR* variant rs1801131 or 1801133 had a significant association. (Adverse Events in Red, No Events in Blue). *p<0.05

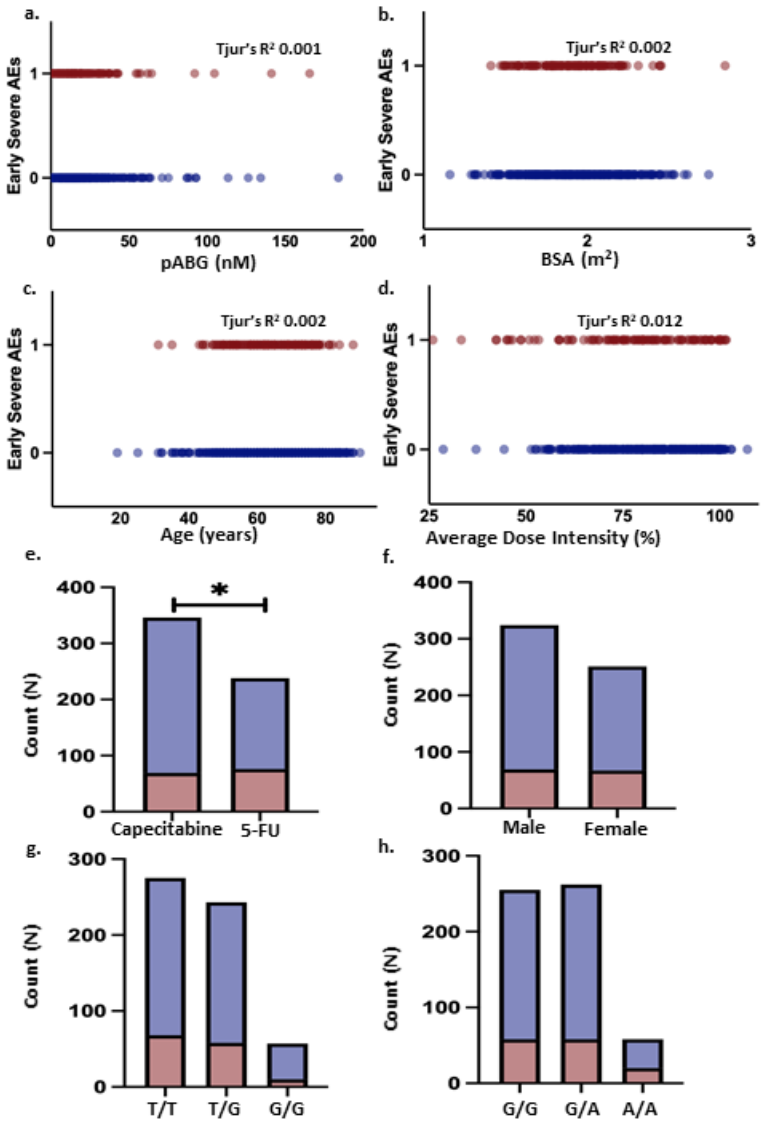


Figure 4.4 Univariate Analysis of Early Adverse Events

Continuous characteristics including pABG concentration (a.), body surface area (BSA) (b.), age (c.), and average dose intensity (d.) were assessed by logistic regression. None of the continuous characteristics had a significant association with the Early AE outcome. Categorical characteristics including drug (e.), sex (f.), *MTHFR* rs1801131 (g.), and *MTHFR* rs1801133 (h.) were assessed by χ^2 tests. Patients treated with 5-FU were at a significantly increased risk compared to capecitabine. Unlike for the Total AE outcome, sex was not significantly associated with the Early AE outcome. Finally, as in the Total AE analysis neither *MTHFR* variant rs1801131 or 1801133 had a significant association. (Adverse Events in Red, No Events in Blue). *p<0.05

4.3.3 Collinearity between pABG and Baseline Characteristics.

To limit the potential confounding of collinearity, the interaction between pABG and other baseline characteristics was tested by simple-linear regression for continuous variables, unpaired t-test or one-way ANOVA for categorical variables (Fig. 4.5). Only baseline characteristics that were to be included in multivariate analysis were considered. There was no relationship between pABG concentration and age (R^2: 0.01) or sex (p=0.808). Neither *MTHFR* rs1801131 (p=0.712) or *MTHFR* rs1801133 (p=0.383) was associated with differences in pABG concentration. This indicates that a multivariate model containing a combination of these variables is permissible.

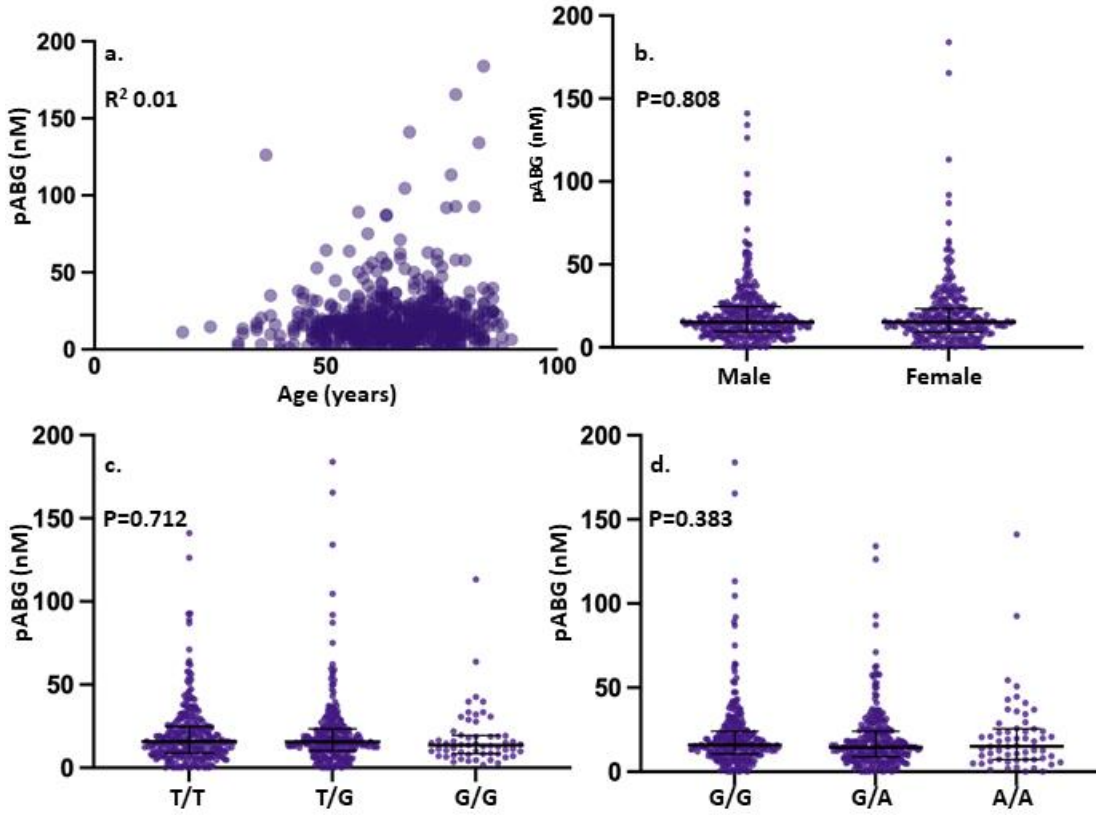
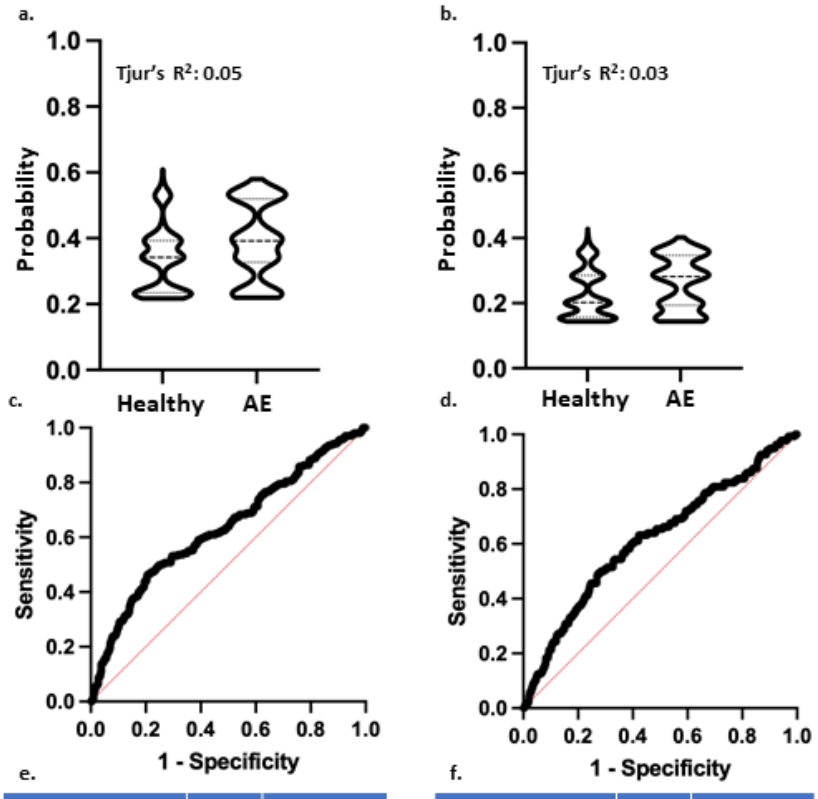


Figure 4.5 Assessing Collinearity between pABG and Baseline Characteristics

Collinearity between plasma pABG concentration and the baseline characteristics that were going to be included in the model was tested prior to multivariate regression analysis. pABG concentration was plotted against age in years and showed no significant correlation by simple linear regression (a). pABG concentration was also compared based on sex, there was no significant difference as tested by an unpaired student's t-test (b). pABG concentration was also compared by the variant status of *MTHFR* rs1801131 (c), and *MTHFR* rs1801133 (d) with no significant differences by one-way ANOVA. This shows there was no significant association between pABG and the baseline characteristics of age, sex, *MTHFR* rs1801131 or *MTHFR* rs1801133.

4.3.4 Multivariate Logistic Regression.

Multivariate logistic regression was completed using both Total AE and Early AE outcomes. The model consisted of four covariates: pre-treatment plasma pABG concentration, age in years, sex (binary male/female), and drug (capecitabine vs 5-FU). The model was not able to discriminate between patients that experienced an AE and those that did not when applied to the Total AE outcome (Tjur's R^2 0.05, Fig.4.6a). The receiver-operator curve (ROC) for this model demonstrates a poor performance of the model for Total AE, with the best cut-off providing approximately 40% sensitivity and 80% specificity (Fig.4.6c). Amongst the covariates neither pABG (OR 1.00; 95% CI 0.99-1.01) nor age (OR 1.00; 95% CI 0.99-1.01) were associated with the Total AE outcome. However as seen in the univariate analysis, both sex (female OR 1.74; 95% CI 1.22-2.47) and drug (capecitabine OR 0.45; 95% CI 0.31-0.65) were predictive of Total AE risk (Fig.4.6e). Multivariate logistic regression using these four variables also demonstrated poor performance when discriminating between patients using the Early AE outcome (Tjur's R^2 0.03, Fig.4.6b). Again, amongst the covariates neither pABG (OR 1.00; 95% CI 0.99-1.01) nor age (OR 1.00; 95% CI 0.99-1.01) were associated with the Early AE outcome. Consistent to the results for the model when applied to Total AE the fluoropyrimidine used (capecitabine OR 0.45; 95% CI 0.31-0.65) was predictive of Early AE risk (Fig.4.6f). However unlike when the model is applied to Total AE, sex was not significantly predictive of Early AE (female OR 1.39; 95% CI 0.93-2.06) in the multivariate analysis, which agrees with the findings of the prior univariate analysis. A sub-analysis was conducted amongst patients treated with capecitabine, compared against the occurrence of hand-foot syndrome. This model included pABG, age and sex and the outcomes were grade $\geq$3 HFS during either early or total follow-up. This demonstrated that pABG was not predictive of experiencing severe HFS in either the early (OR 1.00; 95% CI 0.95-1.02) or total follow-up period (OR 1.01; 95% CI 0.99-1.02).



e.

	OR	95% CI
pABG	1.00	0.99-1.01
Age	1.00	0.99-1.01
Sex: Female	1.74	1.22-2.47
FP: Capecitabine	0.45	0.31-0.65

f.

	OR	95% CI
pABG	1.00	0.99-1.01
Age	0.99	0.98-1.01
Sex: Female	1.39	0.93-2.06
FP: Capecitabine	0.45	0.30-0.68

Figure 4.6 Multivariate Logistic Regression

Multivariate logistic regression was completed using a model that included pABG, age, sex, and drug. The model was applied to both Total AE (a, c, e) and Early AE (b, d, f). Plotting the predicted probability for patients with adverse events (AE) and those without (Healthy) shows that the model was not able to differentiate between these patients for either the Total AE (a) or Early AE (b) outcomes. The receiver operator curves for the model in both the Total AE (c) and Early AE (d) outcomes shows there is not a strong predictive value of the model. The individual estimates for the effect of the covariates are summarized for both the Total AE (e) and Early AE (f) outcomes.

4.3.5 Multivariate Logistic Regression incorporating *MTHFR*.

Multivariate logistic regression was repeated using both Total AE and Early AE outcomes to incorporate variation within *MTHFR*. The model consisted of either *MTHFR* rs1801131 or *MTHFR* rs1801133 and four covariates: pre-treatment plasma pABG concentration, age in years, sex (binary male/female), and drug (capecitabine vs 5-FU). The *MTHFR* rs1801131 model was not able to discriminate between patients that experienced an AE and those that did not when applied to the Total AE outcome (Tjur's R^2 0.05, Fig.4.6a) or Early AE outcome (Tjur's R^2 0.05, Fig.4.7b). The ROCs for this model demonstrate a poor performance of the model for both outcomes (Fig.4.7 c, d). The pre-treatment pABG concentration was not a significant predictor for either Total AE (OR 1.00; 95% CI 0.99-1.01) or Early AE (OR 1.00; 95% CI 0.99-1.01). The second model to incorporate *MTHFR* variation included rs1801133, this model was also was not able to discriminate between patients that experienced an AE and those that did not when applied to the Total AE outcome (Tjur's R^2 0.06, Fig.4.7e) or Early AE outcome (Tjur's R^2 0.04, Fig.4.7f). The ROCs for this model demonstrate a poor performance of the model for both outcomes (Fig.4.7 g, h). The pre-treatment pABG concentration was not a significant predictor for either Total AE (OR 1.00; 95% CI 0.99-1.01) or Early AE (OR 1.00; 95% CI 0.99-1.01). In all adding either *MTHFR* rs1801131 or *MTHFR* rs1801133 to the four baseline covariates did not improve the predictive value of the multivariable regression.

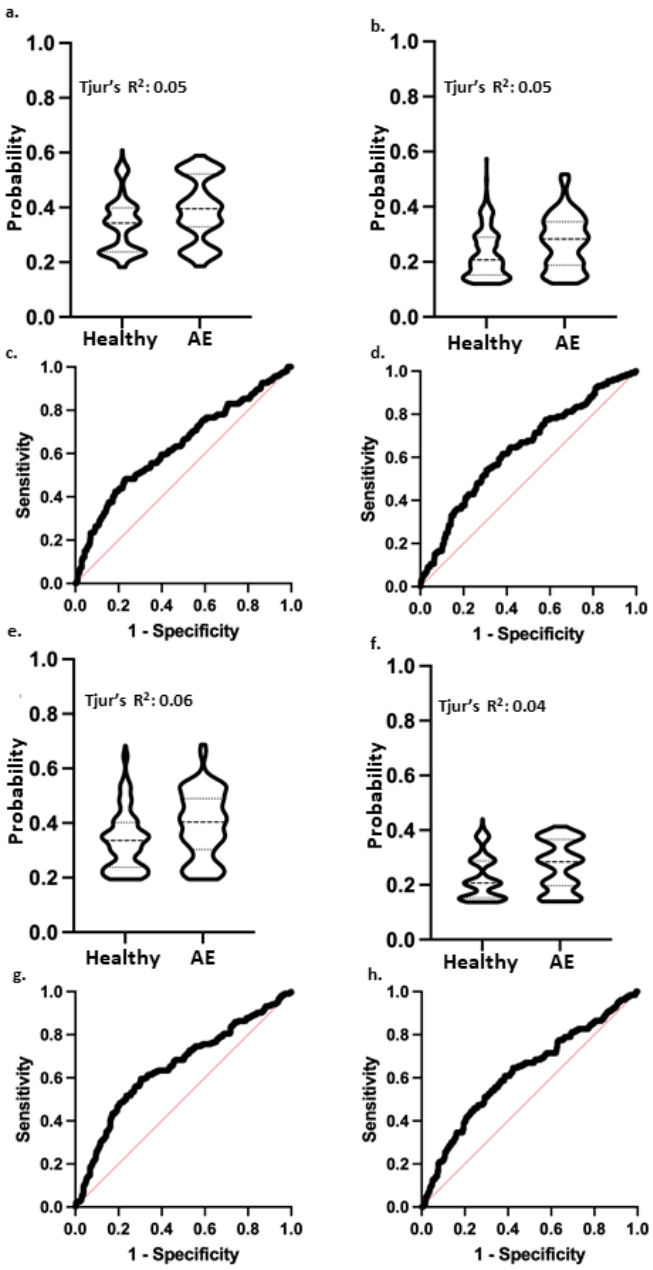


Figure 4.7 Multivariate Logistic Regression including *MTHFR* variants
Multivariate logistic regression was completed using a model that included pABG, age, sex, drug and either *MTHFR* rs1801131 (a-d) or rs1801133 (e-h). The model was applied to both Total AE (a, c, e, g) and Early AE (b, d, f, h). The addition of neither *MTHFR* rs1801131 (a-d) nor rs1801133 (e-h) improved the predictive ability of the model for either Total AE or Early AE outcomes.

4.4 Discussion

This chapter investigated the proposed role of total plasma folate level in the occurrence of fluoropyrimidine-related AEs. The plasma folate level was measured as pABG equivalents in archival plasma samples of known *DPYD* wild-type patients. Both the primary outcome of Total AE and the secondary outcome of Early AEs were graded in a standardized fashion and use the commonly accepted severe AE threshold of CTCAE grade $\geq$3. The sample size was 575 patients representing an 80% power to detect a 1.25 OR difference. This represents the largest single study of plasma folate status and fluoropyrimidine-related AEs completed to date. Furthermore, within the individual fluoropyrimidines this study includes more 5-FU or capecitabine patients alone than any previously published work. The findings of this study do not support a clinically relevant association between high plasma folate status and fluoropyrimidine-related AEs.

The proposed mechanism for an interaction between plasma folate and fluoropyrimidines is through the inhibition of TS. While the underlying molecular relationship is valid there are multiple layers of confounding variables that may make the determination of plasma folate irrelevant to the outcomes of fluoropyrimidine therapy. Firstly, fluoropyrimidines exert cytotoxic effects through methods other than solely the inhibition of TS[4]. The incorporation of fluorouridine triphosphate (FUTP) into RNA results in functional impairments. FUTP incorporation in RNA leads to failure of preRNA processing[35], tRNA modification[36], and polyadenylation of mRNA[37]. When fluorodeoxyuridine triphosphate (FdUTP) is mistakenly incorporated into DNA cells enter repeated cycles of attempted excision repair which ultimately destabilizes the DNA[38]. These mechanisms may be compensatory for small variations in TS that occur due to fluctuations in plasma folate. Secondly, the evidence linking nutritional folate status to fluoropyrimidine chemotherapy arises from two animal models which focused on efficacy and not AEs[14,39]. In these animal models the effect of dietary folate augmentation was seen when animals were folate deficient, rather than experiencing an increased exposure to folate. This finding does not necessitate that the inverse relationship holds true, which complicates the assessment of plasma folate status in Canadian individuals. Canada has mandated the fortification of wheat products with folic acid in an effort to reduce neural tube defects,

this policy has been in place since 1998 and had significant effects on population health[40]. In fact, a study in 2011 confirmed that <1% of Canadians are folate deficient[41]. Therefore, an underlying association between folate exposure and fluoropyrimidine AEs may be obscured by the population level folate supplementation. Finally, this study was limited to a Canadian population in southwestern Ontario, regional effects for fluoropyrimidine-related effects have been proposed[42] and this study cannot rule out that folate is integral to these differences. Both recent articles purporting an association between plasma folate status and fluoropyrimidine AEs report on east-Asian populations[16,17]. While it has been proposed that regulations around folate supplementation play a role in the regional toxicity profiles it is not possible to rule out the other environmental factors or differences in genomic variants. The combination of various mechanisms of action, weak experimental data and regional effects likely make any potential impact of plasma folate level clinically irrelevant.

4.4.1 Conclusion

Total plasma folate level at baseline is an insignificant predictor of fluoropyrimidine-related adverse events in Canadian patients. The prediction or fluoropyrimidine-related adverse events was not improved by the addition of *MTHFR* variants rs1801131 or rs1801133. The proposed interaction between a high endogenous plasma folate concentration and fluoropyrimidine-related adverse events is not clinically relevant in our population.

4.5 Chapter 4 References

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Chapter 5

5 Provincial Burden of Fluoropyrimidine-related Adverse Events in Ontario

5.1 Introduction

Chapters 2-4 have discussed mechanisms of identifying patients at risk for fluoropyrimidine-related AEs and possible interventions that could be implemented to reduce these AEs. While the use of plasma folate status appears to have limited utility the use of *DPYD* genotype testing has demonstrated significant promise. Translating these findings into evidence-based practice within Ontario requires knowledge translation to inform policy makers. Chapter 5 discusses efforts to quantitatively define the burden of fluoropyrimidine therapy and the consequent AEs within the Ontario healthcare system.

In the fall of 2019, the Personalized Medicine lab submitted a request for a health technology assessment (HTA) of prospective *DPYD* genotype-guided fluoropyrimidine dosing. Health Quality Ontario (HQO) conducts HTAs in collaboration with experts in the field in order to inform the recommendations of the Ontario Health Technology Advisory Committee (OHTAC). Recommendations of the committee are used to inform funding decisions of the Ontario Ministry of Health and Long-Term Care, and ultimately which tests or procedures are implemented in practice. The process to complete a HTA involves a thorough review of the literature, expert consultation and a public comment period[1]. Through collaborating with HQO on the HTA we gained better clarity on the priorities of policy makers which highlighted key limitations of the existing literature. The main source of contention HQO identified within the literature was a distinct lack of regional data. The lack of regional data included the efficacy of *DPYD* genotyping, the rate of fluoropyrimidine use, the rate of fluoropyrimidine-related AEs and estimates of the cost of fluoropyrimidine-related therapy within Ontario. Chapter 2 reports the efficacy of pre-treatment *DPYD* genotype-guided dosing at a single-centre in Ontario[2]. However, it could not be assumed that the fluoropyrimidine AE rate in this patient population was representative of the province as whole. As well the study reported in chapter 2 had no means of assessing provincial usage of fluoropyrimidine therapy.

The literature of fluoropyrimidine-related AEs commonly estimates that between 10-30% of patients experience a severe AE[2-7]. A frequent citation for this estimate is an editorial on the toxicity of capecitabine by Mikhail *et al*. from 2010[8], while a helpful review of the subject matter this lacks the statistical rigor to justify the rate for which it is cited. An

alternative strategy has been to directly cite large phase 3 fluoropyrimidine clinical trials or meta-analyses of said trials[9-12]. While this likely more accurately reflects the rate of fluoropyrimidine-related AEs, these trials do not take into consideration regional effects and most of these trials were completed more than 20 years ago. Haller *et al*. used a combination of phase 3 trial data and proprietary prescription pattern data to demonstrate regional differences in capecitabine tolerability[13]. Those authors found that patients in US based trials experienced more frequent severe AEs compared to trials conducted in Europe and Asia. The fallout of this finding appeared to be that American physicians prescribed capecitabine at a lower target dose than counterparts across Europe. This supports the notion that current regional data on fluoropyrimidine use and AEs is needed when looking to make new policy recommendations. Recently a group of French authors conducted a study in an effort to characterize the 'real-life' toxicities that occur during fluoropyrimidine-chemotherapy[14]. First the authors assessed the use of capecitabine and 5-FU within one of the 18 administrative regions in France. To determine the rate of adverse events the authors performed an in-depth chart review of 513 patients. Finally, they extrapolated these regional estimates to determine the likely fluoropyrimidine usage and adverse event burden at the national level. This insight into clinical practice is intriguing but the methodology relies on the extrapolation from less than 10% of the population, and did not determine the cost of these outcomes. Techniques that can directly interrogate the fluoropyrimidine-use, AEs, and cost at a population level would provide a more robust description of the issue that must be addressed at a policy level.

The cost of fluoropyrimidine-related AEs is a key metric for policy makers considering the implementation of preventive strategies. Authors from the Netherlands, Italy, Spain and Ireland have independently estimated *DPYD* genotype-guided therapy is a cost-effective strategy for reducing AEs[4,15-18]. Yet, during the HTA process the translational relevance of these findings to the population of Ontario could not be assumed. Concerns were raised surrounding differences in the populations, healthcare systems and methods of economic analysis previously employed. Given the concerns that arose during the HTA the Personalized Medicine Lab collaborated with ICES to directly assess fluoropyrimidine use, AEs, and cost within Ontario using provincial administrative databases.

5.2 Patients and Methods

This project was completed through collaboration with data scientists and analysts from the Western division of ICES. ICES is a non-profit research organization with the unique ability to access the administrative healthcare-records for patients across the province of Ontario. ICES is able to assess data on health-care services, publicly funded drugs, demographic data, and cancer registries amongst other databases. ICES has the capacity to link these databases in a secure manner allowing the assessment of patient specific data on a population health scale. In order to protect the anonymity of patients there are multiple secure checkpoints in place. Patient data is highly protected and external researchers are not permitted to view individual patient data or small groups that contain less than six members. Therefore, to work with ICES databases external researchers develop dataset creation plans (DCPs) that are reviewed iteratively with ICES collaborators to define the analysis. Once a DCP is completed the ICES analyst follows the stepwise methodology and produces outcome tables that report aggregate outcomes and protect the privacy of individual patients. The DCP for this project can be reviewed in Appendix B, however for simplicity the methods are summarized below.

5.2.1 Cohort Build: Inclusion and Exclusion Criteria

The inclusion criteria for this study was the use of systemic fluoropyrimidine chemotherapy between the dates of December 1st 2014 and December 1st 2018. These patients were identified by using a combination of Drug Identifier Number (DIN) and Cancer Care Ontario (CCO) regimen codes queried within the Cancer Activity Level Reporting data base (ALR). The date of first exposure to a systemic fluoropyrimidine within the study window was defined as the index date. Exclusion criteria assessed in the ALR included past exposure to capecitabine or 5-FU prior to the study period, and/or conflicting CCO regimens on index date. Further exclusion criteria for data cleaning were invalid or missing: age, sex, or resident status recorded from the Registered Persons Database (RPDB), this data set also tested for invalid death date (i.e. death before index date). The final exclusion criterion was a missing cancer diagnosis within the 1 year of index date within the Ontario Cancer Registry (OCR).

5.2.2 Data Collection

The first exposure to systemic fluoropyrimidine chemotherapy within the study window defined the index date (Fig.5.1). Prior to the index date there was a one-year look-back period in which to assess baseline characteristics. Age and sex were determined on index date from the RPDB. Cancer characteristics including primary disease site and stage were collected from the "INCIDENT_CASES" and BEST_STAGE" variables of OCR respectively. The observation window was developed as a rolling timeframe that was specific for each CCO regimen. A rolling window was used to capture multiple continuguos cycles allowing for minor delays whilst stopping follow-up when the patient had discontinued therapy. To achieve this, the observation window for each patient starts at the index date, continuing through the length of the cycle as described in the CCO regimen, then allowing for a 60 day look-forward. If within the look-forward the patient recieves a second cycle of fluoropyrimidine chemotherapy the observation window is recalculated forward from the start of the second cycle, following through the minimum length of the cycle with a new 60 day look-forward. This process was extended for all subsequent cycle, in the event no subsequent cycle is identified the follow-up is censored at 60 days from the beginning of the final cycle. Patients were censored at death or the maximum follow-up date of March 31st, 2019. During the observation window acute care hospital encounters including emergency room (ER) visits and hospital admissions were collected for each patient from the National Ambulatory Care Reporting System (NACRS) and Canadian Institute for Health Information Discharge Abstract Database (CIHI-DAD) respectfullly. As well total health care costs for the full observation window were calculated on a per-person basis and inflated to 2018 Canadian Dollars. The %getcost function employed by ICES includes both acute and continuing care costs compiling data from many databases including NACRS (ER visits), CIHI-DAD (admissions), Hospital outpatient clinics, Same Day Surgery, Ontario Health Insurance Plan (physician billing), Ontario Drug Benefit, National Rehabilitation Reporting System, Continuing Care Reporting System, Long-Term Care, Home Care, Ontario Mental Health Reporting System, New Drug Funding Plan, and the Assistive Devices Program.

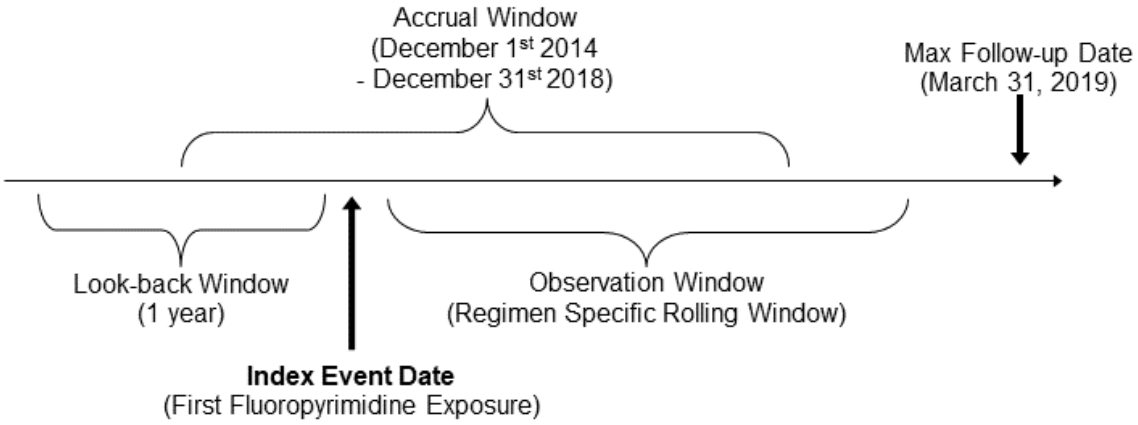

Figure 5.1 Summary of Patient Timeline

The template for the key timepoints during the study and defining the follow-up for individual patients. The accrual window is the period of study enrollment. Patients were included if they were exposed to fluoropyrimidines within the accrual window without any of the exclusion criteria, this exposure marks the index date for the individual patients. The look-back window was used to collect baseline demographics and confirm cancer diagnosis. The observation window defined follow-up time, only events and costs within the observation window were eligible for inclusion in the study.

5.2.3 Outcomes

The primary outcome of interest was the occurrence of fluoropyrimidine-related acute care hospital encouters, termed chemotherapy-related visits (CRVs). To determine the relationship between chemotherapy and hospital encounters the most responsible diagnosis for each encounter was compared against a pre-defined list of chemotherapy related diagnoses. The list of chemotherapy related diagnosis was adapted from the work of Krzyanowska *et al.*, the codes have been previously validated for use within ICES datasets[19]. The adapted list used in this study excluded venous thromboembolism as it is not caused by the fluoropyrimidine component of the regimen, the list of codes used is contained in Appendix C. An exploratory objective in this study was to assess the cost associated with fluorpyrimidine chemotherapy. The cost outcome includes both acute and continuing costs generated by the patient within the full observation window. To explore the difference between the general cost of fluoropyrimidine-chemotherapy and potential added cost of CRVs the total cost of individual paitents were compared between patients with at least one CRV and patients with no CRVs during the observation window.

5.2.4 Statistical Analysis

Descriptive statistics are presented for the baseline characteristics, and primary outcome. Descriptive statistics are shown using number (percentage), mean (SD), and median (interquartile range (IQR)) as applicable. The exploratory outcome comparing cost between patients with and without CRVs was compared using a two-sided unpaired students t-test, $p<0.05$ considered significant. Graphing and statistical analysis of the cost outcome was completed GraphPad Prism version 9.0.0 for Mac OS (GraphPad Software).

5.3 Results

5.3.1 Cohort Build

There was a total of 30,745 patients exposed to systemic fluoropyrimidine chemotherapy within the accrual window. The data cleaning exclusion criteria removed a total of 67 patients for incomplete data. The primary exclusion criterion was a lack of cancer diagnosis within a year prior to the index date, this criterion excluded 6,372 patients. These may represent patients with metastatic disease originally diagnosed in the distant past who are progressing to fluoropyrimidine chemotherapy. The next largest exclusion criterion was past exposure to fluoropyrimidines prior to the accrual window, which excluded an additional 2,215 patients. Excluding patients with prior fluoropyrimidine exposure or distant cancer diagnosis limited the patient sample to first time fluoropyrimidine exposure. The final minor exclusion criterion was multiple fluoropyrimidine-regimens recorded on the same date, which excluded only 9 patients. This exclusion was necessary as it was unclear which regimen would define the observation window for those patients. This cohort build generated a sample of 22,102 patients with a first exposure to fluoropyrimidines within the accrual window (summarized in Table 5.1). The average age of the sample was 61.6 (12.2) years and 53% of the patients were female. The most common disease site was colorectal cancer, representing over half of the sample (N=11,164 (50.5%)). Breast cancers were the next most common disease site (N=4,401 (19.9%)), while the remaining disease sites of pancreatic, gastroesophageal, anal and head/neck tumour represented less than 10% each of the total sample. The majority of patients had advanced stage III/IV disease at the time of enrollment (N= 12,316, 55.7%), however a sizeable proportion of patients lacked sufficient staging data (N= 4,049, 18.3%) (summarized in Table 5.2).

Table 5.1 Cohort Build

Step	Criteria	Description	Included	Excluded
1	Inclusion	All patients in Ontario with valid IKN who received their first capecitabine or 5-FU therapy in ALR between Dec 1, 2014 and Dec 31, 2018.	30,765	
2	Exclusion	Missing or invalid age (<18 or >105) or sex	30,720	45
3	Exclusion	Non-Ontario resident	30,706	14
4	Exclusion	Death on or before index date	30,698	8
5	Exclusion	Restrict to patients who have a cancer diagnosis on or in the year prior to index date	24,326	6,372
6	Exclusion	History of capecitabine or 5-FU therapy prior to index date	22,111	2,215
7	Exclusion	More than 1 distinct CCO regimen on index date.	22,102	9
Final		Study Cohort	**22,102**	**8,663**

Table 5.2 Baseline Characteristics

Characteristic	Mean	SD
Age (years)	61.6	12.2
BSA (m^2)	1.85	0.24
Sex	**N**	**%**
Female	11,707	53
Year	**N**	**%**
2014	1,830	8.3
2015	5,422	24.5
2016	5,444	24.6
2017	5,431	24.6
2018	3,975	18
Primary Cancer	**N**	**%**
Colorectal	11,164	50.5
Breast	4,401	19.9
Pancreatic	1,569	7.1
Esophagus/Gastric	1,153	5.2
Anal	760	3.4
Head and Neck	128	0.6
Other	2,927	13.2
Cancer Stage	**N**	**%**
Missing	4,049	18.3
I	1,107	5.0
II	4,628	20.9
III	7,646	34.6
IV	4,672	21.1

5.3.2 Treatment Characteristics

Patients in this study received a median of 3 (2-6) cycles of treatment equating to a median observation window of 123 (101-220) days. Amongst the treatment regimens the sample was biased toward 5-FU use with over 2/3 of patients treated with a 5-FU based regimen (N=14,662, 66.3%). The top 10 regimens by prevalence reflect the disease sites observed in the baseline characteristics. The most prevalent regimen *MFOLFOX6 is a 5-FU based regimen commonly used in both the adjuvant and palliative treatment of the most prevalent disease site colorectal cancers. The *MFOLFOX6 was used to treat 3,298 (17.8%) patients in the cohort. The second most frequent regimen, *CAPE, is capecitabine monotherapy a widely used regimen in the palliative treatment of both advanced colorectal cancer and metastatic breast cancer. Capecitabine monotherapy was the most common capecitabine containing regimen and was used to treat 3,124 (14.1%) patients. The final example regimen *FEC-D was the third most common and is used in the adjuvant treatment of the second most prevalent disease site, breast cancer. The *FEC-D regimen was the second most frequent 5-FU containing regimen and was used to treat 2,627 (11.9%) patients. The remaining top 10 regimens are commonly used in colorectal (*Cape RT and *FOLFIRI+BEV), pancreatic (*FOLFIRINOX), breast (*FEC-D+TRAS), anal (*FUMTMC+RT) and gastroesophageal (*ECX and *ECF) cancers. The fluoropyrimidine use in this sample is summarized in Table 5.3.

Table 5.3 Treatment Characteristics

Length of Follow-up	**Median**	**IQR**
No. of Cycles	3	2-6
No. of Days	123	101-220
Fluoropyrimidine	**N**	**%**
Capecitabine	7,440	33.7
5-Fluorouracil	14,662	66.3
Top 10 Regimens	**N**	**%**
*MFOLFOX6	3,298	17.8
*CAPE	3,124	14.1
*FEC-D	2,627	11.9
*CAPE(RT)	2,295	10.4
*FOLFIRI+BEVA	1,491	6.8
*FOLFIRINOX	1,167	5.3
*FEC-D+TRAS	934	4.2
*FUMTMC(RT)	750	3.4
*ECX	586	2.7
*ECF	543	2.5

Regimen components: MOFOLFOX6 includes 5-FU, Folinic Acid, and Oxaliplatin; CAPE includes capecitabine; FEC-D includes 5-FU, Epirubicin, Cisplatin and Docetaxel; CAPE(RT) includes Capecitabine concurrent with Radiation; FOLFIRI+BEVA includes 5-FU, Folinic Acid, Irinotecan and Bevacizumab; FOLFIRINOX includes 5-FU, Folinic Acid, Irinotecan and Oxaliplatin; *FEC-D+TRAS includes 5-FU, Epirubicin, Cisplatin, Docetaxel and Herceptin; FUMTMC includes 5-FU, Mitomycin-C concurrent with Radiation; ECX includes Epirubicin, Cisplatin, and Capecitabine; ECF includes Epirubicin, Cisplatin, and 5-FU

5.3.3 Chemotherapy Related Visits

CRVs were identified through review of ER visits and hospital admissions within the observation window of each patient (summarized in Table 5.4). Early events occurring in the first or second cycle are reported as secondary findings. First cycle CRVs include either events before the second cycle start date or those that occurred within 60 days of the index date in patients that had only one cycle of therapy. Second cycle CRVs include events between the start of the second cycle and the start of the third cycle for patients with more than two cycles of therapy. Second cycle CRVs also include events that occurred within 60 days after initiating the second cycle in patients with only two cycles of therapy. While first and second cycle CRVs are unique events they are not mutually exclusive, it is possible for patients to experience both a first and second cycle CRV. Furthermore, the prevalence of CRVs in the full observation window reports patients that have at least one CRV; patients can be counted in all three categories if they had multiple CRVs. In total 6,862 (31%) patients had a CRV in the full observation window. Amongst CRVs during the full observation window ER visits were more frequent (6,403) than hospital admissions (3,185). Within the early cycles, 2,381 (10.7%) patients experienced a CRV during the first cycle and 1,528 (8.5%) experienced a CRV during the second cycle. Interestingly while 22,102 patients were initiated on fluoropyrimidine chemotherapy only 17,988 received at least two cycles of therapy. The discrepancy in patients between the first and second cycle represents a loss of 19% if the total sample. Given that nearly all regimens recommend at least two cycles of therapy, this finding suggests a significant proportion of the population prematurely discontinues therapy. The effect of this discontinuation on the potential efficacy of the fluoropyrimidine chemotherapies cannot be identified from this dataset.

Table 5.4 Chemotherapy Related Visits

	CRVs N (%)	ER CRVs N (%)	Hospitalization CRVs N (%)
Full Observation Window (N=22,102)	6,862 (31.0)	6,403 (29.0)	3,185 (14.4)
First Cycle (N=22,102)	2,381 (10.7)	2,176 (9.8)	1,029 (4.7)
Second Cycle (N=19,988)	1,528 (8.5)	1,397 (7.8)	664 (3.7)
Abbreviations: CRVs, Chemotherapy Related Visits; ER, Emergency Room			

5.3.4 Healthcare Cost within the Observation Window

The healthcare cost metric employed in this study captures all publicly funded healthcare services within the province of Ontario, and was applied to the full observation window for each patient. The mean treatment cost for the cohort was $32,459 ($25,934) adjusted to a per person-year rate the mean treatment cost was $88,191 ($80,342). Furthermore, the median cost was $26,393 ($16,394-$40,469) or adjusted to $69,479 ($50,831-$99,746) per person-year. This data represents aggregates over the observation window and cannot be attributed directly to individual encounters. However, it is possible to compare the healthcare cost between patients that experienced a CRV (N=6,828) and those patients without a CRV (N=15,274) (Fig.5.2). The mean cost during the observation window for patients with a CRV was $44,472 ($31,147) which was significantly higher than that of patients that did not have a CRV at $27,718 ($21,198) ($p<0.001$). The mean difference cost increase for patients that experienced a CRV was $16,754. Across the 6,828 patients that represents a total of over $114,000,000 of additional cost over the four years included in the accrual window. The difference was consistent when costs were adjusted to a per person-year rate with CRV patients having a mean adjusted cost of $109,512 ($93,221) compared to an adjusted cost of $78,821 ($71,915) ($p<0.001$). Adding to the description of this difference the costliest patient in the CRV group was more expensive than in the no CRV group in raw cost ($358,843 vs. $291,487) and more than double the highest no CRV patient within adjusted per person-year cost ($2,854,428 vs $1,440,180).

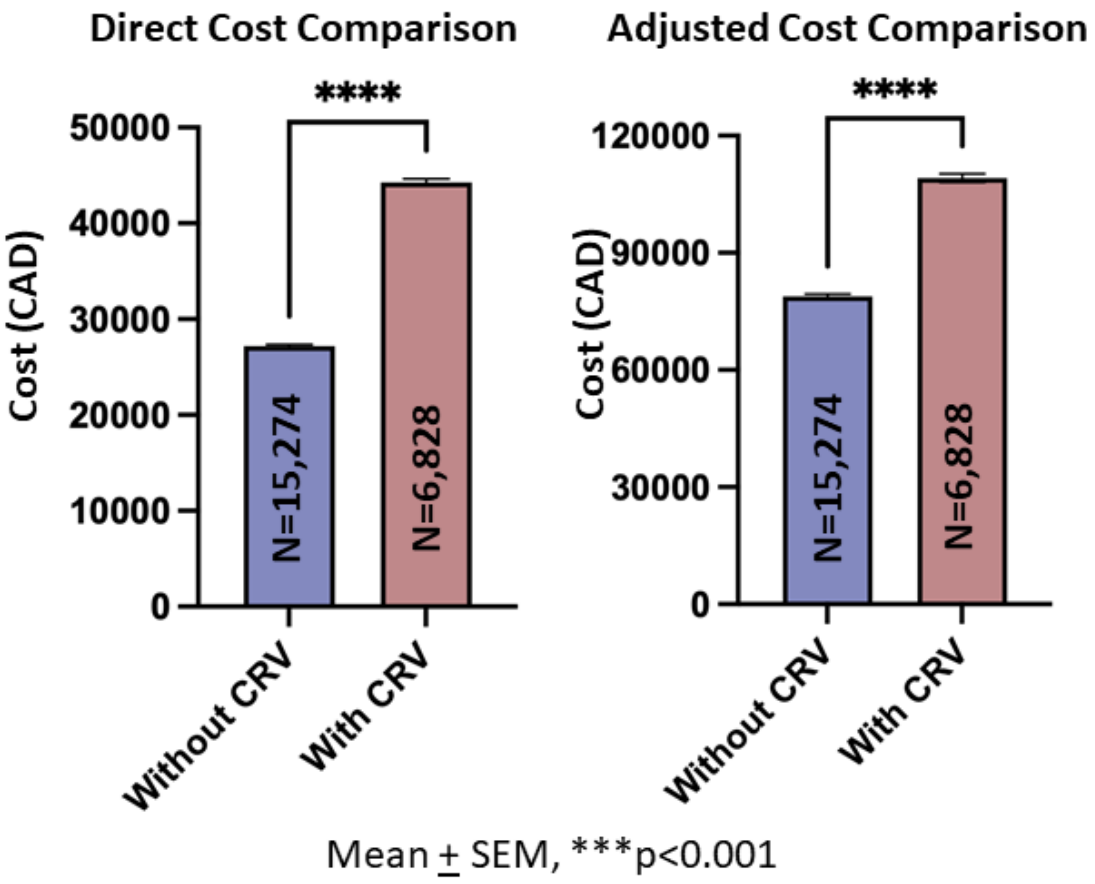

Figure 5.2 Comparison of Healthcare Costs

Comparing the healthcare costs during the full observation window grouped by patients that had at least one CRV (Red) and patients that did not have any CRV (Blue). Direct comparison of raw cost and cost adjusted to per person-year rate both show significantly higher cost for patients that experience a CRV compared to patients that did not experience a CRV. Plots are mean +/- standard error of the mean, compared using unpaired student's t-test.

5.4 Discussion

This chapter leveraged ICES's capabilities to describe the use of fluoropyrimidine chemotherapy in the province of Ontario based upon administrative databases. The results reported in this chapter represent the first description of fluoropyrimidine-related AEs that have been directly assessed at a population level. This data serves both immediate and long-term goals in the clinical pharmacology of fluoropyrimidines. In the immediate future these results will be used in efforts to ensure the rapid adoption *DPYD* genotype-guided dosing. The HTA of *DPYD* genotype-guided dosing was completed in parallel to this study, with open communication between members of the Personalized Medicine lab and HQO staff members. Upon review of the HTA and guidance of the OHTAC, Ontario Health has recently published a draft recommendation which supports publicly funding *DPYD* genotype testing prior to fluoropyrimidine chemotherapy[20]. The results of this study will be provided to the authors of the HTA for inclusion in the final recommendations. In the larger context, the findings presented here demonstrate that fluoropyrimidine-related adverse events continue to represent a significant target for pharmacologic intervention.

Historically the rate of fluoropyrimidine-related adverse events has been estimated at approximately 30% derived from prior clinical trials. The current study found that 31.0% of patients treated with fluoropyrimidines in Ontario required a chemotherapy related hospital visit. Coinciding with this, we found that 31.0% of *DPYD* wild-type patients experienced a severe fluoropyrimidine-related AE during genotype-guided dosing at a single-centre in Ontario (section 2.3.3 Toxicity Outcomes)[2]. The agreement between the historical estimates, our local findings within the London Regional Cancer Program and now the provincial dataset suggest that 30% is likely an accurate estimate in our region given the current regimens and patient population. A similar finding has been reported amongst patients treated with irinotecan-based regimens. Tam *et al*. reported that the rate of serious AEs in clinical practice were the same as those in published clinical trials[21]. These data all suggest that serious adverse event rates from clinical trials literature serve as a reasonable surrogate for clinical practice. However, this generalization cannot be universally applied. For example, in multiple investigations of *DPYD* genotype-guided

dosing in the Netherlands 23% of patients experienced a severe fluoropyrimidine-related AE[3,4]. While a small absolute difference this represents a 23% relative difference between the estimates. This variation between estimates compounded with perceived regional differences represented a significant barrier to the translation of *DPYD* genotype-guided dosing. Undertaking this study to define the current burden from a regional dataset alleviated the hesitancy of knowledge brokers allowing for a smoother transition towards implementation of *DPYD* genotype-guided dosing.

Defining the economic impact of fluoropyrimidine-related AEs is the first step in supporting the claims of cost-effectiveness in a local setting. In this study we were able to assess the total health care cost of individual patients during the follow-up window. This allowed a comparison between the total health care cost for individuals with at least one CRV compared to those without CRVs. The $114,000,000 of increased cost amongst CRV patients represent a large potential savings if even a minority of these events can be prevented. *DPYD* genotype-guided dosing is currently the most applicable method for fluoropyrimidine-related AEs in Canada. In addition to the evidence of efficacy, the standardization of genotype testing and simplicity of the reporting add to its' viability. The final barrier is to demonstrate cost-savings, while cost effectiveness modeling was beyond the scope of the current study multiple efforts have predicted cost savings through *DPYD* genotype-guided dosing in countries across Europe[4,15-18].

5.4.1 Conclusion

The use of fluoropyrimidine chemotherapy is associated with a significant number of chemotherapy related hospital encounters. Patients that require a chemotherapy related visit generated an additional $114 million of health care spending, equating to roughly $28.5 million dollars of increased spending annually. The potential cost savings add to the benefit of patient experience when implementing strategies to reduce fluoropyrimidine-related AEs. Formal cost-effectiveness modeling of *DPYD* genotype-guided dosing can now be completed to further support the rapid adoption of this strategy in Ontario.

5.5 Chapter 5 References

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Chapter 6

6 Discussion

6.1 General Discussion

The fluoropyrimidine class of chemotherapies are broadly used and unlikely to be replaced in the near future. Ensuring their safe and effective use through clinical pharmacology-based risk stratification is an important strategy for improving patient outcomes. The goal of this thesis was to demonstrate the benefit of *DPYD* genotype-guided dosing in Ontario, and test the potential value of plasma folate level as a predictive risk factor. *DPYD* genotype-guided dosing successfully ameliorated the increased risk of fluoropyrimidine-related adverse events amongst carriers of the CPIC recognized clinically relevant variants[1]. On the contrary, plasma folate status did not demonstrate any ability to predict the risk of fluoropyrimidine-related adverse events. These studies suggest that *DPYD* genotype-guided dosing is ready for clinical implementation in Ontario, and that additional research is still required to identify pertinent risk factors for the remaining adverse event risk. In order to support these findings, we directly assessed the fluoropyrimidine adverse event burden in the province through health care administrative databases. We demonstrated that 31% of patients treated with a fluoropyrimidine containing regimen in Ontario require a chemotherapy related hospital visit. This also demonstrated that the cost of treating patients who experience a fluoropyrimidine-related visit was significantly increased. Together these data made convincing arguments to policy makers and garnered support for implementing a publicly funded *DPYD* genotype-guided dosing program in Ontario[2].

Implementing *DPYD* genotype-guided dosing has rapidly gained support in Europe and now has some backing in Canada as well. Physicians in Quebec have recently published their experience implementing *DPYD* genotype-guided dosing[3]. The Centre Hospitalier de l'Université de Montréal (CHUM) began offering *DPYD* c.1905+1G>A testing in 2017 as part of routine clinical practice. Through retrospective assessment of this process these authors demonstrated that implementation was feasible in routine clinical practice and likely cost-effective. Over a one-and-a-half-year period over 2,617 patients were genotyped through CHUM at an average cost of only $18.30 (CAD) per patient or $47,890 total. With an average turn-around time less than one week this testing did not interfere with the planning and delivery of care. Not all patients were sent for testing

prior to therapy and five *DPYD* c.1905+1G>A carriers were identified after experiencing a severe fluoropyrimidine-related AEs. These patients that were identified post-adverse event were hospitalized for an average of 15.4 days, equating to an estimated cost of $15,400 each. Protecting just 4 of these patients would have more than paid for testing all 2,617 patients[3]. Following the publication by Henricks *et al*.[4] CHUM now provides testing for the four clinically relevant variants listed in the CPIC guidelines[1]. The model employed in Quebec leverages the economy of scale through offering testing to a large population in a centralized facility. We believe a similar model employed in Ontario will prove a cost-effective method for improving patient centered outcomes. Chapter 5 consists of the first large scale patient-level assessment of fluoropyrimidine burden in routine clinical practice. This assessment demonstrated that 31% of patients experienced a chemotherapy-related hospital visit with an average increased cost of $16,754 (CAD). When operating at scale we estimate that testing for four SNVs would cost less than $50 (CAD), meaning 335 patients could be tested for $16,754. Given a 7% carrier frequency for clinically relevant variants it is predicted that 23 variant carriers could be found in a sample of 335 patients. Dose modification of these 23 variant carriers would prevent a significant number of adverse events and the costs associated with their hospital utilization. This data supported our application for a health technology assessment to be performed by Health Quality Ontario. This process resulted in the recommendation by the Ontario Health Technology Advisory Committee to publicly fund a *DPYD* genotype testing program in the province[5].

Despite the clear benefit of *DPYD* genotype-guided fluoropyrimidine dosing there remains a significant number of adverse events that will not be prevented using this method alone. The relative rarity of *DPYD* variants has led some institutions to favor DPD phenotype testing in the clinical setting. While direct assessment of DPD activity in peripheral blood mononuclear cells proved too variable for implementation[6], assessing the endogenous DPD substrate uracil is used as an alternative[7]. Endogenously DPD converts uracil to dihydrouracil, therefore measuring the level of these circulating metabolites can give an approximation of the global DPD activity of the patient[8]. There is a significant negative correlation between plasma uracil concentration and DPD activity of peripheral blood mononuclear cells (r^2=0.51, p=0.023)[9]. There is also a significant

association between an elevated plasma uracil concentration and risk for fluoropyrimidine-related adverse events[10]. Meulendijks *et al*. found that patients with plasma uracil concentration in the 97th or greater percentile were at a significantly increased risk of severe adverse events (OR: 5.3; 95%CI: 1.53-18.7), this suggests a cut-off value of 16 ng/mL. Meulendijks *et al.* also found that the results may be extended to include the 94-97th percentile bracket (13.9-16.0 ng/mL) as patients within this range of concentrations also experienced an increased risk for severe fluoropyrimidine-related adverse events (OR: 8.2; 95% CI: 2.55-26.1)[10]. The benefit of a phenotype test compared to a genotype test is the broader applicability of the result. A genotype test is limited to only the variants specifically tested for, and does not account for any other sources of variation in the endpoint of enzymatic activity. The phenotypic test circumvents these limitations by measuring an outcome that is the sum of enzymatic activity. For these reasons some jurisdictions have favoured the implementation of phenotype testing through plasma uracil measurement for pretreatment DPD deficiency screening. Since December 2018 it has been required that plasma uracil level be assessed and considered before administering a systemic fluoropyrimidine chemotherapy in France[7]. A recent analysis of this effort has demonstrated the difficulty of determining the appropriate testing modality. Pallet *et al* retrospectively assessed the correlation between uracil phenotype and *DPYD* genotype amongst 3,680 French patients. In this study phenotypic DPD deficiency was defined as hyperuracilemia defined with plasma levels >16 ng/mL. These authors found that 6.8% of patients had hyperuracilemia, and 4.5% of patients carried a clinically relevant deleterious *DPYD* variant. Carrying a *DPYD* variant was highly specific (95%) for phenotypic DPD deficiency with a strong negative predictive value (93%). Despite this, Pallet *et al*. concluded that uracil-based phenotype testing outperformed *DPYD* genotype testing as it identified a greater number of at-risk patients[11]. This conclusion is limited in scope and applicability by many confounding factors.

The association between plasma uracil concentration, DPD activity and fluoropyrimidine is complex and imperfect. The hyperuracilemia cut-off value of 16 ng/mL is derived from a single Dutch study was merely the 97th percentile rank and not a statistically derived cut-off value[10]. The association between a plasma uracil level >16 ng/mL and

fluoropyrimidine adverse events was demonstrated in the first study but has not yet been confirmed. However, using the 16 ng/mL cut-off Pallet *et* al identified 6.5% of patients as DPD deficient, doubling the 3% cut-off that defined the value in the Dutch study. Doubling the number of patients classified as DPD deficient by hyperuracilemia has significant implications for the implementation of this phenotyping technique. The appropriate cut-off value for predicting increased risk of adverse events may prove to be population and assay specific requiring further studies to validate the clinically relevant plasma uracil concentration. Assay variability has already been demonstrated as a limitation in measuring plasma uracil concentrations[12]. Pallet *et al*. did test for between centre variation and confirmed no significant variation, and they note that determining an improved cut-off value remains an area of interest[11]. Another key distinction is that pre-treatment uracil concentration is not perfectly predictive of DPD deficiency[12]. A major criticism of DPD deficiency screening and fluoropyrimidine dose modification is the potential for subtherapeutic dosing and ineffective chemotherapy. In light of this critique it is essential to ensure a high specificity of the DPD deficiency screening being employed. To classify one technique as preferential on the basis of sensitivity for detecting DPD deficiency alone is premature. The risk of under dosing the fluoropyrimidine component of chemotherapy is pervasive in this literature and has led to the suggestion that therapeutic drug monitoring should be considered as a co-requisite during fluoropyrimidine chemotherapy.

Predicting patients at an elevated risk of fluoropyrimidine toxicity remains a challenging problem and the result is that there remains a large degree of interpatient variability in the pharmacokinetics of fluoropyrimidines. For example, it is well established that the clinical outcomes of 5-FU are strongly associated with the systemic exposure achieved during therapy resulting in a narrow target exposure with tolerable side-effects[13]. Beyond predicting patients at high risk for adverse events the current dosing strategy also results in significant under dosing. 5-FU is dosed using body surface area, a composite of height and weight. Unfortunately, dosing 5-FU by body surface area achieves only 25% of patients within the therapeutic range, another 15% receive a toxic exposure and 60% of patients are effectively underdosed[14,15]. In response to this it has been demonstrated that therapeutic drug monitoring followed by dose titration is possible and improves clinical

outcomes while reducing toxicity[16-23]. Additionally, two assessments have demonstrated that therapeutic drug monitoring of 5-FU can be cost effective[24,25].The cumulation of these findings is a set of therapeutic drug monitoring guidelines by the International Association of Therapeutic Drug Monitoring and Clinical Toxicology (IATDMCT). The IATDMCT guidelines strongly recommend that 5-FU therapeutic drug monitoring be used in patients treated for colorectal cancer and patients with squamous cell head and neck cancers[14]. Despite this recommendation there has been significant lag in the uptake of therapeutic drug monitoring due to concerns over the quality of the evidence available, the practical concerns about the implementation of the procedure and the receptiveness of oncologists. 5-FU is a relatively mature drug in the oncology field and therefore physicians have clinical experience and comfort with managing 5-FU dosing in the face of adverse events. This comfort and trust of clinical experience can limit the uptake of new guidance if the treating physician does not have substantial buy-in for the new technique[14]. A lingering concern over the available data is that only two of the trials have been prospectively collected randomly controlled trials[16,17], with the remaining being uncontrolled or single arm studies. Further the two prospective trials were completed more than 20 years ago using regimens with short infusions (8hr) compared to modern regimens with extended continuous infusions (46hr). This concern limits the potential applicability of these findings in the contemporary context. Updated trial data is needed to appropriately validate therapeutic drug monitoring, however multiple trials have been discontinued due to slow enrollment. The final hurdle for therapeutic drug monitoring is the limitation to 5-FU based regimens, without translation for capecitabine regimens[26]. Capecitabine is a prodrug of 5-FU and although they exert the same mechanisms of action and are subject to the same elimination, capecitabine pharmacokinetics are significantly different than 5-FU. The conversion of capecitabine to 5-FU requires three enzymatic steps, the final reaction occurs in the end target tissues. This means that following capecitabine administration there is not a significant systemic circulation exposure of 5-FU. There is currently a very limited understanding of the exposure response relationship for capecitabine and the data is too immature to suggest the relevancy of therapeutic drug monitoring[26]. These many limitations of therapeutic drug monitoring will require significant efforts to overcome.

6.2 Future Directions

No model is able to perfectly account for all sources of variation in a biologic system however we must demonstrate that the approximations we derive from model systems are accurate enough to provide a meaningful result. The different studies in this thesis provide additional insight into the prevention of fluoropyrimidine-related adverse events but remain limited in their own way. We have demonstrated that *DPYD* genotype-guided dosing reduces the risk of fluoropyrimidine-related adverse events in Ontario. However, this study tested for only four single nucleotide variants and did not account for any other sources of variation when making dose recommendations. Given the highly polymorphic nature of *DPYD* it is implausible that the four assessed variants are the only deleterious *DPYD* variants in the population. The nested case-control study reported in Chapter 3 demonstrates this point through identifying a carrier of the *DPYD* exon 4 deletion who experienced a severe adverse event. The rarity in our sample necessitates screening a large number of patients to estimate the true allele frequency and determine the clinical relevance of this variant. As well in the broader *DPYD* literature novel deleterious variants are commonly discovered although their frequencies may be rare. To this end consideration of different genetic testing approaches should be considered in future studies. Sequencing individual patients at a population scale would be extremely costly and is unlikely a realistic option for improving the detection of deleterious *DPYD* variants. However other techniques such as multiplex ligation dependent probe amplification (MLPA) may prove to balance the increased sensitivity and cost of testing. MLPA allows for 60 probes per reaction and simultaneously detects both CNVs and SNVs[27,28]. This dual action may allow for the improved detection of *DPYD* genetic deficiency and improve the performance of genotype-guided dosing. Furthermore, *DPYD* variant carrying patients in chapter 2 were treated with a dose reduction, but given the narrow therapeutic index of the fluoropyrimidines this places patients at risk of a subtherapeutic exposure. The original follow-up in chapter 2 was related only to time on therapy, a more extensive follow-up for the purpose of assessing survival outcomes is planned. This study will hopefully demonstrate that the dose reduction is balanced by the decreased enzymatic potential of variant carriers and they are therefore experiencing therapeutic systemic exposure and equivalent survival outcomes. The report of genotype-

guided dosing in Chapter 2 also did not account for other endogenous co-factors or predictors of risk. While Chapter 5 demonstrated that plasma folate level assessed through pABG equivalents did not significantly improve the prediction of fluoropyrimidine-related adverse events, it did suggest that sex and the fluoropyrimidine used were significant predictors. Previously it has been demonstrated that sex, age, and which fluoropyrimidine used are all significantly associated with the risk for adverse events. To this end a dosing nomogram was suggested to incorporate these variables with *DPYD* genotype guided dosing[29]. As well the French company Onco Drug Personalized Medicine (ODPM) has created a multi-parametric dosing model that includes *DPYD* genotype, plasma uracil concentration, and clinical characteristics into the dose recommendations for fluoropyrimidines. ODPM has published results suggesting the multi-parametric model outperforms genotyping or phenotyping alone, however this study was of relatively small size and likely confounded by selection bias[30]. Building on this interest of expanding prediction beyond a single variable the Netherlands Cancer Institute is currently conducting the Alpe2U trial using both *DPYD* genotyping and Phenotyping concurrently. Together these various efforts demonstrate the momentum of the field is headed towards a more robust system for predicting the risk of fluoropyrimidine-related adverse events. Efforts to use multiparametric models will need to be replicated and validated in both Europe, North America and Asia individually due to the known variance in fluoropyrimidine-related adverse events.

6.3 Conclusion

We set out to improve the use of fluoropyrimidine chemotherapy in Ontario. From the work completed we have successfully demonstrated that *DPYD* genotype-guided dosing is beneficial in an Ontario population. We continued from this result to work towards knowledge translation and lobby for policy changes to support the implementation of this technology within the publicly funded Ontario health care system. We found that despite *DPYD* genotype-guided dosing unexplained fluoropyrimidine adverse events still impact 30% of patients in Ontario. Therefore, further investigation of fluoropyrimidine pharmacology including multi-parametric dosing models and therapeutic drug monitoring is required.

6.4 Chapter 6 References

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Appendices

Appendix A: R-Script for analysis in Chapter 2

```
################ Library required packages first################
library("readxl")
library("tidyverse")
library("epiR")
library("TOSTER")

################ Import Data and define datasets################

my_data <- read_excel("Data/data1.xlsx",
                      na = "**")

################ Clean data.frame and redefine the data types################

factor.columns <- c("Reason",
                    "Phenotype", ##a factor variable that labels patients as Non-carrier or Carrier
of any variant
                    "Sex",
                    "Site",
                    "Histology",
                    "Stage",
                    "ECOG",
                    "Race",
                    "Genotype", ## a factor variable that uses the *allele system for easier
review of the data
                    "c.1905.1.G.A", ## genotype of this variant factor of GG or GA or AA (no
AA detected)
                    "c.2846.A.T" , ## genotype of this variant factor of AA or AT or TT (no TT
detected)
                    "c.1679.T.G", ## genotype of this variant factor of TT or AT or TT (no TT
detected)
                    "c.1236.G.A", ## genotype of this variant factor of GG or GA or AA (no AA
detected)
                    "Initial.Drug",
                    "Regimen",
                    "Initial.Pharmacy.Intent",
                    "Radiation",
                    "Early.Global", #Presence or absence of this class of event for the patient
(0,1)
                    "Early.GI", #Presence or absence of this class of event for the patient (0,1)
                    "Early.MS", #Presence or absence of this class of event for the patient (0,1)
```

```
                "Early.Cardiac", #Presence or absence of this class of event for the patient
(0,1)
                "Early.HFS", #Presence or absence of this class of event for the patient (0,1)
                "Early.Other", #Presence or absence of this class of event for the patient
(0,1)
                "Early.Discontinued", #Presence or absence of this class of event for the
patient (0,1)
                "Early.Death", #Presence or absence of this class of event for the patient
(0,1)
                "Total.Global", #Presence or absence of this class of event for the patient
(0,1)
                "Total.GI", #Presence or absence of this class of event for the patient (0,1)
                "Total.MS", #Presence or absence of this class of event for the patient (0,1)
                "Total.Cardiac", #Presence or absence of this class of event for the patient
(0,1)
                "Total.HFS", #Presence or absence of this class of event for the patient (0,1)
                "Total.Other", #Presence or absence of this class of event for the patient (0,1)
                "Total.Discontinued", #Presence or absence of this class of event for the
patient (0,1)
                "Total.Death", #Presence or absence of this class of event for the patient
(0,1)
                "Category") #variable summarizing the genotype status generated by
database


my_data[factor.columns] <- lapply(my_data[factor.columns], factor)

int.columns <- c("Age",
              "Cycles")

my_data[int.columns] <- lapply(my_data[int.columns], as.integer)

my_data$c.1905.1.G.A <- factor(my_data$c.1905.1.G.A,
                          levels = c("GG", "GA"))
my_data$c.2846.A.T <- factor(my_data$c.2846.A.T,
                        levels = c("AA", "AT"))
my_data$c.1679.T.G <- factor(my_data$c.1679.T.G,
                        levels = c("TT", "TG"))
my_data$c.1236.G.A <- factor(my_data$c.1236.G.A,
                        levels = c("GG", "GA"))
my_data$Regimen <- factor(my_data$Regimen,
                      levels = c(
                        "FOLFOX",
                        "FOLFIRI/FOLFIRINOX",
                        "5-FU Cis/Carb",
                        "5-FU Other",
```

```
                        "Cape Mono",
                        "Cape Ox",
                        "Cape Cis/Carb",
                        "Cape Other",
                        "Cape RT")
                              )

my_data$Category <- factor(my_data$Category,
                           levels = c(
                            "PreWT",
                            "PreVariant"
                                 )
                                  )
################ Baseline Data ################

table(my_data$Sex, my_data$Phenotype)
table(my_data$Sex)

table(my_data$Race, my_data$Phenotype)
table(my_data$Race)

my_data %>%
  group_by(Phenotype) %>%
  summarize(mean = mean(Age),
            sd = sd(Age),
            min = min(Age),
            max = max(Age))
my_data %>%
  summarize(mean = mean(Age),
            sd = sd(Age),
            min = min(Age),
            max = max(Age))

my_data %>%
  group_by(Phenotype) %>%
  summarize(mean = mean(BSA),
            sd = sd(BSA),
            min = min(BSA),
            max = max(BSA))

my_data %>%
  summarize(mean = mean(BSA),
            sd = sd(BSA),
            min = min(BSA),
            max = max(BSA))
```

```
table(my_data$Site, my_data$Phenotype)
table(my_data$Site)

table(my_data$Regimen, my_data$Phenotype)
table(my_data$Regimen)

table(my_data$Genotype, my_data$Phenotype)
table(my_data$Genotype)

################ Testing for Total Toxicities###############

my_data %>%
  group_by(Category) %>%
  summarize( min = min(Cycles),
             Q1 = quantile(Cycles, 0.25),
             median = median(Cycles),
             Q3 = quantile(Cycles, 0.75),
             max = max(Cycles))

my_data %>%
  group_by(Genotype) %>%
  summarize( min = min(Cycles),
             Q1 = quantile(Cycles, 0.25),
             median = median(Cycles),
             Q3 = quantile(Cycles, 0.75),
             max = max(Cycles))

my_data %>%
  group_by(Genotype) %>%
  summarize(mean = mean(Initial.Intensity),
            sd = sd(Initial.Intensity),
            min = min(Initial.Intensity),
            max = max(Initial.Intensity))

my_data %>%
  group_by(Phenotype) %>%
  summarize(mean = mean(Initial.Intensity),
            sd = sd(Initial.Intensity),
            min = min(Initial.Intensity),
            max = max(Initial.Intensity))


my_data %>%
  group_by(Genotype) %>%
  summarize(mean = mean(Average.Intensity),
            sd = sd(Average.Intensity),
```

```
        min = min(Average.Intensity),
        max = max(Average.Intensity))

my_data %>%
  group_by(Phenotype) %>%
  summarize(mean = mean(Average.Intensity),
        sd = sd(Average.Intensity),
        min = min(Average.Intensity),
        max = max(Average.Intensity))

wilcox.test(my_data$Cycles ~ my_data$Phenotype,
        mu = 0,
        alt = "two.sided",
        conf.int = T,
        conf.level = 0.95,
        paired = F,
        exact = F,
        correct = T)

table(my_data$Phenotype, my_data$Total.Global)
Pre.Total.Global <-table(my_data$Phenotype, my_data$Total.Global)
chisq.test(Pre.Total.Global, correct = F)

table(my_data$Phenotype, my_data$Total.GI)
Pre.Total.GI<-table(my_data$Phenotype, my_data$Total.GI)
chisq.test(Pre.Total.GI, correct = F)

table(my_data$Phenotype, my_data$Total.MS)
Pre.Total.MS<-table(my_data$Phenotype, my_data$Total.MS)
chisq.test(Pre.Total.MS, correct= F)

table(my_data$Phenotype, my_data$Total.Cardiac)
Pre.Total.Cardiac<-table(my_data$Phenotype, my_data$Total.Cardiac)
fisher.test(Pre.Total.Cardiac, conf.int = T, conf.level =0.95)

table(my_data$Phenotype, my_data$Total.HFS)
Pre.Total.HFS<-table(my_data$Phenotype, my_data$Total.HFS)
fisher.test(Pre.Total.HFS, conf.int = T, conf.level =0.95)

table(my_data$Phenotype, my_data$Total.Other)
Pre.Total.Other<-table(my_data$Phenotype, my_data$Total.Other)
fisher.test(Pre.Total.Other, conf.int = T, conf.level =0.95)


table(my_data$Phenotype, my_data$Total.Death)
Pre.Total.Death<-table(my_data$Phenotype, my_data$Total.Death)
```

```
fisher.test(Pre.Total.Death, conf.int = T, conf.level =0.95)

table(my_data$Phenotype, my_data$Total.Discontinued)
Pre.Total.Discontinued <-table(my_data$Phenotype, my_data$Total.Discontinued)
fisher.test(Pre.Total.Discontinued, conf.int = T, conf.level =0.95)

################ Testing for Early Toxicities ###################
table(my_data$Phenotype, my_data$Early.Global)
Pre.Early.Global <-table(my_data$Phenotype, my_data$Early.Global)
chisq.test(Pre.Early.Global, correct = F)

table(my_data$Phenotype, my_data$Early.GI)
Pre.Early.GI<-table(my_data$Phenotype, my_data$Early.GI)
fisher.test(Pre.Early.GI, conf.int = T, conf.level =0.95)

table(my_data$Phenotype, my_data$Early.MS)
Pre.Early.MS<-table(my_data$Phenotype, my_data$Early.MS)
fisher.test(Pre.Early.MS, conf.int = T, conf.level =0.95)

table(my_data$Phenotype, my_data$Early.Cardiac)
Pre.Early.Cardiac<-table(my_data$Phenotype, my_data$Early.Cardiac)
fisher.test(Pre.Early.Cardiac, conf.int = T, conf.level =0.95)

table(my_data$Phenotype, my_data$Early.HFS)
Pre.Early.HFS<-table(my_data$Phenotype, my_data$Early.HFS)
fisher.test(Pre.Early.HFS, conf.int = T, conf.level =0.95)

table(my_data$Phenotype, my_data$Early.Other)
Pre.Early.Other<-table(my_data$Phenotype, my_data$Early.Other)
fisher.test(Pre.Early.Other, conf.int = T, conf.level =0.95)

table(my_data$Phenotype, my_data$Early.Death)
Pre.Early.Death<-table(my_data$Phenotype, my_data$Early.Death)
fisher.test(Pre.Early.Death, conf.int = T, conf.level =0.95)

table(my_data$Phenotype, my_data$Early.Discontinued)
Pre.Early.Discontinued <-table(my_data$Phenotype, my_data$Early.Discontinued)
fisher.test(Pre.Early.Discontinued, conf.int = T, conf.level =0.95)

################ Unadjusted RR by Genotype for Total Global################

table(my_data$c.1905.1.G.A, my_data$Total.Global)
Pre.Total.Global.c.1905.1.G.A.v2 <- matrix(c(3, 6, 426, 959),
                                   nrow=2,
                                   byrow =T)
epi.2by2(Pre.Total.Global.c.1905.1.G.A.v2,
```

```
        method = "cohort.count",
        conf.level = 0.95)

table(my_data$c.2846.A.T, my_data$Total.Global)
Pre.Total.Global.c.2846.A.T.v2 <- matrix(c(5, 14, 424, 951),
                                nrow=2,
                                byrow =T)
epi.2by2(Pre.Total.Global.c.2846.A.T.v2,
        method = "cohort.count",
        conf.level = 0.95)

table(my_data$c.1679.T.G, my_data$Total.Global)
Pre.Total.Global.c.1679.T.G.v2 <- matrix(c(0, 1, 429, 964),
                                nrow=2,
                                byrow =T)
epi.2by2(Pre.Total.Global.c.1679.T.G.v2,
        method = "cohort.count",
        conf.level = 0.95)

table(my_data$c.1236.G.A, my_data$Total.Global)
Pre.Total.Global.c.1236.G.A.v2 <- matrix(c(3, 15, 426, 950),
                                nrow=2,
                                byrow =T)
epi.2by2(Pre.Total.Global.c.1236.G.A.v2,
        method = "cohort.count",
        conf.level = 0.95)


retro.c.1236.G.A.v2 <- matrix(c(14, 27, 432, 944),
                                nrow=2,
                                byrow =T)
epi.2by2(retro.c.1236.G.A.v2,
        method = "cohort.count",
        conf.level = 0.95)


############### Adjusted RR calculations Total Global ###############

model1.1 <- glm(Total.Global ~ c.1905.1.G.A, data = my_data, family = "binomial")
summary(model1.1)
confint(model1.1)

model1.2 <- glm(Total.Global ~ c.1905.1.G.A + Age + Sex, data = my_data, family =
"binomial")
summary(model1.2)
confint(model1.2)
```

```
model1.3 <- glm(Total.Global ~ c.1905.1.G.A + Age + Sex + Regimen + Initial.Intensity, data = my_data, family = "binomial")
summary(model1.3)
confint(model1.3)

model2.1 <- glm(Total.Global ~ c.2846.A.T, data = my_data, family = "binomial")
summary(model2.1)
confint(model2.1)

model2.2 <- glm(Total.Global ~ c.2846.A.T + Age + Sex, data = my_data, family = "binomial")
summary(model2.2)
confint(model2.2)

model2.3 <- glm(Total.Global ~ c.2846.A.T + Age + Sex + BSA + Regimen, data = my_data, family = "binomial")
summary(model2.3)
confint(model2.3)

model3.1 <- glm(Total.Global ~ c.1236.G.A, data = my_data, family = "binomial")
summary(model3.1)
confint(model3.1)

model3.2 <- glm(Total.Global ~ c.1236.G.A + Age + Sex, data = my_data, family = "binomial")
summary(model3.2)
confint(model3.2)

model3.3 <- <- glm(Total.Global ~ c.1236.G.A + Age + Sex + BSA + Regimen, data = my_data, family = "binomial")
summary(model3.3)
confint(model3.3)

################Tests of Equivalence using an inferiority design################
##These tests compare variant carriers against non-carriers of any variant.
##The high_eqbound is set by the minimum significant predicted difference between non-carriers and carriers.
##The difference is found using the low bound of the 95% CI for unadjusted RR for the SNP with the weakest effect in the literature.
##Values for c.1236G>A as defined in the meta-analysis (Meulendijks et al, Lancet, 2015) used in this section.
##As this is a non-inferiority test low bound is not considered in interpretation but required by the TOSTtwo.prop function, therefore it is set to -0.31 for plotting.
## non-inferiority test for variant carriers vs non carriers and risk of severe adverse events in total treatment period
```

TOSTtwo.prop(prop1 = .23, prop2 = .31, n1 = 47, n2 = 1347, low_eqbound = -0.31, high_eqbound = 0.0682, alpha = .050)
non-inferiority test for variant carriers vs non carriers and risk of severe adverse events in early treatment period
TOSTtwo.prop(prop1 = .13, prop2 = .211, n1 = 47, n2 = 1347, low_eqbound = -0.31, high_eqbound = 0.0252, alpha = .050)

Appendix B: ICES Dataset Creation Plan

Dataset Creation Plan

Project Initiation This Section must be Completed Prior to Project Dataset(s) Creation		
Project Title:	Implementation of Pharmacogenomics-based Personalized Medicine- 5-Fluorouracil and Capecitabine --- Feasibility Analysis	
Project TRIM number:	2020 0906 171 004	
Research Program:	KDT	
Site:	ICES Western	
Project Objectives:	*Insert Project Objectives as listed in the approved ICES Project PIA*	
	1. Examine the current 5-FU and capecitabine use in Ontario 2. Provide a descriptive analysis of the demographics, comorbidties and health care utilization amongst the 5-FU and capecitabine cohort	
ICES Project PIA Initial Approval Date:	*The ICES Employee or agent who is responsible for creating the Project Dataset(s) is responsible for ensuring there is an approved ICES Project PIA and verifying the date of approval prior to creating the Project Dataset(s)*	
	2019-Jul-19	
Principal Investigator (PI):	Richard Kim	
Check the applicable box if the PI is an ICES Student/Trainee	☐ ICES Student ☐ ICES Fellow ☐ ICES Post-Doctoral Trainee ☐ Visiting Scholar	
Responsible ICES Scientist:	*Name the Responsible ICES Scientist if the PI is not a Full Status ICES Scientist*	
	Amit Garg	
Project Team Member(s) Responsible for Project Dataset Creation and/or Statistical Analysis and date joined (list all):	*All person(s) (ICES Analyst, Appointed Analyst, Analytic Epidemiologist, PI, and/or Student) responsible for creating the Project Dataset(s) and/or statistical analysis on the Research Analytics Environment (RAE) and the date they joined the project must be recorded*	
	Nivethika Jeyakumar	2019-Jun-01
	Eric McArthur	
Other Project Team Members and date joined (list all):	*All other Research Project Team Members (e.g., Research Administrative Assistants, Research Assistants, Project Managers, Epidemiologists) and the date they joined the project must be recorded*	
	Brandi Povitz	2019-Jun-01
	Robin Legan	
	Theo Wigle	
	VeeraDurga Panuganty	
	Erin Cragg	
	Samantha Medwid	
Confirmation that DCP is consistent with Project Objectives: **NOTE: Project cannot start if this section is not complete.**	*The following individuals must confirm that the ICES Data provided for in this DCP is relevant (e.g., with respect to cohort, timeframe, and concepts) and required to achieve the Project Objectives stated in the ICES Project PIA prior to initial Project Dataset creation: 1) PI; 2) Responsible ICES Scientist if the PI is not a Full Status ICES Scientist, or a second ICES Scientist or the Scientific Program Lead if the PI is creating both the DCP and the Project Dataset(s); 3) ICES Research Practice Staff creating the DCP; and 4) ICES Analytic Staff (ICES Employee or agent responsible for creating the Project Dataset(s)). This may be delegated either verbally or via e-mail.*	
	Principal Investigator	
	Richard Kim ☒	2019-10-30
	Responsible ICES Scientist or Second ICES Scientist/Lead	
	Amit Garg ☒	2019-11-21
	ICES Research Practice Staff Creating the DCP	
	Danielle Nash ☒	2019-Nov-08

Project Initiation This Section must be Completed Prior to Project Dataset(s) Creation		
	ICES Analytic Staff	
	Eric McArthur	☒ 2019-Nov-08
Designated ICES Research Practice Staff accountable for Project Documentation:	*The person named (ICES staff) is accountable for ensuring that the approved ICES Project PIA, ICES Project PIA Amendments, and DCP are saved on the T Drive, ensuring ICES Project PIA Amendments are submitted as required, ensuring DCP Amendments are documented, and sharing the final DCP with the PI/Responsible ICES Scientist at project completion*	
	Nivethika Jeyakumar	
DCP Creation Date and Author:	*Date DCP was finalized prior to Project Dataset(s) creation*	*Name of person who created the DCP*
	Date	***Name***
	2019-Jun-01	Nivethika Jeyakumar

ICES Data This Section must be Completed Prior to Project Dataset(s) Creation	
The ICES Employee or agent who is responsible for creating the Project Dataset(s) must ensure that this list includes only data listed in the ICES Project PIA.Changes to this list after initial ICES Project PIA approval require an ICES Project PIA Amendment.	*Mandatory for all datasets that are available by individual year*
General Use Datasets – Health Services	***Years (where applicable)***
CIHI DAD	2008-2018
CIHI SDS	2008-2018
NACRS	2008-2018
OHIP	2008-2018
CONTACT	2008-2017
ODB	2013-2018
OMHRS	2008-2018
HCD	2012-2018
NRS	2013-2018
General Use Datasets – Care Providers	
IPDB	2012-2018
General Use Datasets – Population	
CENSUS	2018
POP	2016
RPDB	2012-2018
General Use Datasets – Coding/Geography	
DIN	2012-2018
REF	2011
LHIN	2009

ICES Data This Section must be Completed Prior to Project Dataset(s) Creation	
PCCF	2013
General Use Datasets – Other	
AVGPRICE	2013-2018
CAPE	2013-2018
Controlled Use Datasets	
ALR	2005-2018
NDFP	2013-2018
OCR	1993-2018
Other Datasets	
OLIS	2012-2018

Project Amendments and Reconciliation

ICES Project PIA Amendment History (add additional rows as needed):

Privacy approval date	*Person who submitted amendment*	*Note that any changes to the list of ICES Data or Project Objectives require an ICES Project PIA Amendment*
Date	***Name***	***Amendment***
2020-06-23	Nivi	Only use CCO regimens to build cohort
2020-07-13	Nivi	Examine outcomes between cycles instead of visits
2020-07-23	Nivi	Rebuild cohort using CCO drug code/DIN instead of CCO regimens
2020-08-05	Nivi	Rebuild cohort using CO drug code/DIN and restricted to specific CCO regimens

DCP Amendment History:

DCP Amendment History Template.doc

Date Programs/DCP reconciled

The person(s) creating the dataset and/or analyzing the data are responsible for ensuring that the final DCP reflects the final program(s) when the project is completed

yyyy-mon-dd

Study Design and Project Time Frame Definitions

Study Design

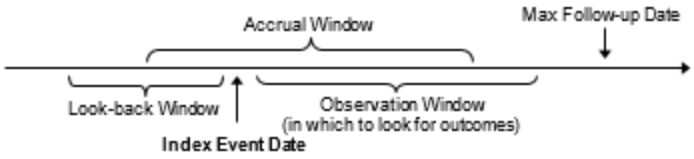
☒ Cohort study ☐ Matched cohort study ☐ Case-control study

☐ Cross-sectional study ☐ Other (specify):

L

Study Design and Project Time Frame Definitions

Project Timeline



Accrual Start/End Dates	December 1, 2014 to December 31, 2018
Max Follow-up Date	March 31, 2019
When does observation window terminate?	• The first day without ta CAPE or 5FU CCO drug code or DIN visit (associated with their index CCO regimemn of interest) in its 60-day look forward window (both end inclusive) (Censor date: Previous dose date + days administered/length + 60 days) • For 5-FU regimens associated with a 14 day cycle, censor on the date that a dose is received < 12 days from the previous dose • For CAPE regimens, censor if the next dose is observed before the associated days administered • Death • March 31, 2019
Lookback Window(s)	• 5 year lookback for comorbidities • 1 year lookback for health care utilization • 120 day lookback for medication use

Cohort Build

Note: Include a cohort build table in appendices.

Index Event / Inclusion Criteria and Index Date	All patients in Ontario with valid IKN who received their first capecitabine or 5-FU therapy (must be a non-missing and non-zero dose) between December 1, 2014 and December 31, 2018. **Index date:** Date of first capecitabine or 5-FU chemotherapy dose **Note:** Capecitabine and 5-FU therapy defined using CCO drug code or DINs and restricted to specific CCO regimens
Estimated Size of Cohort (if known)	N/A

Exclusions (in order)

Step	Description
1	Data cleaning: • Missing or invalid, age (≤ 18 or >105) or sex • Non-Ontario residentd(individuals without RPDB variable "prdcddablk" beginning with "35")
2	Death on or before index date
3	Restrict to patients who have a cancer diagnosis on or in the year prior to index date
4	History of capecitabine or 5-FU chemotherapy at any time prior to index date

M

Cohort Build	
	Note: history will be based on CCO drug code or DIN of interest
	5 More than 1 distinct CCO regimen on index date

Concept Definitions (add additional rows as needed)	
Note: Include concept definition details in appendices.	
Main Exposure or Risk Factor	5-FU or capecitabine chemotherapy
Primary Outcome Definition	Any chemotherapy-related acute care visits
Secondary Outcome Definition(s)	• Chemotherapy-related acute care visits (hospitalizations only) • Chemotherapy-related acute care visits (ER visits only) • Febrile neutropenia related acute care visits • Mortality • Cancer-specific mortality • Hospitalizations • ER visits • Edmonton Symptom Assesment System score • Cancer surgery during follow-up • Healthcare- associated costs See **Appendix C Table C3** for details
Baseline Characteristics	See **Appendix C Table C2** for details

N

Analysis Plan and Dummy Tables
(Below is a guide – please MODIFY/EXPAND as appropriate)

O

Step 0: Cohort codes
Review cohort code list

a. Use %assign to obtain the cohort code list
b. Use %dinexplore to explore the set of DINs provided
c. PI to record date that they reviewed and approved of the cohort code list (quality assurance box below)

***** STOP FOR REVIEW *****

1. Cohort build **(Appendix B: Table 1 Cohort Build). Details provided in Table C1.**
   a. Report the number of patients included and excluded at each step of the cohort build
   b. ODB

***** STOP FOR REVIEW *****

2. Follow-up Time **(Appendix B: Table 2 Follow-Up Time).**
   a. Distribution of days between cycles for full follow-up only considering death and March 31, 2019 as censoring points (min, max, mean (SD), median (IQR)
      Note: only count as a new a cycle if the doses are separated by at least the number days listed in the length variable in **Appendix D Tab CCO regimen_FLU and CCO_regimen CAPE** for each specific regimen type and no more than 60 days
   b. Distribution of follow-up in days for complete follow-up (min, max, mean (SD), median (IQR))
   c. Distribution of days between 1st and 2nd dose cycle (min, max, mean (SD), median (IQR))
      Note: only calculate the distribution of days in 2b for patients who have a 2nd dose cycle recorded in ALR
   d. Distribution of days between 2nd and 3rd CCO dose cycle (min, max, mean (SD), median (IQR))
      Note: Restrict to one dose per day per patient per visit
      Note: only calculate the distribution of days in 2c for patients who have a 3rd dose cycle recorded in ALR

***** STOP FOR REVIEW *****

3. CCO Regimen Characteristics **(Appendix B: Table 3 Regimen Characteristics). Details provided in Table C4.**
   a. Report the number of cycles received during complete follow-up including index date (min, max, mean (SD), median (IQR))
      Note: Resitrict to one dose per day per patient per visit for 3a
   b. Report the distribution of the % ideal dose administered per cycle (min, 5th percentile, mean (SD), median (IQR), 95th percentile, max)
      Note: See Appendix C Table C4 for % ideal dose administered algorithm

***** STOP FOR REVIEW *****

4. Baseline Characteristics (**Appendix B: Table 4 Baselines).** Details provided in **Table C2.**
   - Report baseline characteristics for full cohort

***** STOP FOR REVIEW *****

5. Primary CRV Outcome (**Appendix B: Table 5 Outcomes).** Details provided in **Table C3.**

P

Analysis Plan and Dummy Tables
(Below is a guide – please MODIFY/EXPAND as appropriate)

Report the following for the primary outcome

a. Number of patients with at least one event
b. Total number of CRV events
c. Mean (SD), Median (IQR) for CRV events per person
d. Rate of CRV events (per 1000 person-years)

Note: Aggregate event rate for the primary outcome of chemotherapy related visit with a follow-up between 1st and 2nd treatment dose cycle (for those who have 2nd dose cycle), 1st dose and 60 days from 1st dose (for those who do not have a 2nd dose cycle), 2nd and 3rd treatment dose cycle (for those who have a 3rd dose) , 2nd dose and 60 days from 2nd dose (for those who have a 2nd dose cycle but do not have a 3rd dose cycle), 90 days (for individuals who did not have another dose ≥30 days from index date, will only contribute 60 days from their last dose for this time point) and complete follow-up.

Note: Report the number of patients who only had a code for VTE during their acute care visit in the specified follow-up periods

Note: Amongst individuals who had a CRV , report the proportion of patients who had any CRV event ≤ 4 weeks from the previous chemotherapy dose visit

Note: If there is no second/third dose cycle, report event at last dose + 60 days

Note: Report the number of events/per dose per person for the complete follow-up

***** STOP FOR REVIEW *****

6. Secondary Outcomes (**Appendix B: Table 5 Outcomes).** Details provided in **Table C3.**
   a. Repeat step 5 for secondary outcomes CRV visits (hosp and ER separately), neutropenia,all-cause mortality,cancer-specific mortality, cancer surgeries, hospital admissions, ER visits and ESAS tests

   Note: Report the number of cancer related surgeries that occurred before adjuvant treatment

   Note: Report the number of days between last neoadjuvant dose and cancer surgery date ((min, max, mean (SD), median (IQR))

   Note: For ESAS, report the distribution of days from 1st and 2nd ESAS test dates during follow-up (min, max, mean (SD), median (IQR))

***** STOP FOR REVIEW *****

7. Report composite health care costs (**Appendix B: Table 6 Costs).** Details provided in **Table C3.**
   a. Total Cost
   b. Mean (SD) and median (IQR) cost per person-year

***** STOP FOR REVIEW *****

Additional Analytic Requests for Theo's Thesis Chapter

8. Report composite health care costs stratified by those who experienced a CRV during follow-up vs those who did not
   a. Total Cost
   b. Mean (SD) and median (IQR) cost per person-year

Q

Quality Assurance Activities		
RAE Directory of SAS Programs		
RAE Directory of Final Dataset(s)	*The final analytic dataset for each cohort includes all the data required to create the baseline tables and run all the models. It should include all covariates for all models such as patient risk factors, hospital characteristics, physician characteristics, exposure measures (continuous, categorical) and outcomes. It should include covariates that were considered but didn't make the final cut. This would permit an analyst to easily re-run the models in the future.*	
RAE README file available:	☐Yes ☐No	
Date results of quality assurance tools for final dataset shared with project team (where applicable):		
	%assign	yyyy-mon-dd
	%evolution	yyyy-mon-dd
	%dinexplore	yyyy-mon-dd
	%track / %exclude	yyyy-mon-dd
	%codebook	yyyy-mon-dd
Additional comments:		

Appendices (add appendices as needed)	
Appendix A: Codes	Refer to the "Appendix A DPYD Codes" Excel document Appendix A DPYD Codes.xlsx
Appendix B: Output Tables	Refer to the " Appendix B DPYD Output Tables" Excel document Appendix B DPYD Output Tables_2020
Appendix C: Concept Details	See below (additional details for inclusion/exclusion criteria, exposure, baseline characteristics, outcomes, and other concepts).
Appendix D: DRUGLIST	Refer to the " Appendix D " Excel document Eligible CCO regimens, drugcode

R

Appendix C List of Chemotherapy Related Visit Codes

	Description	ICD-10
Neutropenia	Agranulocytosis- Including drug induced	D70
Fever	Other Specified Fever	R508
	Chills with fever	
	Persistent fever	
	Fever with rigors	
	Fever unspecified	R509
	Fever NOS	
	FUO	
	Hyperpyrexia NOS	
	Pyrexia NOS	
	Pyrexia UO	
Infection	Infectious and parasitic diseases	A00-B99
	Bacteremia	
	Line associated Infection	T82.7
	Bronchitis	J20-J22
	Pneumonia	J12-J18
	Flu	J09-J11
	Kidney Infection	N10, N390
	Acute cystitis	N300
	Cellulitis	L00-L08
	Empyema	J86
	Abscess of lung/mediastium	J85
	Other septicaemia	A41
	Septicaemia unspecified	A419
	Septicaemia septic	
	Septicaemia other	A418
Gastrointestinal	Diarrhea	K52
	Functional diarrhea	K59.1
	Nausea/emesis	R11
	Heartburn	R12
	Constipation	K59.0
	Obstruction	K56
	Stomatitis	K12
	Cachexia	R64.0
	Anorexia	R63.0
Other Systemic Treatment Related	Hyponatremia	E87.1
	Hypokalemia	E87.6
	Electrolyte disorder	E87.0, 2, 3, 4, 5, 7, 8
	Magnesium disorder	E834
	Dehydration/hypovolemia	E86
	Malaise/Fatigue	R53
	Syncope	R55
	Dizziness	R42
	Hypotension	I959
	Fe deficiency anaemia	D50
	Other deficiency anaemia	D51-D53
	Aplastic anemia	D60, D61
	Other and unspecified anemia	D62-D64
	Thrombocytopenia	D69.5, D69.6
	Rash and non-specific skin eruptions	R21
	Hyperglycemia	R73

ICD-10: International Statistical Classification of Diseases and Related Health Problems 10th revision; NOS= not otherwise specified; FUO= fever of unknown origin; UO= unknown origin

Curriculum Vitae

Name: Theodore Wigle

Post-secondary Education and Degrees:

University of Winnipeg
Winnipeg, Mb, Canada
2012-2016 BSc(Hons)

The University of Western Ontario
London, Ontario, Canada
2016-2023 M.D. (In Progress)

The University of Western Ontario
London, Ontario, Canada
2018-2021 Ph.D.

Honours and Awards:

George Tomlinson Scholarship in Biochemistry
2015-2016

Chancellor's Gold Medal for the Highest Standing in Science
2016

Gold Medal for Achievement in Honours Biochemistry
2016

Western Graduate Research Scholarship
2018-2021

Province of Ontario Graduate Scholarship
2020-2021

Publications:

Wigle, Theodore J., *et al*. "Impact of pretreatment dihydropyrimidine dehydrogenase genotype-guided fluoropyrimidine dosing on chemotherapy associated adverse events." Clinical and Translational Science (2021).

Wigle, Theodore J., *et al*. "*DPYD* and fluorouracil-based chemotherapy: mini review and case report." Pharmaceutics 11.5 (2019): 199.

Wigle, Theodore J., *et al*. "Pharmacogenomics guided-personalization of warfarin and tamoxifen." Journal of personalized medicine 7.4 (2017): 20.

Presentations:

CITAC-CSCI Annual General Meeting “Genotype Testing During Chemotherapy: Reducing Adverse Events and Health Care Cost.” Oral Presentation (2019)

Physiology and Pharmacology Annual Research Day Poster Presentation Award “DPYD Genotype testing in Ontario.” Poster Presentation-Awarded Best in Category (2019)

ASCO Annual General Meeting “Prospective cohort study of the impact of hospital-wide dihydropyrimidine dehydrogenase (*DPYD*) genotype testing for fluoropyrimidine-based chemotherapy on adverse events and hospital costs.” Poster Presentation (2019).