IMPLEMENTATION OF PHARMACOGENETIC RISK PREDICTION MODELS IN PEDIATRIC ONCOLOGY

by

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Submitted by Tessa Bendyshe-Walton in partial fulfillment of the requirements for

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in Experimental Medicine

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Abstract

Adverse drug reactions (ADRs) are increasingly recognized as important and sometimes irreversible complications of cancer treatment[1,2]. Anthracyclines and cisplatin, two widely-used chemotherapeutic agents in the treatment of childhood malignancies, have contributed to the increased 5-year survival rates for childhood cancer to over 82% today[3]. Their use, however, is limited by the occurrence of anthracycline-induced cardiotoxicity in up to 57%[4] of treated children and cisplatin-induced ototoxicity in 60-70%[5-7] of treated children. Genetic associations for the susceptibility of these two ADRs have been discovered and replicated[8-12], and clinical practice guidelines have been published[13,14] outlining which associations have sufficient evidence for their use in clinical practice. Based on these clinical practice guidelines, pharmacogenetic risk prediction models that combined several genetic variants into one predicted outcome for anthracycline-induced cardiotoxicity and cisplatin-induced ototoxicity were developed using logistic regression.

In this study, pharmacogenetic risk prediction models for two common ADRs were implemented into clinical practice in pediatric oncology at BC Children's Hospital. Between July 2013 and September 2018, 279 patients were enrolled in the study and have had their pharmacogenetic risk prediction results returned to their treating oncologists. Results have been incorporated into treatment decision-making and have resulted in treatment modifications such as the use of cardioprotective and otoprotective drugs, increased audiological and cardiac monitoring, and the use of results to decide between different treatment protocols. Prospective evaluation of the occurrence of cardiotoxicity and ototoxicity currently demonstrates that pharmacogenetic-tested patients have experienced significantly less cardiotoxicity than previously treated patients that did not receive pharmacogenetic results over the same follow-up period (3.4% versus 11.8%, p=0.0005). Rates of cisplatin-induced ototoxicity in patients that received pharmacogenetic testing were similar to previously-treated patients used to develop the risk prediction model (58.9% versus 66.7%, respectively), and none of the patients that have received treatment modifications as a result of pharmacogenetic testing have developed clinically relevant ototoxicity (≥ grade 2 ototoxicity). Interviews with patients/families (n=11) and oncologists (n=4) demonstrated that patients/families felt more involved in treatment decisions and were reassured by understanding their risk of

toxicity. Oncologists indicated that testing helped ensure that treatment and long-term monitoring were appropriate for each patient.

Lay Summary

Anthracyclines and cisplatin are chemotherapy drugs used in the treatment of a variety of childhood cancers. While these drugs are extremely effective at treating cancer, they also have the ability to cause adverse drug reactions such as heart problems (cardiotoxicity) and hearing loss (ototoxicity) in 50-70% of treated patients. Previous studies have found that certain genes make patients more likely to develop cardiotoxicity and ototoxicity than others. In order to determine who is most at risk of experiencing these adverse drug reactions, genetic tests have been developed and integrated into clinical practice that allow oncologists to test their patients for genes that would put their patients at higher or lower risk. At BC Children's Hospital between July 2013 and September 2018, 279 children diagnosed with cancer were enrolled in this study and received genetic testing for their risk of cardiotoxicity from anthracyclines and/or ototoxicity from cisplatin.

Preface

I wrote the entire dissertation with direction and input from Drs. Bruce Carleton, Colin Ross and Rod Rassekh. This study was approved by the University of British Columbia Clinical Research Ethics Board (certificate numbers H12-02655: Implementation of a Pharmacogenetic ADR Prevention Program in B.C and H04-70358: GATC: Genotype Specific Approaches to Therapy in Childhood: The Canadian Pharmacogenomics Network for Drug Safety).

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List of Abbreviations

5-HT3	Serotonin-Receptor 3
ABC	ATP-binding cassette transporter
ABCC3	ATP-binding cassette transporter, subfamily C, member
ABR	Auditory Brainstem Response
ASSR	Auditory Steady-state Response
ACYP2	Acylphosphatase 2
ADME	Absorption, Distribution, Metabolism, and Excretion
ADRs	Adverse Drug Reactions
AGREE	Appraisal of Guidelines Research and Evaluation Enterprise
ALL	Acute Lymphoblastic Leukemia
APL	Acute Promyolytic Leukemia
ATRA	All-trans Retinoic Acid
AUC	Area Under the Curve
CAT	Catalase Enzymes
CBR	Carbonyl Reductases
CDS	Clinical Decision Support
CI	Confidence Interval
CNV	Copy Number Variant
COG	Children's Oncology Group
COMT	Catechol O-methyltransferase
CPGs	Clinical Practice Guidelines
CPIC	Clinical Pharmacogenetics Implementation Consortium
CTCAE	Common Terminology Criteria for Adverse Events
CTRs	Copper Transporter Proteins
CYP450	Cytochrome P450
CYPOR/POR	Cytochrome P450 reductase
DPD	Dihydropyrimidine Dehydrogenase
DPWG	Dutch Pharmacogenomics Working Group
EF	Ejection Fraction

EMR	Electronic Medical Record
EPV	Events Per Variable
FDA	Food and Drug Administration
G6PD	Glucose-6-Phosphate Dehydrogenase
GLP	Good Laboratory Practice
GST	Glutathione S-transferases
GSTM	Glutathione S-transferase mu
GSTP	Glutathione S-transferase pi
GSTT	Glutathione S-transferase theta
HAS3	Hyaluronan Synthase 3
HMG	High Mobility Group
HNMT	Histamine N-Methyltransferase
LD	Linkage Dysequilibrium
LRP2	Megalin
MEC	Minimum Effective Concentration
MMR	Mismatch Repair
MTC	Minimum Toxic Concentration
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NAT	N-acetyltransferase
NER	Nucleotide Excision Repair
NGS	Next Generation Sequencing
NOS3	Nitric Oxide Synthase 3
OAEs	Otoacoustic Emissions
OAT	Organic Anion Transporter
OCTN	Organic Zwitterions/Cation transporters
OCT	Organic Cation Transporter
OCT2	Organic Cation Transporter 2
OS	Overall Survival
PD	Pharmacodynamics
PharmGKB	Pharmacogenomics Knowledgebase
PK	Pharmacokinetics

POG	Pediatric Oncology Group
QALY	Quality Adjusted Life Year
QR	Quick Response
RAR	Retinoic Acid Receptor
RARG	Retinoic Acid Receptor Gamma
RCTs	Randomized Controlled Trials
ROC	Receiver Operating Characteristic
ROS	Reactive Oxygen Species
SD	Standard Deviation
SLC	Solute Carrier Transporters
SNOMED	Systematized Nomenclature of Medicine
SNP	Single Nucleotide Polymorphism
SULT	Sulfotransferase
SULT2B1	Sulfotransferase Family 2B Member 1
TI	Therapeutic Index
TopII	Topoisomerase II
TPMT	Thiopurine Methyltransferase
UGT	Uridine Diphosphate Glucuronosyltransferase
UGT1A6	UDP-glucuronosyltransferase family 1A, isoform 6
U-PGx	Ubiquitous Pharmacogenomics
XPC	Xeroderma Pigmentosum

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Dedication

To my mom, dad, and sister for their endless love and support.

Chapter 1: Introduction

1.1: Adverse Drug Reactions (ADRs)

Adverse drug reactions (ADRs) are broadly defined as unintended or unwanted consequences experienced after administering or receiving a drug or combination of drugs[15,16]. Each year, there are an estimated 2 million severe ADRs resulting in 100,000-218,000 deaths in the United States, which places ADRs as the 4th leading cause of death[17,18]. In Canada, the prevalence of ADRs is expected to be similar and costs the Canadian healthcare system between $13.7 and $17.7 billion each year[19]. This is also likely an underestimation given that approximately 95% of ADRs are not reported to regulators and causes of drug-related deaths are often misidentified[19]. ADRs can be classified as Type A (intrinsic) or Type B (idiosyncratic)[17]. Type A reactions are usually predictable, dose-related toxicities while Type B occur independent of drug-dose and pharmacological effect. ADRs can vary from lack of therapeutic response to life-threatening and permanently disabling adverse effects. They can also range in timing of onset with some ADRs occurring immediately after administration and some exhibiting effects years after exposure to the causative drug[20].

1.1.2: ADRs in Children

ADRs are often more severe and frequent in children with up to 39% of ADRs being potentially life threatening or fatal[21]. Medications prescribed to children are often done so on an 'off-label' basis as clinical trials done to assess the safety and effectiveness of medications are largely conducted in adult populations. While efforts from the US Food and Drug Administration (FDA) have been made to expand age-appropriate evidence sufficient to provide information for labelling, more than 50% of products still have no pediatric information on their regulatory drug label recommendations[4,22]. The limited safety data coupled with the lack of pharmacokinetic studies in children may result in patients being frequently under- or over-dosed[23]. Additionally, the growth and development of children can result in changes in drug transporters and metabolizing enzymes which can have a significant impact on the therapeutic dose and risk of toxicity in this population[24,25]. Even in clinical trials that are conducted in children, serious ADRs are often rare and generally not observed during the trial period. This is especially true if there is a latency period in the time between when the drug is taken and the adverse effect is

observed—such as a change in growth or delayed-onset cardiomyopathy from anthracyclines. For most drugs, it is impossible to fully investigate rare ADRs before the drug reaches the market as it requires that a large population be exposed to the drug of interest to observe ADRs that occur with low frequency.

1.1.3: ADRs in Pediatric Oncology

Chemotherapeutic drugs exert their effects by either killing cancer cells (cytotoxic) or by preventing their proliferation (cytostatic). Currently, there are very few chemotherapeutic agents that have enough specificity to target solely cancer cells and, as a result, normal cells are also affected leading to toxicity. The therapeutic index (the ratio of the blood concentration at which a drug becomes toxic and the concentration at which the drug is effective) is very narrow for most chemotherapeutic drugs with a fatal dose rarely being more than double its therapeutic dose[26]. The cytotoxic effects on the host cells coupled with the narrow therapeutic range of most chemotherapeutic drugs highlights the need for drug safety information when prescribing and administering these drugs and explains why cancer patients experience the highest rates of ADRs[26].

With the improvement in five-year survival rates for pediatric cancer patients from 30% in the 1960s to over 80% today[3,27,28], the late adverse effects of cancer therapy are now becoming more apparent and a major concern in this population of patients[1,3,27,29]. ADRs account for 22% of all pediatric cancer patient's hospital admissions in the United States[28]. In up to 40% of these patients, these ADRs can cause life-threatening and permanently disabling effects[30-32]. ADRs have become one of the major complications of cancer treatment, and are so common that they are often seen as foreseeable consequences of treatment[33]. These ADRs can provide a major challenge for health care providers and patients, and can have a profoundly negative impact on patients' quality of life. For instance, 10% to 25% of childhood cancer survivors in their 20s to 30s have been found to exhibit an adverse health status including poor general health, poor mental health, functional impairment, activity limitation, cancer-related pain, or cancer-related anxiety[34]. These estimates are shown to continue increasing as the survivor ages[35].

While many clinical factors (age, sex, ethnicity, radiation, etc.) contribute to variable drug response[36-38], and are currently incorporated into clinical decision-making, this traditional approach to drug therapy limits safety and effectiveness as approximately 20-95% of drug variability and ADRs are suggested to be caused by genetic factors[39,40].

1.1.4: History of Genetic Variability in Drug Response

The suggestion that genetic variants could cause variability in drug response was first proposed by British physician Sir Archibald Garrod in 1939 who described "inborn errors of metabolism" that would lead to enzymatic defects affecting drugs concentrations and their subsequent effects[41,42]. One pioneering example of genetically-determined drug variability was elucidated in the 1950s when Kalow discovered that prolonged paralysis of patients after receiving succinylcholine could be caused by a pseudocholinesterase deficiency[39,43-45]. Subsequently, Snyder found that differences in one's sense of taste could be attributed to genetics[46]. The first idiosyncratic ADR was recognized by the Ancient Greeks who observed that some individuals who consumed fava beans were prone to hematuria. This was later found to be due to a deficiency in the enzyme, glucose 6-phosphate dehydrogenase (G6PD) [47]. This condition was not only prevalent in the Mediterranean population at the time, but is common among African populations. During World War II many African American soldiers who served in areas affected by malaria received an anti-malarial medication, primaquine, which causes hemolytic anemia in G6PD deficient individuals[48]. As a result, many of them suffered from hematuria, highlighting the role that ancestry and genetic isolation play in modulation drug response and other clinical phenotypes.

After World War II, huge advancements in medicine were being made, as antibiotics first entered the market. Shortly after the introduction isoniazid, an antibiotic, it was noted to cause peripheral neuropathy in some patients. This was later determined to be caused by impaired activity of the enzyme N-acetyltransferase (NAT) which results in higher levels of the drug circulating in the blood stream[49]. These discoveries attracted the attention of a number of scientists, such as Kalow and Motolsky, who would write seminal textbooks and papers about genetically determined differences in drug response[50]. This would create the basis for Friedrich Vogel to later coin the term 'pharmacogenetics' in 1959[51].

1.2: Pharmacogenetics

Pharmacogenetics and pharmacogenomics describe the role that genetic variation plays in affecting drug response and ADRs[52]. Pharmacogenetics refers to how variation in a single gene influences drug response while pharmacogenomics is a broader term that describes how the entire genome influences drug response[52]. A prime determinant of drug efficacy and toxicity is the concentration of active metabolites at the drug's target site or in the plasma[53]. The therapeutic index (TI) of a drug is the concentration range between the drug eliciting a therapeutic effect and a toxic effect[53]. This range of drug concentration is required for safe and effective drug use, however, concentrations of drugs in the plasma can vary by up to 600 times between individuals that receive the same drug dose[54]. As a result, a drug dose within the average therapeutic window for the majority of a patient population may be too low or too high for some individuals resulting in less optimal drug therapy and, in some cases, toxicity [55,56]. The field of pharmacogenomics aims to discover genetic variants that influence individual drug efficacy and toxicity in the hopes of avoiding the traditional trial and error approach to drug therapy, and to instead cater the dose to the individual patient to limit the exposure of drugs that are not effective or harmful to them[52].

1.2.1: Types of Genetic Variation

Although any two individual's genomes are 99.9% identical, the number of nucleotides (approximately 3 billion) is so large that millions of variant sequences still occur between different individuals. Variants that are found in >1% of the population are known as polymorphisms[54]. The most abundant type of polymorphisms are single nucleotide polymorphisms (SNPs), which cause single base pair substitutions and account for >90% of all human genetic variation[57,58]. The effect of the SNP on the function of the gene depends on where the substitution occurs in comparison to the coding region and regulatory factors of the gene, as well as, whether one or both copies of the gene are affected. While only 24,000 of the approximately 3.6 million SNPs that each individual carries are contained within exons, SNPs both inside and outside of coding regions can have an effect on the function of genes by causing changes to amino acid sequences, mRNA stability, and transcription factor binding affinity[59].

While less common that SNPs, genetic variation can also occur as structural variants such as insertions or deletions (indels), copy number variants (CNVs), and inversions[53]. These structural

variants often have greater repercussions than SNPs because they affect a larger area of the genome rather than a single nucleotide.

1.2.2: Pharmacokinetics and Pharmacodynamics

Drug response is determined by the pharmacokinetics and pharmacodynamics of a drug, which may be directly or indirectly affected by polymorphisms in drug metabolizing enzymes and transporters[59]. Drug pharmacokinetics are determined by drug absorption, distribution, metabolism, and excretion (ADME). Polymorphisms in drug metabolizing enzymes or transporters can results in changes in drug exposure by influencing any of the ADME processes. In contrast, drug pharmacodynamics are dependent on the interaction of the drug on its target through receptor binding, post-receptor effects, and chemical interactions. Polymorphisms that interfere with drug-receptor interactions, such as binding affinity or inhibition of cellular membrane transport, can result in pharmacodynamic alterations.

To date, the bulk of pharmacogenetics research has focused on inter-individual differences in drug metabolism, which can be separated into phase I and phase II metabolism. Phase I metabolism involves the oxidation, reduction or hydrolysis of parent drugs by cytochrome P450 (CYP450) enzymes that insert or unmask polar functional groups (i.e., -OH, -SH, -NH2)[60]. This can result in the metabolites of the parent drug being completely pharmacologically inactive, or in one or more of the metabolites being pharmacologically active but less so that the parent drug. Alternatively, the original parent drug can be pharmacologically inactive (known as a prodrug), but have metabolites that are active. Phase II metabolism involves glucuronidation, acetylation, and sulfation reactions (known as conjugation reactions) that alter the polarity of metabolites to allow for their excretion in urine[61]. Metabolites formed in phase II metabolism are unlikely to be pharmacologically active. Some drugs undergo either phase I or phase II metabolism, but the majority undergo phase I metabolism followed by phase II metabolism.

CYP450 enzymes, involved in phase I metabolism, are responsible for metabolizing the majority of drugs. Genetic polymorphisms in CYP450 genes have been shown to affect the metabolism of 70 to 80% of drug therapies resulting in normal, increased, reduced, or eliminated response [40]. For example, genetic variations in *CYP2D6* have been correlated with different metabolic

phenotypes: ultra-rapid metabolizers, normal metabolizers, intermediate metabolizers, and poor metabolizers. *CYP2D6* is involved in the metabolism of approximately 20%–25% of all administered drugs including antidepressants, antipsychotics and opioids, as well as in the metabolism of endogenous neuroactive substrates (i.e., neuroactive monoamines, endocannabinoids and endomorphines)[40]. The incidence of variants affecting *CYP2D6* function varies drastically between ancestries. For instance, poor metabolizers are uncommon in African (0-5%) and Asian populations (0-1%), but are found in between 5-14% of European populations. Pre-emptive screening for *CYP2D6* variants can allow therapy to be modified to reflect these interindividual and interracial differences. Tamoxifen, for example, is a first-line hormonal therapy used to treat women with estrogen-receptor positive breast cancer. *CYP2D6* is involved in the metabolism of tamoxifen and a number of clinical studies have demonstrated that screening for *CYP2D6* variants can improve the disease-free survival of cancer patients treated with tamoxifen[62].

Polymorphisms in the genes of phase II enzymes (i.e., uridine diphosphate glucuronosyltransferase (UGT), sulfotransferase (SULT), glutathione S-transferases (GST), N-acetyltransferase (NAT), and thiopurine methyltransferase (TPMT)) are also highly involved in variability in drug response. Irinotecan, for example, is a topoisomerase I inhibitor that is first hydrolyzed to SN-38 during phase I metabolism and then inactivated via glucaronidation by UGT1A1[63]. The *UGT1A1*28* polymorphism is responsible for most cases of Gilbert's syndrome and is associated with the development of neutropenia and diarrhea in patients that receive irinotecan[64-66]. Another example, TPMT, is best known for its key role in metabolizing thiopurine drugs used to treat cancers or as immunosuppressants[67]. Patients that inherit a non-functional *TPMT* allele are at significant risk of thiopurine-induced myelosuppression. Those with absent TPMT activity may only tolerate 5% of the average therapeutic dose of thiopurines[67]. The US Food and Drug Administration (FDA) has now introduced a label change to the prescribing information for thiopurines recommending *TPMT* genotyping prior to the initiation of therapy[68], and clinical practice guidelines for the use of *TPMT* information to guide treatment decision-making have been published[57,69].

1.2.3: Pharmacogenomics in Pediatric Oncology

With a variety of treatment options available for different cancer types, pharmacogenomics provides an understanding of the biological mechanisms and genomic contributions to treatment response in order to predict and individualize therapy and improve treatment outcomes[70]. Unlike other diseases, cancer genetics must take into account somatic (acquired) and germline (inherited) genetic variation when evaluating the safety and efficacy of drugs. Somatic mutations are often associated with treatment efficacy while germline mutations are used to identify patients at lower or higher risk of experiencing an ADR[67]. As scientists and clinicians in the past have primarily been concerned with survival, and therefore drug efficacy, somatic markers have been more readily adopted into regular clinical use while germline markers have not[67]. As the survival rates in pediatric oncology have now exceeded 82%[3], the late effects of chemotherapy are now more of a concern and there has been a push towards implementing pharmacogenomic markers to predict and avoid these late effect ADRs[71]. Despite this recognition, very few predictive markers have met a threshold of evidence deemed necessary before they are able to be implemented into clinical use. Additionally, a majority of studies investigating pharmacogenomic markers are conducted in adults rather than children[22,26].

As children grow into adults, changes in body composition occur such as an increase in body fat and protein mass[71]. These factors can affect pharmacokinetic data in relation to pharmacogenomic markers. Additionally, the ontogeny of phase I and II drug metabolizing enzymes, transporters, and target protein can undergo developmental changes causing their activity and effects to be different amongst children versus adults. For example, the enzyme CYP3A7 is involved in oxidative metabolism during fetal development, however, by infancy the expression of this enzyme declines and is replaced by CYP3A4[3]. It has previously been shown that 688 genes are differentially expressed during different phases of growth[67]. Children have been shown to have higher susceptibility to ototoxicity from cisplatin therapy[67], greater effects on neurological development from methotrexate[72], higher clearance of tacrolimus[73], and inability to metabolize codeine during infancy[74]. These differences highlight the need for further pharmacogenomic investigation in pediatric populations—specifically in the area of pediatric oncology where medications have narrow therapeutic indices and a greater potential for causing

adverse effects that can have a lasting impact on the quality of life of this vulnerable population[67].

1.3: Cisplatin

Cisplatin, cisplatinum, or *cis*-diamminedichloroplatinum (II), is a chemotherapy drug discovered by accident in 1965 when a biophysical chemist, Barnett Rosenberg, decided to set up an electromagnetic field experiment using platinum electrodes, which were previously thought to be inert[75]. After adding bacterial cells and turning on the electromagnet, he observed that the microbial population had grown to 300 times their normal size but had stopped dividing. Rosenberg found that his electrodes were corroding and producing cisplatin which was blocking the cell division of the bacteria. Rosenberg published this finding in the journal *Nature* and later published a paper showing that cisplatin could cure tumours in mice[76]. Cisplatin was approved for use in humans by the FDA in 1978 and became most famous for its role in improving the cure rate of testicular cancer from 10% to almost 90% today[77]. Platinum-based drugs are now used in 40% of all chemotherapy treatments[77,78]. In children, cisplatin is used in the treatment of neuroblastomas, germ-cell tumours, osteosarcomas, retinoblastomas, hepatoblastomas, brain tumours (low-grade gliomas and medulloblastoma/PNET), and relapsed and refractory lymphomas[79]. In adults, cisplatin is used to treat ovarian, gastrointestinal, testicular, lung, and head and neck tumours[5].

Since the discovery and early use of cisplatin, several thousand analogs have been synthesized but only one (carboplatin) has demonstrated the same efficacy as cisplatin and achieved worldwide approval[80]. Compared to cisplatin, carboplatin is less potent and depending on the type of cancer may be less effective[5]. It has, however, been shown to have less associated adverse nephrotoxic and ototoxic effects[5]. Cisplatin still remains the mainstay of therapy for the majority of platinum-treated cancers.

1.3.1: Cisplatin's Mechanism of Action

Cisplatin functions by inducing apoptosis in cells which is mediated by various signal transduction pathways. It does so by binding to the N7 reactive center of purine residues creating DNA-protein and DNA-DNA interstrand and intrastrand cross-links known as DNA adducts[5].

These cross-link DNA adducts distort the DNA by unwinding and bending, and are recognized by several cellular proteins involved in DNA repair mechanisms[81]. This activates various signal-transduction pathways involved in DNA-damage recognition and repair, cell cycle arrest, and apoptosis[5,82,83] **(Figure 1.1)**.

Oxidative stress is one of the most important mechanisms involved in cisplatin cytotoxicity. Cisplatin causes the release of reactive oxygen species (ROS) which cause oxidative stress, damage macromolecules, and signal the activation of apoptotic pathways[82]. Additionally, several proteins can recognize DNA lesions caused by cisplatin such as high mobility group (HMG) proteins which bind to cisplatin adducts[82]. Once bound, these proteins shield and protect DNA from common repair mechanisms (i.e., nucleotide excision repair (NER) and mismatch repair (MMR)) resulting in the inability to repair damaged DNA and subsequent downstream activation of apoptosis[5]. It is also known that p53 protein plays a role in cisplatin-induced apoptosis by activating pro-apoptotic genes like PUMA, caspases, PIDD, the MAPK protein family, as well as, through interactions with the Bcl2 family of proteins[82]. Other mechanisms involving dysregulation of calcium homeostasis causing mitochondrial damage have also been implicated[83]. While cisplatin is highly effective at inducing apoptosis in cancer cells it is also cytotoxic to normal, healthy cells thus causing several toxic adverse effects and limiting its efficacy in certain individuals.

1.3.2: Cisplatin-induced ADRs

Some of the most commonly-associated ADRs from cisplatin include nausea, vomiting, ototoxicity, nephrotoxicity, peripheral neuropathy, hypersensitivity reactions, and electrolyte disturbances. Nausea and vomiting are the most frequently occurring ADRs from cisplatin with up to 90% of treated patients being affected[5]. These symptoms are usually managed with the administration of antiemetic drugs such as 5-hydroxytrptamine receptor (5-HT3) antagonists, however, some studies have shown that the rates of nausea prevention with these agents were poor[84,85]. Ototoxicity is often more severe in children (affecting 60-70% of treated children), and manifests as tinnitus and progressive hearing loss beginning in the high frequency ranges[73]. Nephrotoxicity is another common and dose limiting ADR of cisplatin, which commonly presents as an acute kidney injury in 20-30% of patients[86]. Electrolyte disturbances such as

hypomagnesaemia, hyponatremia, hypocalcaemia and hypokalemia are also known to occur[87,88]. Recovery of renal function usually occurs over a 2-4 week period after receiving cisplatin, however, progressive and permanent nephrotoxicity can occur with successive cisplatin treatment despite preventative measures[89,90]. Patients may also experience decreased appetite, alopecia, myelosuppression and immunosuppression from cisplatin[5]. Alopecia has been found to effect around 50% of cisplatin treated patients, however, other studies have shown conflicting results for whether cisplatin is a causative agent for alopecia[86,91,92]. Although less common, hepatotoxicity[93,94], cardiotoxicity[95], and reproductive adverse effects[96,97] have been reported. While the majority of the above ADRs are either preventable or will subside once treatment stops, ototoxicity can cause permanent and irreparable damage to cisplatin-treated patients.

1.4: Cisplatin-induced Ototoxicity

Cisplatin-induced ototoxicity affects between 10-20% of treated adults[5,97,98] and 60-70% of treated children[5-7]. Carboplatin is considered less ototoxic than cisplatin, but when both drugs are used in combination, ototoxicity can affect between 80-90% of treated children[97,99]. Ototoxicity may occur immediately after administration of cisplatin, but may also manifest months or even years after therapy [100,101]. One study evaluated the evolution of hearing loss in 120 childhood cancer survivors and found that progression of hearing loss was observed up to 136 months after the end of treatment and that worsening of hearing was not only evident in patients who sustained hearing loss during treatment but also in patients who had a normal audiometric assessment at the end of cisplatin therapy[102]. The median time to the development of a significant decrease in hearing (at ≥4kHz) in these children was 135 days from their first dose of cisplatin[102].

Symptoms of ototoxicity include hearing loss, ear pain, and tinnitus (ringing in the ear). Hearing loss is often permanent, bilateral, and affects high-frequency first (4000-8000Hz) with lower frequencies being affected with continued exposure (500-2000Hz). Nearly 50% of English consonants require hearing above 2000Hz including relevant sounds in the English language, such as “th”, “f”, “k” and “s”[103]. As a result, even hearing loss restricted to high frequencies can significantly impact word recognition and learning, especially in younger children. In pre-lingual children who do not have the language base to fill in the gaps, even mild loss in high

frequency sounds may permanently affect a child's development and quality of life. In a study of 226 childhood cancer survivors who received platinum chemotherapy for brain tumours, those with significant hearing loss (at frequencies of < 4kHz and above) were at twice the risk of non-independent living, unemployment, and not graduating high school compared to those without significant hearing loss[102]. Even in older children who develop ototoxicity after language acquisition, they have been shown to experience academic difficulties due to impairment in their cognitive ability to gather and process information at school[103]. Persistent tinnitus has been reported in 20-25% of childhood cancer survivors and has been shown to cause strong emotional reactions and interfere with sleep and concentration[104]. Rates of tinnitus are also often under-reported and under-appreciated in children[104]. Ototoxicity may not be readily apparent to care givers and medical providers without proper audiological assessments, and while hearing aid technology can help to mitigate the negative consequences of ototoxicity, they are not a replacement for normal hearing[104].

1.4.1: Pathophysiology of Ototoxicity

Sound waves reach the outer ear and are transmitted towards the tympanic membrane in the middle ear causing it to vibrate. These vibrations are transferred by middle ear bones (incus, malleus, stapes) to fluid in the cochlea of the inner ear[105]. The fluid moves along the organ of Corti, which is lined with hair cells that act as auditory nerve receptors. Movement of the fluid stimulates hair cells and generates nerve impulses which are sent through the cochlear nerve to the brain. The signal eventually gets sent to the auditory cortex of the temporal lobe to be interpreted as sound. Hearing loss can be classified as conductive or sensorineural depending on what area of the ear is affected[105]. Conductive hearing loss is the result of a problem in the outer or middle ear that prevent sound from being conducted properly into the inner ear. Sensorineural hearing loss results from missing or damaged hair cells in the cochlea. Individuals that have hearing loss only in high frequencies are usually the result of hair cells being damaged at the base of the cochlea while those in the apex of the cochlea, which are responsible for lower frequencies, remain intact. As cisplatin is known to cause sensorineural hearing loss affecting the high frequencies first, hair cells at the base of the cochlea are the first to be affected with further cells towards the apex of the cochlea being affected as hearing loss progresses to lower frequencies[73].

1.4.2: Mechanism of Cisplatin-induced Ototoxicity

Mechanisms underlying the development of cisplatin-induced ototoxicity are complex, and the knowledge is evolving. The normal function of the cochlea requires high metabolic activity leading to the leakage of electrons from the mitochondrial electron transport chain which can react with oxygen to form superoxide radicals[106]. Environmental stimuli, such as loud noise, have been shown to increase the oxidative stress in the cochlea[106]. Ototoxic drugs, such as cisplatin, also increase the generation of reactive oxygen species (ROS) by stimulating enzyme systems involved in apoptosis or by inactivating antioxidant systems (glutathione and antioxidant enzymes)[97]. In the absence of antioxidant systems, ROS cause cellular damage by lipid peroxidation leading to increased levels of lipid peroxide, malondialdehyde, and 4-hydroxynonenal. The accumulation of lipid peroxides and ROS can lead to an influx of calcium into cochlear cells and the activation of apoptotic pathways[107]. Additionally, ROS can lead to morphological changes in the structure and length of hair cells[107]. This suggests that the generation and inability to attenuate ROS lead to the promotion of apoptosis and hair cell damage which result in ototoxicity from cisplatin.

1.5: Management and Prevention of Hearing Loss

Cisplatin-induced ototoxicity is often irreversible leading to permanent hearing loss that can worsen after treatment from cisplatin has ended. Depending on the severity of ototoxicity, therapeutic options to manage hearing loss in children include the use of hearing aids, cochlear implants, and educational interventions. Although these therapeutic interventions help to improve patients' ability to cope with their hearing loss, their quality of life is still impacted. In order to inform treatment management and to provide timely interventions as soon as possible after hearing loss begins, audiological monitoring at the start of and throughout treatment is recommended[100]. In order to prevent hearing loss, alternative platinum agents with less ototoxic potential are viable treatment options for number of tumour types[108-110]. Additionally, several protective drugs, discussed below, are in the preclinical and clinical stages of testing to determine their ability to protect against cisplatin-induced ototoxicity.

1.5.1: Audiological Monitoring

Prospective audiological monitoring is important for identifying hearing loss and providing early interventions, and remains the only reliable method for detecting early ototoxicity before it becomes symptomatic[100,111,112]. According to guidelines developed by the American Association of Speech-Language-Hearing Association (ASHA), patients should have a baseline audiological assessment prior to beginning cisplatin therapy to rule out pre-existing hearing loss[111]. They subsequently recommend having audiometric assessment prior to each cycle of cisplatin chemotherapy followed by assessments at one, three, and six months after completing cisplatin therapy. This is consistent with monitoring recommendations followed in Canadian institutions [100]. If the child is very young or very ill, a proper baseline assessment may not always be possible before beginning treatment[100]. Methods for ototoxicity monitoring in children include behavioural pure tone audiometry, speech audiometry, immittance, acoustic reflexes, otoacoustic emissions, extended high-frequency audiometry and electrophysiological testing[101]. Behavioural pure tone audiometry is the most commonly employed audiometric assessment method, however, the quality of the test results may be limited by the child's age, health status, development, cooperation, and energy level[100].

For children where a comprehensive assessment at a large range of frequencies is not possible, an algorithm was developed at the Ototoxicity Monitoring and Grading Workgroup at the 42nd International Society of Pediatric Oncology Congress in Boston in 2010 to guide a minimal test battery to direct testing to those frequencies critical to identifying ototoxicity[113]. Sound field testing is necessary when a child cannot tolerate earphones or headphones[100]. Typically, sound field testing can only be done up to 6kHz and results are not ear-specific which may miss unilateral or asymmetric hearing loss[100]. Auditory brainstem response (ABR) or auditory steady-state response (ASSR) can be used to assess hearing threshold when behavioural audiometry is not possible due to the child's age or health status[100]. This requires that the child be sedated, which could be coupled with other procedures required for their cancer treatment[100]. Measuring otoacoustic emissions (OAEs) to evaluate cochlear outer hair cells may provide an indirect measurement of early ototoxic changes and are a part of the standard pediatric audiological evaluation[100]. Reductions in OAE amplitudes can be observed before changes in hearing are apparent on conventional pure tone audiometry[100]. Tympanometry should be assessed at each

audiometric evaluation to determine middle ear function as otitis media is common among pediatric populations and middle ear pathology can confound audiometric results[100]. Bone conduction audiometry distinguishes sensorineural from conductive hearing losses up to 4kHz, but not all children will comply with wearing bone conduction oscillators[100].

As platinum agents can remain in and exert their effects on the body for more than 20 years[114], patients should receive regular post-treatment audiology assessments to monitor any continued changes in auditory function. The Children's Oncology Group has published Long-Term Follow Guidelines for late effects related to childhood cancer treatment (http://www.survivorshipguidelines.org/). They suggest that patients under 5 years of age be monitored yearly after the end of cisplatin treatment, while patients age 6-12 are suggested to received audiological monitoring every 2 years, and those 13 years and older are suggested to be monitored every 5 years.

1.5.2: Otoprotective Agents

In addition to regular monitoring, there are several otoprotective agents being tested as options for preventing hearing loss. These agents have generally focused on antioxidants, free radical scavengers, agents that facilitate DNA repair, and factors that increase antioxidative enzymes. Some of the agents that have been successfully tested in animals include sodium thiosulfate[100], N-acetylcysteine[100], amifostine[115], D-methionine[115,116], salicylates[117], alpha-tocopherol[118], lipoic acid[119], and ebselen[120]. Clinical trials in humans have only been conducted to evaluate the otoprotective effects of sodium thiosulfate[16,121] and amifostine. A phase III randomized controlled clinical trial comparing the otoprotective effect of sodium thiosulfate to a placebo has shown that the likelihood of hearing loss was significantly lower in patients treated prophylactically with sodium thiosulfate (OR 3.1; 95% CI: 0.13, 0.73; p=0.0036). They also evaluated overall survival (OS) over 3 years to determine if sodium thiosulfate was exhibiting a tumour protective effect and found no significant differences between the sodium thiosulfate treated group (OS:87%; 95% CI: 76,93) versus placebo controls (OS: 70%; 95% CI: 56,80) when it came to localized disease (p=0.88). They did note significantly lower overall survival in those with disseminated disease that were treated with sodium thiosulfate (p=0.009), however, the sample size of those with disseminated disease was small (n=47). The overall survival estimates

for controls versus sodium thiosulfate-treated groups were 84% (95% CI: 62,94) versus 45%(95% CI:23,65), respectively. Another randomized controlled trial produced similar findings regarding the otoprotective effect of sodium thiosulfate with a 48% lower incidence of hearing loss observed in those who received sodium thiosulfate (RR: 0.52; 95% CI:0.33,0.81; p=0.002)[121]. In these patients, the 3-year OS rates were not significantly different at 98% (95% CI: 88,100) versus 92% (95% CI:81,97) for the sodium thiosulfate treated group versus controls, respectively. Additional studies with larger sample sizes are needed to understand whether there is a tumour protective effect in subsets of patients, such as those with disseminated disease.

Adult studies have demonstrated the ability of amifostine to reduce various toxicities associated with cisplatin (e.g., ototoxicity, neurotoxicity, nephrotoxicity) without altering therapeutic efficacy[122-124], however, two randomized controlled trials in pediatric patients treated for hepatoblastomas and germ cell tumours demonstrated no protective effect against ototoxicity, nephrotoxicity or myelosuppression[16,125,126]. A study conducted in children treated for medulloblastoma, did report an otoprotective effect with higher doses of amifostine (1200mg/m2/day) than those used in the randomized controlled trials (825 and 740mg/m2/day)[16]. One year after treatment, 13 (37.1%) of the control group compared to 9 (14.5%; p=0.005) of the amifostine-treated group had developed ototoxicity that required a hearing aid in at least one ear[108]. A meta-analysis of four randomized, controlled trials showed a trend towards decreased ototoxicity in patients that received amifostine, however, the result did not reach statistical significance (OR: 0.54; 95% CI: 0.27, 1.11)[127]. Hypokalemia and hypotension and are commonly report ADRs associated with amifostine that are usually transient but do require monitoring before and for 24 hours after administration, and can require hydration and calcium supplementation in order to manage[108]. In comparison, sodium thiosulfate is easier to administer and has not been associated with these toxicities, which is why it has been the focus of more recent randomized controlled trials[16].

N-acetylcysteine is a promising emerging agent that is well established as being safe in humans and has shown otoprotective effects in animal and small cohort studies in humans[125,126]. Delayed administration has been suggested as concurrent administration with cisplatin have been found to ameliorate antitumour activity of cisplatin in rats[128]. When N-acetylcysteine was administered 4 hours after cisplatin, anti-tumoural activity was uninhibited. A phase 1 clinical trial

(NCT02094625) is currently underway to determine the dose of N-acetylcysteine needed for otoprotection, as well as, how it is tolerated in combination with chemotherapy. The use of local delivery for the administration of N-acetylcysteine has been investigated to try to circumvent issues with antitumour activity, but results have been mixed in preclinical and clinical studies[129]. For example, a 2% solution of N-acetylcysteine injected trans-tympanically in head and neck cancer patients found no statistically significant difference in otoprotection compared to a control group[129]. In contrast, another clinical study that used a 10% solution rather than 2% found a statistically significant difference (p=0.005) in hearing between N-acetylcysteine treated and untreated patients, however, the difference was only significant at the 8kHz frequency[109]. Efforts to improve local delivery techniques are underway and may provide viable treatment options to protect against otoprotection without compromising the anti-tumour efficacy of cisplatin[130].

1.5.3: Alternative Platinum Compounds

In terms of its structure, carboplatin differs from cisplatin in that it has a bidentate dicarboxylate leaving group instead of two chloride ligands which act as leaving groups in cisplatin. Due to the alternative leaving group, carboplatin exhibits lower reactivity and slower DNA binding kinetics[5]. Relative to cisplatin, carboplatin has been shown to be less ototoxic, however, it is also less potent than cisplatin and depending on the cancer type may only be 1/8 to 1/45 as effective[5]. Some studies have reported similar outcomes in pediatric patients treated with carboplatin rather than cisplatin for the treatment of germ cell tumours[108]. The partial substitution of cisplatin for carboplatin has also been suggested as an option with similar survival outcomes in patients treated for neuroblastomas[109].

In adults, cisplatin is not often substituted for carboplatin as it has been found to be less effective for several tumours such as testicular, bladder, and head and neck cancers [131,132]. Oxaliplatin is an alternative to cisplatin that has been investigated and shown to be as effective as cisplatin at treating advanced gastric cancers and advanced non-small cell lung cancer[110]. Ototoxicity has rarely been associated with oxaliplatin treatment, however, it has been commonly associated with neurotoxicity[133]. Neurotoxicity occurs in between 60 to 90% of oxaliplatin-treated patients with around 20% of cases being grade 3 or 4 neurotoxicity[134]. These

effects are usually reversible with the majority of cases resolving after a few days, however, some have reported chronic symptoms lasting up to four years[134].

Better strategies for the administration and delivery of platinum agents are been investigated that limit their toxic effects on healthy tissue including the use of nanoparticles, polymers and macrocycles as delivery vehicles[135,136] and active targeting of the drugs through the use of antibodies and aptamers[137,138]. Preclinical studies using nanoparticle formations of cisplatin have demonstrated promising results, however, clinical trials to date have not shown the same level of efficacy over free cisplatin as novel formulations have been unable to overcome off-target effects and tumour resistance[139]. As toxicities are associated with each of these platinum derivatives it is important to weigh the risks and benefits of different treatment options before making a decision to treat with an alternative platinum agent.

1.6: Clinical Risk Factors for Cisplatin-induced Ototoxicity

Several clinical risk factors such as dose, age, cranial radiation, exposure to other ototoxic drugs, and previous hearing loss have been reported to play a role in the development of cisplatin-induced ototoxicity[107]. Patients that receive higher cumulative doses of cisplatin are at significantly higher risk of ototoxicity. In one study, testicular cancer patients that received doses in excess of 400mg/m^2 experienced rates of ototoxicity greater than 50% while only 10-20% of those that received doses lower than 400mg/m^2 experienced ototoxicity[97,140-144]. The majority of pediatric cancers are treated with doses of cisplatin at, or above, 400mg/m^2, and patients treated at these doses have been shown to experience worsening hearing loss long after treatment compared to those that received lower doses[97,145]. There is also evidence to suggest that individual doses and varied dose schedules of cisplatin may affect susceptibility to ototoxicity[102,145,146]. For example, patients with germ cell tumours experience less ototoxicity than other tumour types, which has been suggested to be due to the 5 days consecutive dosing protocols of 20mg/m^2/day that they receive compared to other tumour type protocols that use single day dosing regimens of 100 or 120mg/m^2/day[102,145]. While the overall cumulative doses of these different regimens may be the same, the dose schedules may cause differences in ototoxicity susceptibility. Children less than 5 years of age and patients that have been treated with cranial radiation have also been shown to be at higher risk[141]. Additionally, exposure to

other ototoxic medications such as carboplatin, aminoglycosides, and loop diuretics increase patients' risk of experiencing ototoxicity, which can be further exacerbated by cisplatin [97 145]. Noise exposure and low serum albumin and anemia have also been shown to increase the risk of developing ototoxicity[107]. There is also evidence that individuals with higher melanin content in the cochlea, which manifests as darkness around the eyes, are at greater risk of ototoxicity as the melanin causes retention of platinum within the cochlea[147]. While these clinical risk factors contribute significantly to the development of ototoxicity, there is still large variability in hearing loss between patients who are treated with similar age, doses, and cancer types[100,101]. This suggests that there are other factors, such as genetics, that contribution to the susceptibility of cisplatin-induced ototoxicity.

1.7: Pharmacogenomic Studies of Cisplatin-Induced Hearing Loss

While clinical risk factors contribute to cisplatin-induced ototoxicity, genetic variation in the genes involved in the biotransformation, transport, and targets of drugs have been recognized to influence patient drug response and susceptibility to ototoxicity. The heritability for susceptibility to cisplatin-induced cytotoxicity has been estimated to be approximately 38-47%[99,148]. Specifically, genetic variants in thiopurine S-methyltransferase (*TPMT*) and acylphosphatase 2 (*ACYP2*) currently have the strongest evidence for involvements in the development of cisplatin-induced ototoxicity having been replicated with odds ratios (OR) ranging from 3.6 to 31. Additional variants in genes encoding catechol-O-methyltransferase (COMT), transporters (ABCC3, OCT2), glutathione-S-transferases (GSTs), megalin (LRP2) and DNA repair genes (XPC) have been significantly associated with the development of cisplatin-induced ototoxicity and are discussed below, however, their strength of association and levels of evidence require further investigation.

1.7.1: Thiopurine S-methyltransferase (*TPMT*)

Three thiopurine S-methyltransferase (*TPMT*) variants (rs12201199, rs1800460 and rs1142345) were associated with increased risk of developing cisplatin-induced ototoxicity have been discovered and replicated in three independent pediatric cohorts (n=53, n=109, n=155) with odds ratios ranging from 3.6 to 14.3[9]. These risk variants include the *TPMT*3B, *3C* and **3A* loss of function alleles, which lead to rapid degradation of the TPMT proteins. In a cohort of 317

children treated with cisplatin, 43 (91.5%) *TPMT* carriers developed clinically significant hearing loss compared to 4 (8.5%) carriers that did not (OR: 9.3; CI: 3.1, 27.4 ; p=5.5 x 10^{-5}). Three additional studies did not replicate these findings, however, there were key differences in the patients' demographics and treatment protocols between these studies[144,149,150]. For example, Yang et al.'s cohort of patients all received craniospinal radiation which is a known risk factor for hearing loss[149]. Additionally, majority of the patients received amifostine as a prophylaxis for cisplatin ototoxicity, which may have influenced the ability to observe an association for *TPMT*[149]. When the analysis was restricted to patients that did not receive cranial radiation or amifostine, a strong trend in association of *TPMT* functional variants (rs1800460, rs1142345) was observed, but their analysis was underpowered to reach statistical significance.

TPMT encodes the enzyme, thiopurine S-methyl transferase, which catalyzes the transfer of a methyl group from S-adenosylmethionine (SAM) to the sulfur residue of thiopurines (i.e., 6-mercaptopurine, azathioprine) for their metabolism and detoxification. Variants in *TPMT* are hypothesized to alter cisplatin toxicity by influencing the binding of cisplatin to purines in DNA, thereby modulating cisplatin cross-linking[9,144,149,150]. While TPMT is directly involved in the metabolism of thiopurines, there have been mechanistic studies demonstrating its indirect role in the metabolism and detoxification of cisplatin. The overexpression of wild type *TPMT* (TPMT*1) in two murine ear cell lines was shown to significantly mitigate their susceptibility to cisplatin toxicity while, in contrast, cytotoxicity was increased in *TPMT**3A variant-expressing cells in response to cisplatin[144]. Additionally, indirect evidence demonstrating the upregulation of *TPMT* expression in response to cisplatin has been reported[9]. Cisplatin treatment has also been shown to increase the concentration of TPMT's substrate, SAM[06]. Given that increased SAM levels have been shown to increase cisplatin ototoxicity in mice[151], it has been hypothesized that *TPMT* variants that are transcriptionally upregulated by cisplatin and destabilized by *3A mutations are unable to utilize SAM. This suggests a potential mechanism that involves a combination of enhanced cisplatin cytotoxicity, SAM metabolite accumulation, and diminished cisplatin detoxification[140].

1.7.2: Acylphosphatase 2 (ACYP2)

Another genetic variant *ACYP2* (rs1872328) was recently identified using a genome-wide association study of 238 pediatric patients being treated for brain tumours (hazard ratio (HR) = 4.5, CI: 2.63, 7.69, $p = 3.9\times10^{-8}$) with an independent replication in 68 similarly treated children[152]. All of the 20 patients (100%) carrying the A allele at rs1872328 developed ototoxicity, regardless of whether a patient was heterozygous or homozygous for the risk allele while only 57.3% of patients that did not carry the risk allele developed ototoxicity. The risk variant at rs1872328 was more common among individuals of African descent, however, the association remained significant after restricting the analysis to Europeans (HR=3.85; CI:1.72,8.33; p=0.001). An additional two studies in pediatric cancer patients (n=156 and n=119) and one study in adult patients treated for testicular cancer replicated the association (n=229)[152]. The association was found to have an odds ratio ranging from 1.9 to 31 with a meta-analysis demonstrating a pooled odds ratio of 5.9 (95% CI: 1.51, 23.16; p=0.01)[153-155].

ACYP2 encodes an acylphosphatase that hydrolyzes phosphoenzyme intermediates of different membrane pumps, particularly the Ca^{2+}/Mg^{2+}-ATPase from sarcoplasmic reticulum of skeletal muscle. ACYP2 is thought to effect Ca^{+2} homeostasis, and while originally thought to be muscle specific, *ACYP2* has also be found to be expressed in the cochlea. While the mechanism of how *ACYP2* affects the cochlea remains unclear, ATP-dependent Ca^{+2} signaling is essential for hair cell development and dysregulation of Ca^{+2} has been implicated in hair cell damage[153]. Additionally, other polymorphisms within *ACYP2* have been associated with severe neuropathy related to oxaliplatin suggesting a broader relationship between *ACYP2* and platinum toxicities. At the gene level, the expression of *ACYP2* has been shown to positively correlate with cisplatin cytotoxicity in lymphoblastoid cell lines *in vitro* ($p=6.5\times10^{-5}$).

1.7.3: Catechol-O-Methyltransferase (*COMT*)

Two variants in Catechol-O-Methyltransferase (*COMT*) (rs9332377 and rs4646316) have been significantly associated with cisplatin-induced ototoxicity in a discovery (n=53; OR:2.5 and 5.1; p=0.044 and 0.024) and replication (n=109; OR:2.5 and 6.2; p=0.0059 and p=0.0087)[9] cohort of pediatric cancer patients. The *COMT* risk allele, rs9332377, was found in 14 out of 16 discovery

patients with ototoxicity and 22 out of 24 patients in the replication cohort group, and a total of 36 out of 40 patients in the combined group. Another study in pediatric cancer patients showed a similar trend in association, but it was not statistically significant (n=155, OR:1.3 and 1.4; p=0.33 and 0.28) [11]. While no significant association was found, the study retained *COMT* rs4646316 in their combined logistic regression because it added significantly to the prediction model[11]. A meta-analysis of these studies showed a combined OR of 1.52 (95% CI: 1.16,1.99) [144].

As COMT and TPMT enzymes utilize the same substrate, S-adensylmethionine, ototoxicity may result from increased SAM levels due to reduced COMT and TPMT activity. Recent studies of LRTOMT2, an enzyme that has 60% similarity with COMT, including the substrate-binding region, demonstrate that the enzyme functions as a COMT and is essential for proper auditory function in mice and humans[156,157]. A novel gene, *COMT2,* was reported to produce an isoform of COMT-COMT2 which is expressed in inner and outer cochlear hair cells[156]. These mechanistic findings coupled with genetic associations suggest that a loss of COMT activity may result in increased susceptibility to ototoxicity when patients are treated with cisplatin.

1.7.4: Transporters (ABCC3, OCT2)

An association between a synonymous variant in *ABCC3* (rs1051640) and ototoxicity was discovered (n=166, OR 2.1, p=0.0092) and replicated (n=155 OR 1.8, p=0.036) in pediatric cancer patients[9,11]. In each study, the associations were no longer significant after correcting for multiple testing, however, when the cohorts were combined the association was significant after multiple testing corrections (OR 2.0; p=0.00078,). ABCC3 is a transporter that mediates the efflux of organic anions, xenobiotics, and glutathione S-conjugates, including glutathione S-conjugated cisplatin[9,11]. Platinum drugs are detoxified through conjugation of the active metabolite to glutathione enabling cisplatin to be more readily exported through an ATP-dependent pump[158,159]. Reduced activity of ABCC3 may thus affect cisplatin detoxification through reduced transport of toxic compounds out of the cell. Studies have shown that ABCC2 and ABCC3 protein levels and mRNA expression are increased in response to cisplatin in rat hepatocytes[160]. Additional studies have shown that *ABCC3* mRNA expression levels are significantly correlated with resistance to platinum drugs[160]. Functional validation studies are

required to assess the exact mechanisms by which variants in *ABCC3* affect cisplatin-induced hearing loss and additional studies in adult populations are required to determine if a similar association is also observed in adult patients.

Another transporter, organic cation transporter 2 (OCT2) **(***SLC22A2*), has been shown to mediate the cellular transport of cisplatin[161]. In pre-clinical models, *SLC22A2* was associated with cisplatin induced ototoxicity, as well as, nephrotoxicity[161]. In a candidate gene study of pediatric cancer patients (n=64), a *SLC22A2* polymorphism (rs316019; Ser270Ala) was significantly associated with protection from cisplatin-induced ototoxicity (p=0.022). This finding was replicated in an independent cohort of adult patients (n=66, p=0.048)[162]. This result was confirmed by multiple logistic regression analysis accounting for age as a covariate with an OR of 0.12 (95% CI: 0.02,0.58; p=0.009). In mouse studies, reduced expression of SLC22A1 and 2 was shown to protect from cisplatin induced ototoxicity providing additional support for its role in the transport and ototoxicity of cisplatin[163].

1.7.5: Glutathione-S-transferases (GSTs)

Glutathione s-transferases (GSTs) detoxify cisplatin by catalyzing the conjugation of active platinum metabolite to glutathione resulting in its export from the cell[164]. This protects the cell from increased oxidative stress associated with cisplatin therapy. In the cochlea, the activity of glutathione is decreased when hearing loss develops from cisplatin therapy, which suggests that it may play a role in the development of ototoxicity[164]. Several studies have examined polymorphisms in *GST* genes (*GSTM, GSTP, GSTT*) for their association with cisplatin-induced ototoxicity[9,165-168].

One study in pediatric patients (n=39) found a higher frequency of the *GSTM3*B* allele in patients with normal hearing compared to those that developed ototoxicity (OR 5.37, p=0.023)[165], however, a second study in ovarian cancer patients (n=69) could not replicate this finding[169]. Differences in the age of patients (children versus adults) and the grading criteria for hearing loss (Muenster Criteria versus National Cancer Institution Criteria) could have contributed to the inability to replicate these findings. One study in testicular cancer patients (n=238) reported significant associations of *GSTM1* with cisplatin induced ototoxicity (OR 1.81,

p=0.025)[166]. Aside from this study, one study in ovarian cancer patients[170] and four studies in pediatric cancer patients[165,167,168] have failed to find an association and one even observed a trend towards an association in the opposite direction[9] (OR 0.78, *p*=0.51). Further studies are required to investigate whether age and differences in hearing grading criteria are reasons for these conflicting results.

A study in testicular cancer patients found an association between a non-synonymous variant in *GSTP1* (rs1965) and hearing loss (n=173, OR 0.24, *p<0.001)*, however, it was not statistically significant in an extended patient cohort (n=238, OR 0.81, p=0.055) [163,166,171]. An additional study in pediatric medulloblastoma patients (n=69) reported the opposite association for *GSTP1* and hearing loss (OR 4.0, *p*=0.03)[168] while five other studies reported no significant associations[9,11,165,166,170]. All 69 medulloblastoma patients received craniospinal radiation and patients were considered cases of ototoxicity if they required hearing aids. Comparatively, in the study that found a protective effect for *GSTP1* cases were by ranking patients into 10th, 25th, 75th and 90th percentiles at 4000Hz. These differences in clinical characterization may provide a reason for opposing associations observed between these studies.

In a cohort of 68 pediatric patients, individuals with wild-type GSTT1 were found to have an increased risk of cisplatin-induced ototoxicity compared to those with null genotypes (OR 10.1, p=0.023)[172]. Three studies in children[165,167,168] and two studies in adults[166,170] were unable to replicate these findings. Current evidence is inconsistent as to whether or not variants in GSTs are associated with cisplatin-induced ototoxicity and differences in grading criteria for hearing loss and clinical factors may be reasons for the lack of replication between studies.

1.7.6: Megalin (LRP2)

Megalin, a multi-ligand endocytic receptor abundantly expressed in absorptive epithelia such as renal proximal tubules and epithelia of the inner ear, is involved in the endocytosis of various ligands including drugs such as aminoglycosides[173]. Aminoglycosides, as well as, cisplatin are well known for their nephro- and oto-toxic effects, and both show the same manifestation of organ toxicity[174]. A non-synonymous variant in *LRP2* (rs2075252) was found to be associated with hearing loss in 50 pediatric cancer patients where cases had a higher frequency of the minor

A allele compared to controls (0.32 versus 0.14) (OR 3.45; 95% CI:1.11,11.2; p=0.016)[172]. Another study in pediatric cancer patients (n=68) found an association between a polymorphism in the SNP rs222817 of the *LRP2* gene and ototoxicity (OR 4.33, 95%CI: 1.22,5.82, p=0.034), but found no association for rs2075252[9,165,168]. While both of these studies were conducted in pediatric patients with similar cancer types, one study considered cases of hearing loss to be those that experienced grade 2-4 hearing on the Muenster grading scale (conferring a minimum hearing loss of >20dB at 4kHz and above)[175] while the other considered cases to be grade 1-4 on the Brock grading scale (conferring a minimum hearing loss of >40dB at 8kHz and above)[172]. These variations in grading criteria would have created a much different case control designation between the two studies which may explain the differences in their findings.

1.7.7: DNA repair genes (XPC)

Nucleotide excision repair genes play a key role in reversing DNA damage. Since cisplatin causes DNA lesions through cross linking, several studies have examined whether genes in these repair pathways play a role in the development of ototoxicity. Xeroderma pigmentosum (XPC) encodes a protein involved in the recognition of bulky DNA adducts in nucleotide excision repair. A small study in pediatric osteosarcoma patients (n=32) detected a marginally significant association between ototoxicity and *XPC* (rs2228001) (p=0.042)[172]. Ototoxicity was observed in 27% of patients with the AA genotype compared with 80% in the CC genotype (OR 17.16; 95% CI: 1.10, 266.8). Additional studies have failed to replicate these findings and have not found any associations between DNA repair genes and the development of ototoxicity.

1.8: Anthracyclines

Anthracyclines are a class of chemotherapeutic agents that were originally derived from *Streptomyces* bacteria and were introduced to the market in the 1960s. Daunorubicin was the first agent in this drug class to be developed followed by doxorubicin, however, when it was discovered that tumours could become resistance to these drugs and that they were associated with several ADRs, chemists tried to develop analogs with wider activity and lower toxicity. More than 2000 analogs have been developed and studied, however, only a select few (i.e., epirubicin, idarubicin, mitoxantrone) in addition to daunorubicin and doxorubicin have been approved for clinical use in North America[176].

Anthracyclines have greatly improved the survival rate of cancer patients since their introduction in the 1960s, and are now used in the treatment of around 32% of breast cancer patients, 57-70% of elderly lymphoma patients and 50-60% of pediatric cancer patients[177,178]. While daunorubicin was the first anthracyclines approved and is still used to treat leukemias, doxorubicin is the most widely used anthracycline[179]. Doxorubicin is one of the most effective drugs for treating solid tumours such as breast cancer, small-cell lung cancer, and ovarian cancer[180]. It is also used in combination with other agents to treat bladder, stomach, liver and thyroid tumours in adults. In pediatrics, doxorubicin is highly effective at treating Ewing's and osteosarcomas, soft tissue sarcomas, neuroblastomas, Wilms tumours, as well as, several types of leukemias and lymphomas[181]. Epirubicin is used in the treatment of gastrointestinal cancers, breast cancer, lung, ovarian, esophageal and prostate cancers, while idarubicin is used in the treatment of acute myelogenous leukemia[177].

1.8.1: Mechanism of Action

Anthracyclines enter the cell through passive diffusion and bind to proteasomes in the cytoplasm before being translocated as a drug-proteasome complex into the nucleus[177]. Proteasomes are found more abundantly in the nucleus of malignant proliferating cells than non-proliferating normal cells thus providing a reason for the higher rate of transport of anthracyclines into cancerous cells compared to normal cells[182,183]. Once inside the nucleus, anthracyclines can bind to DNA and exert their anti-cancer effects through multiple pathways by inhibiting DNA synthesis, inhibiting topoisomerase II (TopII) enzymes, and promoting free radical formation.

Anthracyclines' mechanism of action involves the intercalation of anthracyclines with DNA leading to the inhibition of macromolecule biosynthesis[184]. Through intercalation, anthracyclines can also displace nuclear protein from transcriptionally active chromatin resulting in the unfolding and aggregation of chromatin[184]. As a result, DNA damage response, transcription, and epigenetic regulation are dysregulated in anthracycline exposed cells. Anthracyclines have been shown to affect gene regulation by inhibiting or promoting the binding of various transcription factors (e.g., SP-1)[185]. Additionally, anthracyclines have been shown to affect the initiation of the elongation phase of DNA synthesis and have been shown to inhibit RNA polymerase activity[186].

Some anthracyclines, however, do not exhibit a strong affinity for binding with DNA, suggesting that other mechanisms are involved in the anti-cancer activity of these drugs[187].

Anthracyclines have also been shown to interact with TopII, an enzyme involved in the relaxation of DNA supercoils to promote transcription[188]. Anthracycline rings that do not intercalate into DNA appear to stabilize the complex between TopII and the nicked DNA. This results in the inability to reseal the DNA and the accumulation of damaged DNA subsequently causing the cell cycle to arrest in the G1 and G2 phases[189]. Rapidly replicating cells, such as tumour cells, exhibit greater sensitivity to the DNA damage resulting in the drug's chemotherapeutic effect.

Additionally, anthracyclines have been shown to interact with the tumour suppressor protein, p53, to induce apoptosis[190]. There are some conflicting reports regarding the relationship between p53 and anthracyclines, however, more DNA breaks have been observed in p53 proficient cells compared to p53 deficient cells irrespective of TopII levels[190]. It has, therefore, also been proposed that p53 exerts its activity by binding and inhibiting TopII's ligase activity[190]. Alternative apoptotic pathways that do not require p53 have also been shown to be triggered by anthracyclines[191-193]. Enzymatic reduction of anthracyclines can also produce free radicals which damage healthy and tumour tissue and lead to the further activation of pro-apoptotic pathways[194,195]. Free radical damage appears to be more closely associated with cardiotoxic rather than anti-tumour effects[196].

Lastly, anthracyclines have also been shown to inhibit cell growth through anti-angiogenic pathways[197]. They have been shown to inhibit the transcription factor, HIF-1, from binding to DNA in hypoxic human cells and inhibit tumour growth in human prostate cancer xenografts[197]. This inhibition leads to decreased vascular endothelial growth factor, stromal-derived factor 1, and stem cell factor expression resulting in decreased tumour vascularization and growth.

1.8.2: Anthracycline-induced ADRs

Anthracyclines, like other conventional chemotherapeutics, exert their cytotoxic effects on both malignant and normal cells resulting in ADRs. The most commonly-associated ADRs include

nausea and vomiting, myelosuppression, cardiotoxicity, mucositis, alopecia, and increased skin pigmentation.

Nausea and vomiting has been estimated to occur in up to 70% of the pediatrics and continues to be one of the most distressing ADRs resulting in a significant deterioration in quality of life[198]. Similar rates of nausea and vomiting are reported in adults and are described as the first and third most distressing ADR, respectively[199]. Antiemetic medications and protocols are in place to prevent nausea and vomiting; however, they are not always sufficient at controlling these symptoms—especially in breast cancer patients treated with anthracyclines and cyclophosphamide[200].

Two of the most serious and use limiting ADRs associated with anthracyclines are myelosuppression and cardiotoxicity. Myelosuppression occurs in between 60-80% of patients treated with anthracyclines by depleting bone marrow progenitor cells resulting in anemia, neutropenia, and thrombocytopenia[201]. Patients suffering from myelosuppression can experience fatigue, dizziness, bruising, hemorrhage, and potentially fatal infections[202,203]. To avoid this, drug doses may be held which in turn can compromise the effectiveness of the cancer treatment[202,203]. Cardiotoxicity can occur in between 50-70% of anthracycline-treated patients and can range in severity with some cases manifesting as arrhythmias and tachycardia and other resulting in much more severe congestive heart failure[182,204,205]. Cardiotoxic effects are often irreversible and induce long term sequelae years after treatment with anthracyclines has completed[175,197]. As cardiotoxicity has a life-threatening and lasting impacts to the quality of life and health outcomes of patients, substantial research has been done to better understand the mechanisms and risk factors associated with anthracycline-induced cardiotoxicity—discussed in detail below.

Mucositis is the second most frequent dose-limiting factor in patients receiving chemotherapy[200,206]. While it is more commonly induced by methotrexate and 5-fluorouracil, alkylating agents such as anthracyclines and taxanes are also known to cause mucositis[207,208]. Alopecia is common among those treated with anthracyclines with hair re-growth occurring 2-3 months after stopping anthracycline therapy[209]. Studies have published scalp cooling techniques shown to be effective at reducing alopecia during anthracycline treatment[209]. Doxorubicin has

also been shown to cause severe local tissue necrosis in some patients[210]. Additional studies have found that anthracyclines may increase the risk for developing cancer-related cognitive impairments ("chemobrain")[211,212].

1.9: Anthracycline-induced Cardiotoxicity

Anthracyclines are an effective and widely used class of chemotherapeutic agents used in the treatment of childhood and adult cancers. However, they have the potential to cause cardiotoxic effects ranging from asymptomatic changes in myocardial structure and function in approximately 57% of patients to more severe cardiomyopathy and heart failure in up to 16% of patients[4,213]. Congestive heart failure can result in the requirement of a heart transplant or life-long treatment for chronic cardiac failure, with mortality rates greater than 50%[213]. These adverse effects not only place a huge financial burden on the healthcare system, but also impact patients' quality of life significantly.

Anthracycline-induced cardiotoxicity is often irreversible and can be categorized by the timing of onset as (i) acute, occurring during therapy, (ii) early, within one year of anthracycline treatment or (iii) late, occurring one or more years after anthracycline therapy[4,214]. Incidence of acute clinically significant cardiotoxicity is rare (<1%), and the majority of cardiotoxicity cases present after anthracycline therapy has completed[215]. Evidence of delay-onset of cardiotoxicity can be observed in studies looking at the long-term outcomes of childhood cancer survivors, where up to 65% of those treated with doxorubicin exhibited echocardiographic evidence of left ventricular contractile abnormalities years after treatment[216]. In the Childhood Cancer Survivor Study which looked at 14,358 childhood cancer survivors, the use of anthracyclines at doses of <250mg/m^2 were associated with a 2.4-fold higher risk of developing congestive heart failure compared to untreated children[4]. This risk increased to 5.2-fold in those treated with doses of anthracyclines >250mg/m^2 [217]. In one study in adult patients with breast cancer, the median change in left ventricular ejection fractions from baseline was found to be -5.5% seven years after receiving anthracyclines with 12% of patients having ejection fractions (EFs) below normal (EF<50%) following anthracycline treatment[218]. In a retrospective analysis of three trials of doxorubicin treatment in breast cancer or small cell lung cancer, 5.1% of patients had evidence of congestive heart failure or a significant decline in left ventricular function[218]. Additionally, in

contrast to some studies that have suggested that doses <300mg/m^2 are unlikely to cause cardiac dysfunction, they found that 16.2% and 6.5% of patients treated at doses of 300mg/m2 and 150mg/m2, respectively, experienced a cardiac event[219]. Histopathological changes have been observed in endomyocardial biopsy specimens from patients who have received as little as 240mg/m^2 of doxorubicin[220].

In addition to early and late onset cardiotoxicity, a rare form of acute cardiotoxicity has been observed in case reports and small patient series and can manifest as pericarditis and arrhythmias in addition to left ventricular dysfunction[220]. In contrast to late-onset cardiotoxicity, improvements in cardiac function have been shown to occur in some patients[220]. Additionally, the mechanism responsible for acute toxicity is thought to involve an inflammatory response, which differs from the mechanisms observed in early and late onset chronic cardiotoxicity, discussed below[221]. Many anthracycline derivatives have been developed to try to maximize anti-cancer efficacy while limiting cardiotoxicity, however, all of the most commonly used anthracyclines (doxorubicin, epirubicin, daunorubicin, idarubicin and mitoxantrone) are known to cause cardiotoxicity[217]. Given that cardiac symptoms and dysfunction do not usually manifest until after anthracycline treatment, predicting which patients are most likely to develop cardiotoxicity is an important goal, especially since these agents are frequently used in children and young adults.

1.9.1: Pathophysiology of Anthracycline-induced Cardiotoxicity

Morphological changes in the myocardium following anthracycline therapy include myocardial cell loss either by necrosis or apoptosis, as well as, interstitial fibrosis[217]. Late-onset cardiotoxicity generally exhibits no evidence of inflammation while acute cardiotoxicity involves an inflammatory response. Early morphological changes observed in cardiomyocytes via electron microscopy include the dilation of the sarcoplasmic reticulum and mitochondrial swelling leading to cytoplasmic vacuolization and myofibrillar loss[222,223]. Nuclear deformation, increased number of mitochondria, and intracellular edema have also been observed[224].

The heart consisted of three layers: the outer pericardium (heart sac), the muscle layer (myocardium), and the inner lining (endocardium). The myocardium is the thickest layer and

consists of cardiac muscle with intervening connective tissue, blood vessels, and nerves. Between the endocardium and the myocardium is the subendocardial layer where nerves and the impulse-conducting system (Purkinje fibers) are located. Myocardial damage is most common in the subendocardial layer of the left ventricular wall of the heart[225]. This is consistent with other diseases such as ischemic heart disease, hypertension, and diabetes which have been found to predominantly affect the subendocardium of the left ventricle[225]. Since the Purkinje fibers responsible for signaling contraction are located in this layer, damage results in the deterioration of left ventricular function. In some patients with anthracycline-induced cardiomyopathy, extensive endocardial fibrous thickening leading to restrictive (rather than dilated) cardiomyopathy has been reported[225].

1.9.2: Mechanisms of Anthracycline-induced Cardiotoxicity

Various molecular mechanisms have been postulated for the development of anthracycline-induced cardiotoxicity **(Figure 1.2).** The majority of evidence suggests a mechanism involving the generation of radical oxidation species (ROS) either through the mitochondrial respiratory chain or a non-enzymatic pathway involving iron[226]. The basic structure of anthracyclines consists of a tetracyclic aglycone linked with an amino sugar[225]. The ROS mechanism suggests that anthracyclines may promote the formation of ROS through redox cycling of their aglycones[227-229]. To confirm this hypothesis several studies have investigated whether antioxidants could exert a cardioprotective effect by limiting the production of ROS but the results have been inconsistent[229]. As a result, alternative mechanisms have since been suggested.

The iron hypothesis proposes that anthracycline-induced cardiotoxicity may be due to the generation of ROS from the anthracycline-iron complex[229]. In support of this hypothesis, dexrazoxane, the most commonly used cardioprotective agent has iron-chelating activity, which has been thought to be how it can protect patients from anthracycline-induced cardiotoxicity[229-232]. However, this theory has been questioned because other iron-chelating agents have not shown the same cardioprotective effect[233,234]. Therefore, the ability for dexrazoxane to chelate iron is not a major determinant of its cardioprotective effect.

Other mechanisms suggested to cause anthracycline-induced cardiotoxicity include transcriptional changes to ATP production in cardiomyocytes[234,235]; reduction of mRNA expression for sarcoplasmic reticulum Ca^{+2}-ATPase leading to decreased contractility[235]; prolonged drug-related depression in cardiac glutathione peroxidase activity and respiratory chain defects associated with mitochondrial DNA damage, which can lead to free radicals[236]. None of the above mechanisms have been able to provide any conclusive findings on how cardiotoxicity occurs. Alternative mechanisms involving topoisomerase II (TopII) have since been proposed which expand on the above observations, and provide a more detailed account of how anthracycline-induced cardiotoxicity may be occurring.

It has been well established that TopII is a molecular target of anthracyclines[237]. DNA topoisomerases are essential for the modification of DNA supercoiling during cellular processes[238]. There are two TopII isozymes in humans: TopIIα and TopIIβ[239]. TopIIα is highly expressed in rapidly dividing cells such as cancer cells while TopIIβ is expressed in quiescent cells and has no significant change in expression at different stages of the cell cycle[240]. TopIIα has been shown to be the primary target for anthracycline's anticancer effect based on the fact that TopIIα expression is elevated in rapidly dividing cancer cells[241], and because only TopIIα is essential for cell proliferation as it is required for chromosomal segregration[242]. TopII exerts its normal function by creating ATP-dependent double-stranded breaks in DNA allowing strands to be underwound or overwound to allow different cellular processes such as replication and transcription to occur[242].

It has previously been shown that TopIIβ is the only TopII present in heart tissue in appreciable amounts[243]. As TopIIβ is a target of anthracyclines and is expressed in the heart, it has been postulated that TopIIβ has a critical role in the development of anthracycline-induced cardiotoxicity. This has been supported by previous studies, which have shown that embryonic mouse fibroblasts lacking TopIIβ were protected from anthracycline-induced cardiotoxicity[190]. Anthracyclines disrupt the normal activity of TopIIβ causing double-stranded DNA breaks and activating the apoptotic pathway[244]. Additionally, anthracyclines have been shown to induce transcriptome changes in a TopIIβ-dependent manner, which selectively affect oxidative phosphorylation, mitochondrial biogenesis, and the p53 pathway[245]. Additionally, gene variants

in *RARG,* which have been shown to modulate TopIIβ expression have been associated with anthracycline-induced cardiotoxicity[204]. Anthracyclines, therefore, have been shown to cause the death of cardiomyocytes through the induction of cell apoptosis, reduction of energy production, and the generation of ROS. The accumulation of cardiomyocyte cell death following each cycle of anthracycline eventually leads to heart failure. These observations are all dependent on the presence of TopIIβ in cardiomyocytes. Without TopIIβ, cardiomyocytes have been shown to be protected from anthracycline-induced cardiotoxicity[246]. Additionally, the cardioprotectant dexrazoxane has been shown to decrease TopIIβ levels[246].

1.10: Management and Prevention of Cardiotoxicity

In order to maximize the benefit of anthracyclines much effort has been spent to try to mitigate the associated cardiotoxic risk. Existing guidelines are in place for limiting the dose of anthracycline[247,248], however, even patients receiving low doses of anthracyclines (<150mg/m^2) have been shown to develop cardiotoxicity[220]. The clinical signs of cardiotoxicity may also present as a much later date requiring long term follow up and making it difficult to establish dosing recommendations that achieve therapeutic goals without causing cardiotoxicity[247]. As a result, cardioprotective agents, anthracycline analogs, and different drug delivery mechanisms, highlighted below, have been developed to prevent cardiotoxicity while maximizing drug efficacy. Guidelines for cardiac monitoring and suggestions for how to manage echocardiographic abnormalities have also been developed and are outlined in the follow sections.

1.10.1: Cardioprotectants

Dexrazoxane is the only cardioprotectant that has shown marked efficacy in reducing cardiotoxicity when administered prior to anthracyclines therapy. It's iron-chelating activity is thought to be how it protects patients from cardiotoxicity by reducing free radical formation, however, other iron-chelators have not shown the same cardioprotective effect leading to alternate theories on its mechanism of action. It has also been shown to decrease TopIIβ levels[249-252], thereby decreasing the molecular target for anthracyclines to bind to, however, the mechanism is still not fully understood. Several randomized controlled trials, primarily among women with breast cancer, have demonstrated the cardioprotective efficacy of

dexrazoxane[10,204,253,254]. A meta-analysis showed that dexrazoxane administration alongside doxorubicin or epirubicin reduced the rates of clinical cardiotoxicity (OR 0.21, 95% CI: 0.13, 0.33; $p<0.001$)[245]. In a meta-analysis among 3385 pediatric patients treated with dexrazoxane there was also a significant reduction in anthracycline-induced cardiotoxicity (RR=0.29, 95% CI: 0.14, 0,61; $p=0.001$)[255]. The long-term cardioprotective effect of dexrazoxane was further evaluated in a study of 134 high-risk ALL survivors who received dexrazoxane or placebo with their doxorubicin and showed that left ventricular function was significantly better 5 years post treatment in those that received dexrazoxane in female patients but not male patients ($p=0.04$)[256]. While the cardioprotective effects of dexrazoxane have been shown by numerous studies, there is some suggestion that dexrazoxane may limit the cytotoxic effect of anthracyclines against cancer cells or cause secondary malignancies. A meta-analysis of three randomized controlled trials in children did not show any significant differences in the occurrence of secondary malignancies between children treated with and without dexrazoxane (RR 1.16, 95% CI: 0.06 - 22.17; p=0.92) [256]. However, the Pediatric Oncology Group (POG) studies 9426 and 9425 evaluated the cardioprotective potential and the rates of secondary malignancies in 239 dexrazoxane-treated patients compared to 239 patients that did not receive dexrazoxane and found that the standardized incidence rate of secondary malignancy was 41.86 in dexrazoxane treated patients compared to 10.08 in non-dexrazoxane treated patients ($p=0.0231$)[257]. The authors of the study concluded that the differences in the number of secondary malignancies might be attributed to a combination of three topoisomerase II inhibitors (doxorubicin, etoposide, dexrazoxane) rather than due to the effect of a single agent. Based on these findings, the European Medicines Agency in 2011 restricted the use of dexrazoxane to patients over the age of 18 years due to concerns about the risk of secondary acute myeloid leukemia.

In support of the assertion made in the POG 9426 and 9425 studies regarding the contribution of etoposide in the development of secondary malignancies, a retrospective study found that etoposide was associated with secondary AML (OR 2.36; 95% CI: 1.48, 3.79) while dexrazoxane was not (OR 0.38, 95% CI: 0.12, 1.27)[258]. Other studies have also noted high rates of secondary AML with etoposide[259]. A systematic review of 6 studies examining rates of secondary malignancies in dexrazoxane-treated patients reported no studies identifying significantly increased risk[260]. A retrospective study of 15,532 cancer patients identified

secondary AML rates of 0.21% in dexrazoxane-treated patients compared to 0.55% in those that did not receive dexrazoxane[258]. In a subgroup analysis of patients with lymphoma, the secondary AML rate in dexrazoxane treated versus untreated also did not differ (0.87% versus 0.56%, p=0.67). These findings are consistent with a pooled analysis of 553 children with high and very high risk ALL who all received dexrazoxane and had a mean 5-year incidence of secondary malignancies of 0.24% (Standard deviation, SD: 0.24%)[259]. The perceived risk of secondary malignancies with dexrazoxane use in children is based on the results of a single analysis (POG P9425 and P9426) and no study since then has been able to confirm this risk. The only studies that have shown a non-significant increase in the risk of secondary malignancies have been in trials which also included the use of etoposide which has been associated with increased secondary malignancies[260].

An ongoing Children's Oncology Group (COG) study (ALTE11C2) is examining the long-term efficacy (>10 years from treatment) and impact on secondary malignancies of dexrazoxane across a range of anthracycline exposures (100 to 360mg/m^2). Based on the clinical evidence that has emerged since 2011, the European Medicines Agency as of 2017 only contraindicates dexrazoxane use in children who are receiving less than 300mg/m^2 of doxorubicin or an equivalent dose of another anthracycline. In the United States, there is no labelled indication for dexrazoxane use in children and its safety and efficacy has not been established. In 2014, the US FDA granted dexrazoxane pediatric orphan drug status allowing its use in children[261]. Despite this, the American Society of Clinical Oncology currently only recommends dexrazoxane administration for adults with metastatic breast cancer who have already received 300mg/m^2 of anthracyclines and would benefit from additional anthracycline therapy. Determining who would be most likely to benefit from dexrazoxane would allow treatment to be catered to those individuals at high risk of developing cardiotoxicity while limiting exposure from those individuals at low risk of cardiotoxicity.

Other protective agents, such as coenzyme Q1057 and amifostine have not been shown to be effective in small pediatric randomized controlled trials[255]. Another potential cardioprotectant, probucol, has been studied in pre-clinical settings with promising results, however, its viability in humans remains unknown[262]. Despite initial promising results in *N*-acetylcysteine's

cardioprotective ability, a randomized trial concluded that it was not cardioprotective[262,263]. Several studies have reported that carvedilol could be given in parallel with anthracyclines as a cardioprotective agent[264]. A COG randomized, placebo-controlled trial (ALTE1621) is currently underway in childhood cancer patients who will be given low dose carvedilol or a placebo during and for two years after treatment to evaluate its effect on cardiac and therapeutic outcomes. Dexrazoxane currently remains the only widely used cardioprotective agent.

1.10.2: Anthracycline Analogs and Alternative Drug Delivery Methods

More than 2000 analogs of anthracyclines have been studied, but few have been shown to have similar efficacy and less cardiotoxicity than doxorubicin and daunorubicin [265]. A meta-analysis of 15 studies compared other anthracyclines with mitoxantrone in women with advanced or metastatic breast cancer, multiple myeloma, non-Hodgkin's and Hodgkin's lymphomas and found that anthracycline containing regimen increased the risk of clinical cardiotoxicity (OR 2.88; 95% CI: 1.29, 6.44; $p = 0.01$) compared with a chemotherapy regimen containing mitoxantrone[266]. The same meta-analysis also examined 13 studies comparing doxorubicin with epirubicin in women with advanced or metastatic breast cancer, ovarian cancer, or non-Hodgkin's lymphoma and found that epirubicin significantly decreased the risk of clinical cardiotoxicity (OR 0.39; 95% CI: 0.20, 0.78; $p = 0.008$) compared with doxorubicin. One study of each doxorubicin and epidoxorubicin compared with idarubicin found little difference in risk of cardiotoxicity with comparable therapeutic efficacy in patients with non-Hodgkin's lymphoma[267,268].

In adults, liposomal-encapsulated doxorubicin has demonstrated equivalent efficacy and less cardiotoxicity in randomized controlled trials[269-271]. Their release of anthracyclines is slower which may result in low peak concentration thereby lowering cardiotoxicity[272-276]. A meta-analysis of 4 studies comparing liposomal formulations of doxorubicin with conventional doxorubicin in metastatic breast cancer patients and in men and women with multiple myeloma found a significant decrease in the risk clinical cardiotoxicity (OR 0.18, 95% CI: 0.08, 0.38; $p < 0.0001$) in those treated with liposomal doxorubicin[266]. Moreover, there was no evidence of differences in tumour response rate or survival between the liposomal doxorubicin and conventional doxorubicin[266]. Despite the positive results seen in adult patients, data is limited

for pediatric patients and liposomal formulations of anthracyclines have not been widely used in children[277-279].

Longer infusion rates have also been proposed as a potential mechanism to prevent cardiotoxicity. In adults, continuous infusions given over a 24 to 96 hour time period have been shown to reduce acute cardiotoxicity compared to bolus dosing[280]. A meta-analysis of 4 studies comparing bolus with continuous infusion (6, 48, 72, and 96 hours) in women with breast cancer, ovarian cancer, and adults with recurrent or metastatic soft-tissue sarcomas found a significant increase in clinical cardiotoxicity (OR 4.13; 95% CI: 1.75, 9.72; $p = 0.001$) in those given bolus dosing[266]. A systematic review of the benefits and risks of liposomal anthracyclines in childhood cancer patients, however, found no difference between conventional and liposomal anthracyclines or between different liposomal anthracycline derivates[281]. Continuous infusions may only provide benefits in adult patients, however, more evidence is needed to determine the impact of continuous infusion rather than bolus dosing in children.

1.10.3: Monitoring and Management

In both pediatric and adult patients potentially receiving anthracyclines, the American Heart Association's class I recommendation is routine echocardiography at baseline and recurrent re-evaluation. The Children's Oncology Group also recommends serial monitoring by echocardiography at baseline and throughout treatment. The European Society of Medical Oncology and the American Society of Echocardiography have also published a consensus statement in 2014 of the multimodality imaging assessment of patients during and after cancer therapy and the European Society of Medical Oncology has published algorithms for the serial cardiac monitoring of patients based on whether or not biomarkers are being used[282]. Although there is a consensus on the need for serial monitoring before and after treatment, there are conflicting recommendations for the mode and frequency of monitoring. Currently, there are also no cardiac biomarkers that have been validated as predictors of late cardiotoxicity to indicate when and in which individuals anthracycline therapy should be stopped and how long monitoring should continue in each individual.

All recommendations begin with a baseline left ventricular assessment, which is most frequently conducted using echocardiography due to widespread availability and the absence of radiation exposure[283]. Cardiac magnetic resonance imaging and gated radionucleotide angiography can also be used for serial assessment of cardiac function, but it is important that the same technique used for the baseline cardiac assessment be utilized for that patient's follow-up imaging[283]. If the baseline left ventricular function is abnormal, then a cardiology consultation should be obtained before initiating therapy. If the baseline echocardiogram is normal, the recommendations for the timing of subsequent follow-up imaging in children depend on both the recommendations specified in certain treatment protocols and long term follow up recommendations made by the Children's Oncology Group (http://www.survivorshipguidelines.org/) which recommend that children who received doses <250mg/m2 should receive an echocardiogram every 5 years, while those that received ≥250mg/m2 should receive an echocardiogram every 2 years. The recommended frequency for screening adult survivors is every 5 years with patients who have abnormal results followed up in a year[222]. Alternatively, the algorithms designed by the European Society of Medical Oncology suggest serial monitoring every 3 months for the first year following anthracycline therapy and then monitoring yearly subsequent to that[282]. While majority of these recommendations take into account clinical risk factors such as the patient's age, radiation, anthracycline dose, more recent recommendations have focused on the need for a comprehensive history and physical examination with specific emphasis on cardiac symptoms such as dyspnea, chest pain, palpitations, and exertion tolerance during each follow up assessment[284].

As there are no treatments that are specific to anthracycline-induced cardiotoxicity, standard therapies to treat congestive heart failure such as beta-blockers, ACE inhibitors, and loop diuretics are used[285]. In a study of childhood cancer survivors who developed anthracycline-induced cardiotoxicity, treatment with enalapril (ACE inhibitor) and carvedilol (beta blocker) resulted in a normalization in left ventricular function in 42% of patients[286]. ACE inhibitors reduce the afterload on the myocardium and improve survival in adults, however, their effect in children remains unclear[182]. Growth hormone treatments have been shown to increase LV wall thickness and improved anthracycline-induced cardiotoxicity, however, these improvements appear to be transient[205]. Prevention and risk reducing strategies remain the most effective

management solutions. Survivorship programs managed by oncology or hematology help coordinate long-term follow-up for cancer survivors. Cardio-oncology follow up programs work with such survivorship programs and are being implemented into regular clinical care to ensure the proper monitoring and follow up of patients that receive cardiotoxic chemotherapies[182].

1.11: Clinical Risk Factors of Anthracycline-induced Cardiotoxicity

Clinical risk factors including cumulative dose, anthracycline infusion rate, age, sex, type of anthracycline, and chest radiation have all been associated with anthracycline-induced cardiotoxicity[287]. Higher cumulative doses of anthracyclines have been reported to be the most important risk factor in the development of cardiotoxicity. The recommended lifetime dose of doxorubicin is 450 to 550mg/m^2 [245]. The incidence of congestive heart failure at this level is between 5-16%[245], however, this number approaches 50% when doses of 1000mg/m^2 are reached[288,289]. Weekly or continuous infusion rates over >24 hours have been shown to decrease the incidence of cardiotoxicity in adults[266,290,291], however, there are some conflicting reports regarding whether this applies to children[288,292]. A blinded, randomized trial in children with acute lymphoblastic leukemia compared bolus administration to 1 hour to 48 hour infusion rates and found no significant benefit[293]. The risk of cardiotoxicity is higher in children less than 15 years of age, with those less that age 4 being at a particularly high risk[294]. Female patients, elderly patients, and patients with pre-existing cardiac disease have also been found to be more susceptible to anthracycline-induced cardiotoxicity[293]. Additionally, patients that receive mediastinal or chest wall radiation are at an increased risk[295]. All anthracyclines have been shown to be cardiotoxic, however, epirubicin and mitoxantrone are expected to be less cardiotoxic than doxorubicin[295]. A meta-analysis showed lower rates of clinical cardiotoxicity (OR 0.39, 95% CI: 0.2, 0.78, p=0.008) from epirubicin compared to doxorubicin without compromising anti-tumour efficacy, and higher rates of clinical cardiotoxicity in doxorubicin treated patients compared to mitoxantrone (OR 2.88, 95% CI: 1.29, 6.44; p = 0.01) [266]. Concomitant treatment with other cardiotoxic agents (i.e., trastuzumab, cyclophosphamide, etoposide, melphalan, paclitaxel) has also been found to augment anthracycline-induced cardiotoxicity[295]. Patients of African ancestry and those with trisomy 21 are at a higher risk of developing cardiotoxicity[266].

1.12: Pharmacogenomic Studies of Anthracycline-induced Cardiotoxicity

While clinical risk factors contribute to patients' susceptibility to anthracycline-induced cardiotoxicity, some patients who receive low doses and do not have other risk factors develop cardiotoxicity, suggesting that other factors such as genetics are involved. Candidate gene and genome-wide association studies have identified genetic variants significantly associated with anthracycline-induced cardiotoxicity with variants in retinoic acid receptor gamma (RARG), solute carrier (SLC) transporters, and UDP-glucuronosyltransferase family 1A, isoform 6 (UGT1A6) currently having the strongest evidence. Each of these variants have been replicated in three or more worldwide populations with odds ratios of >3.0 or <0.3. Genetic variants in ATP binding cassette (ABC) transporters and nicotinamide adenine dinucleotide phosphate (NADPH) multi-enzymes complex have also been significantly associated with anthracycline-induced cardiotoxicity, however, the individual risk provided by any of these candidate genes were moderate only (OR: 1.5–2.8). Additional variants in glutathione S-transferase (GST) enzymes, catalase (CAT) enzyme, sulfotransferase family cytosolic 2B member 1 (SULT2B1) enzyme, hyaluronan synthase 3 (HAS3) enzyme, histamine N-methyltransferase (HNMT) enzyme, human haemochromatosis (HFE) protein, cytochrome P450 oxidoreductase (CYPOR/POR), and nitric oxide synthase 3 (NOS3) enzyme have also been reportedly associated with anthracycline-induced cardiotoxicity, however, the evidences are limited and too heterogeneous for a significant conclusion to be drawn.

1.12.1: Retinoic Acid Receptor Gamma (RARG)

RARG belongs to a family of retinoic acid receptors (RARs) that can co-regulate expression of downstream gene products in response to their agonist, all-trans retinoic acid (ATRA)[266]. RARG has been shown to both activate and repress transcription in response to ATRA[266,296]. A study conducted in 456 pediatric cancer patients reported a 5-fold increased risk in the likelihood of developing anthracycline-induced cardiotoxicity in patients who are carriers of a specific non-synonymous coding variant (p.Ser427Leu; rs2229774) in *RARG* (OR 4.7; 95% CI: 2.7, 8.3; $p=5.9\times10^{-8}$)[204]. This association was replicated in European, African, East Asian, Hispanic and Indigenous Canadian patient populations[204]. They then went on to show that in HEK293T cells there was a 17% decrease in RARG activity for those that carried the rs2229774 variant, which is likely an underestimate as the cell assay contained endogenous RARs[204]. *RARG* expression has

been shown to be especially high in cardiomyocytes[204]. Additionally, RARG has also been shown to bind to the TopIIβ promoter providing a potential target for the mechanism of $RARG^{S427L}$ dysregulation[204]. *TopIIβ* expression was significantly reduced with the addition of RARG in rat cardiomyocytes[297]. In contrast, the $RARG^{S427L}$ variant was unable to repress *TopIIβ* to the same extent as wild-type RARG[298] . In summary, RARG is able to repress the expression of *TopIIβ*, while rat cardiomyocytes expressing the $RARG^{S427L}$ variant show higher basal level expression of *TopIIβ*. As mentioned previously, higher levels of TopIIβ have been associated with the development of anthracycline-induced cardiotoxicity.

1.12.2: Solute Carrier (SLC) Transporters

A significant association for the synonymous coding variant rs7853758 (L464L) in the solute carrier family 28, member 3 (*SLC28A3)* gene was discovered (n=156; p=0.0071) and replicated (n=188; p=0.0072) in pediatric cancer patients from across Canada, which remained significant after correcting for multiple testing in the combined Canadian cohort ($p=1.0x10^{-4}$)[299]. In both cohorts the minor A allele was observed more frequently in control than cases of cardiotoxicity (20% versus 7.7%). This association was further replicated in an independent cohort of 73 pediatric cancer patients from the Netherlands (OR 0.42, p=0.067) with a combined odds ratio from all three cohorts of 0.35 (95% CI: 0.21,0.59; $p=1.8x10^{-5}$)[10,12]. Two additional variants in *SLC28A3* (rs885004 and rs4877847) were associated with anthracycline-induced cardiotoxicity with rs885004 having a similar effect size to that of rs7853758 (OR 0.31, $p=2.1x10^{-4}$). The two variants (rs885004 and rs7853758) are in high linkage disequilibrium with each other (R^2=0.83) and the association disappeared after the analysis was adjusted for the effect of rs7853758. The effect of *SLC28A3* may be specific to pediatric patients, as two studies in adult cancer survivors did not replicate these associations[204]. Additional genetic associations in *SLC22A17* (rs4982753) and *SLC22A7* (rs4149178) have been discovered in 344 pediatric cancer patients with ORs of 0.52 (p=0.0078) and 0.41 (p=0.0034*)*, respectively[253]. These findings were replicated in 218 pediatric patients with an OR of 0.39 for both *SLC22A17* (rs4982753; p=0.0071) and *SLC22A7* (rs4149178; p=0.047).[253]

Several studies have provided supportive functional evidence for the importance of *SLC28A3* variants. The rs7853758 variant has been associated with reduced mRNA expression of *SLC28A3* in monocytes (p=0.001) suggesting a functional effect related to this synonymous variant (L461L)[10,12]. Additionally, rs7853758 has been associated with an increased survival after gemcitabine treatment for pancreatic cancer[300]. Both SLC28A3 and SLC28A1 can transport several anthracyclines into cells, providing a potential mechanism by which these variants could affect susceptibility to cardiotoxicity[300,301]. This association was only observed in populations treated with doxorubicin and daunorubicin, but not in patients treated with epirubicin which is metabolized by a different pathway (UGT2B7)[253]. The *SLC22* gene family is comprised of more than two dozen members that encode organic cation transporters (OCTs), organic cation/carnitine transporters (OCTNs) and organic anion transporters (OATs)[302]. Two members of this family, SLC22A16 (OCT6) and SLC22A4 (OCTN1), have been found to be involved in the transport of doxorubicin into cells[303]. Another member, SLC22A15 has been shown to downregulate doxorubicin-resistant cells[304]. *SLC22A17* (brain-type OCT) and *SLC22A7*(OCT2) are both expressed in the heart, and SLC22A7 transports nucleobases and guanine analogs with considerable substrate overlap with SLC28A3[305-307]. It has, therefore, been hypothesized that the proteins encoded by these genes could be responsible for transporting anthracyclines into cardiomyocytes leading to increased cardiotoxicity.

1.12.3: UDP-glucuronosyltransferase family 1A, isoform 6 (UGT1A6)

A synonymous coding variant (V209V) in *UGT1A6* (rs17863783) associated with anthracycline-induced cardiotoxicity has been discovered (OR 3.69; p=0.0059) and replicated (OR 7.98; p=0.0062) in independent pediatric populations. This association was further replicated in an independent mixed age cohort (median age: 43.3 (cases), 46.9 (controls) years old) of hematopoietic cell transplant patients with an OR of 19.5 (95% CI: 3.5, 110.5; p<0.001)[308]. This variant tags a specific haplotype (*UGT1A6*4*), which has been reported to cause a 30–100% reduction in enzyme activity. Two additional variants in *UGT1A6* (rs6759892 and rs4261716) had been found to be associated with anthracycline-induced cardiotoxicity[306], but with smaller odds ratios of 1.77 (p=0.0038*)* and 1.76 (p=0.0043), respectively[10]. UGT1A6 is an enzyme of the glucuronidation pathway that biotransforms molecules into water-soluble metabolites for excretion. While doxorubicin and daunorubicin are unlikely to be metabolized through

glucuronidation, it has been shown that some of their metabolites are glucuronidated[10]. Therefore, alterations in glucuronidation may result in the accumulation of toxic metabolites in patients carrying *UGT1A6*4* leading to cardiotoxicity[10].

1.12.4: ATP Binding Cassette (ABC) transporters

Fourteen variants in genes that encode ABC transporters have been reported for their association with anthracycline-induced cardiotoxicity including: *ABCC1* (rs45511401: OR 2.5, *p*=0.016; rs3743527: *p* = 0.001; rs246221: *p* = 0.027; rs4148350: OR 3.44, *p*=0.0012; rs246214:*p*=0.0014), *ABCC2* (rs8187694-rs8187710 haplotype: OR 1.9, *p*=0.071; rs4148391:*p*=0.056; rs4148399:*p*=0.095), *ABCC5* (rs7627754: *p*=0.001), *ABCB1* (rs2235047: OR 2.92, *p*=0.0087) and *ABCB4* (rs1149222: OR 1.87, *p*=0.0054; rs4148808: OR 1.86, *p*=0.0093). Three of these associations in *ABCC1* (rs246221: OR 1.6, p=0.02), *ABCC2* (rs8187694-rs8187710 haplotype: OR 5.22, p=0.02; OR 4.3, p=0.021) and *ABCC5* (rs7627754: p=0.04) have been replicated. A meta-analysis of ABCC2 rs8187710 demonstrated a pooled OR of 2.2 (95% CI: 1.36, 3.54, p=0.001). Alternatively, a meta-analysis of three studies in European populations revealed no significant associations for ABCC1 rs45511401 (OR 1.8; 95% CI 0.65, 5.07; p=0.26) or ABCC2 rs8187694 (OR 1.7, 95% CI: 0.95, 3.02; p=0.07). ABC transporters are ATP-dependent membrane proteins that are expressed in the heart, liver intestine, blood brain barrier, placenta, and kidney and play an essential role in the transport of anthracyclines and a variety of other drugs, which provides a potential mechanism for how they could be associated with the development of anthracycline-induced cardiotoxicity. Reduced expression and function of certain ABC transporters have been found to increase the intracellular accumulation of drugs and their metabolites.

1.12.5: Nicotinamide Adenine Dinucleotide Phosphate (NADPH) Multi-enzyme Complex

Genetic associations with polymorphisms in NADPH oxidase subunits that are involved in the production of ROS have been shown. Associations for NCF4 rs1883112, CYBA rs4673 and RAC2 rs13058338 have been discovered and replicated at least once in independent studies[309,310]. Neutrophil cytosolic factor 4 gene (*NCF4*) encodes the p40phox subunit of the NAD(P)H oxidase and the rs1883112 polymorphism at the putative promoter of NCF4 blocks oxidase activation of the enzyme thus reduces the formation of reactive oxidant intermediates.

An initial study of the association between rs1883112 and cardiotoxicity demonstrated an OR of 0.39 (95% CI: 0.24,0.64; $p = 1.4x10^{-4}$)[311], however, a meta-analysis of two studies in North American and European populations revealed no significant association (pooled OR: 0.94; 95% CI: 0.64,1.38; $p = 0.75$)[312]. Cytochrome B-245, alpha polypeptide gene (*CYBA*) encodes the primary component of the microbicidal oxidase system of phagocytes and in a meta-analysis the rs4673 variant was found to increase the odds of developing anthracycline-induced cardiotoxicity (pooled OR: 1.55; 95% CI: 1.05, 2.30; $p = 0.03$) with moderate heterogeneity ($I^2 = 33\%$)[312]. Ras-Related C3 Botulinum Toxin Substrate 2 gene *(RAC2*) encodes the protein regulating diverse processes including secretion, phagocytosis, cell polarization and generation of ROS, and is thought to induce NADPH complex assembly by activating signaling pathways such as cytosolic protein kinases[10,204,249,313]. The A allele of the rs13058338 intronic variant has been associated with high mRNA expression of *RAC2* and *NACF4* in granulocytes[224,249,254,314,315]. Three studies have found significant associations between rs13058338 and anthracycline cardiotoxicity with two of them reporting ORs of 1.84 (95% CI: 1.1,3.10; p=0.019)[254] and 2.61 (95% CI: 1.46,4.69; p=0.02)[249,314]. A meta-analysis of the rs13058338 variant in four studies showed that *RAC* mutation increased the risk of cardiotoxicity by nearly two times (pooled OR: 1.79; 95% CI: 1.27,2.52; $p < 0.001$)[312]. Recent studies have indicated that NADPH oxidase-derived ROS play a pivotal role in regulating several key components of cardiac remodeling, such as myocyte hypertrophy, contractile dysfunction, apoptosis, and fibrosis[316,317]. NADPH oxidase activity and expression is increased in heart failure patients[318], and one study has shown that mice deficient in NADPH oxidase activity were protected from anthracycline-induced cardiotoxicity compared to wile type[311]. This provides a potential mechanism for how these genetic polymorphisms in subunits of NAPDH oxidase may be related to the development of anthracycline-induced cardiotoxicity.

1.13: Role of Pharmacogenomics in Clinical Care

As the field of genetics advances rapidly, the identification and use of genetic biomarkers are becoming increasingly integrated into clinical care in order to move away from a "trial-and-error" population-based approach towards a more individualized approach to drug therapy. It is estimated that only 50% of patients achieve the desired therapeutic response from their medications[182]. In addition to this ADRs are among the top leading cause of death in North

America. As such, identification of genetic factors that predispose individuals to an ADR would help to increase drug safety and efficacy. Implementing pharmacogenomic testing into routine clinical care may also be cost-effective due to reductions in ADR-related hospitalizations and treatments. For instance, ADRs have been reported to account for 4.2-30% of hospital admissions in the USA and Canada[319]. Additionally, between 2.1% and 5.2% of ADRs in children lead to hospitalization, and up to 39% of ADRs in pediatric patients can be life-threatening or fatal[23]. One study found that emergency department visits and hospital admissions due to ADRs among seniors in Canada costs an estimated $35.7 million per year with more than 80% of those costs arising from hospitalization[320]. An additional prospective study showed that ADRs increased the mean hospital stay of 8 days in patients without ADRs to 20 days in patients with ADRs[321]. Although pharmaceutical companies have been reluctant in the past to incorporate pharmacogenomics into drug development and evaluation[322], they are now using pharmacogenomic markers in phase 2A and 2B clinical trials to reduce associated risks of treatment[322,323]. Pharmacogenetic testing may also benefit pharmaceutical companies by identifying patients who will respond safely to medications that were previously not approved because of toxicities or treatment inefficacies, potentially expanding market share[322].

While there has been substantial progress in the field of pharmacogenetics with the number of publications having risen sharply over the last 10 years, translation into routine clinical practice remains a challenge. Several papers have been published regarding barriers to implementation with commonly identified barriers summarized in **Figure 1.3**[182,324-327]. In order to overcome some of the barriers associated with the lack of understanding of what genes to test, how to interpret pharmacogenetic results, and what to do with results once you have them, several resources have been developed such as the Pharmacogenomics Knowledgebase (PharmGKB), which is designed to be a comprehensive resource on pharmacogenes and their effects on drug-related phenotypes. They currently have the most extensive searchable database for drugs with associated pharmacogenetic information. Additionally, they provide links to clinical practice guidelines from the Clinical Pharmacogenetics Implementation Consortium (CPIC), the Royal Dutch Association for the Advancement of Pharmacy- Pharmacogenetics Working Group (DPWG), the Canadian Pharmacogenomics Network for Drug Safety (CPNDS), and other professional societies. The U.S. Food and Drug Administration (FDA) has also made label

change recommendations for a number of pharmacogenetic variants with high levels of evidence. In addition to these resources, several large pharmacogenomic initiatives are working to overcome these hurdles in order to expand pharmacogenomic testing into routine clinical practice. Pharmacogenetic testing has been adopted to varying degrees in several clinical fields including cardiology (i.e., warfarin-*CYP2C9/VKORC1* and clopidogrel-*CYP2C19*), psychiatry (i.e., various psychotropics/*CYP2D6*), infectious diseases (i.e., abacavir-*HLA B*5701*), as well as, various somatic and germline pharmacogenetic marker in cancer, discussed in the following section.

1.14: The Current Landscape of Pharmacogenomics Implementation in Oncology—A Scoping Review

Despite the fact that implementation of pharmacogenomics into clinical use has been slow, patients in oncology stand to benefit highly from pharmacogenomic-informed prescribing decisions as many oncological diseases now have several treatment options with similar efficacy and some cancers can now be successfully treated into chronic diseases (e.g., chronic myelogenous leukemia, androgen-sensitive prostate cancer, node-positive breast cancer, surgically-resectable colon cancer, and others). These patients, like patients with diabetes or cardiac disease, often require complex care management and long-term medications to prevent disease recurrence, manage disease-related symptoms, and treat long term drug induced toxicities. As such, incorporating pharmacogenomics into their care can help to improve the long-term management of these patients. Knowledge about an individual's drug response or toxicity susceptibility can allow a physician to choose one treatment option over another if the risk of toxicity is higher, or to select an alternative therapy if the likelihood of response in a given patient is higher. In situations where more than one treatment option does not exist, pharmacogenetic information allows the physician to weigh the toxicity risks with the potential benefits of a given treatment. This is especially relevant in the case of palliative treatments where impact to the patient's quality of life as a result of treatment-related toxicities are weighed against the decreased tumour load and increased life expectancy conferred from a given treatment. Pharmacogenomics can also be of high utility in facilitating patient-specific dose modifications for patients who are known to be poor or rapid metabolizers of a certain drug. For example, specific dose-reduction pharmacogenomic prescribing recommendations are available

for thiopurines (i.e., mercaptopurine, azothioprine, thioguanine) based on the variants an individual carries in the genes: *TPMT* and *NUDT15*[328].

In order to assess the current landscape of pharmacogenomics being implemented into clinical care in oncology, we conducted a scoping review of the literature, which is discussed in the following sections. To our knowledge, there is no systematic or scoping review to date that provides an overview of germline pharmacogenomics being implemented into routine clinical use in oncology. Given the clinical importance and benefits of predicting who is at greatest risk of suffering from chemotherapy-related toxicities, we discuss the strategies and methods that others have found successful, as well as, the opportunities for improvements moving forward. We specifically restricted our considerations to germline pharmacogenomic markers and excluded discussions related to somatic tumour genomics.

1.14.1: Methods

The Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines for a scoping review were followed to reduce bias and the corresponding checklist was followed to ensure completeness of the review. A study protocol was written prior to the initiation of the review, but was not registered.

A literature search was performed using Medline (1946-August 19, 2018) and Embase (1974-August 19, 2018) databases to extract any publications describing germline pharmacogenomic markers that had been implemented into clinical care in oncology. This was done using the keywords: "pharmacogen*" or "PGx*", implement*, and "onco* or cancer*" with a complete search strategy described in **Table 1.1**. Inclusion criteria limited search results to include only publications written in English and limited the publication type to include peer-reviewed articles, observational studies, clinical studies, multi-center studies, and practice guidelines. Two authors (TBW and JL) independently screened titles and abstracts of all retrieved publications to determine if they met the inclusion criteria. If a decision could not be made based on the title and abstract, the publication was reviewed in its entirety to evaluate its inclusion. After independent review, any conflicting opinions regarding the inclusion or exclusion of an article were discussed as a group until a consensus was reached.

For all articles that met the inclusion criteria, each was reviewed (including supplemental materials) and the following data points were extracted: name and number of sites that the program or research project involved, the period of time over which implementation of pharmacogenomics occurred, the research project's eligibility and exclusion criteria, the genotyping platforms that were used, whether any clinical practice guidelines for testing were followed, what genetic variants were being tested, how the results were being delivered to the utilizing parties, whether testing was reactive or pre-emptive, whether results were being uploaded to electronic medical records (EMRs), whether any clinical decision support (CDS) tools were being used, what outcomes each research project were measuring, any challenges and barriers to implementing pharmacogenomics, and the number of patients enrolled or tested in each research project. If the research project did not have a given name, the main institute where the study was conducted was used to identify them. The number of sites that the research project involved referred to sites or institutions that were directly involved in returning pharmacogenomics results. The genetic variants being tested included only germline variants that were being returned for use in clinical care in oncology. Utilizing parties refers to either clinicians, pharmacists, or patients that received pharmacogenomics results. Challenges and barriers identified were specific to the program/research project and not based on what the article identified as general barriers that had previously been reported. Programs that had more than one publication were grouped by year to provide a complete overview of their findings and progress over time.

1.14.2: Results

The Ovid Embase and MEDLINE search identified 769 unique articles. During the title and abstract review, 695 were excluded due to reasons identified in **Figure 1.4**. The majority of excluded publications were review articles (n=407). An additional 117 articles discussed their future plans and the importance of implementing pharmacogenomics, but were not yet at the stage of doing so. 102 articles only discussed somatic gene variants rather than germline variants, 46 articles were related to pharmacogenomics of drugs not regularly used in oncology practice, and 23 publications were Clinical Practice Guidelines discussing recommendations and available evidence for implementing pharmacogenomics. The remaining 74 publications underwent a full

review of the article. From this, an additional 44 were excluded for similar reasons—13 were review articles, 18 were not yet at the stage of implementing pharmacogenomic findings, 7 were not germline variants, and 6 implemented variants associated with drugs that aren't used in oncology. This left a total of 30 papers that met the eligibility criteria for inclusion and described the implementation of germline pharmacogenomic variants related to drug response in oncology.

Of these 30 articles, 23 (77%) were implemented in hospitals and academic centers in the United States **(Figure 1.5)**. The remaining 7 articles described several observational studies in the Netherlands[324,329-332], France[333-335], Germany[336,337], and Korea[338], a centralized pharmacogenomics service at a cancer institute in Serbia[339], and one large scale multi-center implementation program across Europe initiated by the Ubiquitous Pharmacogenomics (U-PGx) consortium[340]. Three of these observational studies were conducted to assess the cost effectiveness and impact of screening for dihydropyrimidine dehydrogenase (DPD) deficiency prior to receiving fluoropyrimidine-based chemotherapy treatment[341]. Two studies examined the cost-effectiveness and feasibility of genotyping patients for *UGT1A1* prior to treatment with irinotecan[342]. One additional article described a centralized pharmacogenomics service at the Institute for Oncology and Radiology of Serbia where, in addition to tumour biomarkers, they were genotyping patients for the following pharmacogenomic drug-gene pairs: *CYP2D6/*tamoxifen, *DPYD*/5-fluorouracil, and *MTHFR/*methotrexate[336-338]. While several countries are moving towards the implementation of pharmacogenomics, both Europe and the United States have a much more developed framework for implementation with several large networks working together to overcome previously identified barriers to implementation (i.e., eMERGE-PGx, IGNITE, PGRN, U-PGx). These strategies for overcoming barriers and the findings from each of these research projects are discussed in the following sections.

1.14.3: Study Aims and Outcomes

The majority of publications related to these implementation projects were focused on describing their methods for implementation and strategies for overcoming barriers. However, some projects discussed their future goals to determine the cost-effectiveness of implementing pharmacogenetics, as well as, the impact on patients' health outcomes. For instance, Indiana's IGENIOUS project is prospectively enrolling 6,000 patients into a pharmacogenetics testing arm

or a standard treatment arm and comparing the healthcare costs of both arms and the impact on adverse events[343]. U-PGx's PREPARE study also aims to assess the cost-effectiveness of pharmacogenetics testing, process indicators for implementation, and provider adoption[342]. Additionally, they aim to assess the knowledge of pharmacogenetics before and after an educational program is implemented to determine the gaps in knowledge of providers. More specifically, the University of Chicago's 1200 Patients Project aims to evaluate the clinical utility and feasibility of implementing pharmacogenomics[344]. Their initial results show that 90% of the top 21 diseases can be treated with a drug for which pharmacogenetic information is available[344]. They are also measuring prescriber habits in terms of whether pharmacogenetics results are leading to appropriate changes in medication prescribing and whether their online portal for results delivery is being accepted and utilized by providers[345]. Another example is Vanderbilt's PREDICT program that aims to evaluate their algorithm for determining who to pre-emptively genotype. They also compared reactive versus pre-emptive genotyping, and found that out of the 9,589 patients that were pre-emptively genotyped, 91% have as least one actionable genotype and 5% have at least one high risk genotype[346]. Lastly, the PIPseq program aimed to assess clinical utility of tumour and germline genetic testing and found that results were clinically impactful 66% of the time and that actionable genotypes were found in 38% of patients[347]. With implementation still in its early stages, these projects aim to further assess outcomes regarding clinical utility and pharmacogenomics once their programs are fully integrated into clinical care.

1.14.4: Reactive or Pre-emptive Testing

Some projects, such as the Personalized Medicine Programs in the University of Florida and Cleveland Clinic in Ohio are using a reactive approach where patients are genotyped for *TPMT* once they have received a cancer diagnosis that requires treatment with thiopurines. Arguments have been made against this reactive approach due to the time it takes to receive results before they can be incorporated into treatment decision-making. As a result, many have moved towards a pre-emptive approach whereby multiple genes are tested at once prior to a drug being prescribed. The results are then available at the point of care if the patient were to be prescribed a drug influenced by one of the genes tested for. The remaining majority of projects identified are pre-emptively genotyping patients likely to benefit from the results in the near future **(Table 1.2)**. For instance, the eMERGE-PGx network has 10 academic medical centers

across the United States aimed at genotyping patients who are likely to be prescribed a drug of interest within the next 3 years[339,340]. Each site has different strategies for doing so, for example, Vanderbilt University's Hospitals and Clinics and the Mayo Clinic have each developed a predictive algorithm to determine who is most likely to receive a drug of interest[341]. Alternatively, at the Children's Hospital of Philadelphia, they are genotyping patients who are taking more than three medications at once and those who have previously experienced a serious adverse event[348]. The University of Chicago's "1200 Patients Project" is pre-emptively genotyping any patient taking between 1 to 6 regularly used medications[346,349], while several other projects are genotyping patients for multiple pharmacogenomic variants if the patient is planning on receiving any of the drugs of interest[348]. In U-PGx's PREPARE Study, for example, patients are genotyped using a custom panel of 50 variants corresponding to 43 drugs when they receive their first prescription for a drug of interest[327,344]. Many programs and implementation projects are moving towards a pre-emptive approach to genotyping as physicians are reluctant to wait for the results before prescribing. This is especially true in oncology, where treatment is initiated rapidly after diagnosis in most cases.

1.14.5: Genotyping Platforms and Drug-gene Pairs Selected for Implementation

As genotyping technologies have advanced, genotyping strategies used by each of these implementation projects have evolved. At the time of these articles were published, the majority of projects were using Real-Time PCR Systems to characterize Absorption, Distribution, Metabolism, and Excretion genes (ADME; e.g., QuantStudio, Illumina VeraCode ADME Core Panel, Affymetrix DMET Plus) (**Table 1.2**. Some projects have moved towards using custom next generation sequencing (NGS) platforms. For example, three sites that are part of the eMERGE-PGx network were using the PGRNseq platform, which is a custom-capture panel of 84 genes. Columbia University's PIPseq program has also designed a custom NGS platform (Agilent SureSelectXT library), targeting 467 genes.

The majority of publication described projects that are implementing pharmacogenomics across multiple medical specialties, not solely oncology, with the exception being the PIPseq program, which performs next generation sequencing on the tumour and normal tissue of pediatric cancer patients with high risk disease[342,343,348,350]. The other projects and multi-site networks are

implementing drug-gene pairs across multiple specialties with a main focus on implementing those with clinical practice guidelines (i.e., CPIC or DPWG) and those with enough evidence to warrant an FDA label change recommendations. The majority of project described selecting drug-gene pairs for implementation in accordance with CPIC guidelines (10/12 projects)[347]. Five out of 12 projects also mentioned that they selected drug-gene pairs based on FDA recommendations for label changes, and 2 out of 12 projects mentioned selecting drug-gene pairs based on DPWG guidelines[326,343,344,348-353]. The PIPseq program did not mention how they selected which drug-gene pairs to test. Based on the above selection criteria, the most common drug-gene pair implemented in relation to oncology was *TPMT/*thiopurines (11/12 projects) (**Table 1.2**). This was followed by *UGT1A1/*irinotecan (4/12 projects), *DPYD/*fluoropyrimidine (3/12 projects), and *CYP3A5*/tacrolimus (3/12 projects). Additionally, *CYP2D6/*tamoxifen was implemented at the Mayo Clinic as part of the RIGHT protocol study and throughout multiple sites in Europe as part of the U-PGx PREPARE study. Lastly, *ITPA/*thiopurines was implemented as part of Indiana University's INGENIUS study, and *NT5C2,* which has been associated with chemotherapy resistance, was tested as part of Columbia University's PIPseq Program[347].

1.14.6: Electronic Medical Record (EMR) Integration and Clinical Decision Support (CDS) Tools

All of the published projects, except the University of Chicago's 1200 Patients Project, are integrating patients' pharmacogenomic results into EMRs (**Table 1.2**). Instead, at the University of Chicago, they have designed a stand-alone custom interface, the Genome Prescribing System (GPS), to transform patients' pharmacogenomic data into Clinical Decision Support (CDS) summaries[326,327,349,350,352]. Additionally, all described projects had some form of CDS tool or system to help providers interpret results and improve the implementation process. In 10 projects, interruptive pre-test alerts are being presented to physicians when a drug linked to a drug-gene pair is prescribed, informing physicians that the patient does not yet have a documented genotype[327,342]. In the case of the Vanderbilt PREDICT Program, pre-test alerts fire if the patient is identified as an ideal candidate based on their predictive algorithm for who would be most likely to benefit from testing[354]. Four pre-test alert systems give you the option of ordering the pharmacogenomic test directly from the EMR alert[342,343,349-353,355]. Indiana

University's INGENIOUS project's pre-test alert gives physicians the option of enrolling their patient in the study, which randomizes the patient to either receive or not receive pharmacogenomic testing[355].

In addition to pre-test alerts, post-test alerts were presented when a drug was ordered for which genotyping information was available for all projects except PIPseq, which did not incorporate pre- or post-testing alerts into their EMR/CDS system[349,352,355,356]. All of these post-test alerts provide therapeutic recommendations with 5 projects also providing specific dose adjustment suggestions directly within the alert[343]. Drugs which should be avoided were also placed in the post-test alert system for 2 projects[347]. Links to clinical practice guidelines and additional information regarding patients' pharmacogenomic results were also incorporated into alerts in several projects [342,344,350,352,355].

1.14.7: Return of Results Format

Result reports were either generated in the form of consult notes that were delivered to ordering physicians and then uploaded to the EMR[349,352], or stored directly within sections of the EMR or an online portal[343,349,351,352,355,357]. The majority stored their pharmacogenomic results within the EMR as a summary report with phenotypic interpretations (e.g., "CYP2D6 ultrarapid metabolizer") [343,347,351,357]. Some also stored raw genotyping data in the lab section of the EMR without associated phenotypic information[326,342,350,352-354,358]. The GPS online portal system used by the University of Chicago delivers their results using a traffic light system whereby a red light beside the drug name indicates a high risk of experiencing an adverse event, whereas a green light indicates that there is no evidence that the patient is at an increased risk of experiencing an adverse event from the drug given the genetic findings[326,343,349,350,357]. A yellow light indicates that there is some evidence that the patient is at an increased risk of experiencing an adverse event from the drug due to the genetic findings for the patient. The GPS system also provides links to alternative medication recommendations. Results for this interface are designed to be read in 30 seconds or less. Majority of projects reported genotyping results with star-allele nomenclature and a phenotypic interpretation[326,349,352,357] with recommendations for alternative medications included in the result reports for 2 projects[354].

In addition to results reports, 4 projects summarized actionable pharmacogenomic results in a problem list within the EMR to make it easier for users to view and interpret results[342,343,350-353,355,358]. For 4 projects, inbox messages were sent to physicians when a new pharmacogenomic result was available in the EMR[343,350]. Another CDS feature offered by 5 projects was the option to request a consult with a pharmacist with expertise in pharmacogenomics either in person[349,350,352,357,358] or via a virtual consult system[350-352,358]. The University of Chicago's 1200 Patients Project also designed a search engine that physicians could use to search for disease-specific drug information with corresponding pharmacogenomic information to use when making prescribing decisions[343,351,358]. Alternatively, U-PGx's PREPARE Study has implemented the use of quick response (QR) codes as part of a mobile-based CDS system to store individual patients' pharmacogenomic results[352,354]. This was done to enable quick retrieval of patient information and allow for communication of results without the need for existing technology infrastructure or a central data storage system.

The method of returning results to patients varied between projects with the majority of publications not addressing how results were explained or given to patients. Letters describing individuals' pharmacogenomic results were generated for the Children's Hospital of Philadelphia[344] and St. Jude's Children's Research Hospital's PG4KDS program with contact information for a clinical pharmacist[342]. Alternatively, the PREPARE study produced "Safety Code Cards" for patients with their specific genotyping results and phenotypic interpretations as well as a QR code that could be scanned for more information[357]. The GPS system from the University of Chicago and Vanderbilt's EMR interface provided the option for physicians to print a results summary for their patients[350]. Additionally, an online portal for patients to access their pharmacogenomic results was implemented for both the Vanderbilt PREDICT Project and the Mayo Clinic RIGHT protocol study[342].

1.14.8: Barriers to Implementation

While many of these projects and networks are have developed strategies to overcome previously identified barriers (i.e., through the integration of EMRs and CDS tools), significant barriers still remain. Lack of education and understanding of pharmacogenomic for both providers and patients was a barrier identified in almost every project. Some projects have

developed educational programs for physicians[326,354] and others have implemented a consult service for clinical pharmacists to provide support to physicians[349,355]. U-PGx's PREPARE study is in the beginning stages of developing an e-learning based knowledge platform to distribute pharmacogenomics knowledge to physicians and pharmacists[342,350-353]. Mayo Clinic's RIGHT protocol has also developed educational resources complementary to the drug-gene pairs in an "FAQ" format[343]. These resources were designed to be delivered at the point of care by linking them to the CDS alerts within the EMR. In terms of patient education, St. Jude's Children's Research Hospital PG4KDS project has developed a video with details about pharmacogenomics, the goals of the project, and how pharmacogenomics is being used in clinical care[342]. Several projects have also developed educational material published online or through patient handouts[358].

With rapidly advancing technology, additional barriers exist in terms of information technology infrastructure and data storage. Alert fatigue, for example, whereby alerts are being sent to EMR users too frequently was identified as a common problem with CDS systems[359]. To overcome this, most projects have incorporated the ability to suppress alert triggers until the medication of interest is prescribed[350]. Another identified barrier was the ability to store large amounts of data from whole genome sequencing in a secure manner. In order to deal with this issue, all sequencing data is stored in a separate database and only subsets of clinically relevant data are placed within the EMR. Additionally, the variability in EMR interfaces between hospitals and communities creates a barrier in the ability to share data. Many projects are moving towards larger EMR platforms such as Epic or Cerner to promote interoperability between institutions[324]. Alternatively, University of Chicago's online GPS platform allows results to be accessed irrespective of which EMR system their hospital uses to avoid this barrier[349-351,353,358]. The lack of standard nomenclature and terminology when reporting pharmacogenomic results and interpretations was another barrier identified[349,351,352,354]. Do to the fact that current terminologies widely used in the EMRs (i.e., Systematized Nomenclature of Medicine (SNOMED) terminology) do not adequately describe phenotypes, projects have created their own terminology[354] or have integrated multiple terminology datasets into an online portal outside of the EMR to avoid this limitation[350,352,354].

With genotyping technologies and pharmacogenomic discovery advancing rapidly, it is difficult to remain up to date with evolving evidence. To overcome this barrier, pre-emptive testing of patients using whole genome sequencing and large NGS panels is being done to allow new drug-gene pairs to be implemented easily once further evidence becomes available[324]. This approach also eliminates the issue of turn-around time which is a barrier for those using a reactive implementation approach. With whole genome sequencing approaches, however, some physicians are concerned about knowing how to deal with incidental findings in the case of results revealing variants for increased susceptibility of disease. Some projects have incorporated the return of incidental findings into their consenting process as optional for those who wish to receive them, and have developed strict processes and support systems for how to deal with incidental findings[350,352].

The issue of provider buy-in and the need for evidence of clinical utility remains a challenge for all projects that are implementing pharmacogenomics. As mentioned above, some groups are working towards demonstrating clinical utility by randomizing patients to receive or not receive pharmacogenomic testing and then comparing several outcomes of both groups[354]. Additionally, another group is comparing the adverse drug reaction outcomes and cost of pharmacogenomic-guided therapy to a historic cohort of patients that did not receive pharmacogenomic testing[343,350]. Pediatric centers that are implementing pharmacogenomics have highlighted that the lack of pediatric specific drug safety research and the lack of CPIC guidelines related to childhood prescribing remains a barrier towards implementation in pediatrics[343].

Reimbursement is another ongoing barrier that is proving difficult to solve. Research has shown that insurance companies and public health care systems are reluctant to reimburse testing because of the lack of evidence and incentives[360]. In the United States and Europe, where all of these published projects are being conducted, the reimbursement system is complex and only covers certain genetic tests requiring patients to pay out of pocket for the majority of pharmacogenomic tests. Some projects, as a result, are only implementing gene-drug pairs that insurance companies have agreed to cover[342]. Other researchers have proposed that the solution to this problem requires a shift in the attitude of insurance companies by increasing the amount of evidence that demonstrates the cost-effective benefits of pharmacogenomic testing. As

mentioned above, several projects are aiming to demonstrate the cost-effectiveness and improvement to patients' quality of life of pharmacogenomic testing going forward, which will hopefully go towards demonstrating the value of reimbursing pharmacogenomic testing[347,348].

1.14.9: Discussion and Conclusion

We conducted a systematic scoping review to examine the landscape of germline pharmacogenomics being implemented into routine clinical practice in oncology. While somatic biomarkers of tumour response have been incorporated into clinical use, the list of germline pharmacogenomic variants being used routinely in oncology remains small. Some have argued that before pharmacogenomics can be implemented, prospective, randomized validation should be necessary[361]. Others argue that randomized studies are unfeasible given that the greatest benefits will usually be observed in a small percentage of the population who harbor a risk-variant, and the control groups often do not reflect a real-world population[362]. For many pharmacogenomic traits, the mechanisms are well understood[363]. Additionally, many pharmacogenomic variants affect the pharmacokinetics of a drug, and recommendations can, therefore, be made based on pharmacokinetic evidence (e.g., *TPMT*/thiopurines)[363]. In cases like these, where sufficient supporting evidence exists, not only is there not a need to generate evidence via randomized trials (e.g., by comparing reduced doses to normal doses in patients with high risk variants), but it would also likely be unethical[363]. Clinicians continue to debate the level of evidence deemed appropriate and realistically achievable[53,364,365], and based on the lack of consensus, payers continue to decline reimbursement for pharmacogenomic testing despite the fact that at times the tests are less expensive than the drugs that are covered[360,366].

Previous economic evaluation studies have shown that pharmacogenomics has a positive impact on health-care quality and costs[367]. In a review of published economic evaluations of drugs that have FDA label recommendations for pharmacogenomic testing, over half of the 44 economic evaluations took a favourable view of the pharmacogenomic-guided strategy: in 12 studies (27%) it was dominant (cost-saving) and in 13 studies (30%) it was cost-effective[367]. This included favourable economic evaluations for *TPMT*/thiopurine testing (7 out of 9 studies) and *UGT1A1*/irinotecan testing (3 out of 3 studies)[367]. However, only 13% of the FDA-listed drugs and only 27% of available genetic tests have been the subject of a published economic

evaluations to assess the cost-effectiveness of implementing testing[367]. This highlights the need for more pharmaco-economic studies, which will help to overcome the barrier of reimbursement by some insurance companies who often require a favourable economic assessment in addition to clinical utility to support the application of a pharmacogenomic test in clinical practice. With an even larger projected benefit as the cost of genotyping continues to decrease, pharmacogenomic testing has the potential to be a cost-effective or even cost-saving intervention.

Based on our scoping review we determined that several hospitals and academic centers are implementing pharmacogenomics into routine clinical care across multiple medical specialties with the majority of programs being based in the United States and Europe. Choosing when to genotype, which drug-gene pair to implement, along with how to translate and deliver results, were critical considerations discussed in majority of the publications. Many programs and implementation projects are moving towards a pre-emptive approach to genotyping as physicians are reluctant to wait for the results before prescribing. This is especially true in oncology, where treatment is initiated rapidly after diagnosis in most cases. Additionally, projects have focused on implementing gene-drug pairs with substantial evidence that have associated clinical practice guidelines and/or FDA recommendations for label changes in most cases. For example, *TPMT/*thiopurines and *UGT1A1/*irinotecan have the most consistent, strong supporting evidence in favour of their routine use which have led to CPIC and DPWG guidelines being published and FDA recommended label changes. For *UGT1A1,* several prospective studies have demonstrated that patients with high risk genotypes are significantly more like more likely to experience neutropenia, with two of these studies corroborating the relationship with pharmacokinetic data[368]. An additional large study (n=250) in colorectal cancer patients demonstrated an association between *UGT1A1* and neutropenia with an OR of 8.63 (95% CI:1.31,56.55)[369]. For *TPMT,* 170 clinical annotations based on published studies exist, and a meta-analysis of all published studies reported a pooled OR of 4.19 (95% CI: 3.20, 5.48) for it its association with the development of myelosuppression[370]. Similarly, *DPYD/*fluoropyrimidines have an FDA label change recommendation and published CPIC and DPWG guidelines. *CYP3A5/*tacrolimus and *CYP2D6/*tamoxifen both have CPIC/DPWG published guidelines but no recommended label changes. *ITPA/*thiopurines has 6 clinical annotations in PharmGKB, but no published guidelines or label change recommendations. Lastly, *NT5C2* has 2 clinical annotations on PharmGKB for

its association with gemcitabine clearance, however, the program testing for this gene only specifies that they are testing for its association with chemotherapy resistance[347]. According to FDA label recommendations 10 germline genes with associated with drugs used in oncology are considered clinically actionable (https://cpicpgx.org/genes-drugs/; accessed December 7, 2018). Based on our review, 7 unique drug-gene pairs related to oncology have been implemented across all implementation sites examined with only 3 of these 7 being noted as being clinically actionable according to the FDA. This highlights the need for well performed studies and the validation of pharmacogenomic findings. Additionally, this calls into question what level of evidence is required before a pharmacogenomic test is considered actionable. An assertion has been made that there is not necessarily a need for more evidence, rather there is a disconnect between our knowledge of how medications should be prescribed and a health care system that is not designed to accommodate acting on that knowledge[363]. The lack of clinician and patient understanding of pharmacogenomics, comprehensive EMRs, interoperability among health care systems, and patient-centered coordination of health care management are all factors impeding the movement of pharmacogenenomics into clinical use[363]. Through the use of physician and patient education training programs, EMR integration and standardization, CDS tools, and studies designed to evaluate the clinical and economic utility of testing these implementation projects are tackling these challenges to advance the integration of pharmacogenomics into our health care systems.

With the widespread adoption of EMRs, we found that the large majority of implementation projects are utilizing EMRs to store and disseminate pharmacogenomics information to providers and patients. CDS tools and systems have also been a major advancement and goal of the majority of these projects in order to provide support to ordering physicians and improve the scope of implementation. In terms of CDS features, pre- and post-testing alerts to notify physicians to order the test, and that pharmacogenomics information is available, were the most commonly reported features. To avoid alert fatigue, many sites have implemented specific rules within their CDS system to only alert physicians to clinically actionable results when it is necessary (i.e., when the drug is being prescribed). Additional CDS features such as online patient portals to access results, search engines for providers, inbox messages sent to providers, and QR codes to store pharmacogenomics data have been implemented at certain sites.

Implementation into routine practice at these sites has been hindered by lack of provider and patient knowledge regarding pharmacogenomics, rapidly advancing genotyping technologies and discoveries, turn-around time of results, the lack of inter-institutional data sharing or common EMR platforms, the lack of standardization of pharmacogenomics terminology, concerns over what to do with incidental findings, and the lack of evidence of clinical utility, among others. With genotyping become less expensive, pre-emptive whole genome sequencing is likely to surmount barriers associated with turnaround time and cost-effectiveness, as well as, to facilitate implementation as new evidence is discovered. Several projects are implementing educational programs and developing educational resources for both physicians and patients. Many are also utilizing clinical pharmacists to provide guidance and education to physicians ordering pharmacogenomic testing. Many have asserted that pharmacology and pharmacogenomic education needs to be incorporated more heavily into the medical education curriculum to overcome this barrier moving forward[371]. New technology and EMR platforms are being developed for easier incorporation of pharmacogenomic information. As well, large EMR softwares (i.e., Cerner and Epic) are being implemented at multiple sites across the country to allow for easier sharing of data between hospitals and cities. Several groups are also working on standardizing the terminology for phenotypic interpretation of pharmacogenomics for easier integration within the EMR and for billing and diagnostic purposes[372]. Additionally, projects like Indiana's IGENIUS project and U-PGx PREPARE study are focused on demonstrating the clinical utility of implementing pharmacogenomics testing.

A limitation of this scoping review is that we limited our search to include only articles written in English due to lack of language translation resources, which may have eliminated relevant publications in areas where English is not the primary language. We used the keywords "pharmacogen*" or "PGx*", implement*, and "onco* or cancer*" for our search as they are the most common terms in the literature to encompass our search topic. It is possible that these keywords did not cover all relevant publications, however, in our opinion using these terms provided us with a broad range of search results that were screened carefully to ensure that no relevant articles were missed. While we are aware of other sites implementing pharmacogenomics in oncology, this review was restricted to those that have published their

findings. We did not assess the risk of bias and therefore cannot assess the validity of the individual studies included in this review.

Cancer patients stand to benefit greatly from the incorporation of pharmacogenomic-informed prescribing decisions[362]. In oncology, somatic pharmacogenomic testing for the selection of targeted therapies has been broadly applied, while germline pharmacogenomic testing has been used less frequently. These germline tests are currently ordered and performed independently, despite both having an impact on response to cancer therapy[362]. As genomic technology advances and the evidence supporting pharmacogenomic continues to grow, the momentum for clinical implementation of pharmacogenomics is expected to accelerate[323,360,365]. Future studies identifying relevant variants (somatic and germline), and developing and integrating pharmacogenetic tests and models into clinical practice to further individualize therapy for patients with cancer are warranted.

1.15: Hypothesis and Thesis Objectives

This study hypothesizes that pharmacogenomic risk prediction models designed to predict an individual's genetic risk of experiencing two serious ADRs in pediatric oncology (anthracycline-induced cardiotoxicity and cisplatin-induced ototoxicity) can be integrated into clinical care to improve therapeutic decision-making and drug safety.

The specific objectives of the study were:

Objective 1: To implement polygenic risk prediction models for anthracycline-induced cardiotoxicity and cisplatin-induced ototoxicity into routine clinical practice in pediatric oncology

Objective 2: To evaluate the impact that implementing polygenic risk prediction models have had on patients and families, clinical decision-making, and patient outcomes

Objective 3: To develop a plan for the validation of both pharmacogenetic risk prediction models

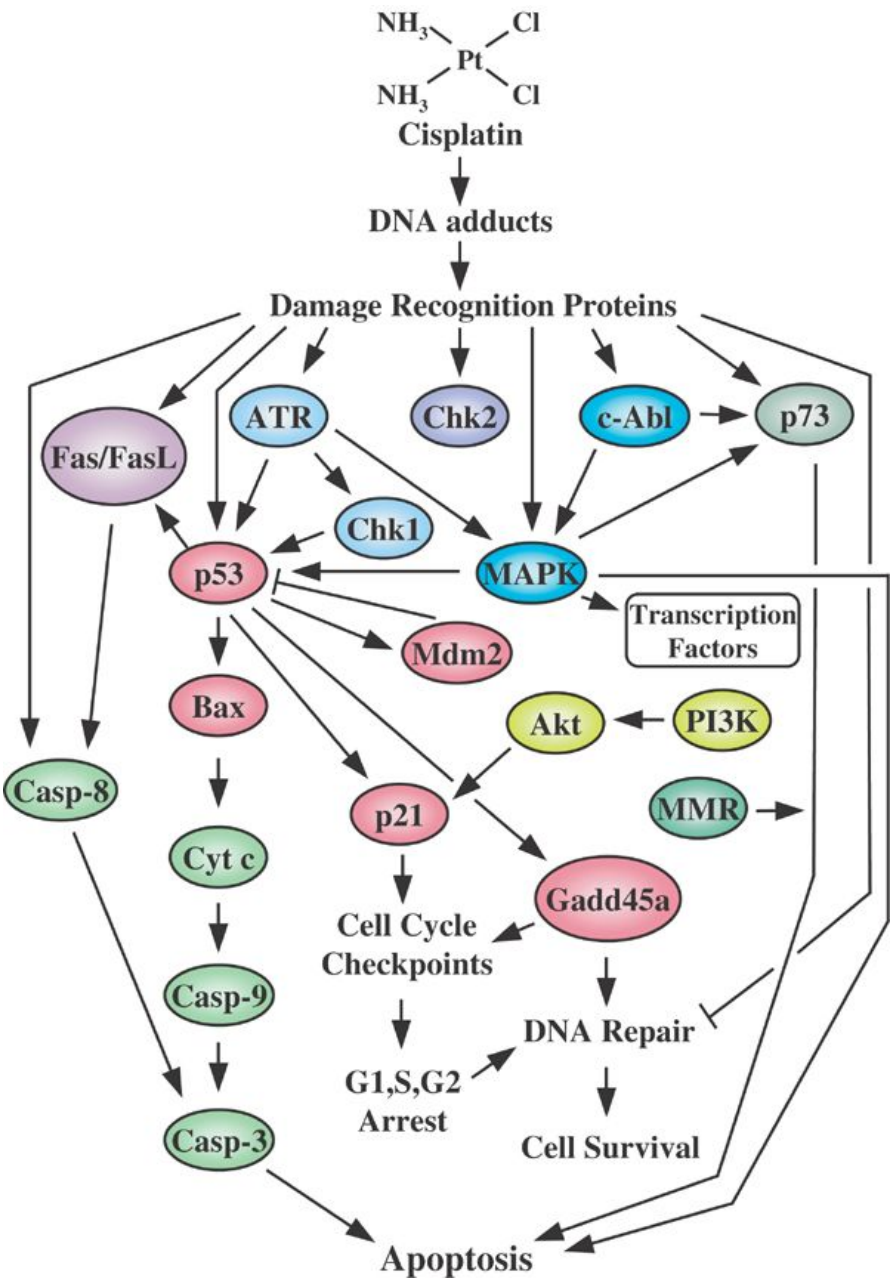


Figure 1.1 Cisplatin's Mechanism of Action

The formation of cross-link DNA adducts distort DNA and activate various signal transduction pathways involved in DNA-damage recognition and repair, cell cycle arrest, and apoptosis. From Siddik, Z.H., Cisplatin: mode of cytotoxic action and molecular basis of resistance. Oncogene, 2003. 22(47): p. 7265-79. Reprinted with permission from Springer Nature.

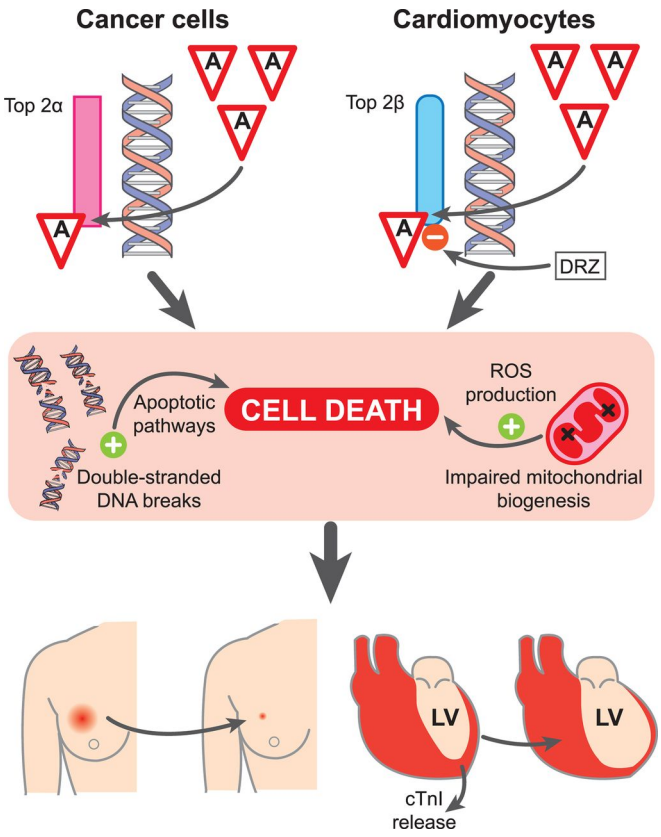


Figure 1.2: Mechanism of anthracycline-induced cell injury and death in cancer cells and cardiomyocytes

The therapeutic effect of anthracyclines is mediated through the inhibition of TopIIα. In cardiomyocytes, TopIIβ is inhibited. TopII inhibition in both cell types causes accumulation of double-stranded DNA breaks and mitochondrial dysfunction leading to activation of cell death pathways and accumulation of ROS. Early cardiac injury can be detected through quantification of circulating cardiac troponin (cTnI). The protective activity of dexrazoxane against anthracycline cardiotoxicity is now thought to be largely mediated through inhibition of anthracycline binding to TopIIβ. From Henriksen, PA., Anthracycline cardiotoxicity: an update on mechanisms, monitoring and prevention. Heart, 2018. 104(12):p. 971-977. Reprinted with permission from BMJ Publishing Group Ltd.

Examples of identified barriers to clinical implementation of pharmacogenetics

Test-related barriers

- Pharmacogenetic test available in CLIA/CAP-compliant laboratory
- Remembering to order test or identifying patients for whom test is appropriate
- Turnaround time for test results
- Cost of test and potential lack of reimbursement for test

Knowledge barriers

- Insufficient knowledge of pharmacogenetic data
- Uncertainty about pharmacogenetic genetic test interpretation
- Uncertainty about drug therapy decision based on pharmacogenetic test

Evidence barriers

- 'Genetic exceptionalism' for genetic and pharmacogenetic tests
- Lack of randomized controlled trials documenting superiority of pharmacogenetic-guided treatment approach

ELSI barriers

- Concerns about inclusion of genetic information in the medical record and potential for genetic discrimination
- Questions about importance of sharing pharmacogenetic findings with family members
- Defining importance of ELSI in pharmacogenetics versus disease genetics

CAP: College of American Pathologists; CLIA: Clinical Laboratory Improvement Amendments; ELSI: Ethical, legal and social implications.

Figure 1.3: Commonly identified barriers towards the implementation of pharmacogenetics in clinical practice

Adapted from Johnson JA. Pharmacogenetics in clinical practice: how far have we come and where are we going?. Pharmacogenomics. 2013 May;14(7):835-43 with permission of Future Medicine.

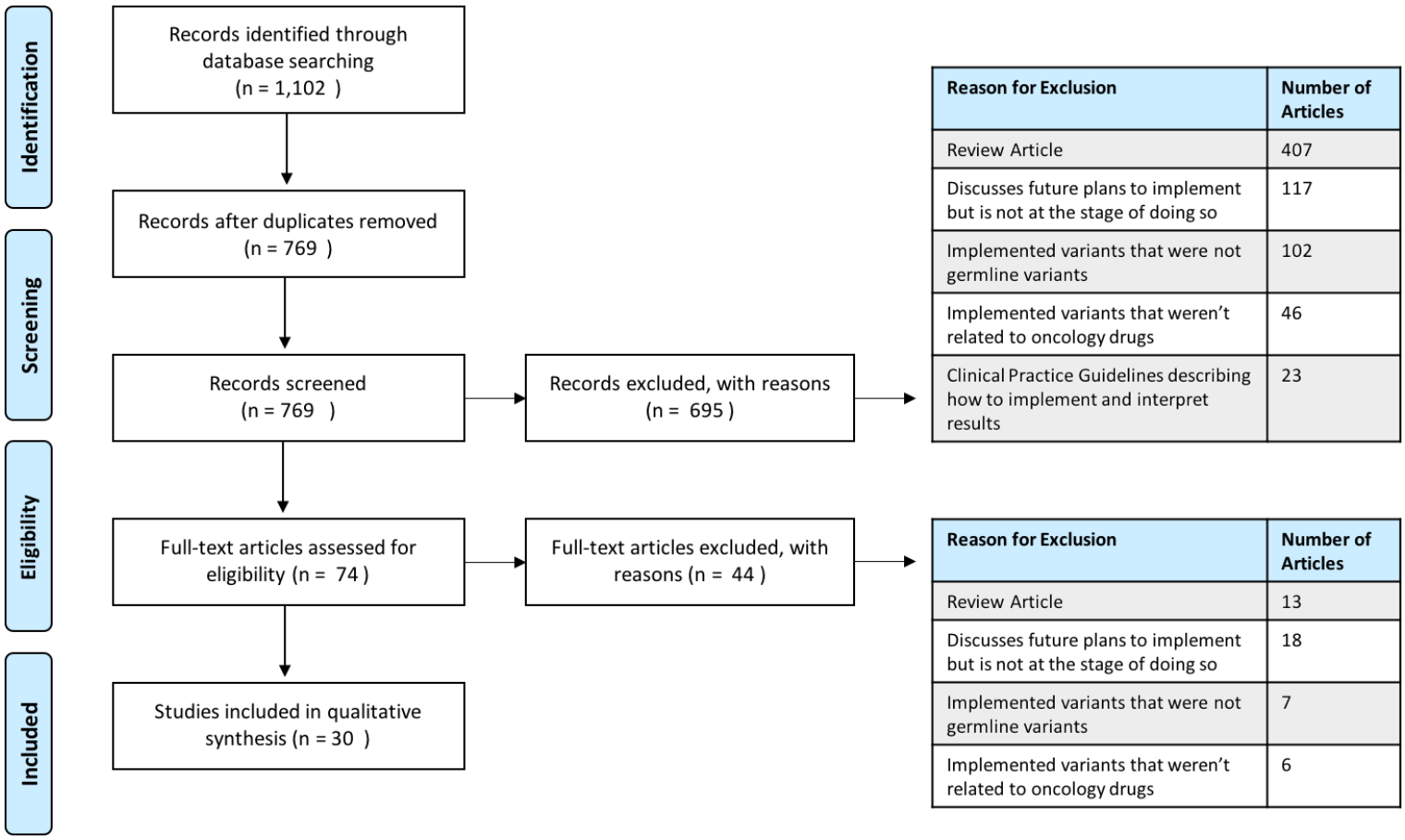


Figure 1.4. Scoping Review PRISMA Flow Diagram of Search and Selection Process

A literature search was performed using Medline (1946-August 19, 2018) and Embase (1974-August 19, 2018) databases to extract any publications describing germline pharmacogenetic markers that had been implemented into clinical care in oncology. Two authors (TBW and JL) independently screened titles and abstracts of all retrieved publications. If a decision could not be made based on the title and abstract, the publication was reviewed in its entirety to evaluate its inclusion.

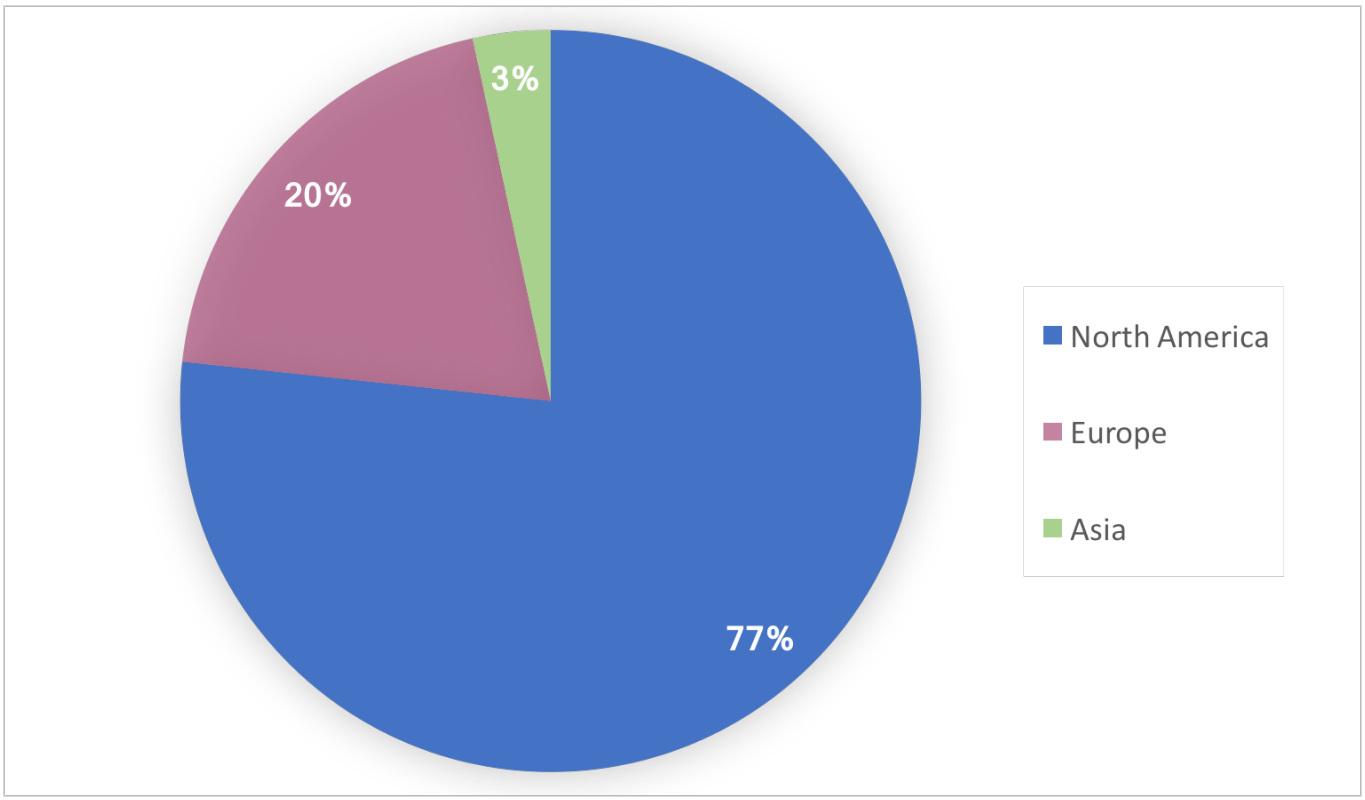


Figure 1.5: Global distribution of publications describing the implementation of germline pharmacogenetics markers in oncology

Distributions were based on a literature search performed using Medline (1946-August 19, 2018) and Embase (1974-August 19, 2018) databases to extract any publications describing germline pharmacogenetic markers that had been implemented into clinical care in oncology. This was done using the keywords: “pharmacogen*” or “PGx*”, implement*, and “onco* or cancer*”.

Table 1.1. Systematic Scoping Review Embase and MEDLINE Search Strategy

Ovid Embase (1946-present) and MEDLINE (1974-present) Search Criteria
1) (pharmacogen* or PGx*).mp.
2) implement*.mp
3) (oncolog* or cancer* or onco*).mp.
4) #1 AND #2 AND #3
5) limit #4 to english language
6) limit #5 to article or article in press or clinical study or introductory journal article or journal article or multicenter study or observational study or practice guideline
7) remove duplicates from #6

'multi-purpose' (.mp.) fields for Embase= abstract, candidate term word, device manufacturer, device trade name, drug manufacturer, drug trade name, floating subheading word, heading word, keyword, original title, title
'multi-purpose' (.mp.) fields for MEDLINE= abstract, keyword heading word, name of substance word, original title, protocol supplementary concept word, rare disease supplementary concept word, subject heading word, synonyms, title, unique identifier

Table 1.2. Overview of programs implementing germline pharmacogenetics in oncology

Study Site (Project Name)	Network(s)	Gene/drug pair implemented (specific to oncology)	Genotyping Platform	Reactive or Pre-emptive testing	EMR Integration	CDSS Features	Citation(s)
University of Chicago ("1200 Patients Project")[a]	-	TPMT/thiopurines UGT1A1/irinotecan DPYD/fluoropyrimidines	Real-Time PCR (OpenArrays, Lifetechnologies)	Pre-emptive	No	• web-based portal with printable summary report for physicians • post-testing alerts • search engine for drug-gene interactions • option to request a virtual consult with a PGx expert	Hussain et al. (2014) O'Donnell et al. (2014) O'Donnell et al. (2017) Danahey et al. (2017)
Vanderbilt University ("PREDICT Program")	• eMERGE PGx • PGRN Translational PGx Program (TPP) • IGNITE	TPMT/thiopurines CYP3A5/tacrolimus	ADME Panel (Illumina VeraCode)	Pre-emptive	Yes	• pre- and post-testing alerts • online portal for patients to access results	Peterson et al. (2013) Rasmussen et al. (2014) Shuldiner et al. (2013) Weitzel et al. (2015) Van Driest et al. (2014) Pulley et al. (2012)
Boston Children's Hospital ("Clinical Pharmacogenomic Service")	• eMERGE PGx	TPMT/thiopurines	Custom NGS Platform (PGRNseq)	Pre-emptive	Yes	• pre- and post-testing alerts	Rasmussen et al. (2014)
Children's Hospital of Philadelphia (CHOP)	• eMERGE PGx	TPMT/thiopurines	Custom NGS Platform (PGRNseq)	Pre-emptive	Yes	• pre-and post-testing alerts • summary report sent to physician • consult letter for patients	Rasmussen et al. (2014)
Mayo Clinic ("RIGHT Protocol")	• eMERGE PGx • PGRN Translational PGx Program (TPP)	CYP2D6/tamoxifen TPMT/thiopurines	Custom NGS Platform (PGRNseq)	Pre-emptive	Yes	• pre- and post-testing alerts • online portal for patients to access results • inbox message to physician when new PGx info uploaded to EMR • option to request consult with PGx expert	Rasmussen et al. (2014) Bielinski et al. (2014) Shuldiner et al. (2013) Cabarello et al. (2016)
Cincinnati Children's Hospital Genetic Pharmacology Service	• eMERGE PGx	TPMT/thiopurines	Real-Time PCR (ABI-7500)	Reactive	Yes	• pre- and post-testing alert	Rasmussen et al. (2014) Ramsey et al. (2018)
St.Judes Hospital ("PG4KDS")	• PGRN Translational PGx Program (TPP)	TPMT/thiopurines UGT1A1/irinotecan[b]	DMET Panel (Affymetrix)	Pre-emptive	Yes	• pre- and post-testing alerts • summary report sent to physician • inbox message to physician when new PGx info uploaded to EMR • consult letters for patients	Hoffman et al. (2014) Bell at al. (2013) Crews et al. (2011) Shuldiner et al. (2013)
University of Florida ("Personalized Medicine Program")	• PGRN Translational PGx Program (TPP) • IGNITE	TPMT/thiopurines	Real-Time PCR (QuantStudio, Luminex xTAG, GenMark or ViiA 7)	Reactive	Yes	• pre- and post-testing alerts • inbox message to physician when new PGx info uploaded to EMR • option to request consult with PGx expert	Weitzel et al. (2018) Johnson et al. (2012) Cavallari et al (2017) Weitzel et al. (2015) Shuldiner et al. (2013)
Indiana University ("INGENIOUS Trial")	• IGNITE	DPYD/fluoropyrimidines TPMT/thiopurines ITPA/thiopurines CYP3A5/tacrolimus	Real-Time PCR (QuantStudio)	Pre-emptive	Yes	• pre-and post-test alerts • summary report sent to physician • option to request consult with PGx expert	Eadon et al. (2016) Weitzel et al. (2015)
Columbia University ("PIPseq")	-	UGT1A1/irinotecan NT5C2/chemotherapy resistance	Custom NGS Panel (Agilent SureSelectXT)	Reactive	Yes	• summary report sent to physician	Oberg et al. (2016)
Cleveland Clinic ("Personalized Medicine Program")	-	TPMT/thiopurines	Real-Time PCR (Prometheus Laboratories)	Reactive	Yes	• pre-and post-test alerts • physicians contacted if patients' results are actionable • inbox message sent to physcian when new PGx info uploaded to EMR • option to request a virtual consult with a PGx expert	Hicks et al. (2016) Teng et al. (2014)
Multiple European Centers ("The PREPARE Study")	• U-PGx	TPMT/thiopurines DPYD/fluoropyrimidines UGT1A1/irinotecan CYP3A5/tacrolimus CYP2D6/tamoxifen	Real-Time PCR (LGC SNPline)	Pre-emptive	Yes	• pre-and post-test alerts • QR codes to access results	van der Wouden et al. (2017)

a) Program did not provide a comprehensive list of all drug-gene pairs implemented, therefore, they may have implemented additional drug-gene pairs that we are unaware of

b) Program has since stopped testing for this drug-gene pair routinely

Chapter 2: Implementation of Pharmacogenetic Risk Prediction Models in Pediatric Oncology

2.1: Introduction, Aim, Rationale

Two widely-used drugs in pediatric oncology, anthracyclines and cisplatin, are extremely effective but their use is limited by their ability to cause life-threatening and disabling ADRs. Anthracyclines are used in the treatment of over 50% of pediatrics cancers, but have been shown to cause cardiotoxicity which can range from asymptomatic cardiac dysfunction in about 57% of patients to much more severe cardiomyopathy and heart failure in up to 16% of patients[4,290,291,373]. Cisplatin, on the other hand, is used to treat between 10-20% of all cancer patients, but is known to cause permanent hearing loss in 60-70% of pediatric cancer patients depending on their treatment regimen[100,101,374,375]. Genetic variants associated with anthracycline-induced cardiotoxicity and cisplatin-induced ototoxicity have been discovered, which could allow us to predict, prevent, and better treat these serious ADRs[376,377]. While the benefits of pharmacogenomic testing to optimize drug safety is recognized, implementation into clinical practice has been extremely slow relative to pharmacogenomic findings and the advancement of genotyping technology. The aim of this study is to describe the development of pharmacogenetic risk prediction models and their implementation in pediatric oncology to predict the likelihood of patients experiencing ADRs prior to the start of chemotherapy.

Cisplatin-induced ototoxicity and anthracycline-induced cardiotoxicity were selected as the first ADRs to implement pharmacogenetic testing for as strong associations between these ADRs and genomic biomarkers have been discovered and replicated, and robust clinical practice guidelines for their use in clinical practice have been published[9-12,204,376,378].

2.2: Clinical Practice Guidelines (CPGs)

A commonly identified barrier towards the clinical uptake of pharmacogenetics testing is the lack of clear and robust clinical guidelines for translating genomic findings into actionable recommendations. Many guideline-generating groups in the past have focused on evaluating whether a clinician should, or is obligated to, order a pharmacogenetic test[379-382]. However, as the price of ordering multi-gene panels continues to decrease, the question has shifted from whether to order a test for a specific gene or variant to how the available genetic results should be

interpreted and used in clinical decision-making. In order to overcome this barrier, CPGs for the genomic prediction of anthracycline-induced cardiotoxicity and cisplatin-induced ototoxicity were previously created and published detailing who should be tested, which variants to be tested for, and information on how to best interpret these results in order to implement them into clinical care[13,14]. These CPGs were developed from a systematic review of peer-reviewed published genetic associations of anthracycline-induced cardiotoxicity and cisplatin-induced ototoxicity. A standard clinical practice recommendation development process was followed based on the Appraisal of Guidelines Research and Evaluation Enterprise (AGREE). All retrieved publications were critically appraised, taking into consideration the consistency of results, magnitude of effect, as well as the number and quality of the studies conducted. Each recommendation made by the CPNDS Clinical Practice Recommendations Group was assigned one of three levels of strength based on the strength of evidence that the recommendation was based on, the balance between benefits and risks of testing and genotype-guided treatment, as well as, the likelihood of variability in the individual values and preferences of patients. The CPGs have been published in peer-reviewed journals and recommendations from these CPGs have been annotated in PharmGKB (https://www.pharmgkb.org/guidelines). PharmGKB is the most comprehensive central repository for pharmacogenetics data including information about drug pathways, gene summaries, and relationships amongst genes, drugs, and diseases.

2.3: Pharmacogenetic Risk Prediction Model Development

Polygenic risk prediction models were previously developed to assess an individual patient's overall genomic risk of developing either anthracycline-induced cardiotoxicity or cisplatin-induced ototoxicity. The threshold of evidence for the inclusion of a predictive variant in the development of the risk prediction models required that each variant be replicated in three independent populations with an odds ratio (OR) of ≥3. The models were developed using logistic regression analyses (Golden Helix SVS version 8.8.3) to identify key transition points in genotype risk scores based on data collected from previously-treated patients with known genotypes **(Figure 2.1)** [9,12,204]. Genotype combinations (i.e., presence of risk or protective variants) and rates of observed toxicity in previously treated patients were used to define percentage risk thresholds (anthracyclines, n=595; cisplatin, n=283) **(Figure 2.2, 2.3)**. The polygenic risk prediction models

therefore combine information across several genetic variants into one predicted outcome for each patient.

2.3.1: Anthracycline-induced Cardiotoxicity Risk Prediction Model

In total, 595 patients who had previously received anthracyclines were used to create the risk prediction model for anthracycline-induced cardiotoxicity. The model incorporated predictive variants that were identified and replicated in three independent cohorts with odds ratios ranging from 0.29-0.46 for the *SLC28A3* protective variant and odds ratios ranging from 4.0-7.98 for the *UGT1A6* and *RARG* risk variants **(Table 2.1)**. These patients were grouped into one of five risk stratification groups (14%, 21%, 39%, 45%, and 89% risk) based on their combination of predictive variants, and whether or not they developed cardiotoxicity **(Figure 2.2)**. Cardiotoxicity was classified as grades 2-5 on a modified version of the CTCAEv3 grading criteria, which adjusted grade 2 cardiotoxicity to include patients with a shortening fraction of 15-26% rather than the original range of 15-24% **(Figure 2.4)**. This adjustment was made based on recommendations by the head of pediatric cardiology at BC Children's Hospital who stated that a shortening fraction of ≤ 26% is the point at which pediatric cardiologists would begin to prescribe cardiac interventions for patients (e.g., increase echocardiographic monitoring frequency, consider dose modifications to anthracycline therapy, use of a cardioprotectant). Patients who had grade 1 cardiotoxicity at the time of the model development were excluded to better differentiate between cases and controls. Clinical characteristics of the patients used to create this model separated into cases (those that developed cardiotoxicity) and controls (those that did not develop cardiotoxicity) can be seen in **Table 2.2**.

Patient cases and controls used to create the predictive model were found to have differences in clinical characteristics similar to what has previously been reported[287,290,383]. Cumulative anthracycline dose is a well-known risk factor[287,383,384] in the development of anthracycline-induced cardiotoxicity and was seen to be significantly higher in cases versus control patients in the model, as expected (p=1.45x10^{-10}). However, after stratifying patients into genetic risk groups and comparing doses among cases, those who carried a genetic risk variant (pharmacogenetic risk of 39% to 89%) received a lower dose on average (median of 250mg/m^2 versus 217mg/m^2) than those that did not carry a risk variant (pharmacogenetic risk of 14% to

21%), and still experienced higher rates of cardiotoxicity. This supports the assertion that dose, while a contributing factor, is not the sole determinant in the development of cardiotoxicity.

While younger age has also been suggested as a risk factor in the development of anthracycline-induced cardiotoxicity[8,182,290], the patients used in the development of the risk prediction model show cases to be significantly older than controls (Median: 8.5 years versus 5 years, p=0.0001). This is likely the result of certain cancer types that require higher cumulative doses of anthracyclines being more prevalent in older children (e.g., AML, Ewings). Additionally, the majority of patients diagnosed with ALL who are young in age (<10 years old) are given a standard risk protocol with relatively low doses of anthracyclines (75mg/m^2) compared to other cancer types, increasing the likelihood that controls were younger patients. Patients treated with the anthracenedione derivate similar to anthracyclines, mitoxantrone, were found to have more instances of cardiotoxicity despite its previously reported ability to reduce cardiotoxic effects (p=8.94x10^{-5}) [385]. As mitoxantrone is used in the treatment of high risk AML, majority of model patients (19/20; 95%) who received mitoxantrone were also treated with an additional anthracycline, and therefore had a larger cumulative dose of anthracyclines on average (Median±sd: 454±114mg/m^2). Patients with Ewings sarcomas and AML/APL were more likely to be cases as their protocols require higher cumulative doses of anthracyclines (p=0.00177; p=0.0005, respectively). These clinical risk factors were included as covariates in the logistic regression of the original discovery and replication studies that identified the predictive variants included in the development of the model[10,12,204].

2.3.2: Cisplatin-induced Ototoxicity Risk Prediction Model

The development of the cisplatin-induced ototoxicity risk prediction model was based on 283 patients who previously received cisplatin. The model incorporates three risk variants (*TPMT*: rs12201199, rs1142345, and rs1800460) that were identified and replicated in three independent cohorts of pediatric patients with odds ratios ranging from 3.6-14.3 **(Table 2.3)**. These patients were stratified into two risk groups (62%, 91% risk) based on their combination of predictive variants and whether they developed ototoxicity or not **(Figure 2.3)**. Audiometric data was used to quantify ototoxicity due to cisplatin by two independent audiologists and a clinical pharmacologist. These patients were classified as having clinically significant ototoxicity if they

developed grades 2-4 hearing loss on the CTCAEv3 grading criteria[386] conferring a hearing loss of >20dB at frequencies of 4-8kHz. To better differentiate cases and controls, patients with hearing loss defined as grade 1 were excluded. Controls were defined as having normal hearing at frequencies from 0-8kHz.

Clinical characteristics of the patients used to create the pharmacogenetic risk prediction model can be found in **Table 2.4**. Consistent with previous findings, cases were significantly younger (p=0.001), were more likely to have received cranial radiation (p=0.049), and were more likely to be treated with carboplatin in addition to cisplatin (p=0.004). Additionally, more patients diagnosed with medulloblastomas (p=0.00997) became cases while less patients with germ cell tumours (p=1.57x10^{-6}) became cases. There is evidence that individual doses and varied dosing schedules can have an effect on incidence of ototoxicity[102,146,387]. Germ cell tumour protocols consist of a 5-day dosing schedules of 20mg/m^2 each day while other cancer types (neuroblastoma and osteosarcomas) have single day dosing of 100mg/m^2, which may account for the lower incidence of ototoxicity observed in these patients. Patients with medulloblastomas, neuroblastomas, osteosarcomas, and hepatoblastomas had the highest incidence of ototoxicity which again may be related to both cumulative cisplatin dose and dosing schedule, and are consistent with previous findings[9,97,101,142,143,149,153]. These clinical risk factors were included as covariates in the logistic regression of the original discovery and replication studies that identified the predictive variants included in the development of the model[9,11].

2.4: Implementation of Pharmacogenetic Risk Prediction Models in Pediatric Oncology

Once the risk prediction models were developed, a Precision Medicine Program was initiated and began enrolling patients for pharmacogenetic testing in the Division of Hematology and Oncology at BC Children's Hospital. The knowledge translation approach to implementation was iteratively developed in consultation with oncologists to ensure that the information users of the test understood the goals of the program and how they were to be achieved.

2.4.1: Strategies for Implementation

In order to gain clinician acceptance, the Precision Medicine Program was first introduced to oncologists at a divisional protocol rounds to familiarize them with how to order testing and how

they could utilize test results in treatment decision-making. One of the key factors identified in successful pharmacogenetic implementation studies[388] is the development of a strong interdisciplinary team consisting of clinicians and scientists with a relevant background in the field, and a physician champion to advocate and educate other physicians. An oncologist with an interest and knowledge in pharmacogenetics became a clinical champion to answer questions other oncologists had about the program or how to interpret and apply the results. Additionally, a clinical pharmacologist lead was able to provide interpretation expertise and a geneticist lead managed Good Laboratory Practice (GLP) standards for genomic data analyses. An audiologist who interpreted audiological changes induced by cisplatin as compared to other factors, and a cardiologist with a strong understanding of anthracycline-induced cardiomyopathy were also involved in the clinical use oversight of these models.

Diffusion of Innovation Theory, developed by E.M. Rogers in 1962 originated in communication to explain how an idea or product gains momentum and is adopted by a population or social system[389]. Adoption meaning that a person does something differently than what they had previously (i.e., acquires a new behaviour such as using pharmacogenetic testing to inform decision-making). This does not happen simultaneously; rather it is a process whereby some people are more likely to adopt the innovation than others. Rogers' Law of Diffusion of Innovations[390] separates users of new technologies into five groups (i.e., innovators, early adopters, early majority, late majority, and laggards) based on the rate and degree to which they adopt new ideas; with innovators being quick to adopt and laggards being the last to adopt. The law posits that once innovators and early adopters are using a new technology, the remainder of the groups will follow suit. This is based on the understanding that innovators and early adopters tend to utilize new technologies (in this case, pharmacogenetic testing) that they see as advantageous, while late adopters and laggards tend to adopt new technologies because opinion leaders have already adopted and found utility in the new technology[390]. Based on Rogers' Law of Diffusion of Innovation[390], we anticipated that strong support from an oncologist who understood the value of pharmacogenomics in predicting drug-induced harm and was comfortable informing patients of these risks, would lead to other oncologists adopting the program over time.

2.4.2: Enrollment

This study was approved by the Research Ethics Board of the University of British Columbia. Written informed consent was obtained from all patients, parents, and legal guardians prior to testing. Patients scheduled to receive cisplatin or anthracycline chemotherapy were eligible for enrolment. Patients who consented provided either a saliva sample (DNA Genotek, Ottawa, Canada) or blood sample (EDTA Vacutainer®, BD Diagnostics, New Jersey, USA) and gave permission for the collection of relevant clinical data. Genomic DNA was extracted using the QIAamp DNA purification system (Qiagen, Venlo, Netherlands) and genotyped using a custom array (Illumina, San Diego, USA). Pharmacogenetic results were returned to the primary oncologist within one week via a written consult.

Between the launch of the Precision Medicine Program in July 2013 and the time of this analysis in January 2019, 297 patients have received testing and had their pharmacogenetic risk prediction results returned to their treating oncologist. Of the 297 patients who have received testing, 219 patient's initial treatment protocols included anthracyclines, 35 included cisplatin, and 43 included both anthracyclines and cisplatin. As expected, having a clinical champion increased test provider buy in, with the majority of patients in the beginning of implementation being requested by the clinical champion oncologist. By 5 months into the study period 6/12 oncologists had requested testing, and by 12 months 100% of oncologists had requested testing. Enrollment has steadily increased with an enrollment rate of 95% of eligible patients in the most recent study year.

2.4.3: Turn-around Time and Return of Results Format

Turn-around time from when the test was ordered to when the oncologists received the results is extremely important in order for the results to be used in clinical decision-making[388]. This is especially true in oncology where a delay in therapy is not always possible and treatment is sometimes initiated within a day of receiving a diagnosis[391,392]. In order to ensure that results are returned to oncologists within a time frame that would allow the results to be clinically useful, the turnaround time from receiving the DNA sample to delivering the risk prediction results to

oncologists is less than a week with a high priority turnaround time of less than 24 hours if the results were needed urgently. In situations or medical settings where it is possible, pre-emptive testing (i.e., before the drug is prescribed) is usually preferred to reactive testing (i.e., after the drug is prescribed) as it allows results to be available at the point of care, thereby eliminating any turn-around time. However, as it is impossible to determine who will need these medications prior to them receiving a cancer diagnosis, turn-around time has instead been optimized to allow results to be made available rapidly depending on the needs of the oncology team. Additionally, because some of these patients experience relapse or secondary malignancies, the results are stored and made available to the oncologists at the point of care when they are deciding on additional treatments for these patients in the future.

Results are returned using a medical consult note format with individual genotyping results and interpretations for each variant, as well as, patient specific risk prediction figures indicating where their patient's pharmacogenetic risk lies compared to all studied patients **(Figure 2.5a, 2.5b).** The oncologist is then able to review the results and to obtain a consult with a clinical pharmacologist with pharmacogenomic expertise, if necessary, to discuss the results further. Published CPG's are also available to oncologists with information on how to manage their patient's treatment given their pharmacogenomic risk results[13,378]. The pharmacogenomic information included in these reports does not include clinical risk factors or recommendations for any changes to therapy; these elements were left out of predictive modeling so that clinical oncology rounds could be used to align pharmacogenomic results with other relevant factors before making a decision about how to use these results in therapeutic decision-making. In this way, genomic information becomes part of the overall risk prediction in a given patient, not the sole determinant. In addition, by not providing recommendations for changes to therapy, this provided an opportunity to generate multi-disciplinary discussion and dialogue for complex cases between the clinical and research teams, as necessary.

2.5: Utilization of results in treatment decision-making

2.5.1: Anthracyclines

The genetic variant combinations observed in patients tested for their pharmacogenetic risk of anthracycline-induced cardiotoxicity was consistent with the expected distribution based on

previously reported genotyping frequencies[10,12,204]. Several treatment modifications were made when the risk of the adverse drug reaction outweighed the benefits of continuing with the initial treatment plan **(Figure 2.6)**. For example, one patient tested in the highest pharmacogenetic risk group for anthracycline-induced cardiotoxicity (89% risk group). This patient was an 11-month old male with a high-risk Stage IV myc-amplified paraspinal neuroblastoma planning on being treated with a protocol that included anthracyclines. In addition to this patient's pharmacogenetic risk of cardiotoxicity, they had a high clinical risk of cardiotoxicity given their young age and the need for posterior mediastinal radiotherapy where the heart would be within the radiation field. The pharmacogenetic results were presented at a Tumour Board Meeting to discuss both the patient's clinical and genomic risk factors of cardiotoxicity and review alternative treatment protocols for this tumour type. A protocol (ANBL12P1) was found with similar cure rates, where only a single dose of anthracycline was needed and a decision was made to drop this single dose of anthracycline. The parents were fully informed of the therapeutic options (including use of cardioprotectants), risks and benefits of each option, and they had a significant voice in the final decision. This patient has now completed therapy and is alive and relapse free five years later with normal cardiac function.

For the 30 patients that carried pharmacogenetic risk variants for anthracycline-induced cardiotoxicity (39%, 45%, 89% risk groups), dexrazoxane was added as a cardioprotectant for 8 patients and 5 patients received more frequent cardiac monitoring and follow-up as a result of the higher risk of toxicity. In one patient with left cerebellum undifferentiated sarcoma who carried a protective variant for anthracycline-induced cardiotoxicity (14% risk group), a decision was made to treat them with a high-dose anthracycline salvage protocol after a relapse (75mg/m^2 of doxorubicin every 3 weeks) given their lower pharmacogenetic risk of cardiotoxicity. Testing has also been requested often for patients who have relapsed, especially in those with acute leukemias, in order to decide whether to give additional anthracycline doses beyond the cumulative dose threshold designed to minimize the probability of cardiotoxicity.

2.5.2: Cisplatin

The distribution of patients that tested in each risk group for cisplatin-induced ototoxicity was consistent with previously reported genotyping frequencies. [9,11,393]. For patients receiving

cisplatin, pharmacogenetic risk prediction results are being incorporated into treatment decision making and treatments have been modified accordingly **(Figure 2.7)**. Results were mainly used to decide between cisplatin versus carboplatin-based treatment protocols, where replacement with carboplatin was an option, as carboplatin is considered less ototoxic than cisplatin[9,101,142] . For two patients in the highest risk group (91% risk), a carboplatin based protocol was chosen to try to minimize the ototoxic risk. Another patient in the 91% risk group was diagnosed with a pineoblastoma and was planning on being treated with both cisplatin and cranial radiation. As cranial radiation is an additional risk factor for ototoxicity and this patient was at high genetic risk of developing hearing loss, sodium thiosulfate (an otoprotectant) was added to mitigate the risk of ototoxicity. This patient is alive and relapse free five years later with grade 1 ototoxicity (based on the CTCAEv4 grading criteria). Results have also been used to help decide between cisplatin-based chemotherapy protocols and proton beam radiation in two patients with infant brain tumours. Neither patient carried a risk variant so treatment with a cisplatin was used to avoid the late effects of radiation such as growth and developmental delays and secondary malignancies[394,395].

2.5.3: Secondary Findings—Thiopurines

TPMT variants included in the risk prediction model for cisplatin-induced ototoxicity are also strongly associated with the development of myelosuppression from thiopurines with odds ratios ranging from 4.6 to 18.6 depending on the variant[396]. The dose of thiopurines is modified according to well established guidelines that describe the risk variants and the potential for hematological toxicity[397]. In total, 12 patients who were carriers of *TPMT* risk variants had their dose of thiopurines lowered due to pharmacogenetic testing. For example, one 14 year old patient diagnosed with acute lymphoblastic leukemia (ALL) was found to carry one low activity *TPMT* variant. This patient received 6-mercaptopurine (6-MP) during the treatment consolidation phase and as a result of testing their dose of 6-MP was reduced by 50%. Despite this dose modification, the patient ended up profoundly neutropenic with *Candida* sepsis requiring admission to the intensive care unit. Had the patient been treated with 100% of the original dose he likely would have had prolonged neutropenia and might not have survived the infection.

2.6: Drug Therapy Outcomes of Tested Patients

Patients were also followed prospectively to monitor their cardiac and hearing function at baseline, throughout, and after treatment to determine how many patients in each pharmacogenetic risk stratification group developed cardiotoxicity and/or ototoxicity. The timing of cardiac and audiological monitoring differed between patients according to the recommendations made in their treatment protocols. Only patients with baseline and at least one echocardiogram or audiogram since beginning anthracycline or cisplatin therapy were evaluated for these outcomes. Patients who were deceased from their disease prior to experiencing any cardiotoxicity or ototoxicity were also excluded. Only echocardiographic data ≥ 21 days from an anthracycline dose were used due to the known transient effects of anthracyclines on cardiac function. The time to cardiotoxicity was evaluated for pharmacogenetic-tested patients and compared to the patients used to develop the risk prediction model for anthracycline-induced cardiotoxicity using the Kaplan-Meyer method (R 3.5.2 for Statistical Computing). Cardiac follow up data for the cohort of patients who were used to develop the risk prediction model was restricted to 5 years to match the follow up data that is currently available for the patients who had pharmacogenetic testing conducted. Patients with grade 2-4 ototoxicity based on the CTCAE version 4 grading criteria [398] were considered to have clinically relevant ototoxicity.

2.6.1: Anthracycline-induced Cardiotoxicity Outcomes

Of the 166 patients that received cardiac monitoring following anthracycline therapy at the time of this analysis, 5 (3.0%) had developed clinically significant anthracycline-induced cardiotoxicity (SF≤ 26%) **(Figure 2.8)**. One of these patients carried the *RARG* risk variant (rs2229774; 39% risk), while the remaining 4 carry no risk and no protective variants (21% risk). An additional 17 patients currently have grade 1 cardiotoxicity (SF of 27-29%) which due to the variability in echocardiometric measurements is still considered borderline normal cardiac function. Due to the delayed onset of anthracycline-induced cardiotoxicity, which can sometimes take years or even decades to develop[182,257], we expect more patients to exhibit the cardiotoxic effects of anthracyclines over time. However, comparing pharmacogenetic-tested patients

(n=166) to the cohort of patients used to create the risk prediction model who did not receive their pharmacogenetic test results (n=553) over the same period of time (5 years), there are significantly fewer cases of cardiotoxicity in patients who received pharmacogenetic testing (n= 5; 3.4%) compared to the group of patients used to create the model (n=65; 11.8%) (p=0.0005) **(Figure 2.9)**. To date, no patients in the 45% and 89% risk groups have developed cardiotoxicity. The majority of these patients (4/5;80%) had their treatments modified due to their pharmacogenetic risk, which may account for the lower incidence of cardiotoxicity observed. Comparatively, in the group of patients used to create the risk prediction models, 22.2% and 44.5% of patients in the two highest risk groups (45% and 89% risk, respectively) had developed cardiotoxicity within the same time frame. The median follow-up periods of pharmacogenetic-tested patients and patients used to create the models were 1.03 (IQR: 2.01) years and 9.5 (IQR: 8.22) years, respectively.

2.6.2: Cisplatin-induced Ototoxicity Outcomes

Of the 56 patients who have received audiological assessments since beginning cisplatin therapy, 33 (58.9%) experienced significant ototoxicity (grade 2 or above on the CTCAEv4) (**Figure 2.10)**. This is similar to previously reported incidence of cisplatin-induced ototoxicity which has been shown to affect between 60-70% of children treated with cisplatin[6,7,73]. One of these patients carried a risk variant (91% risk) while the remaining 32 carried no risk variants (62% risk). As cranial radiation is a well-known risk factor for the development of ototoxicity, the higher proportion of patients in the 62% risk group that received cranial radiation in addition to cisplatin (18 patients; 35.3%) may account for the increased rate of ototoxicity compared to the 91% risk group where no patients received cranial radiation **(Figure 2.10).** Additionally, 3 out of 5 patients that tested in the 91% risk group had their treatment modified due to their increased pharmacogenetics risk, and none of the patients whose treatment was modified have developed ototoxicity. The median follow-up periods of pharmacogenetic-tested patients and untested model patients were 2.03 (IQR: 2.7) years and 4.7 (IQR: 5.6) years, respectively.

2.7: Patient Perspectives on Pharmacogenetic Risk Prediction Results

In order to determine how the test results were perceived by patients and families, participants were asked to participate in an interview regarding their pharmacogenetic results. After the first

100 patients were enrolled, families that received their results from their oncologist were interviewed. Interviews were semi-structured, open-ended, and qualitative to assess the family's understanding of the results and to determine the value of the test from their perspective. Consistent with previously established methods, interviews were iterative and flexible to allow investigators to explore topics that arose during the course of the interview. These interviews were recorded and transcribed verbatim. Applying a grounded theory approach, interview transcripts were then coded by two independent researchers and recurring themes were identified. 11 interviews were conducted until data saturation was reached where additional interviews were yielding no new ideas. Interviews were conducted with 10 parents of children aged 2-17 and 1 adolescent patient aged 16 to evaluate the utility of pharmacogenetic testing from patient and family perspectives to determine how to best provide results and improve the delivery of results in the future.

As interviews were conducted after the first 100 patients were enrolled, the time between when the patients and families received their pharmacogenetic results and when they were interviewed ranged from 94 to 402 days depending on how recently they were enrolled. Common themes emerged regarding perceived benefits of test results, challenges to understanding the utility of the results, and suggestions for improving the interpretation of results **(Figure 2.11)**. Families and patients indicated that test results ensured the treatment plan took into account the child's risk of harm and that potential therapeutic options were considered, when appropriate. They also felt more involved in the discussion regarding their child's treatment plan and the decision-making process with their oncologist when pharmacogenetics test results were incorporated into the discussion. Families and patients also said that having the pharmacogenetic results helped them prepare for the future in knowing what symptoms to look out for and what monitoring recommendations to follow. They reported that the test results helped instill confidence in their child's treatment plan and provided reassurance and psychological benefit to them. They also suggested that their perception of what is meant by low or high risk of drug induced harm may differ from what oncologists consider low or high risk. They, therefore, found that the results provided them with less subjective and more quantifiable information about the level of risk to expect.

Interviewees had the following to say about the benefits of testing:

> *"having to deal with all the side effects after and then being surprised about it. I'd rather go into it prepared and knowing what could happen"*
>
> *" if you can determine any risks then you can try to mitigate them. We all want our kids treated to the best of everyone's ability, but you don't want to see them endangered more than they already are"*
>
> *"I want to live a long healthy life without any risks of any heart problems or effects so I think it's important to know what you need to look out for"*
>
> *"If there is one little piece of the puzzle that can help you through this long journey—that's a crucial piece that can help you."*
>
> *"it is important for me to let my son know as he grows older what risk he is at for various diseases with regard to any major organ in his body"*

Four out of 11 of the patients and families that were interviewed expressed that they had difficulty remembering their child's specific pharmacogenetic results due to being overwhelmed and inundated with information at the time of diagnosis. They also expressed that they were unaware of how common ADRs from these chemotherapeutic agents were prior to receiving their pharmacogenetic risk results. An additional challenge expressed by 4 families was difficulty balancing the chance of survival with the risk of ADRs and an implicit change in quality of life that adverse effects might create.

In order to make the test results more useful for families moving forward, patient and parent interviewees suggested value in having a written copy of the results to refer to. They also recommended that results be re-explained to them at a later date when they are less overwhelmed by their child's diagnosis. Some interviewees (4/11; 36%) also mentioned that they found the discussion about their pharmacogenetic results complicated and recommended the results be explained in lay terms for individuals without a medical background.

2.8: Physician Perspectives on Pharmacogenetic Risk Prediction Results

Oncologists who received pharmacogenetic results were also asked to participate in interviews to gain a multi-stakeholder view of the clinical utility of the pharmacogenetic tests, and to identify facilitators and barriers to incorporating pharmacogenetic results into treatment decision-making.

Interviews with 4 oncologists who were early adopters of pharmacogenetic testing were conducted. Results from these interviews demonstrated that pharmacogenetic test results led to appropriate changes in therapy for patients at a high ADR risk before they occurred. These changes included: more frequent monitoring of audiological and cardiac function, use of protective drugs (i.e., dexrazoxane and sodium thiosulfate), and the use of results to weigh the risks and benefits of multiple treatment options (i.e., carboplatin versus cisplatin-based treatment protocols). For patients in the low risk groups, oncologists indicated that they felt reassured that they could treat patients as they planned based on their clinical risk alone, using the full doses of cisplatin and anthracyclines as outlined in the chemotherapy protocols. Furthermore, oncologists suggested that in the transfer of care from pediatric to adult oncology, the pharmacogenetic test results help ensure follow-up care and long-term monitoring are appropriately planned.

Oncologists had the following to say about the value of the test results, and how they have been helpful when treating patients:

> *"I think it is important to know especially if your patient is predicted to be at high risk of getting these toxicities, and trying to incorporate that into our algorithm of how we modify treatment."*
>
> *"Very valuable. The value is that it enables me to continue with the chemotherapy with less worry about damaging the hearing or the heart."*
>
> *"We talk toxicity all the time when we're talking about …treatment … this makes it a little more concrete in how you describe the risk versus how we typically do it without genetic risk. Everyone is at some risk but quantifying that risk which to us as clinicians—what we think is high risk may not be the same as what a parent or family thinks is high risk, so I think it helps with the discussion with families."*

> "*I've had a couple [high-risk] patients…one where we omitted anthracyclines completely. We had one where we actually used an otoprotectant for cisplatin which is a little more experimental but with the family we felt very strongly that they didn't want cisplatin at all, so we modified and added the otoprotectant to their treatment which was a little off of standard care. We have given dezrazoxane to some families who have received anthracyclines."*

2.9: Summary of Key Findings

Three key performance indicators have previously been identified as important in the successful implementation of pharmacogenetic testing: (1) turn-around time, (2) service utilization/tests ordered, and (3) provider and patient adoption or buy-in.[388] These indicators were used to evaluate current workflow and overall efficiency to identify areas that need improvement. As mentioned previously, test turnaround time can take place in 24 hours to meet the needs of oncologists who need results quickly. Service utilization refers to how often the tests were ordered for eligible patients, and how often the test results were utilized. As discussed, the number of patients enrolled has increased each year since the initiation of testing as familiarity with test ordering and interpretation increased. The vast majority of eligible patients in British Columbia are being tested, and the oncologists are referring all of their patients to our study team for enrollment.

In addition to the number of tests ordered, the following patient outcomes have been collected to evaluate the utility of the test: modifications made to therapy as a result of testing and the occurrence and severity of cardiotoxicity and ototoxicity. As discussed, treatment modifications have been made when appropriate based on the patients pharmacogenetic risk. As enrollment is ongoing, assessments of cardiac and audiology outcomes are limited by the amount of follow up data available for each patient. Cardiotoxicity can occur many years after treatment[215], and cisplatin can remain in the body for 20 years and further exacerbate hearing loss[114]. In order to full capture the impact of pharmacogenetic testing, outcomes will continue to be evaluated as more follow up data becomes available for recently tested patients.

Interviews with patients and oncologists were used to gauge patient and provider adoption and delineate barriers to implementation such that they could be resolved. Based on feedback from

patient and oncologists' interviews, strategies to address challenges and suggestions for how to improve the utility and return of results process have been developed. Since the start of implementing testing, the consult note detailing patients' pharmacogenetics results has been modified to include visual aids detailing individual patients' risk results to improve ease of understanding and aide in the explanation of results to patients. As patients expressed that they were overwhelmed and inundated with information at the beginning of their treatment, oncologists are now revisiting results at a later date to remind patients of their pharmacogenetics risk results and how they factored into their treatment decisions. Pharmacogenetic risk prediction models are helping to facilitate the discussion of ADRs between oncologists and patients, and to quantify risk in order to overcome differing perceptions of what low and high risk may mean to different individuals. Additional interviews with both patients and oncologists are currently underway to explore topics that arose during the first round of interviews.

Data collected from patients and oncologists in this study indicate that pharmacogenetic tests have been of high utility in facilitating treatment plan decisions, especially in high and low genetic risk patients by encouraging a more active discussion of the risks and benefits of drug therapy for each individual patient. These resulting discussions between oncologists and patients, as well as, multidisciplinary Tumour Board discussions have led to changes in therapy, changes in the frequency of adverse event monitoring, and the use of concomitant protective agents to minimize toxicity where appropriate. An additional finding for those patients with no protective or risk variants was that these pharmacogenetic test results provided reassurance to patients and parents that their risk of drug-induced harm was comparable to other similarly-treated patients. Oncologists were also reassured that for patients experiencing relapse or with elevated risk of toxicity due to clinical risk factors (e.g., young age, high dose) that the genetic risk of harm was not elevated as well. An unintended but beneficial outcome of these genetic results is that the cisplatin risk prediction model contains information with respect to TPMT, which affects the response to 6-mercaptopurine or azathioprine. The availability of this information through our pharmacogenomic testing has been an added benefit to oncologists who were previously having this testing done in select patients, off-site at a considerable cost.

Pharmacogenetic testing can be implemented successfully into clinical practice by providing patient-specific risk of drug-induced harm. The specific risk of cisplatin-induced ototoxicity and anthracycline-induced cardiotoxicity for each patient allows the treating clinician to combine that information with their assessment of the patient's clinical risk to determine the best treatment strategy. Pharmacogenetic testing ensures patients and families are more informed about their child's risk of drug-induced harm which facilitates appropriate and necessary discussion between clinicians and patients

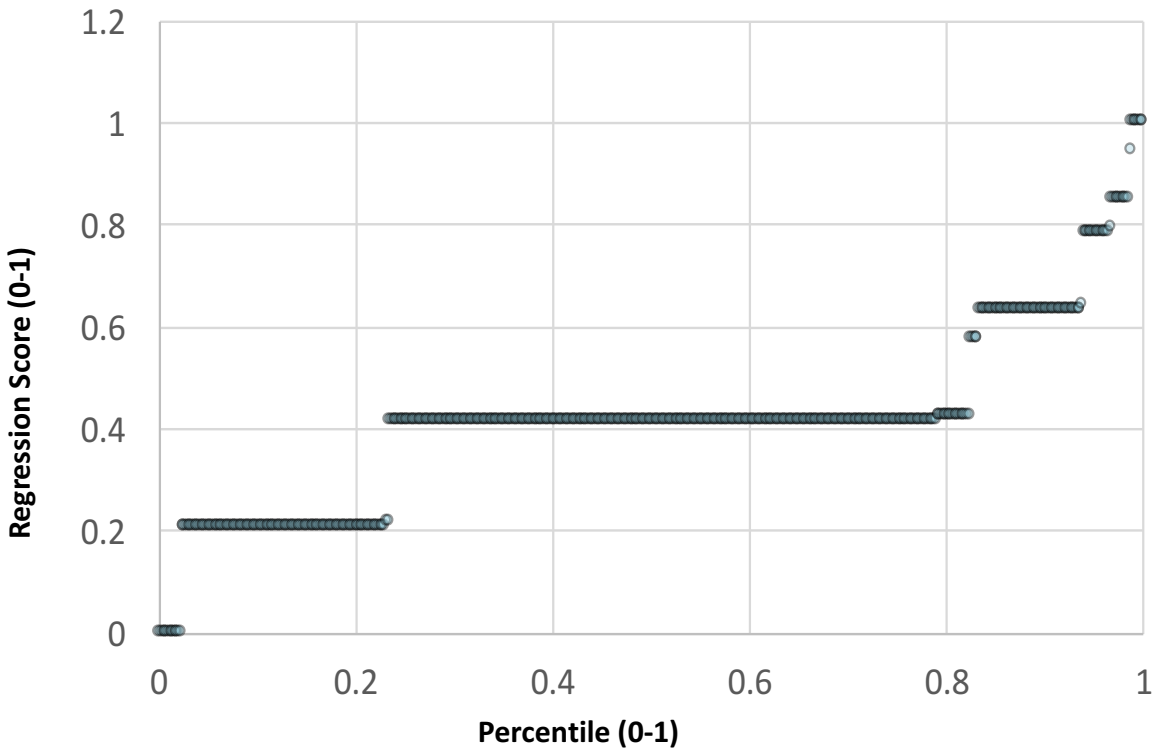


Figure 2.1. Polygenic risk prediction model development using logistic regression

Probabilities generated from logistic regression analysis using predictive variants were used to distinguish patients into different risk groups. The key transition points were determined by calculating the inter-quintile ranges for cases and controls independently.

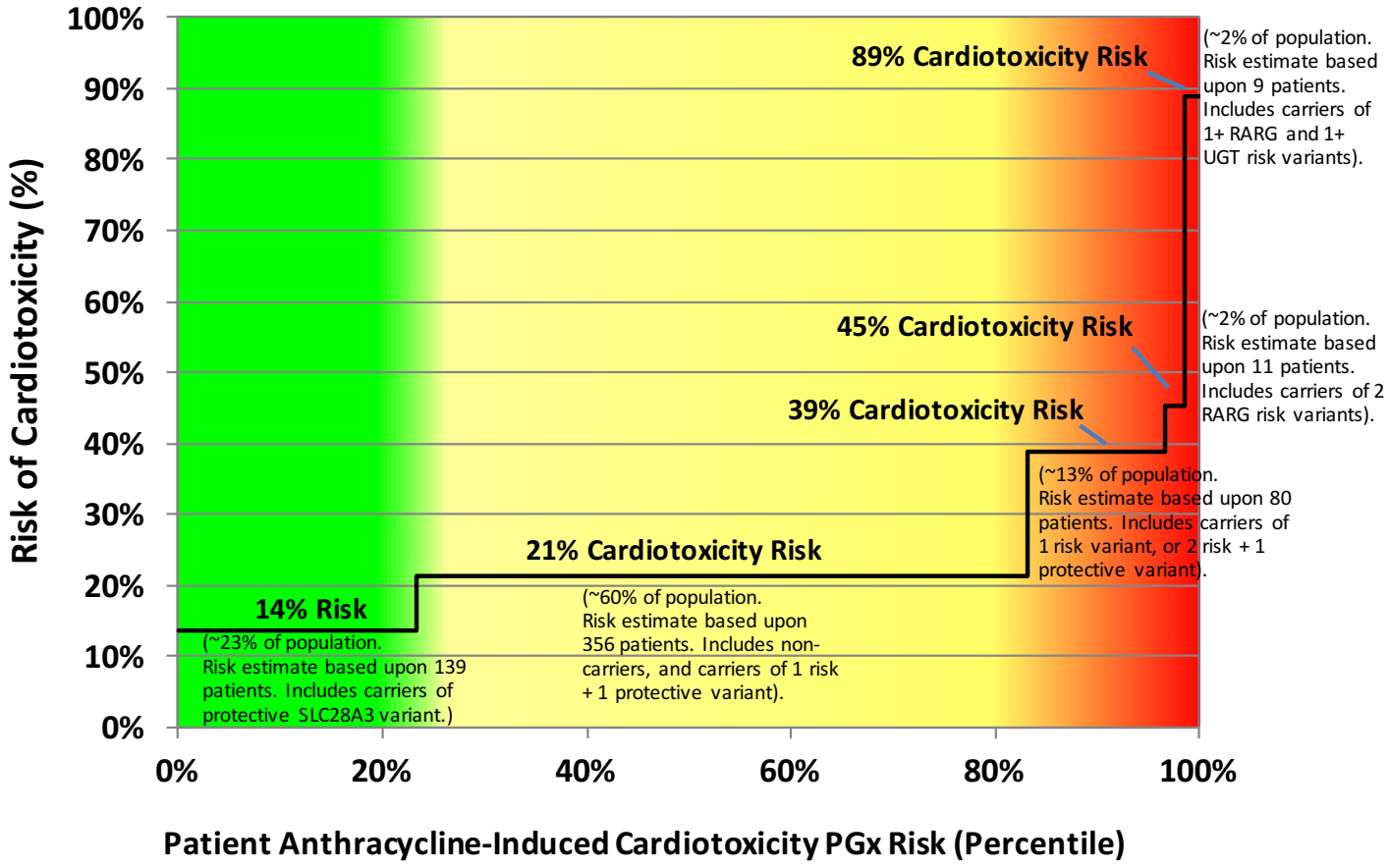


Figure 2.2. Anthracycline-induced Cardiotoxicity Pharmacogenetic Risk Prediction Model

Risk estimates developed using linear regression of 595 patients were used to stratify patients into one of 5 pharmacogenetic risk groups (14%, 21%, 39%, 45%, 89%). The size of the population that results are based on and proportion of patients that carry each predictive variant combination are specified in brackets below/beside each risk group. The x-axis refers to the percentage of the population that fit into each risk stratification group. The y-axis refers to the percentage of studied patients that developed cardiotoxicity (Shortening fraction; SF ≤26%).

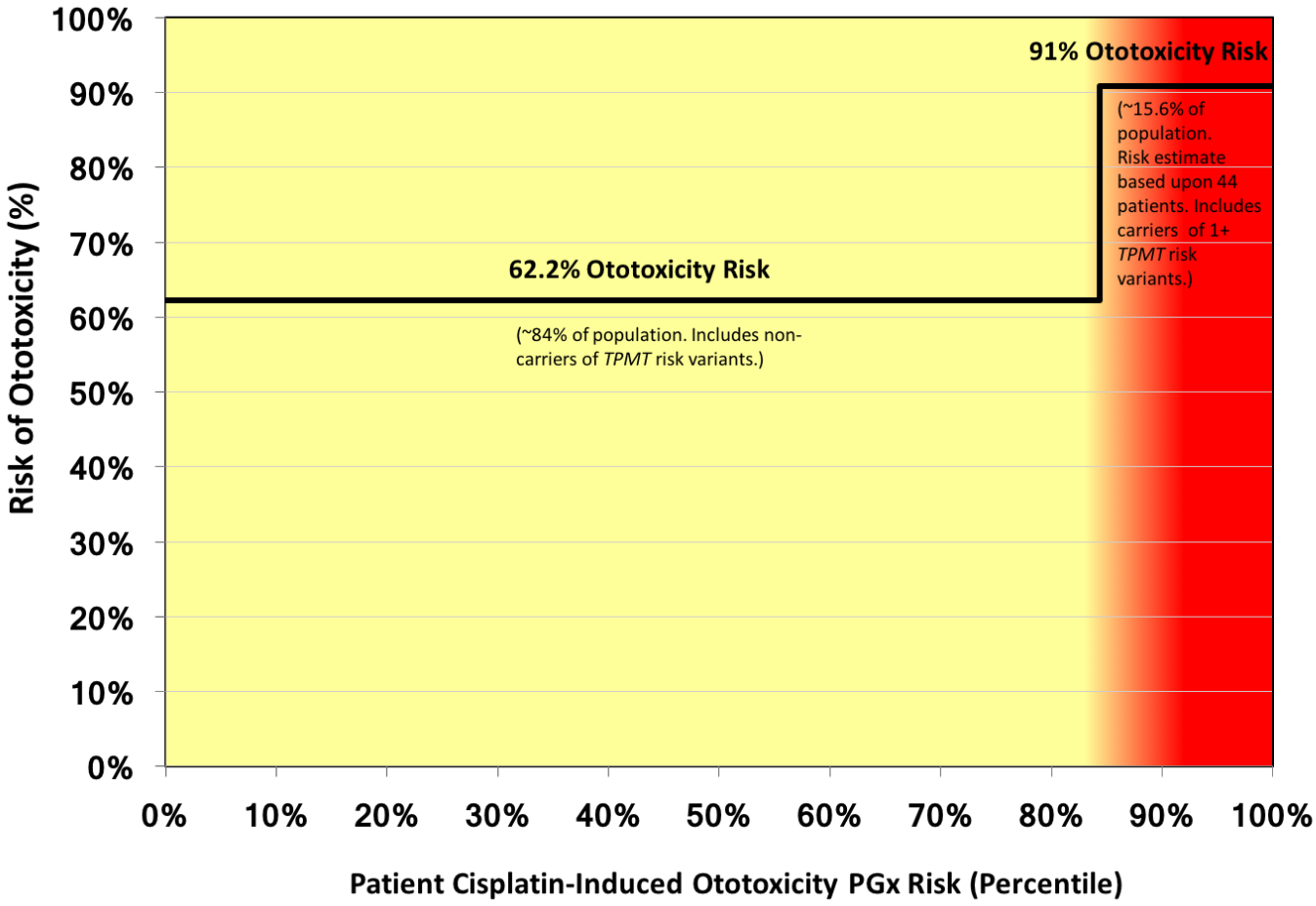


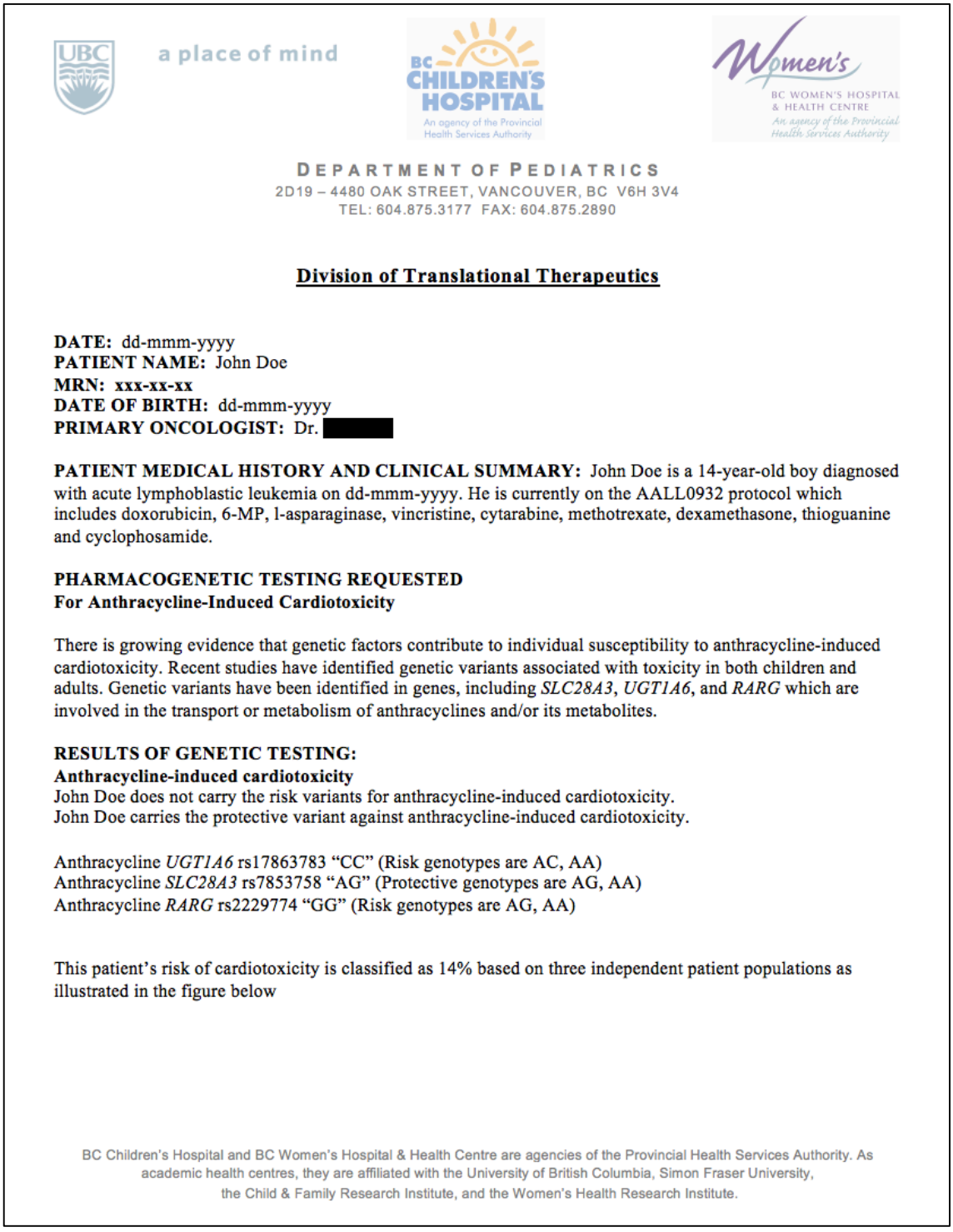
Figure 2.3. Cisplatin-induced Ototoxicity Pharmacogenetic Risk Prediction Model

Risk estimates developed using linear regression of 283 patients were used to stratify patients into one of 2 pharmacogenetic risk groups (62%, 91%). The size of the population that results are based on and proportion of patients that carry each predictive variant combination are specified in brackets below/beside each risk group. The x-axis refers to the percentage of the population that fit into each risk stratification group. The y-axis refers to the percentage of studied patients that developed ototoxicity (≥ grade 2 on the CTCAEv3).

Patients	National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) v 3.0 (Modified version with grade 2 adjustment)
No anthracycline-induced cardiotoxicity (control)	**Grade 0: No toxicity** - Shortening fraction ≥30%, ≥5 yr follow-up
Anthracycline-induced cardiotoxicity (case)	**Grade 1 toxicity** - Shortening fraction: 27-29% or - Ejection fraction: 50-60% **Grade 2 toxicity: Moderate to severe cardiotoxicity** - Shortening fraction: 15-26% or - Ejection fraction: 40-50% **Grade 3 toxicity: Symptomatic congestive heart failure** - Shortening fraction: <15% or - Ejection fraction: <40% **Grade 4 toxicity: Congestive heart failure requiring heart transplant or ventricular assist device** - Ejection fraction: <20%

Figure 2.4. Modified Common Terminology Criteria for Adverse Events Version 3 (CTCAEv3) used for the clinical characterization of anthracycline-induced cardiotoxicity

Grade 2 toxicity was modified to include shortening fractions from 15-26% rather than the original definition of 15-24% based on cardiologists' recommendations.



a place of mind

BC CHILDREN'S HOSPITAL
An agency of the Provincial Health Services Authority

Women's
BC WOMEN'S HOSPITAL & HEALTH CENTRE
An agency of the Provincial Health Services Authority

DEPARTMENT OF PEDIATRICS
2D19 – 4480 OAK STREET, VANCOUVER, BC V6H 3V4
TEL: 604.875.3177 FAX: 604.875.2890

Division of Translational Therapeutics

DATE: dd-mmm-yyyy
PATIENT NAME: John Doe
MRN: xxx-xx-xx
DATE OF BIRTH: dd-mmm-yyyy
PRIMARY ONCOLOGIST: Dr. [redacted]

PATIENT MEDICAL HISTORY AND CLINICAL SUMMARY: John Doe is a 14-year-old boy diagnosed with acute lymphoblastic leukemia on dd-mmm-yyyy. He is currently on the AALL0932 protocol which includes doxorubicin, 6-MP, l-asparaginase, vincristine, cytarabine, methotrexate, dexamethasone, thioguanine and cyclophosamide.

PHARMACOGENETIC TESTING REQUESTED
For Anthracycline-Induced Cardiotoxicity

There is growing evidence that genetic factors contribute to individual susceptibility to anthracycline-induced cardiotoxicity. Recent studies have identified genetic variants associated with toxicity in both children and adults. Genetic variants have been identified in genes, including *SLC28A3*, *UGT1A6*, and *RARG* which are involved in the transport or metabolism of anthracyclines and/or its metabolites.

RESULTS OF GENETIC TESTING:
Anthracycline-induced cardiotoxicity
John Doe does not carry the risk variants for anthracycline-induced cardiotoxicity.
John Doe carries the protective variant against anthracycline-induced cardiotoxicity.

Anthracycline *UGT1A6* rs17863783 "CC" (Risk genotypes are AC, AA)
Anthracycline *SLC28A3* rs7853758 "AG" (Protective genotypes are AG, AA)
Anthracycline *RARG* rs2229774 "GG" (Risk genotypes are AG, AA)

This patient's risk of cardiotoxicity is classified as 14% based on three independent patient populations as illustrated in the figure below

BC Children's Hospital and BC Women's Hospital & Health Centre are agencies of the Provincial Health Services Authority. As academic health centres, they are affiliated with the University of British Columbia, Simon Fraser University, the Child & Family Research Institute, and the Women's Health Research Institute.

Figure 2.5a. Pharmacogenetic Risk Prediction Results Consult Note Format (Page 1)

The pharmacogenetic risk prediction results are returned to the treating oncologists in the consult note format seen above with a summary of the patient's cancer diagnosis and treatment plan, as well as, the patients genotype results for the variants that are included in the relevant risk prediction model. Page 2 of the consult note is displayed below.

RESULTS OF GENETIC TESTING:
Anthracycline-induced cardiotoxicity
John Doe does not carry the risk variants for anthracycline-induced cardiotoxicity.
John Doe carries the protective variant against anthracycline-induced cardiotoxicity.

Anthracycline *UGT1A6* rs17863783 "CC" (Risk genotypes are AC, AA)
Anthracycline *SLC28A3* rs7853758 "AG" (Protective genotypes are AG, AA)
Anthracycline *RARG* rs2229774 "GG" (Risk genotypes are AG, AA)

This patient's risk of cardiotoxicity is classified as 14% based on three independent patient populations as illustrated in the figure below

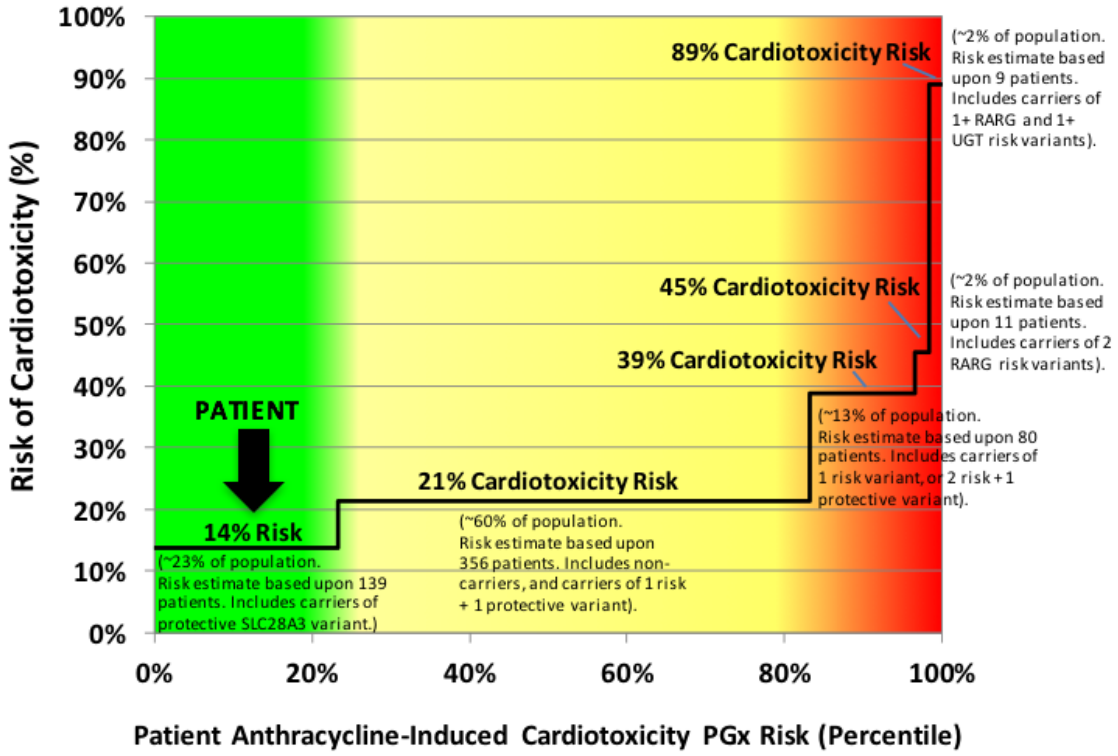
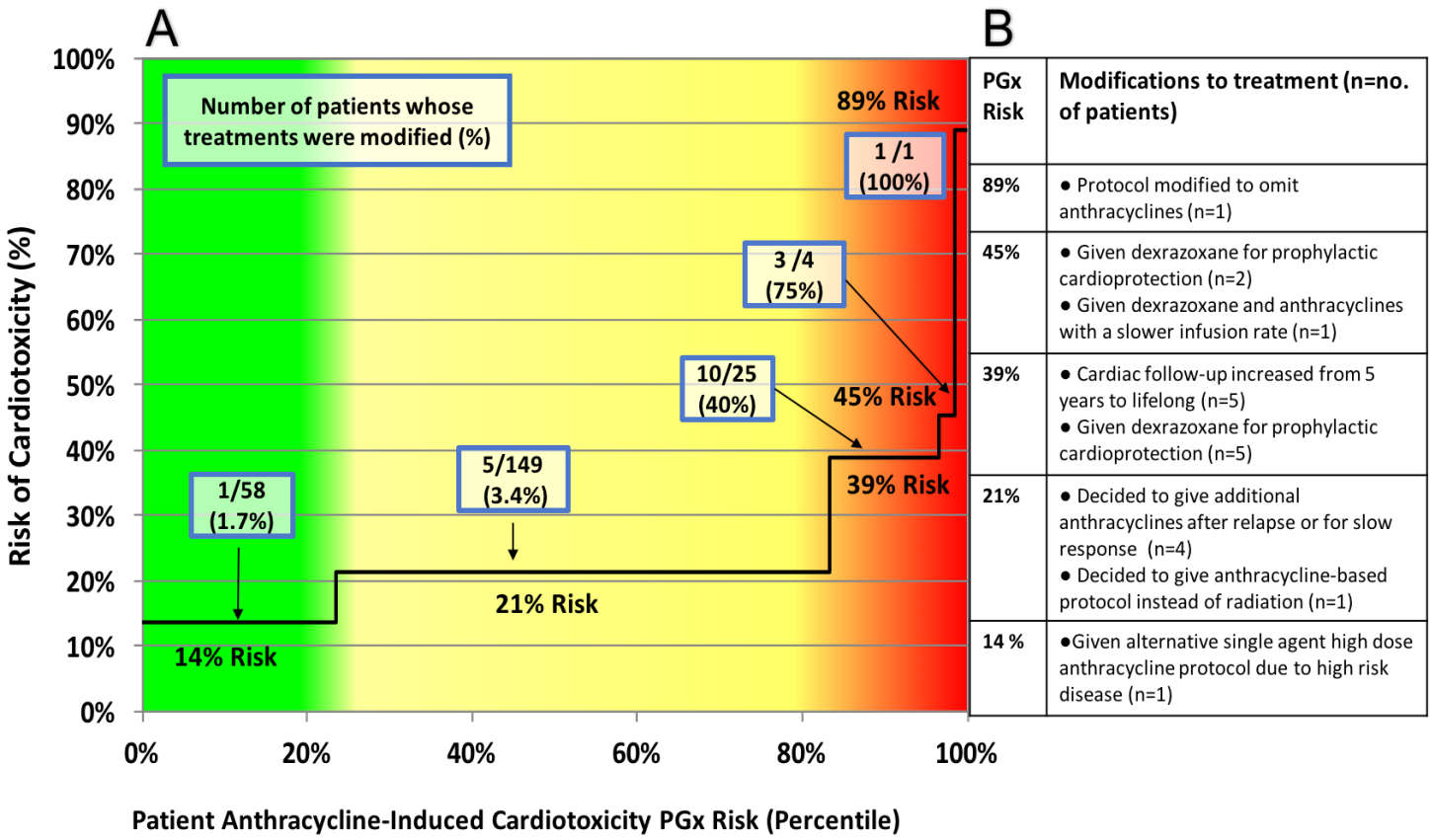


Figure 2.5b. Pharmacogenetic Risk Prediction Results Consult Note Format (Page 2)

The second page of the consult note includes a risk stratification graph with an arrow indicating which pharmacogenetic risk group the individual patient fell in based on their combination of predictive variants.



PGx Risk	Modifications to treatment (n=no. of patients)
89%	• Protocol modified to omit anthracyclines (n=1)
45%	• Given dexrazoxane for prophylactic cardioprotection (n=2) • Given dexrazoxane and anthracyclines with a slower infusion rate (n=1)
39%	• Cardiac follow-up increased from 5 years to lifelong (n=5) • Given dexrazoxane for prophylactic cardioprotection (n=5)
21%	• Decided to give additional anthracyclines after relapse or for slow response (n=4) • Decided to give anthracycline-based protocol instead of radiation (n=1)
14 %	•Given alternative single agent high dose anthracycline protocol due to high risk disease (n=1)

Figure 2.6. Treatment modifications of patients tested for their pharmacogenetic risk of anthracycline-induced cardiotoxicity.

A) The number of treatment modifications are represented in blue boxes as a fraction of the total number of patients that tested in each risk stratification group
B) Types of treatment modifications are indicated for each risk group

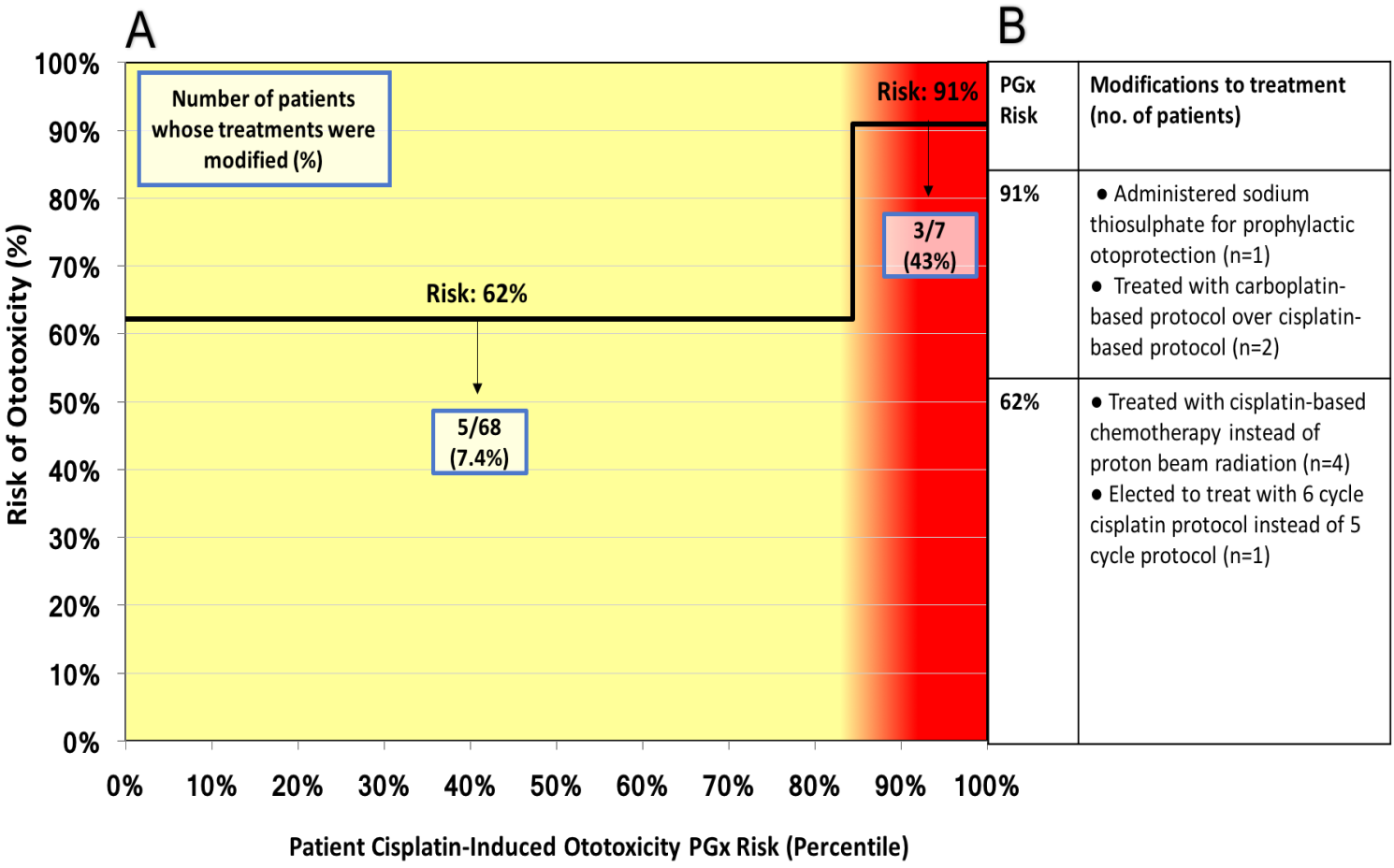


Figure 2.7. Treatment modifications of patients tested for their pharmacogenetic risk of cisplatin-induced ototoxicity

A) The number of treatment modifications are represented in blue boxes as a fraction of the total number of patients that tested in each risk stratification group
B) Types of treatment modifications are indicated for each risk group

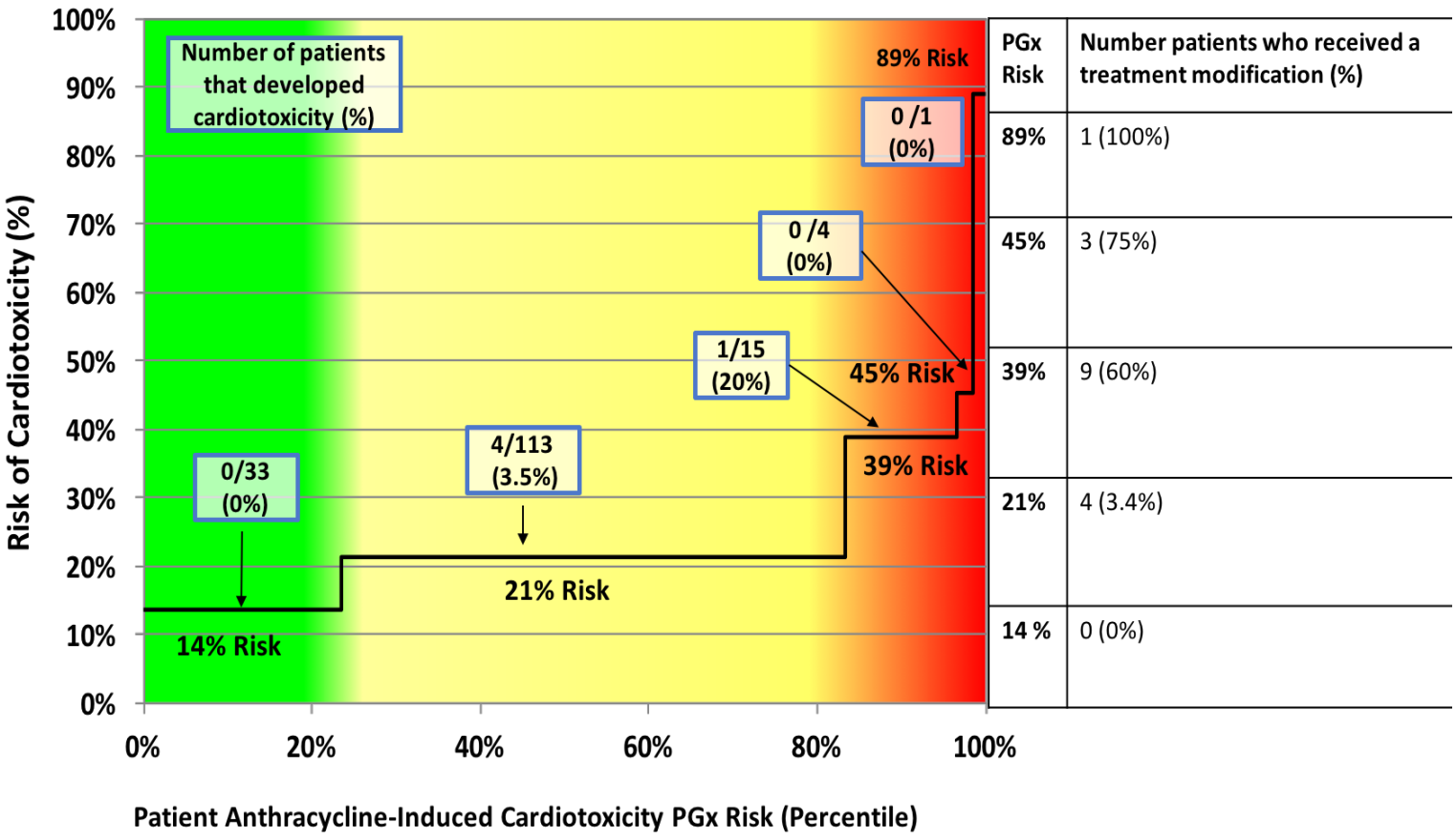
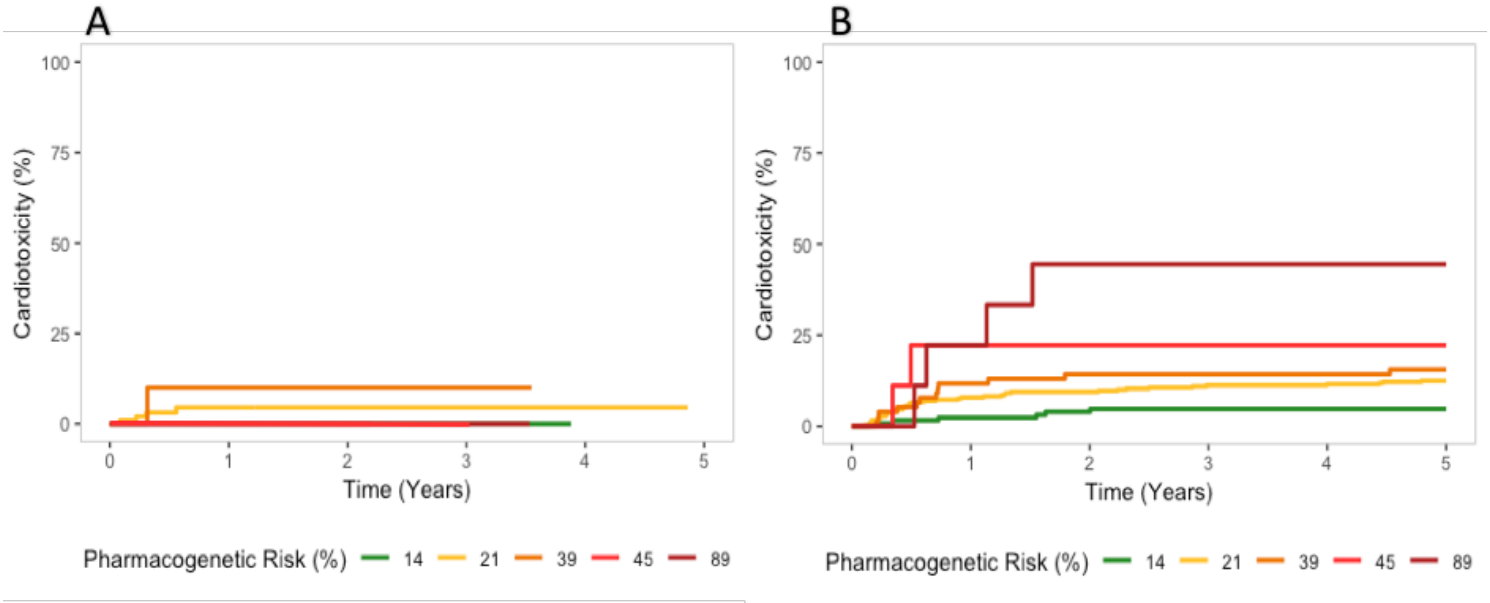


Figure 2.8. Cardiac outcomes of patients tested for their pharmacogenetic risk of anthracycline-induced cardiotoxicity

The number of patients in each risk stratification group that developed cardiotoxicity are presented as a fraction of the total number of patients in that risk group for which cardiac follow up data is available. Cardiotoxicity was defined as grade 2 or greater on the modified version of the CTCAEv3. Only patients with at least one echocardiogram assessment since the start of anthracycline therapy were included in the totals presented. The number of treatment modifications also only included patients with follow up cardiogram assessments. Types of treatment modifications are presented in Figure 2.6. Patients that were deceased from their disease prior to experiencing any cardiotoxicity (n=10) were also excluded from this analysis.



	Number of cases of cardiotoxicity that developed within 5 years (%)		P-value
PGx Risk	**A) Pharmacogenetic-tested Patients (n=166)**	**B) Model Development Patients (n=553)**	
14% Risk	0/33 (0%)	6/128 (4.7%)	0.204
21% Risk	4/113 (3.5%)	41/330 (12.4%)	0.134
39% Risk	1/15 (6.7%)	12/77 (15.6%)	0.187
45% Risk	0/4 (0%)	2/9 (22.2%)	**0.003**
89% Risk	0/1 (0%)	4/9 (44.4%)	**1.9×10^{-6}**

Figure 2.9. Cumulative incidence of cardiotoxicity in pharmacogenetic-tested patients compared to the cohort used to create the polygenic risk prediction model

A) Time to toxicity of patients that received pharmacogenetics testing which was incorporated into treatment decision-making. Only echocardiograms within 5 years since the start of anthracyclines were included. Patients that were deceased and had not experienced any cardiotoxicity at the time of their death were excluded (n=10), patients with no echocardiogram assessments since the start of anthracyclines were excluded (n=50), and patients who had grade 1 cardiac function at the time of their last assessment were excluded (n=17)

B) Time to toxicity of patients that were used to create the pharmacogenetic risk prediction model and did not receive pharmacogenetic testing prior to therapy. Only echocardiograms within 5 years from the start of anthracycline therapy were included. Patients that did not have an echocardiogram assessment within 5 years since the start of anthracyclines were excluded (n=42).

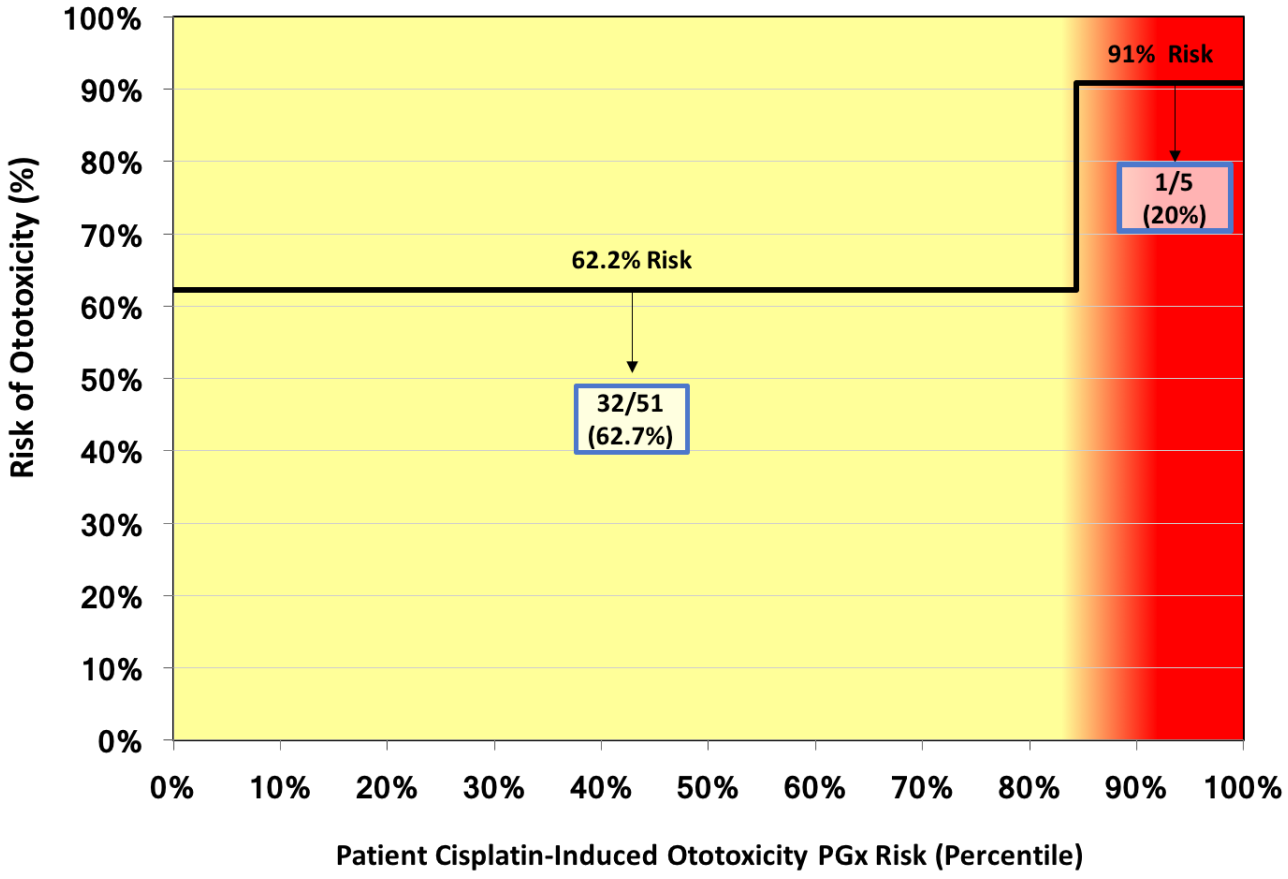


Figure 2.10. Ototoxicity outcomes of patients tested for their pharmacogenetic risk of cisplatin-induced ototoxicity

The number of patients that developed ototoxicity (≥ grade 2 on the CTCAEv4) are presented in blue boxes as a fraction of the total number of patients in that risk group for which audiometric follow up data is available. Patients that were deceased from their disease prior to experiencing ototoxicity (n=5) were excluded from this analysis. Cranial radiation, which is also known to cause ototoxicity, was given to 18/51 patients in the 62% Risk group. No patients in the 91% risk group received cranial radiation.

Key Themes	Sub-themes
Perceived benefits of PGx testing	• PGx results help to prepare families for the future and how to manage their risk of cardiotoxicity or ototoxicity. • Using PGx results to modify patients' treatment plans helps to mitigate risk and improve patients' outcomes. • Patients and families felt more involved in the treatment decision making process when PGx results were included in the discussion. • PGx testing informs treatment decision parameters and therefore instills confidence in the child's treatment plan thereby providing reassurance and psychological benefit to patients and families.
Challenges and barriers to understanding the utility of PGx results	• Patients and families felt overwhelmed with information at beginning of their child's diagnosis and treatment which lead to difficulty remember their specific PGx results. • Patients and families were unaware of the substantial risks of toxicity from their child's chemotherapy leading to differing perceptions of what 'low' and 'high' risk are compared to clinicians. • Patients and families had difficulty weighing their child's risk of survival from their cancer versus risk of suffering from an ADR.
Patients/families suggestions for improving the return and interpretation of PGx results moving forward	• Patients and families expressed an interest in receiving a written copy of results for their reference. • Patient and families suggested that it would be helpful to revisit/re-discuss risk results at a later date to remind individuals of their risk when they are not as overwhelmed by their child's diagnosis. • Patients and families also suggested that clinicians discuss results in more lay terms for individuals without a medical background.

Figure 2.11. Patient perspectives on pharmacogenetic testing in pediatric oncology

A grounded theory approach was used to identify themes and subthemes from text that was independently coded by two reviewers. This was based on feedback from 10 parents and 1 adolescent patient who received pharmacogenetic results.

Table 2.1. Pharmacogenetic variants included in the risk prediction model for anthracycline-induced cardiotoxicity

Genetic Marker	Studies	Association
RARG rs2229774	Discovery Cohort: Aminkeng *et al*, 2015	OR = 7.0 (95% CI: 2.9,17.0) $P = 4.1 \times 10^{-8}$
	Replication – European patients : Aminkeng *et al*, 2015	OR = 4.1 (95% CI: 1.5,11.5) $P = 0.0042$
	Replication – non-European patients: Aminkeng *et al*, 2015	OR = N/A $P = 2.2 \times 10^{-5}$
UGT1A6*4 rs17863783	Discovery Cohort Visscher *et al*, 2012	OR = 4.1 (95% CI: 1.03,16.17) $P = 0.040$
	Replication Cohort Visscher *et al*, 2012	OR = 4.0 (95% CI: 0.92,17.02) $P=0.075$
	Replication Cohort Visscher *et al*, 2013	OR=7.98 (95% CI: 1.85,34.4) $P=0.0062$
SLC28A3 rs7853758	Discovery Cohort Visscher *et al*, 2012	OR = 0.29 (95% CI: 0.11,0.81) $P = 0.0071$
	Replication Cohort Visscher *et al*, 2012	OR = 0.33 (95% CI: 0.13,0.80) $P = 0.0072$
	Replication Cohort Visscher *et al*, 2013	OR=0.46 (95% CI: 0.20,1.08) $P = 0.058$

Table 2.2. Clinical characteristics of patients used to the pharmacogenetic risk prediction model for anthracycline-induced cardiotoxicity

Patient Characteristics [a]	**Case (n=140)**	**Control (n=456)**	**P**
Age at start of treatment in yrs, median (IQ range)	8.5 (3.95-13.3)	5.0 (2.4-9.8)	**0.000115**[e]
Sex, Female/male (% female)	66/73 (47.5%)	214/242 (46.9%)	0.909
Anthracycline type, n (%)			
Doxorubicin	109 (77.9%)	383 (84.0%)	0.09438
Daunorubicin	30 (21.4%)	116 (25.4%)	0.3345
Mitoxantrone	12 (8.6%)	8 (1.8%)	**8.94×10^{-5}**
Epirubicin	11 (7.9%)	23 (5.0%)	0.2093
Idarubicin	2 (1.4%)	12 (2.6%)	0.6149
Cumulative anthracycline dose in mg/m2[b], median (IQ range)	300 (219.5-390.0)	199.5 (149.0-300.0)	**1.45×10^{-10}**
Primary Diagnosis (Tumour type), n (%)			
ALL	20 (14.3%)	161 (35.3%)	**2.23×10^{-6}**
AML/APL	16 (11.4%)	17 (3.7%)	**0.0005**
Non-Hodgkins lymphoma	10 (7.1%)	31 (6.8%)	0.888
Hodgkins lymphoma	5 (3.6%)	31 (6.8%)	0.161
Neuroblastoma	3 (2.1%)	39 (8.6%)	**0.0163**
Osteosarcoma	6 (4.3%)	22 (4.8%)	0.792
Ewings	12 (8.6%)	12 (2.6%)	**0.00177**
Hepatoblastoma	6 (4.3%)	19 (4.2%)	0.951
Wilms	13 (9.3%)	34 (7.5%)	0.482
Other	6 (4.3%)	7 (1.5%)	0.0513
Unknown	43 (30.7%)	83 (18.2%)	**0.00152**
PCA- determined ancestry, n (%)[c]			
European	109 (77.9%)	362 (79.4%)	0.698
Asian	7 (5.0%)	23 (5.0%)	0.983
Southeast Asian	8 (5.7%)	23 (5.0%)	0.755
South Asian	2 (1.4%)	13 (2.9%)	0.347
African	3 (2.1%)	9 (19.7%)	0.901
First Nations	4 (2.9%)	11 (2.4%)	0.988
Unknown	7 (5.0%)	15 (3.3%)	0.348
Radiotherapy involving the heart[d], n (%)	16 (11.4%)	39 (8.6%)	0.304
Use of dexrazoxane, n (%)	6 (4.3%)	21(4.6%)	0.874
Duration of follow up in years, median (range)	21.9 (4.5-49.9)	19.3 (6.9-52.1)	0.162

Age, dose and duration of follow-up were analyzed by Wilcoxon-Mann-Whitney U test. Sex, anthracycline type, tumour type, ancestry, and radiotherapy involving the heart and use of cardioprotectant were analyzed by Fisher exact test. b) Cumulative anthracycline dose in doxorubicin isotoxic equivalent doses. c) Ancestry was determined via principal component analysis (PCA) using pruned ADME genotypes and minor allele frequency <0.01 incorporating the 1,000 Genomes Project samples[399] as a reference. d) Includes mantle and mediastinal radiation, whole lung radiation, whole or upper abdominal radiation, left sided flank radiation and total body irradiation. e) Bold font indicates statistically significant P-value ($P < 0.05$) and covariates for logistic regression.

Table 2.3. Pharmacogenetic markers included in the risk prediction model for cisplatin-induced ototoxicity

Genetic Marker	Studies	Association
TPMT **(rs12201199)**	Discovery Cohort Ross *et al.* (2009)	OR 14.3 (95% CI: 0.81,251.74) *P*=0.0097
	Replication Cohort Ross *et al.* (2009)	OR 10.0 (95% CI: 1.31,76.36) *P*=0.0071
	Replication Cohort Pussegoda *et al.* (2013)	OR 6.1 (95% CI: 1.8,20.9) *P*=0.0013
TPMT **(rs1142345)**	Discovery Cohort Ross *et al.* (2009)	OR 11.0 (95% CI: 0.61,197.64) *P*=0.022
	Replication Cohort Ross *et al.* (2009)	OR 5.8 (95% CI: 0.73,45.72) *P*=0.044
	Replication Cohort Pussegoda *et al.* (2013)	OR 4.5 (95% CI: 1.3,15.7) *P*=0.011
TPMT **(rs1800460)**	Discovery Cohort Ross *et al.* (2009)	OR 11.0 (95% CI: 0.61,197.64) *P*=0.022
	Replication Cohort Ross *et al.* (2009)	OR 8.1 (95% CI: 0.46,143.37) *P*=0.046
	Replication Cohort Pussegoda *et al.* (2013)	OR 3.6 (95% CI: 1.0,12.8) *P*=0.038

Table 2.4. Clinical characteristics of patients used to create the pharmacogenetic risk prediction model for cisplatin-induced ototoxicity risk

Patient Characteristics[a]	**Case (n=188)**	**Control (n=94)**	**P**
Age at start of treatment in yrs, median (IQ range)	4.20 (1.8- 8.4)	9.9(2.2- 13.1)	**0.0001**[b]
Sex, Female/male(% female)	78/109 (41.7%)	47/47 (50.0%)	0.175
Cumulative cisplatin dose in mg/m2, median(IQ range)	400 (300-480)	400 (300-480)	0.914
Adjuvant therapy with carboplatin, n(%)	63 (33.5%)	13 (13.8%)	**0.0004**
Primary Diagnosis (Tumour type), n (%)			
Neuroblastoma	48 (25.5%)	15 (16.0%)	0.0688
Osteosarcoma	34 (18.1%)	26 (27.7%)	0.0640
Hepatoblastoma	37 (19.7%)	10 (10.6%)	0.0548
Germ cell	15 (8.0%)	28 (29.8%)	**1.57×10^{-6}**
Medulloblastoma	36 (19.1%)	7 (7.4%)	**0.00997**
PNET	8 (6.0%)	0 (0%)	0.0992
Pinealoblastoma	4 (3.0%)	2 (2.1%)	1.0
Nasopharynx carcinoma	3 (2.3%)	1 (1.1%)	1.0
Other	3 (2.3%)	5 (5.3%)	0.163
PCA-determined Ancestry, n (%)[c]			
European	130 (69.1%)	74 (78.7%)	0.0902
East Asian	15 (8.0%)	11 (11.7%)	0.203
South Asian	13 (6.9%)	3 (3.2%)	0.0494
Admixed	27 (14.4%)	6 (6.4%)	0.170
Unknown	3 (1.6%)	0 (0%)	N/A
Cranial Radiation, n (%)	52 (27.7%)	16 (17.0%)	**0.0490**
Duration of follow up in years, median (range)	14.43 (4.017-117.50)	14.25 (7.93-28.08)	0.859

a) Age, dose and duration of follow-up were analyzed by Wilcoxon-Mann-Whitney U test. Sex, Adjuvant carboplatin, tumour type, ancestry, and cranial radiation were analyzed by Fisher exact test. b) Bold font indicates statistically significant P-value ($P < 0.05$) and covariates for logistic regression. c) Ancestry was determined via principal component analysis (PCA) using pruned ADME genotypes and minor allele frequency <0.01 incorporating the 1,000 Genomes Project samples[399] as a reference.

Chapter 3: Discussion and Future Directions

Cisplatin and anthracyclines are widely used chemotherapy drugs in the treatment of pediatric cancers, however, their use remains limited by their ability to cause ototoxicity and cardiotoxicity, respectively. Both of these ADRs have significant implications for patients, often restricting further use of the chemotherapy drug with potential reductions in survival, as well as, harmful and expensive long-term consequences for patients and their families[2,31,37,248]. Pharmacogenetic studies examining the genetic associations for the development of these two serious ADRs have been conducted and published, and Clinical Practice Guidelines have been published to outline which genetic variants have strong evidence for their use in clinical decision-making and to provide recommendations for what to do with the results once you have them[376,378]. Polygenic risk prediction models that incorporated genetic variants associated with anthracycline-induced cardiotoxicity and cisplatin-induced ototoxicity have been developed, and their implementation into clinical care in pediatric oncology at BC Children's Hospital is described here. By evaluating the use of pharmacogenetic risk prediction results in clinical decision-making for the first 297 patients that received testing since the program began in 2013, this study has shown that patients' pharmacogenetic results helped to facilitate discussions about drug harm and treatment options between clinicians and families. Additionally, this study found that pharmacogenetic results led to appropriate treatment modifications when the risk of experiencing an ADR outweighed the benefits of continuing with the original treatment plan. This study has provided evidence that patients whose pharmacogenetic risk prediction results were incorporated into treatment decision-making have experienced lower rates of ADRs than patients who did not. This study also evaluated families' and clinicians' perspectives of the utility of testing, and found that both groups found the results to be of high clinical utility and value. Finally, this is the first study to assess the use of polygenic risk prediction models as a method of integrating pharmacogenetics into clinical practice in oncology. This study was able to demonstrate both the feasibility and value of incorporating results from polygenic risk prediction models into clinical decision-making to improve patient-clinician discussions and drug therapy outcomes.

3.1: Overcoming Previously-identified Barriers

3.1.1: Interpretation of Pharmacogenetic Results

As discussed in Chapter 1, pharmacogenetic testing is being conducted and implemented into clinical practice in oncology in hospitals and academic centres globally. However, several barriers towards more wide-spread adoption and the use of pharmacogenetic results in clinical decision-making still exist. A major barrier towards the successful implementation of pharmacogenetic information remains the interpretation of genetic results for appropriate clinical decision-making[323,360]. In order to overcome this barrier, the polygenic risk prediction models presented in this study combined multiple genetic variants into one predicted risk estimate in order to provide clinicians with clear and easily interpretable results. Additionally, as clinical decisions are often made based on weighing the risks and benefits of different interventions, the risk estimates based on the described pharmacogenetic risk prediction models provided more informative and quantitative results than a dichotomization of risk (i.e., high versus low risk).

As pharmacogenetic risk factors are known to play a large role in the development of these ADRs[400,401], incorporating genetic risk into the overall discussion of risk factors and treatment options allows for more informed treatment decisions to be made. With this in mind, the polygenic risk prediction models for anthracycline-induced cardiotoxicity and cisplatin-induced ototoxicity were designed to provide an easily interpretable estimate of genetic risk alone, in order to facilitate the inclusion of genetics in multi-disciplinary discussions of risk factors and treatment options. Results for individual gene variants using multi-gene panel testing have largely been the method of how pharmacogenetic results have been returned to clinicians and patients to date, however, these results are often complex; yielding results for multiple variants with poorly defined risk, combinations of risk variants with uncertain significance, and recommendations that are limited to a single drug-gene pair[323,360,388]. The polygenic risk prediction models described here, instead, used cohorts of previously treated patients (cisplatin n=283, anthracycline n=595) with various combinations of predictive variants to define risk thresholds. The predicted risk estimates are, therefore, based on ADR outcomes of patients that carried that same genotype combinations as the patients receiving testing. Additionally, by defining genetic risk as a percentage, the risk prediction models avoid the previously identified

challenge of providing interpretations of results that are universally recognized and easy for both clinicians and patients to understand. For instance, as discussed in Chapter 1, many hospitals conducting pharmacogenetic testing are returning results with phenotypic interpretations (e.g., metabolizer status), however, there has been a lack of standardization of phenotype nomenclature[360]. Genetic testing laboratories, for example, have been shown to report a *TPMT*3A* allele as leading to "low function", "low activity", "null allele," "no activity," or "undetectable activity"[402]. The use of inconsistent terms can be confusing to both clinicians and patients. As percentages are used frequently in oncology to describe risk and outcomes, and are something that patients are familiar with in everyday life, providing pharmacogenetic risk prediction results as a risk percentage allows the results to be easily understood and incorporated into discussions between clinicians and patients[403].

3.1.2: Pharmacogenetic Education and Training

As described in Chapter 1, another significant barrier towards the successful adoption of pharmacogenetic information in clinical practice remains the education and understanding amongst clinicians of how pharmacogenetic results can be incorporated into clinical decision-making. Clinicians reportedly feel increasing pressure to counsel patients about their pharmacogenetic results and given the many competing management priorities for newly diagnosed patients and limited genetic experience or training, this is a major barrier toward clinicians opting to utilize pharmacogenetic test results in their treatment decision-making[388]. The need for pharmacogenetic training was highlighted in a survey of more than 10,000 physicians in the United States, which showed that despite 98% of respondents agreeing that the genetics of a patient could influence a drug therapy decision, only 29% had received pharmacogenetics education during their medical training and only 10% felt like they were adequately trained to apply the knowledge in clinical practice[382]. Risk prediction models are already commonly used in oncology practice, for example, through prognostic estimates used for terminal illnesses to help facilitate planning the remaining lifetime or to give hope for recovery if the disease prognosis is good[404]. As such, the pharmacogenetic risk prediction models discussed here help to overcome the barriers of lack of pharmacogenetic education and training by providing the information in a format that is easily understood by clinicians. Additionally, clinical practice guidelines have been published to assist clinicians in answering who should be

tested, what genes should be tested, and most importantly what to do with the results. Lastly, a clinical pharmacologist with expertise in pharmacogenetics is available to provide interpretational support and guidance to clinicians and families in order to further facilitate the utility of the test results.

3.1.3: Clinician Buy-in and Acceptance of Pharmacogenetic Testing

Another potential hurdle reported by other groups implementing pharmacogenetics has been gaining clinicians' acceptance of testing. Previous studies have found that clinicians were concerned, not only about their knowledge of pharmacogenetics, but also about the increased burden that pharmacogenetic testing would place on their management and care of patients[405,406]. As mentioned above, several steps were taken to make the pharmacogenetic risk prediction models clear and easily interpretable, and support services (i.e., clinical practice guidelines, pharmacology consultation) were available to overcome previous concerns expressed by clinicians. In addition to this, an oncologist with a keen interest and understanding of the pharmacogenetic risk prediction models was available to act as a clinician champion to provide support and demonstrate the value of test results in clinical decision-making in order to promote buy-in from other clinicians. Identifying a clinician champion has previously been found to be essential in overcoming lack of provider acceptance of pharmacogenetic testing[388].

3.1.4: Defining and Evaluating Clinical Utility

Establishing the clinical utility of implementing pharmacogenetics has been advocated for in order to ensure that its use is appropriate, cost-effective and improves health outcomes[407]. However, a consensus on the level of evidence required for proof of clinical utility that is scientifically appropriate as well as realistically achievable has yet to be agreed upon[331,332,408]. While the gold standard for demonstrating the clinical utility of an intervention remains the use of randomized controlled trials (RCTs), they are expensive and time-consuming, not often feasible for rare outcomes, and considered by many to be unethical when it comes to providing pharmacogenetic information that could spare a patient from experiencing a harmful ADR[409]. Instead, researchers are looking to alternative non-randomized approaches to determining clinical utility, and other ways of defining clinical utility in the context of pharmacogenetic-informed prescribing. Although the term can be narrowly defined as the ability to prevent

adverse health outcomes, others prefer to broadly define clinical utility as any test result used to inform clinical decision-making[408]. Several groups agree that clinical utility should not be restricted to health outcomes, and instead should be expanded to include biological, psychological, and social impacts to the individual, family, and society[408,410-413].

The value of pharmacogenetic information is to better understand the individual patients' risk of developing an ADR and has been viewed as important and beneficial even in the absence of a measurable change to therapy[408]. For instance, a group of cancer patient's screened for their genetic risk considered their results valuable even if they resulted in no changes to their treatment plan or outcome, such as when no alternate treatment was available[414]. As shown in Chapter 2, similar findings were observed from the patients and families that were interviewed, which were consistent with previous studies that have described patients' perceptions of the benefits of pharmacogenetic testing[405,415-417]. For example, patients and families who were interviewed universally agreed that results from the pharmacogenetic risk prediction models were valuable at informing treatment decisions to maximize drug safety and efficacy, and helped facilitate in-person discussions with their clinicians about the risk of ADRs. The results from patient and family interviews indicated that the promise of precision medicine through pharmacogenetics is perceived and understood by most participants. Clinicians also described the results as being important to clinical care and emphasized the importance of providing and communicating the results in a clear way that could be used to facilitate conversations between patients and clinicians, and aid in the decision-making process.

Additional evidence of the clinical utility of implementing pharmacogenetic testing has been demonstrated through previous cost-effectiveness analyses that have evaluated whether test results led to improvements in patients' health and quality of life, and what the costs of implementing testing were per quality adjusted life year (QALY)[367]. Pharmacogenetic testing offers potential benefits by decreasing the risk of adverse drug events thereby lowering the treatment costs associated with that event and improving response[367]. Previously published literature reviews of pharmacogenetic-guided treatment decision-making have demonstrated that the majority of pharmacogenetic strategies were cost-effective[418-420], with one recent literature review finding that 57% of economic evaluations drew conclusions in favour of pharmacogenetic

testing, of which 30% were cost-effective and 27% were dominant (cost-saving)[367]. They further concluded that if there were no costs attached to genetic testing, the number of economic evaluations in support of pharmacogenetic-guided treatment would increase to 75% with 25% being cost-effective and 50% being dominant. The economic value of incorporating pharmacogenetic risk of anthracycline-induced cardiotoxicity into treatment decision-making has been evaluated and predicted to lead to a 17% reduction in deaths due to anthracycline-induced cardiotoxicity[421]. This represents 11.8 deaths avoided and 625 years of life gained for every 1000 patients treated with anthracyclines. Assuming that the cost of testing for pharmacogenetic risk of anthracycline-induced cardiotoxicity was $100 per patient, pharmacogenetic-guided decision-making was found to have an average value savings of $495 per patient with a 5.7% overall reduction in costs[421]. For patients at low genetic risk of experiencing cardiotoxicity, testing was found to further decrease costs due to less monitoring and follow-up being required (average of $420 per patients, 16.9% overall reduction). As the costs of genomic testing continue to decline, evidence of the cost-effectiveness of pharmacogenetic testing will further increase, and evaluation of the clinical utility of pharmacogenetics can instead focus of how the information from testing is utilized to improve clinical decision-making and health outcomes.

3.2: Added Predictive Value of Genetic Risk Factors

As discussed in Chapter 1, many clinical risk factors have been identified for both anthracycline-induced cardiotoxicity (e.g., anthracycline dose, cardiac irradiation, younger age, female sex) and cisplatin-induced ototoxicity (e.g., younger age, vincristine treatment, germ-cell tumour, cranial irradiation), however, these factors alone have been insufficient at accurately determining who is most likely to experience these ADRs[400,401]. Previous studies have demonstrated the added value of incorporating predictive genetic markers of anthracycline-induced cardiotoxicity and cisplatin-induced ototoxicity into risk prediction models compared to models based on only clinical factors [11,12,299]. For instance, a predictive model for cisplatin-induced ototoxicity that combined variants in *TPMT* (rs12201199), *ABCC3* (rs1051640), and *COMT* (rs4646316) with clinical variables (age, vincristine treatment, germ-cell tumour, and cranial irradiation) was shown to significantly improve the prediction of ototoxicity development as compared with using clinical risk factors alone (area under the curve (AUC) 0.786 vs. 0.708, $p = 0.00048$) **(Figure 3.1)**[11]. The combination of genetic and clinical factors predicted the risk of ototoxicity

with a sensitivity of 50.3% and a specificity of 92.7%. Similarly, a risk prediction model for anthracycline-induced cardiotoxicity which combined nine SNPs (*UGT1A6* rs6759892, *ABCB4* rs1149222, *ABCC1* rs4148350, *HNMT* rs17583889, *SLC28A3* rs7853758 and rs4877847, *FMO2* rs2020870, *SPG7* rs2019604, *SLC10A2* rs9514091) with clinical risk factors (age at start of treatment, cumulative anthracycline dose, sex, radiation involving the heart region) was found to perform significantly better than clinical factors alone (AUC 0.87 vs. 0.68, p=4.6x10^{-6}) **(Figure 3.2)**[10]. A prediction model than included only genetic predictors of anthracycline-induced cardiotoxicity was found to be significantly more predictive than a risk prediction model of clinical factors alone (AUC 0.81 vs. 0.68) **(Figure 3.2)**[10]. An additional model for anthracycline-induced cardiotoxicity that included five SNPs (*SLC28A3* rs7853758, *UGT1A6* rs17863783, *SULT2B1* rs10426377, *SLC28A1* rs2305364, and *ABCB4* rs4148808) and clinical variables (age at start of treatment, cumulative anthracycline dose, sex, radiation involving the heart region) was shown to discriminate significantly better between cases and controls than clinical factors alone in both an original (AUC 0.77 vs. 0.68, $p = 0.0031$) and replication cohort (AUC 0.77 vs. 0.69, $p = 0.060$)[12]. Compared to clinical factors alone, genetic variables were found to be significantly better at predicting patients risk of experiencing both cisplatin-induced ototoxicity and anthracycline-induced cardiotoxicity. As clinical risk factors for both of these ADRs are already included treatment discussions and the clinical decision-making process, and given the above evidence of the improved predictive power of including genetic factors in risk prediction models, there is strong evidence in support of including genetic risk factors that are highly predictive of ADR outcomes in treatment decision-making.

3.3: Incorporating Pharmacogenetics into Drug Discovery and Development

Further opportunities to generate evidence of the clinical utility of implementing pharmacogenetics lie in the drug development process[422]. If, for example, preclinical studies have determined that a drug is metabolized by a CYP450 enzyme or is a substrate for a specific transporter, phase I, II, and III clinical trials can incorporate pharmacogenetic testing to examine the effects of altered gene expression or transporter function on drug deposition[422]. Pharmacogenetic-guided clinical trials would allow for a more informative and efficient drug development process, which in the past has required a multitude of retrospective and prospective studies on drugs that have existed for decades[422,423]. Additionally, failure to show efficacy in

phase II studies is the most common reason for terminating drug development, and large phase III studies, required to establish safety and efficacy, are extremely expensive[422]. Therefore, if more information could be determined from earlier, smaller phase II studies which incorporate pharmacogenetics then valuable time and resources could be saved during phase III trials[424].

With increased capabilities to easily obtain DNA and perform these studies, pharmacogenetic-guided clinical trials are increasing and have been shown to improve the drug-development process by enhancing patient selection, contributing to dose optimization and therapy selection, reducing ADRs, and decreasing overall health care costs[422]. For example, a phase I clinical trial involving *UGT1A1* genotype-guided dosing in metastatic colorectal cancer patients demonstrated that patients lacking the high toxicity genotype (*UGT1A1*28/*28)* were able to tolerate higher doses (310-370mg/m^2) compared to the standard dose (180mg/m^2) in the FOLFIRI protocol (5-fluorouracil, leucovorin, and irinotecan)[425]. Had this genetic association information been available during the initial drug development phase, clinical studies could have focused on evaluating the improved survival benefit and impact on rates of toxicity of tailoring the dose by genotype. Additionally, drug candidates that have previously failed clinical trials based on toxicity or lack of efficacy can be resurrected if proven safe and effective in sub-groups of patients based on pharmacogenetic determinants of toxicity and response[70,422,423].

Drug targets (e.g., transporters, receptors) have many polymorphisms leading to variations in drug effect, which can lead to inconsistent results in pre-clinical and clinical studies[422]. Using pharmacogenetics to decide on drug targets for further investigation can allow ineffective targets to be avoided thus leading to a more efficient drug discovery and development process. Pharmacogenetic studies of cisplatin-induced ototoxicity and anthracycline-induced cardiotoxicity have elucidated potential drug targets to aid in the identification of protective agents that may prevent ototoxicity and cardiotoxicity without compromising antitumour activity. For example, a study in 188 testicular cancer patients treated with cisplatin identified a protective role of a variant in *SLC16A5* (rs4788863; OR 0.06; 95% CI: 0.02, 0.2; p=2.2x10^{-7})[426]. Cimetidine, a histamine H2 receptor antagonist commonly used to treat heart burn and peptic ulcers, has been shown to inhibit SLC16A5 and therefore holds the potential to act as an otoprotective agent to prevent cisplatin-induced ototoxicity[163,427]. It has been shown to prevent

the occurrence of cisplatin-induced ototoxicity in mice and rat cochlear cells without compromising the anti-tumour activity of cisplatin treatment[428,429].

Based on the association between *RARG* (rs2229774) and anthracycline-induced cardiotoxicity, potential drug targets for the prevention of cardiotoxicity have also been identified. As mentioned in Chapter 1, RARG has been shown to bind to the promotor and repress the expression of *TopIIβ*, and higher levels of TopIIβ have been found to be associated with the development of anthracycline-induced cardiotoxicity[204,245]. As such, drugs that reduce the expression of *TopIIβ* or increase the expression of wild-type *RARG* have been shown to confer a cardioprotective effect[245]. Dexrazoxane, currently the only drug widely used for cardioprotection against anthracyclines, has been shown to reduce the expression of *TopIIβ*[245]. All-trans retinoic acid (ATRA), which is a natural ligand of retinoic acid response elements, is a potent activator of RARG and has been shown to significantly protect rat cardiomyocytes from anthracycline-induced cardiotoxicity[430]. These results, along with support clinical evidence of dramatically reduced rates of anthracycline-induced cardiotoxicity in acute promyolytic leukemia (APL) patients treated with ATRA[431], suggest that activators of RARG confer a cardioprotective effect. Based on this evidence, additional activators of RARG could be further explored to determine their cardioprotective effect against anthracyclines. Additionally, given the fact that anthracyclines can bind both TopIIα and TopIIβ to exert their cytotoxic effects, strategies to improve the specificity of anthracyclines to bind solely TopIIα have been proposed in order to prevent anthracycline-induced cardiotoxicity[383].

These examples highlight the utility of pharmacogenetics to uncover drug targets for preventing toxicity, which can be further tested in model organisms and clinical studies. Model organisms to investigate otoprotectants against cisplatin-induced ototoxicity have previously been established in mouse cochlear explants, as well as, zebrafish and murine models[432,433]. Well established models of anthracycline-induced cardiotoxicity using human cardiomyocytes[434], zebrafish[435], and mice[436] can be used to investigate cardioprotective agents.

3.4: Future Directions

This work highlights the feasibility and value of incorporating pharmacogenetic risk prediction models into clinical decision-making in pediatric oncology to inform therapeutic decisions and improve drug therapy outcomes. Through patient and clinician interviews, these risk prediction models were found to aid in therapeutic decision-making, assist in patient-clinician discussions about ADRs and drug safety, and facilitate in the long-term planning and monitoring of ADRs. The findings of the current study provide support for additional studies aimed at implementing strongly predictive pharmacogenetic markers and developing strategies for overcoming persisting barriers towards the implementation of pharmacogenetics. Additionally, given the demonstrated value of genetic determinants of drug response, this study highlights the importance of further pharmacogenetic association studies to help predict and prevent serious ADRs. Highly predictive pharmacogenetic markers, such as the ones incorporated into the pharmacogenetic risk prediction models discussed here, have provided potential drug targets for future exploration as potential mechanisms to prevent cisplatin-induced ototoxicity and anthracycline-induced cardiotoxicity from occurring.

3.4.1: Updating Clinical Practice Guidelines and Risk Prediction Models

As genotyping technologies are rapidly advancing and new genetic discoveries continue to emerge, an immediate next step in this project would be to determine whether any additional genetic associations for both anthracycline-induced cardiotoxicity and cisplatin-induced ototoxicity have sufficient evidence for their use in clinical practice. Previously-created clinical practice guidelines may then be updated to reflect new findings and provide recommendations based of the current levels of evidence for each genetic association.

An initial comprehensive systematic review has already been conducted for new studies examining pharmacogenetic associations with anthracycline-induced cardiotoxicity and cisplatin-induced ototoxicity with a full search strategy shown in **Figures 3.3**, **3.4**. The retrieved articles have been reviewed by two independent researchers (AD and TBW), and relevant articles that described pharmacogenetic associations for either ADR have been isolated for further review. Based on the initial screening of retrieved articles, 12 articles describing 6 new genetic variants associated with cisplatin-induced ototoxicity were found and included: *ACYP2*

(OR 12.06, 95% CI: 0.66, 221.98 and OR 14.7, 95% CI: 2.6, 84.2)[154,155], *SLC16A5* (OR 0.06, 95% CI: 0.02,0.22)[426], *SLC22A2* (OR 0.12, 95% CI:0.02, 0.58 and OR 0.479, 95% CI: 0.236, 0.976)[437,438], *NFE2L2* (OR: 0.416; 95% CI: 0.181, 0.957)[438], *WFS1* (β=-0.34, SE=0.06)[158], and *ERCC2* (OR 4.1, 95% CI: 1.43, 11.52)[439]. The association between cisplatin-induced ototoxicity and ERCC2 was discovered in pediatric cancer patients, while associations with *SLC16A5, WFS1,* and *NFE2L2* were discovered in adult cancer patients. Associations between cisplatin-induced ototoxicity and *SLC22A2* and *ACYP2* were found in adult cancer patients, as well as, mixed age cohorts (*SLC22A2*: 5-22 years old, *ACYP2*: 5-43 years old). An additional 3 genetic associations were replicated from the original clinical practice guidelines for cisplatin-induced ototoxicity and included: *COMT* (OR 6.49, 95% CI: 2.01, 20.9)[440], *GSTT1* (OR 6.3, 95% CI: 1.4, 28.7; OR 3.53, 95% CI: 1.07, 11.6)[439,440], *GSTP1* (OR 9.39, 95% CI: 0.93, 93.9)[439], *XPD* (12.3, 95% CI: 1.2, 126.0)[441]. Each study identifying these genetic associations was assessed for their sample size, study design, clinical demographics (i.e., ancestry, age, grading criteria, clinical covariates), and genotyping technology to allow comparisons to be made about the validity of their findings. All of these factors are reviewed using a standard clinical practice guideline process (discussed below) in order to provide evidence-based recommendations about the use of these genetic associations in clinical practice.

A systematic review of studies describing genetic associations with anthracycline-induced cardiotoxicity found 7 articles describing 16 new genetic associations including: *ETFB* (OR 6.17, 95% CI: 1.61, 27.7)[442], *CELF4* (OR 2.26, 95% CI: 1.2, 4.0)[443], *ATP2B1* (OR 0.26, 95% CI: 0.07, 0.96)[444], *ABCG2* (OR 5.3, 95% CI: 1.7-16.5)[445], *PLCE1* (OR 0.36, 95% CI: 0.18, 0.76)[444], *HLA-C* (OR 8.61, 6.56, 5.41; 95% CI: n/a)[446], *NFKBIL1* (OR 6.83, 8.87, 7.99; 95% CI: n/a) [446], *ATP6V1G* (OR 6.83, 4.12; 95% CI: n/a) [446], *C6orf10* (OR 3.89, 95% CI: n/a) [446], *TNF-a* (OR 5.67, 95% CI: n/a) [446], *MSH5* (OR 11.58, 95% CI: n/a) [446], *MICA* (OR 4.5, 95% CI: n/a) [446], *LTA* (OR 6.83, 95% CI: n/a) [446], *BAT1* (OR 4.13, 95% CI: n/a) [446], *NOTCH4* (OR 0.22, 95% CI: n/a) [446], and *PRDM2* (β=-4.97, SE=1.10)[447]. Associations between anthracycline-induced cardiotoxicity and *CELF4, ATP2B1* and *PLCE1* were discovered in pediatric cancer patients, while the association with *ETFB* was discovered in adult cancer patients and replicated in a pediatric cohort. The association between *ABCG2* and anthracycline-induced cardiotoxicity was discovered patients 14 years of age and above, while the association with

PRDM2 was discovered in patients aged 40-61. The associations between anthracycline-induced cardiotoxicity and *HLA-C, NFKBILI, ATP6V1G, C6org10, TNF-a, MSH5, MICA, LTA, BAT1,* and *NOTCH4* were discovered in adult cancer patients (median age: 53.1 years old; range: 35-76 years old)[249]. An additional 3 genetic associations were replicated from the original clinical practice guidelines including: CBR3 (β=0.84, p=0.004)[448], *CYBA* (OR 0.3, 95% CI: 0.1-0.9)[445], and *NCF4* (OR 0.35, p=0.011)[448]. Similar to the systematic review for cisplatin-induced ototoxicity, information about each study's sample size, study design, clinical demographics (i.e., ancestry, age, grading criteria, clinical covariates), and genotyping technology was collected to assess and provide recommendations based on the strength of evidence of each genetic association.

While an initial overview of the retrieved findings from systematic reviews of published genetic associations with cisplatin-induced ototoxicity and anthracycline-induced cardiotoxicity are presented here, a standard clinical practice recommendation process based on quality criteria suggested by the Appraisal of Guidelines Research and Evaluation Enterprise (AGREE) is currently underway to further review the retrieved articles and produce high quality clinical practice guidelines[449,450]. This process was followed during the development of the original clinical practice guidelines, as described in Chapter 2, and involved the critical appraisal of retrieved articles to evaluate the consistency of results, the magnitude of effect, and the number and quality of the studies conducted[376,378]. Clinical practice recommendations developed during a workshop meeting of clinical recommendation group members were then assigned one of three levels of strength based on the strength of available evidence upon which the recommendation was based, the balance between benefits and risks of genetic testing and genotype-guided treatment, as well as, the likelihood of variability in the individual values and preferences of patients. Following a similar process would help to ensure that the clinical practice guidelines build on the guidelines that have already been published, are clinically useful, and provide recommendations based on strong levels of evidence[449,450].

3.4.2: Validating the Predictive Strength of the Risk Prediction Models

Risk prediction modelling research typically involves three steps: (1) the development of the model, (2) testing the model in an independent population (external validation), and (3) assessing the model's impact on therapeutic decision-making and outcomes[404,451,452]. While this study was aimed solely at describing the impact of implementing pharmacogenetic risk prediction models in pediatric oncology, an external validation of the risk prediction models may be conducted in future studies to provide further evidence of their clinical utility and improve the predictive strength of the models. As described in Chapter 2, each of the predictive variants included in these models has been independently validated with high reproducibility (replicated in 3 independent populations) and predictability (OR≥3). An external validation of the models would aim, therefore, not to validate the strength of the individual predictive markers, but the predictive performance of the model in a group of new individuals. An analysis plan for the external validation of both pharmacogenetic risk prediction models has been developed to provide a potential method for future investigation and is described below.

3.4.2.1: Study Design

Types of External Validation

Different types of external validation studies have been conducted and published including: temporal, geographical, and domain validation[451,453]. Temporal validation involves taking new individuals from the same institutions or study sites that the patients used to design the original model were recruited from over a different, usually later, period of time. Temporal validation allows the generalizability of the model to be assessed over time to determine whether changes in the healthcare system (e.g., new medications, new treatments, changing populations) have an effect on the predictive performance of the models[451]. Alternatively, geographical validation assesses the transportability or generalization of the models to other institutions or countries. Domain validation is a more rigid form of geographic validation that uses individuals that are very different from those used to develop the original model[451]. For example, domain validation may assess the transportability of a model in adults compared to children or in patients with a different medical condition[451].

Temporal Validation of Pharmacogenetic Risk Prediction Models

The original risk prediction models were developed using patients enrolled using active surveillance at multiple pediatric hospitals and academic centers across Canada between July 2005 and June 2010. Implementation of the models into clinical practice then started in July 2013. A temporal validation approach using patients that were enrolled at the same study sites as the originally model between the time that the original model development patients were enrolled and the time that the models were implemented into clinical practice (July 2010-June 2013) **(Figure 3.5)** would allow the models to be validated using similarly treated patients and measure the generalizability of the models in a cross-Canadian population of patients. As these models have been implemented and aim to provide pharmacogenetic information for children with cancer in Canadian hospitals, a validation study conducted in Canadian children would be more appropriate than one conducted in patients from another country (geographical validation) or in patients with vastly different clinical demographics (domain validation).

Between July 2010 and June 2013, 777 and 209 pediatric patients receiving an anthracycline and cisplatin, respectively, were enrolled and eligible to be included in a validation cohort for each risk prediction model. As with the patients included in the original model development, patients were recruited using active surveillance by the Canadian Pharmacogenetics Network for Drug Safety (CPNDS) from 7 pediatric oncology units across Canada (Hospital for Sick Children (Toronto), Winnipeg Health Services Centre (Winnipeg), Children's Hospital of Eastern Ontario (Ottawa), Sainte Justine Hospital (Montreal), Children's Hospital of Western Ontario (London), Alberta Children's Hospital (Calgary), and B.C. Children's Hospital (Vancouver)). Patients were included in the totals presented for eligible validation patients if they were under the age of 19 at the time of their treatment, and they received either an anthracycline or cisplatin as part of their cancer treatment.

Proposed Strategy for Future Model Validation: Anthracycline-induced Cardiotoxicity

Future steps needed to proceed with this external validation would involve the collection of relevant clinical information from each patients' health record including their age, sex, anthracycline dose, concomitant medications, radiotherapy information, and information

pertaining to whether or not they experienced anthracycline-induced cardiotoxicity. Provided the same criteria used to define cardiotoxicity during the development of the model is applied during an external validation, patients should be classified as cases if they had an echocardiogram with a shortening fraction (SF) of ≤26% during or after anthracycline therapy. Additionally, echocardiograms within 21 days of a dose should be excluded to account for the transient acute cardiotoxic effects of anthracyclines[10,215]. Control patients should have normal echocardiograms (SF≥30%) during and after therapy, with enough follow-up time to ensure that both early- (within one year of anthracycline treatment) and the majority of late-onset (one or more years after treatment) cases are captured and not misclassified as controls. A follow-up time of ≥5 years after completion of anthracycline therapy was previously selected for controls used in the development of the risk prediction model as this amount of time was found to capture the majority of cases **(Figure 3.6)**. The median time to developing cardiotoxicity was found to be 1.1 years (IQR: 0.4-5.8 years) in the original model patients. This was similar to a previous study which reported 76% of cases of cardiotoxicity to occur within the first year of anthracycline therapy (median: 0.84 years, range: 0.1 to 20.9 years). Several studies have demonstrated that the risk of cardiotoxicity increases over time[182,248,454-456] with one study citing that the risk of experiencing severe congestive heart failure from anthracyclines increased from 3.3% two years after the start of anthracyclines to 9.8% twenty years after the start of anthracyclines[456]. This highlights the importance of selecting an adequate follow-up time to ensure that patients who develop late-onset cardiotoxicity are not misclassified as controls. Additionally, cases with mild echocardiographic changes characteristic of lower cardiac function (SF≥27-30%) were previously excluded during the development of the model to ensure optimal separation between cases and controls and to account for variability in echocardiographic measurements[457].

Proposed Strategy for Future Model Validation: Cisplatin-induced Ototoxicity

Clinical information from each patients' health record including their age, sex, cisplatin dose, concomitant medications, cranial radiation treatment, and information pertaining to whether or not they experienced cisplatin-induced ototoxicity would need to be collected. Provided that the same clinical characterization process and case definitions used for the development of the pharmacogenetic risk prediction model for cisplatin-induced ototoxicity are used for validation, all subject data should be reviewed blind to genotype data by a clinical

pharmacologist, an audiologist, an oncologist, and an ADR surveillance clinician who reviews and collects audiogram test results from the patients' medical records. Based on the definitions used during model development, patients should be considered cases of ototoxicity if they exhibit grades 2-4 hearing loss based on the CTCAEv4 criteria which confers a minimum hearing loss of >25 dB at frequencies ≥ 4 kHz. Patients should be considered controls if they exhibit normal hearing function between 4-8kHz with an adequate enough follow-up time to prevent the misclassification of late-onset cisplatin-induced ototoxicity cases as controls. As cisplatin is a heavy metal that can remain in circulation in the body for more than 20 years[114], toxicity can sometimes develop after treatment with cisplatin has stopped. An audiometric follow up time of 8 months since the start of cisplatin was used during the development of the model as this was the amount of time it took for 90% of cases to develop ototoxicity **(Figure 3.7)**[393]. During model development, patients exhibiting grade 1 hearing loss at the time of the analysis were excluded to better differentiate between cases and controls. The same strategy may be applied during a validation of the risk prediction model to ensure that similar patient cases and controls are being used to test the model.

3.4.2.2: Sample Size

When multivariate prediction models are developed, the sample size is often based on the ratio of the number of individuals that experience an outcome of interest to the number of candidate predictors, referred to as the events per variable (EPV) [453]. When the number of outcomes is smaller than the number of predictors, there is a risk of overfitting the predictive models[453]. Some studies have suggested, as a general guideline, to select a sample size of at least 10 events (i.e., cases of cardiotoxicity or ototoxicity) per each predictive variable (i.e., predictive genetic variant)[458,459]. Conversely, more recent studies have suggested that an EPV criteria of >10 may be too strict in particular settings, showing several examples where predictive models showed strong predictive performance with an EPV <10[460,461]. Given that some of the predictive variants used in the risk prediction models are rare within the population, a large number of patients may be needed for validation to ensure that enough patients in rare risk groups are included. For example, the incidence of patients who carry both a copy of the *RARG* (rs2229774) risk variant and the *UGT1A6*4* (rs7853758) risk variant (89% risk group), is approximately 0.61% based on genotyping frequencies from the 1000 Genomes Project (PharmGKB) and given

the distribution of ancestries observed in our original risk prediction models (79.2% European, 5.0% Asian, 5.2% Southeast Asian, 2.5% South Asian, 2.0% African, 2.5% First Nations). However, as this genotype combination is associated with a much higher risk of developing cardiotoxicity, by using active surveillance to capture the majority of cases of cardiotoxicity at study sites across Canada, we would expect to see a higher incidence of patients that carry these rare genotype combinations. Based on genotyping frequencies observed in the original model, we would expect 2% of enrolled patients to carry the rarest genotype combination (89% risk group). We would, therefore, need to include approximately 500 patients in the validation cohort for the anthracycline-induced cardiotoxicity risk prediction model to observe 10 patients in the 89% risk group. For the cisplatin-induced ototoxicity risk prediction model, the incidence of carrying a *TPMT* risk variant in the original cohort used to develop the model was 15.6%. Using the same active surveillance process that was used to develop the model, we would require a smaller cohort of <100 patients to observe at least 10 carriers of a *TPMT* variant. As larger sample sizes provide a more reliable assessment of the predictive performance of the model, including as many patients as possible that have clear and well defined clinical data would be optimal during a validation of the risk prediction models. As discussed above, there are 777 patients that received anthracyclines and 209 patients that received cisplatin enrolled through active surveillance that are eligible for inclusion in a validation cohort. Based on the observed frequencies of genotyping combinations, both of these sample sizes should sufficiently capture enough patients in rare genotype groups needed to validate both risk prediction models.

3.4.2.3: Evaluating Predictive Performance

Overall performance measures could be assessed in the validation cohorts by measuring the distance between the predicted outcomes, as determined using the developed risk prediction models, and the actual outcomes that were observed. For binary outcomes, such as those presented in this study, the R^2 or Brier score could be used to measure the predictive performance[462]. The Brier score is a quadratic scoring rule, where the squared differences between actual binary outcomes (Y) and predictions (*p)* are calculated (Y - *p*)[462]. Performance could be further quantified in terms of calibration and discrimination. Calibration is related to the agreement between the predicted and the actual outcome (i.e., do close to *x* of 100 patients with a

risk prediction of *x%* have the outcome?)[463]. A Hosmer-Lemeshow goodness-of-fit test for binary outcomes could be done and visually represented as a calibration plot with the predicted probabilities on the x-axis and the observed outcomes on the y-axis with perfect calibration creating a 45º line[463]. The Hosmer-Lemeshow test output returns a chi-square value and a *p*-value with a *p*-value <0.05 indicating that the model has poor calibration[463].

Discrimination refers to the ability of a prediction model to differentiate between two outcomes (i.e., do patients who have the outcome have higher risk predictions than those who do not?)[462]. Similar to what was previously done to evaluate the discrimination of genetic risk prediction models of both cisplatin-induced ototoxicity[11] and anthracycline-induced cardiotoxicity[10,12], statistical measures, including sensitivity and specificity analysis and receiver operating characteristic (ROC) curves with the area under the ROC curve (AUC), could be used in an external validation of the models. The concordance statistic (c-index), which is mathematically identical to the AUC for binary outcomes, is the most widely accepted measure of discriminatory ability[464]. It can be interpreted as the probability that predicting the outcome using the model is better than by chance. A c-index value of 0.5 indicates that the model has no discriminatory ability while a value of 1.0 indicates that the model has perfect discrimination[464]. In general, a c-index of 0.7 or above is considered adequate, with a strong predictive model having a c-index of >0.8[465]. As mentioned earlier in this chapter, previously developed genetic risk prediction models for cisplatin-induced ototoxicity and anthracycline-induced cardiotoxicity have produced c-index (or AUC) values ranging from 0.7 to 0.81 with even higher values when clinical risk factors were incorporated (c-indices of 0.79 to 0.87). While ROC curves are widely used, the predicted risk can differ from the observed risk even when discrimination is perfect[462,464]. It is, therefore, important to consider both discrimination and calibration when evaluating the predictive performance of the models.

3.4.2.4: Updating Pharmacogenetic Risk Prediction Models

Poorer performance is often observed when prediction models are tested in new individuals compared to the original cohort from which they were developed[403]. This likelihood is increased with more stringent validation techniques such as geographical or domain validation compared to temporal validation, which is another reason that temporal validation may be a preferred

validation strategy[452]. If a lower performance is observed, investigators often reject and create new models; many times using the same methods that were applied during the original model development[452]. This has led to multiple models being created for the same outcomes, causing confusion amongst physicians about which models to use[452]. Additionally, dismissing scientific findings and information gained from the original model developed rejects the notion that inferences to enhance evidence-based medicine should be based on as much information as possible. In order to retain and build upon the risk prediction models already developed, alternative strategies to adjust and recalibrate models based on findings in an external validation have been suggested[404,452]. This would allow the updated models to combine information captured in both the original model and from new individuals in the validation cohort, and improve the transportability and generalizability of the models to other individuals.

A simple updating method could, for instance, focus on re-calibrating the model if there is a difference in outcome frequency (i.e., rates of cardiotoxicity or ototoxicity) observed in the validation cohort compared to the original model cohort[403,404]. This may involve adjusting the intercept and regression coefficients using a correction factor and calibrated slope, respectively (Method 1 and 2) (**Table 3.1)**. The correction factor is added to the intercept from the original model while the regression coefficients from the original are multiplied by the calibration slope[404]. A calibration slope equal to 1 means that the regression coefficients do not need adjustment. This re-calibration approach, known as logistic calibration, has been successfully applied to previously developed models for risk adjustment and prediction[403]. Alternatively, model revision (Method 3) involves re-estimating more parameters in the model to test whether predictors have an effect that is clearly different in the validation cohort[403]. In order to do this, likelihood ratio tests of model extensions are performed in a stepwise manner, considering the predictor with the strongest difference first until all differences in effects have a $p > 0.5$ for each predictor. Model extension (Methods 5 and 6) involves considering additional predictors (i.e., predictive variants) based on the findings in the validation cohort for potential inclusion in an updated model if found to improve the predictive power of the model. For example, as additional predictive variants with strong associations for the development of cisplatin-induced ototoxicity and anthracycline-induced cardiotoxicity are discovered, they may be incorporated and tested in the validation cohort to determine whether they improve the predictive power of the models.

These methods have been successfully used previously to update risk prediction models used in clinical practice, and could be used in future studies aimed at validating and updating the current risk prediction models to improve their predictive power and clinical validity[403,404,451,452].

3.5: Strengths and Limitations of Implementing Pharmacogenetic Risk Prediction Models

Compared to previous studies implementing pharmacogenetic in oncology, which have primarily returned results for individual genetic variants associated with drug response, the pharmacogenetic risk prediction models in this study integrated multiple genetic variants into one predicted risk estimate to be returned to clinicians and integrated into clinical decision-making. This is important because it allowed multiple genetic determinants of the same ADR to be combined to provide an overall estimate of an individual's genetic risk of developing that ADR. This helped to facilitate the integration of pharmacogenetics as an important risk factor in treatment decision-making. Additionally, as opposed to other studies which have largely returned pharmacogenetic results as genotypes (i.e., star allele nomenclatures) with phenotypic interpretations (i.e., metabolizer status), the pharmacogenetic results in this study were returned using a risk stratification graph that highlighted the individual's risk compared to all studied patients. This return of results' format was shown to aid in discussions between clinicians and families about ADRs, and allowed issues surrounding lack of standardized nomenclature and difficulty interpreting results to be avoided.

A key limitation of this study is the number of patients that tested in risk stratification groups containing rare genotypes. For example, the 45% cardiotoxicity risk group included patients who were homozygous carriers of the *RARG* risk variant, which is estimated to occur in 0.4% of European patients. It is, therefore, difficult to assess the impact and long-term outcomes of implementing pharmacogenetic testing in these groups of patients. Additionally, as these ADRs sometimes develop years after treatment (especially in the case of anthracycline-induced cardiotoxicity)[215], patients will need to be monitored continuously to determine whether they experience ototoxicity or cardiotoxicity as more follow-up data becomes available. As allelic frequency, linkage disequilibrium (LD) and confounding environmental factors differ across populations, inclusion of diverse populations in genomic studies is important for evaluating the

accuracy and wider relevance of findings[466]. Genetic variants included in the risk prediction models were discovered and replicated in patient populations of largely European descent (i.e., 80-85% European), which may affect the generalizability of results to patients of other ancestries. While there is a need for further studies examining genetic associations in non-European patients, the association between *RARG* (rs2229774) and anthracycline-induced cardiotoxicity was replicated in African, East Asian, Hispanic and Indigenous Canadian patient populations[204]. Additionally, in a subgroup analysis of non-European patients, *SLC28A3* (rs7853758) remained significantly associated with anthracycline-induced cardiotoxicity[12]. This, in conjunction with strong mechanistic evidence[140,204,467], suggests that the SNPs included in the risk prediction models play an important role in the development of anthracycline-induced cardiotoxicity and cisplatin-induced ototoxicity in European and non-European patients. Given that pharmacogenetics is a rapidly evolving field, new information about genetic associations for these ADRs will continue to emerge. For instance, strong evidence for an association between the gene *ACYP2* and ototoxicity has been discovered and replicated [153,468]. Systematic reviews of the literature to review new evidence and updates of the models to include relevant findings will be necessary to ensure that the risk prediction models are comprehensive and scientifically accurate.

3.6: Conclusions

The value of improving drug safety and effectiveness through pharmacogenetic testing has been recognized as an essential component of precision-medicine for over six decades[48]. With decreasing costs and rapid technological advances for genetic analyses, the number of genetic variants implicated in drug action are much higher than previously thought—further highlighting the promise that pharmacogenetics has in improving drug outcomes and guiding therapeutic decision-making[469]. Clinical implementation of pharmacogenetics has seen significant advances in recent years with many multi-site networks in the United States, Europe and Canada implementing testing in hospitals and academic centers. In 2014, 7% of US hospital were offering pharmacogenetic testing[470], and since then this number has increased substantially. In oncology, specifically, patients stand to benefit highly from pharmacogenetic-informed prescribing decisions given that the therapeutic index of drugs is often narrow and the consequences of drug toxicity can be life-threatening[70]. Implementing pharmacogenetic testing

in oncology that extends beyond somatic tumour genomics and incorporates knowledge about patients' germline variation in drug response should be the new paradigm which we strive for.

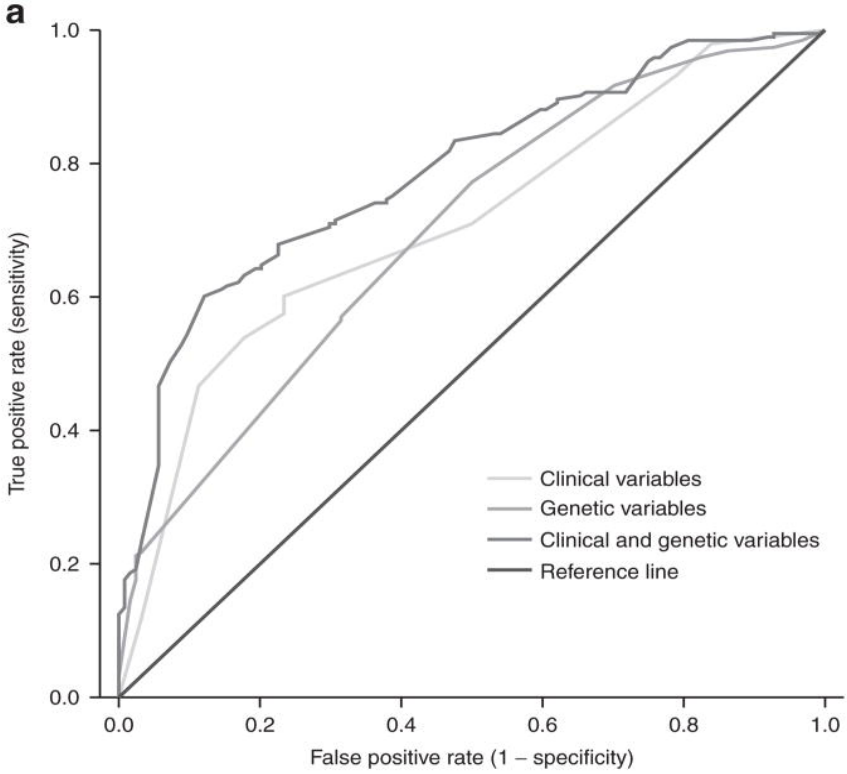
This study describes the implementation of pharmacogenetic risk prediction models for two well known, serious ADRs in pediatric oncology: anthracycline-induced cardiotoxicity and cisplatin-induced ototoxicity. These models incorporated pharmacogenetic variants with strong levels of evidence into one predicted outcome for each tested individual. The use of risk prediction models to return pharmacogenetic results was found to overcome previously-identified barriers towards implementing pharmacogenetics in clinical practice, such as difficulty interpreting and understanding pharmacogenetic results. Test results for each patient were returned to their treating oncologist to allow genomic risk to be incorporated into treatment decision-making. In this way, oncologists and families were able to make more informed decisions about their patient's or child's treatment options taking into consideration genomic risk in addition to clinical risk factors that are already a part of clinical care. Appropriate treatment modifications were made when patients' pharmacogenetic risk of experiencing either anthracycline-induced cardiotoxicity or ototoxicity outweighed the benefits of continuing with the original treatment plan, and included: additional audiological or cardiac monitoring, the use of a protective agent, and selecting an alternative treatment protocol, among others.

Interviews with families and oncologists who received test results from pharmacogenetic risk prediction testing indicated that they found high utility in the results. Oncologists described the information as being useful in facilitating discussions between patients and clinicians about their treatment options and risk, and helped ensure that the most appropriate treatment plan and follow up recommendations were made for each individual patient. Families and patients felt more involved in the discussions and decisions regard theirs or their child's treatment, and felt reassured by understanding their child's genetic risk of experiencing an ADR.

Patients will continue to be monitored prospectively to determine what their long-term drug outcomes are in comparison to patients that didn't receive pharmacogenetic testing. Currently, pharmacogenetic-tested patients have experienced significantly less cardiotoxicity than patients that did not receive testing over the same follow up period (3.4% versus 11.8%, p=0.0005). Rates

of cisplatin-induced ototoxicity in patients that received testing were similar to previously-treated patients used to develop the model (58.9% versus 66.7%, respectively), and none of the patients that have received treatment modifications as a result of pharmacogenetic testing have developed clinically relevant ototoxicity. As enrollment continues and more corresponding follow-up data becomes available, the outcomes and impact of implementing pharmacogenetic risk prediction models will become more apparent. Furthermore, as additional evidence of pharmacogenetic markers of drug response become available, the risk prediction models will be updated to include relevant predictive variants that have strong levels of evidence. Future studies aimed at validating the pharmacogenetic risk prediction models in an independent cohort of patients could be performed to determine the predictive power of the risk prediction models and provide further evidence of their clinical validity and utility. Recognition of the value of pharmacogenetic-guided treatment decision-making and its application into routine clinical care has the ability to improve drug safety and effectiveness—especially in this vulnerable population of patients.

a



b

Model	AUC (95% CI)	*P*-value
Clinical variables	0.708 (0.651–0.765)	–
Genetic variables (three SNPs)	0.696 (0.638–0.754)	0.77
Clinical and genetic variables	0.786 (0.736–0.836)	0.00048

Figure 3.1. Receiver operating characteristic curves of clinical and genetic variables for the prediction of cisplatin-induced ototoxicity in 317 pediatric oncology patients

(**a**) Clinical variables are age, vincristine treatment, germ-cell tumour, and cranial irradiation, whereas genetic variables combine the effect of *TPMT* rs12201199, *COMT* rs4646316, and *ABCC3* rs1051640.
(**b**) The area under the curve (AUC) for the combined cohort for each model. The *p*-values indicate the statistical significance between the curves for the combination of genetic and clinical variables as compared with clinical variables alone. Higher AUC shows better discriminatory ability.
From Pussegoda K, Ross CJ, Visscher H, Yazdanpanah M, Brooks B, Rassekh SR, Zada YF, Dubé MP, Carleton BC, Hayden MR. Replication of TPMT and ABCC3 genetic variants highly associated with cisplatin-induced hearing loss in children. Clinical Pharmacology & Therapeutics. 2013 Aug 1;94(2):243-51. Reprinted with permission from John Wiley and Sons.

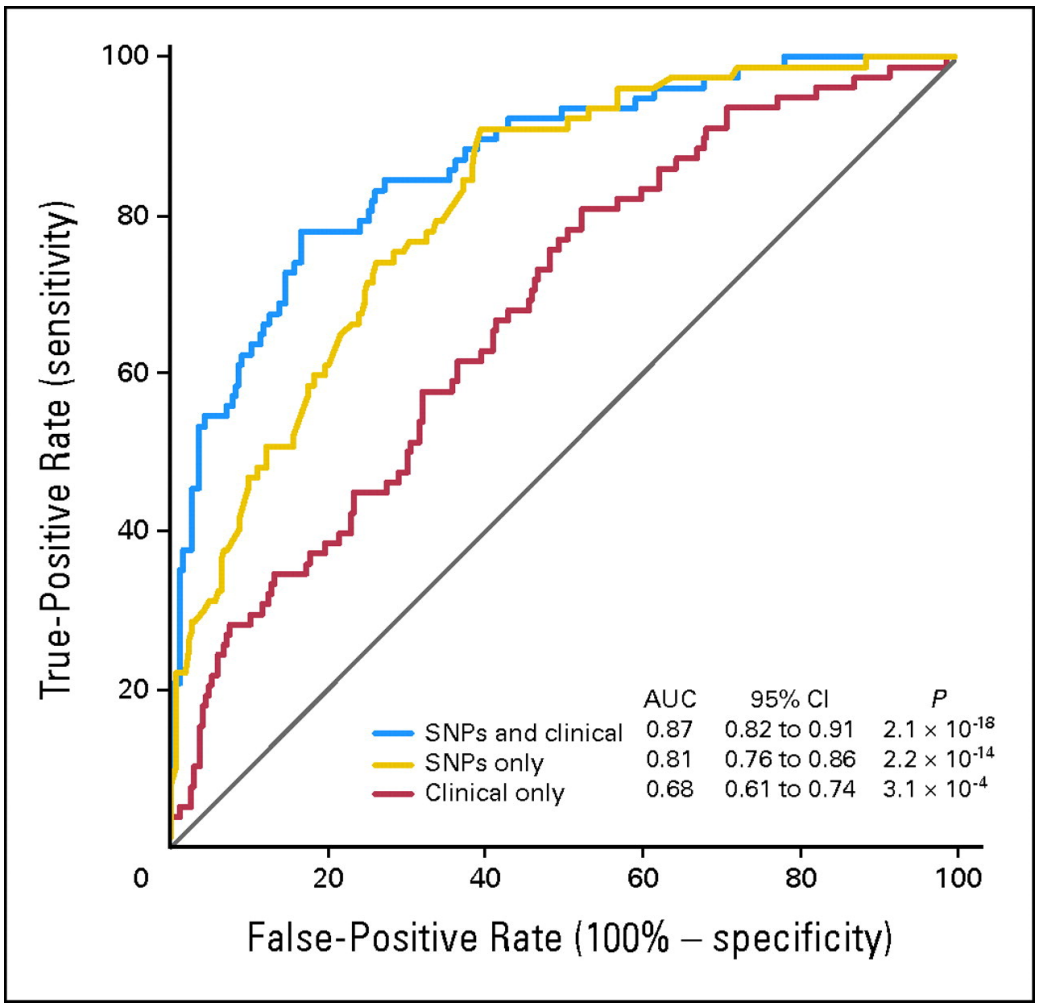


Figure 3.2. Receiver operating characteristic (ROC) curves of three different models to predict risk of anthracycline-induced cardiotoxicity.

ROC curves of three models: (1) genetic and clinical, (2) genetic only, and (3) clinical only. Models were constructed by plotting false positive rate (1-specificity) versus true positive rate (sensitivity). Area under curve (AUC) or c-statistic were calculated as well as *p*-values of full logistic regression models. Higher AUC shows better discriminatory ability.

From Visscher H, Ross CJ, Rassekh S, Barhdadi A, Dubé MP, Al-Saloos H, Sandor GS, Caron HN, van Dalen EC, Kremer LC, van der Pal HJ. Pharmacogenetic prediction of anthracycline-induced cardiotoxicity in children. Journal of clinical oncology. 2012 May 1;30(13):1422-8. Reprinted with permission from the American Society of Clinical Oncology.

Ovid Embase (1946-present) and MEDLINE (1976-present) Search Criteria
1. (anthracycline* or doxorubicin or daunorubicin or epirubicin or idarubicin or valrubicin).ab,ti.
2. (pharmacogen* or genetic* or genom* or gene varia* or genotype* or polymorphism*).ab,ti.
3. (heart or cardi*).ab,ti.
4. 1 and 2 and 3
5. remove duplicates from 4
6. limit 5 to (editorial or letter or note or "review" or short survey or comment) [Limit not valid in Embase,Ovid MEDLINE(R),Ovid MEDLINE(R) Daily Update; records were retained]
7. 5 not 6
8. limit 7 to human
9. Limit year: 2016 to present

ab=abstract, ti=title

Figure 3.3. Systematic review search strategy for the update of clinical practice guidelines for anthracycline-induced cardiotoxicity

Literature search performed using Medline (1946-August 19, 2018) and Embase (1974-August 19, 2018) databases to extract any publications describing genetic associations for anthracycline-induced cardiotoxicity.

Ovid Embase (1946-present) and MEDLINE (1976-present) Search Criteria
1. (ototoxic* or toxic* or hearing or ear* or oto* or audiomet* or adverse event or adverse reaction or adverse effect* or adverse drug or side effect* or deaf*).mp.
2. (cisplatin or cis-platinum* or ddp or cddp or platinol*or platinum* or carboplatin).ab,ti
3. (pharmacogen* or genetic* or genom* or gene varia* or genotype* or polymorphism*).mp
4. 1 and 2 and 3
5. Remove duplicates from 4.
6. Limit 5 to (editorial or letter or note or "review" or short survey or comment)
7. 5 not 6
8. limit 7 to humans
9. limit to year: December 2014 to present

ab=abstract, ti=title

Figure 3.4. Systematic review search strategy for the update of clinical practice guidelines for cisplatin-induced ototoxicity

Literature search performed using Medline (1946-August 19, 2018) and Embase (1974-August 19, 2018) databases to extract any publications describing genetic associations for cisplatin-induced ototoxicity.

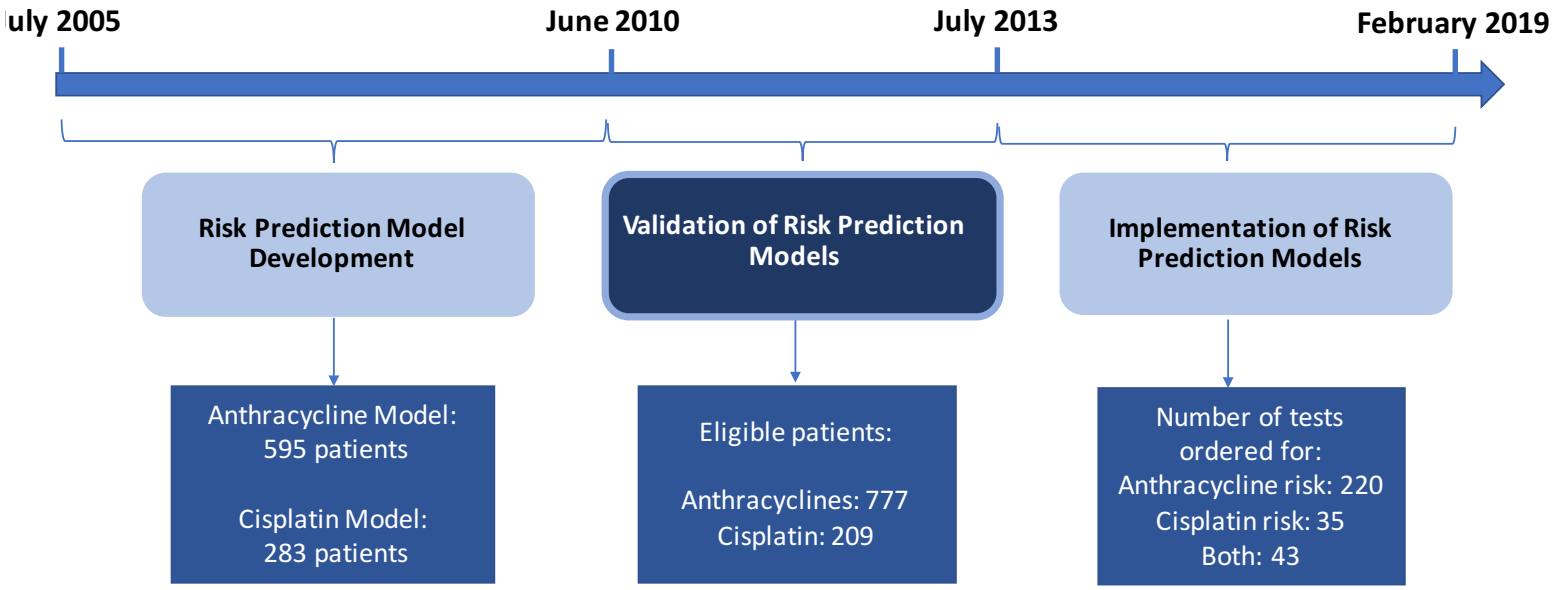
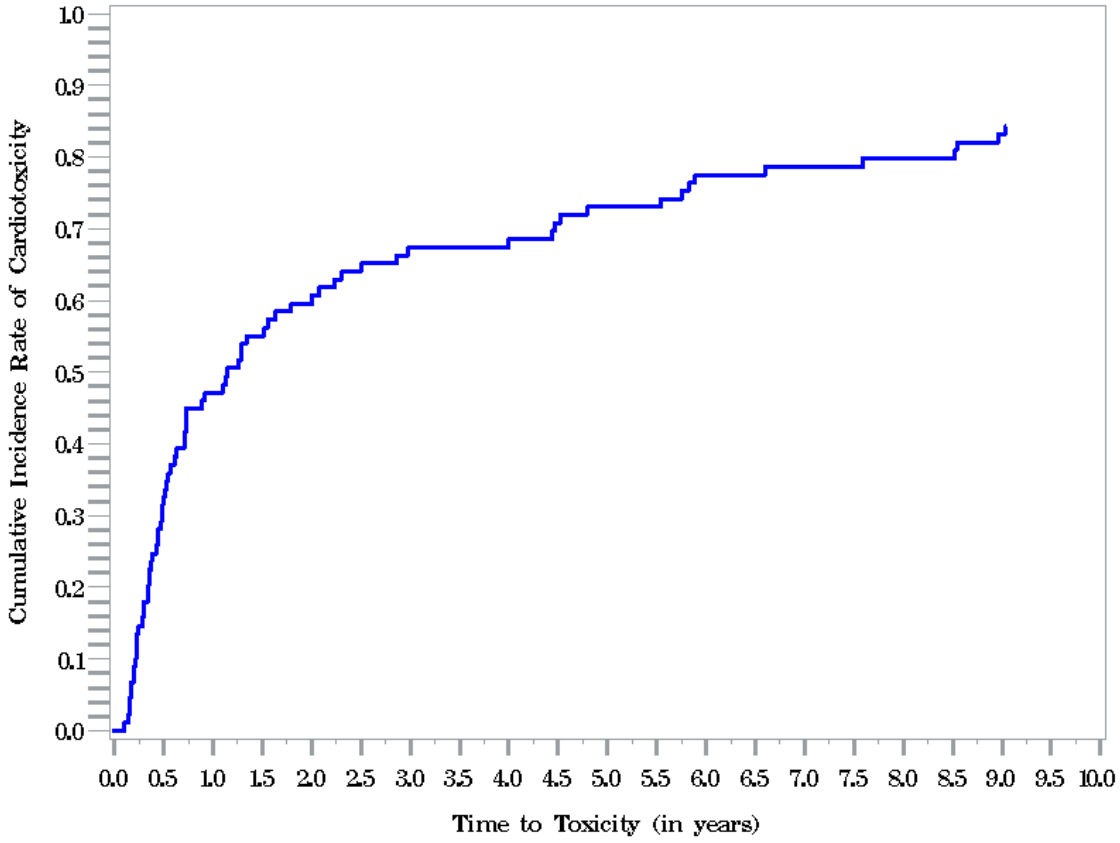


Figure 3.5. Timeline of patient selection for pharmacogenetic risk prediction model development, implementation, and potential future temporal external validation

Patients were enrolled using active surveillance between July 2005-June 2010 were included in the development of pharmacogenetic risk prediction models. Pharmacogenetic testing using risk prediction models was implemented into clinical practice in July 2013. Patients enrolled through active surveillance between the time of model development and the time that models were implemented into clinical practice provide a potential cohort for external validation of the risk prediction models using a temporal validation strategy.



	Number of Patients	Minimum Time (years)	Maximum Time (years)	Mean	Standard Deviation	Median	Lower Quartile	Upper Quartile
Cases	89	0.1	18.2	3.7	4.9	1.1	0.4	5.8

Figure 3.6. Kaplan-Meyer analysis of the time to cardiotoxicity (SF≤26%) for cases used in the development of the pharmacogenetic risk prediction model of anthracycline-induced cardiotoxicity

Time to toxicity was defined as the time between the date of the first dose of anthracyclines and the first echocardiogram with a shortening fraction of ≤26%.

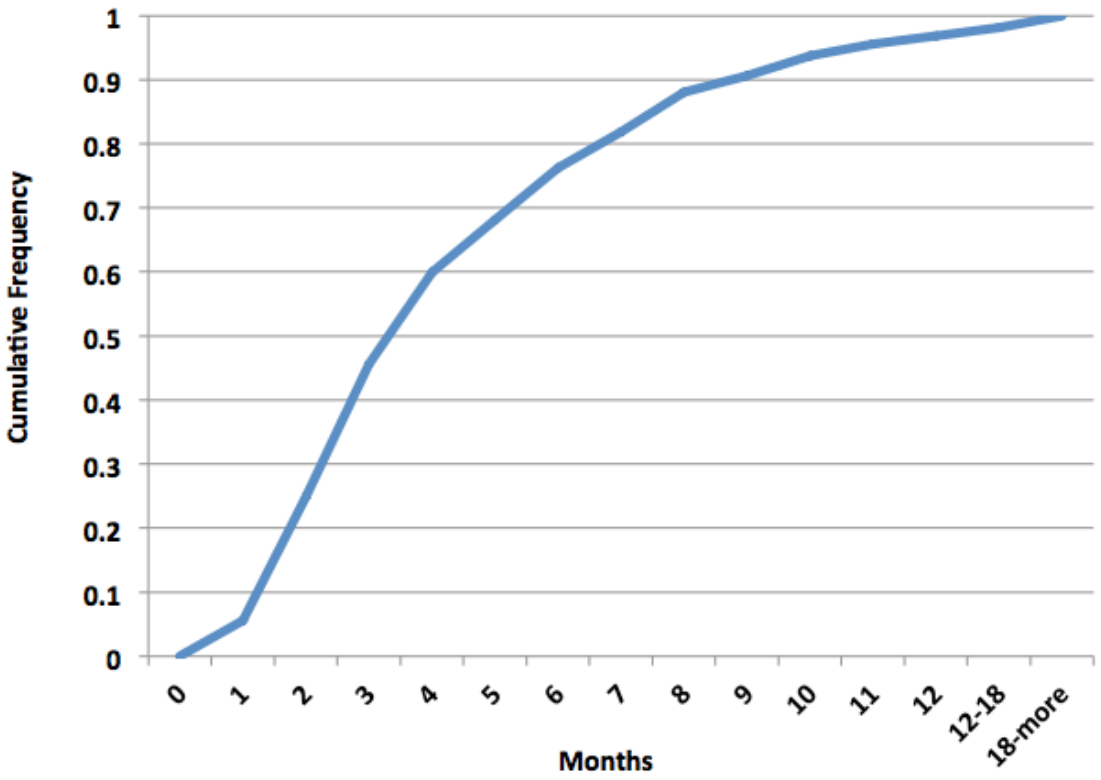


Figure 3.7. Kaplan-Meyer Analysis of the Time to First Audiometric Symptoms of Grade 2 or Higher Ototoxicity in Cases of Cisplatin-treated Pediatric Patients (n=160)

Audiogram dates indicating the first sign of hearing loss were used to identify time to ototoxicity. Patients that had no audiogram data within the first 6 months of treatment were excluded from this analysis as the specific date ototoxicity first occurs could not be accurately captured (n=28). From Lee, J. W. (2014). *A genome-wide association study of cisplatin-induced hearing loss in children* (T). University of British Columbia. Retrieved from https://open.library.ubc.ca/collections/ubctheses/24/items/1.0167553.

Table 3.1. Potential Methods for Updating Risk Prediction Models

Method	Updating method	Reason for updating
0	No adjustment (the original prediction model)	—
1	Adjustment of the intercept (baseline risk)	Difference in the outcome frequency (prevalence or incidence) between development and validation sample
2	Method 1 + adjustment of all predictor regression coefficients by one overall adjustment factor	Regression coefficients of the original model are overfitted (or underfitted)
3	Method 2 + extra adjustment of regression coefficients for predictors with different strength in the validation sample as compared with the development sample	As in method 2, and the strength (regression coefficient) of one or more predictors may be different in the validation sample
4	Method 2 + stepwise selection of additional predictors	As in method 2, and one or more potential predictors were not included in the original model, or a newly discovered marker may need to be added
5	Re-estimation of all regression coefficients, using the data of the validation sample only	The strength of all predictors may be different in the validation sample, or the validation sample is much larger than the development sample
6	Method 5 + stepwise selection of additional predictors	As in method 5, and one or more potential predictors were not included in the original model

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