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A Canadian Study of Cisplatin Metabolomics and Nephrotoxicity (ACCENT): A Clinical Research Protocol

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Abstract

Background: Cisplatin, a chemotherapy used to treat solid tumors, causes acute kidney injury (AKI), a known risk factor for chronic kidney disease and mortality. AKI diagnosis relies on biomarkers which are only measurable after kidney damage has occurred and functional impairment is apparent; this prevents timely AKI diagnosis and treatment. Metabolomics seeks to identify metabolite patterns involved in cell tissue metabolism related to disease or patient factors. The A Canadian study of Cisplatin mEtabolomics and NephroToxicity (ACCENT) team was established to harness the power of metabolomics to identify novel biomarkers that predict risk and discriminate for presence of cisplatin nephrotoxicity, so that early intervention strategies to mitigate onset and severity of AKI can be implemented.

Objective: Describe the design and methods of the ACCENT study which aims to identify and validate metabolomic profiles in urine and serum associated with risk for cisplatin-mediated nephrotoxicity in children and adults.

Design: Observational prospective cohort study.

Setting: Six Canadian oncology centers (3 pediatric, 1 adult and 2 both).

Patients: Three hundred adults and 300 children planned to receive cisplatin therapy.

Measurements: During two cisplatin infusion cycles, serum and urine will be measured for creatinine and electrolytes to ascertain AKI. Many patient and disease variables will be collected prospectively at baseline and throughout therapy. Metabolomic analyses of serum and urine will be done using mass spectrometry. An untargeted metabolomics approach will be used to analyze serum and urine samples before and after cisplatin infusions to identify candidate biomarkers of cisplatin AKI. Candidate metabolites will be validated using an independent cohort.

Methods: Patients will be recruited before their first cycle of cisplatin. Blood and urine will be collected at specified time points before and after cisplatin during the first infusion and an infusion later during cancer treatment. The primary outcome is AKI, defined using a traditional serum creatinine-based definition and an electrolyte abnormality-based definition. Chart review 3 months after cisplatin therapy end will be conducted to document kidney health and survival.

Limitations: It may not be possible to adjust for all measured and unmeasured confounders when evaluating prediction of AKI using metabolite profiles. Collection of data across multiple sites will be a challenge.

Conclusions: ACCENT is the largest study of children and adults treated with cisplatin and aims to reimagine the current model for AKI diagnoses using metabolomics. The identification of biomarkers predicting and detecting AKI in children and adults treated with cisplatin can greatly inform future clinical investigations and practices.

Abrégé

Contexte: Le cisplatine, un agent utilisé en chimiothérapie pour traiter les tumeurs solides, entraîne de l'insuffisance rénale aiguë (IRA); un facteur de risque connu de néphropathie chronique et de mortalité. Le diagnostic de l'IRA repose sur des biomarqueurs qui ne sont mesurables qu'après l'apparition d'une lésion rénale et d'une déficience fonctionnelle; ce qui empêche le diagnostic et le traitement précoce de la maladie. La métabolomique s'efforce d'établir le profil des

métabolites impliqués dans le métabolisme des tissus cellulaires en relation avec des facteurs liés à la maladie ou au patient. Une étude canadienne portant sur la métabolomique et la néphrotoxicité du cisplatine (ACCENT) s'est amorcée, elle explore la puissance de la métabolomique dans l'identification de nouveaux biomarqueurs permettant de prédire le risque de néphrotoxicité du cisplatine et d'en distinguer la présence. L'objectif étant de mettre en œuvre des stratégies d'intervention précoce, dès l'apparition de l'IRA, et de limiter la gravité de la maladie.

Objectifs: Décrire la conception et la méthodologie de l'étude ACCENT. Cette étude vise à établir et à valider des profils métabolomiques, dans l'urine et le sérum, associés au risque de néphrotoxicité médiée par le cisplatine chez les enfants et les adultes.

Type d'étude: Étude de cohorte prospective.

Cadre: Six centres canadiens d'oncologie (trois centres pédiatriques, un centre pour adultes et deux centres mixtes).

Sujets: L'étude porte sur 300 adultes et 300 enfants pour qui un traitement par cisplatine est prévu.

Mesures: L'IRA sera confirmée par mesure de la créatinine et des électrolytes dans le sérum et l'urine au cours de deux cycles de perfusion de cisplatine. De nombreuses variables relatives au patient et à la maladie seront recueillies prospectivement avant et pendant le traitement. Les analyses métabolomiques des échantillons de sérum et d'urine seront effectuées par spectrométrie de masse. Une approche métabolomique non ciblée sera utilisée pour analyser les échantillons avant et après les perfusions de cisplatine pour identifier les biomarqueurs candidats d'une IRA découlant du traitement par cisplatine. Les métabolites candidats seront validés dans une cohorte indépendante.

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Méthodologie: Les patients seront recrutés avant le premier cycle de cisplatine. Le sang et l'urine seront recueillis à des moments précis, soit avant et pendant le traitement; plus précisément lors de la première perfusion, puis d'une perfusion subséquente au cours du traitement contre le cancer. Le principal critère d'évaluation est la présence d'IRA, laquelle sera établie selon la définition classique fondée sur la mesure de la créatinine sérique et d'une autre définition fondée sur les anomalies électrolytiques. Un examen des dossiers trois mois après la fin du traitement par cisplatine sera effectué afin de documenter la santé rénale et la survie des patients.
Limites: Il pourrait être impossible de corriger tous les facteurs confusionnels mesurés et non mesurés lors de l'évaluation de la prédiction de l'IRA à l'aide de profils de métabolites. La collecte de données sur plusieurs sites sera un défi.
Conclusion: ACCENT est la plus vaste étude portant sur des enfants et des adultes traités avec le cisplatine; cette étude tente de revoir le modèle actuel en utilisant la métabolomique pour diagnostiquer l'IRA. L'identification de biomarqueurs permettant de prédire et de détecter l'IRA chez les enfants et les adultes traités par cisplatine pourrait grandement éclairer les futures études et pratiques cliniques.
Renseignements sur l'enregistrement de l'essai clinique: ClinicalTrials.gov, insuffisance rénale induite par le cisplatine, NCT04442516

Keywords
acute kidney injury, metabolomics, cisplatin nephrotoxicity, pediatrics, cohort study

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Introduction

Cisplatin is a commonly used chemotherapeutic agent for multiple adult and pediatric cancers.[1] One of the most common adverse effects of cisplatin is nephrotoxicity.[2,3] Acute kidney injury (AKI) occurs in up to 30% of children and 33% of adults treated with cisplatin.[4,5]AKI is an established risk factor for short- and long-term morbidity, chronic kidney disease (CKD), cardiovascular disease, and mortality in adults.[6-14] In the Applying Biomarkers to Minimize Long-Term Effects of Childhood/Adolescent Cancer Treatment (ABLE) Nephrotoxicity Study, a national cohort of children treated with cisplatin (n = 159), 40% showed signs of CKD at 12-months post cisplatin therapy (unpublished data).[15] CKD is an established potent cardiovascular risk factor.

A challenge in detecting nephrotoxicity is the suboptimal performance of current diagnostic methods. Currently, the clinical diagnosis of AKI is based on acute serum creatinine (SCr) rise and decreased urine output—biomarkers only evident after kidney damage has progressed to functional impairment whereupon mitigation strategies are ineffective.[16] Biomarkers that can predict cisplatin nephrotoxicity risk or diagnose nephrotoxicity early may substantially increase the opportunity for early intervention, which might mitigate AKI severity and its long-term effects.

Metabolomics seeks to identify metabolite patterns involved in cell or tissue metabolism related to disease or patient factors like lifestyle and genetics. Unlike proteomics, metabolomics represents the end-products of processes occurring due to alterations of the genome and proteome. Serum and urine are ideal for sampling the metabolome and can reveal disease-related changes earlier than existing diagnostic methods.[17] As cancer survival improves, chemotherapy-associated morbidity prevention is important to improve quality of life in survivors. For cisplatin, tests that could predict AKI risk or diagnose AKI before significant functional loss may afford opportunities for prevention or early intervention including individualized dosing protocols to mitigate cisplatin nephrotoxicity.[18]

We describe the design and methods of ACCENT (**A** **C**anadian study of **C**isplatin m**E**tabolomics and **N**ephro**T**oxicity), a multidisciplinary, prospective observational cohort study using metabolomics to identify urine and serum metabolite profiles that could predict the risk for cisplatin-induced AKI and identify early-stage cisplatin-mediated nephrotoxicity in children and adults treated for cancer.

Study Team and Infrastructure

ACCENT is a Canada-wide, multicenter study leveraging a network of experts (Nominated Principal Applicant is Dr Brad Urquhart [clinical pharmacologist, University of Western Ontario (UWO)]; co-Principal Applicants are Dr Michael Zappitelli and Dr Tom Blydt-Hansen [pediatric nephrologists, University of Toronto and University of British Columbia, respectively]). Three core groups led by the principal investigators will oversee (1) metabolomics analysis, (2) data/biorepository & central study coordination, and (3) data & statistical analysis. The main coordinating center is at The Hospital for Sick Children (SickKids) in Toronto, ON, Canada where the database and study specimens will be housed. The organization of collaborators, their roles, and site investigators are shown in Figure 1. Principal investigators and their study coordinators meet biweekly to discuss study roadmap, track progress, and troubleshoot.

Rationale

Cisplatin-mediated kidney tubule injury often causes AKI, characterized by decreased glomerular filtration rate (GFR),

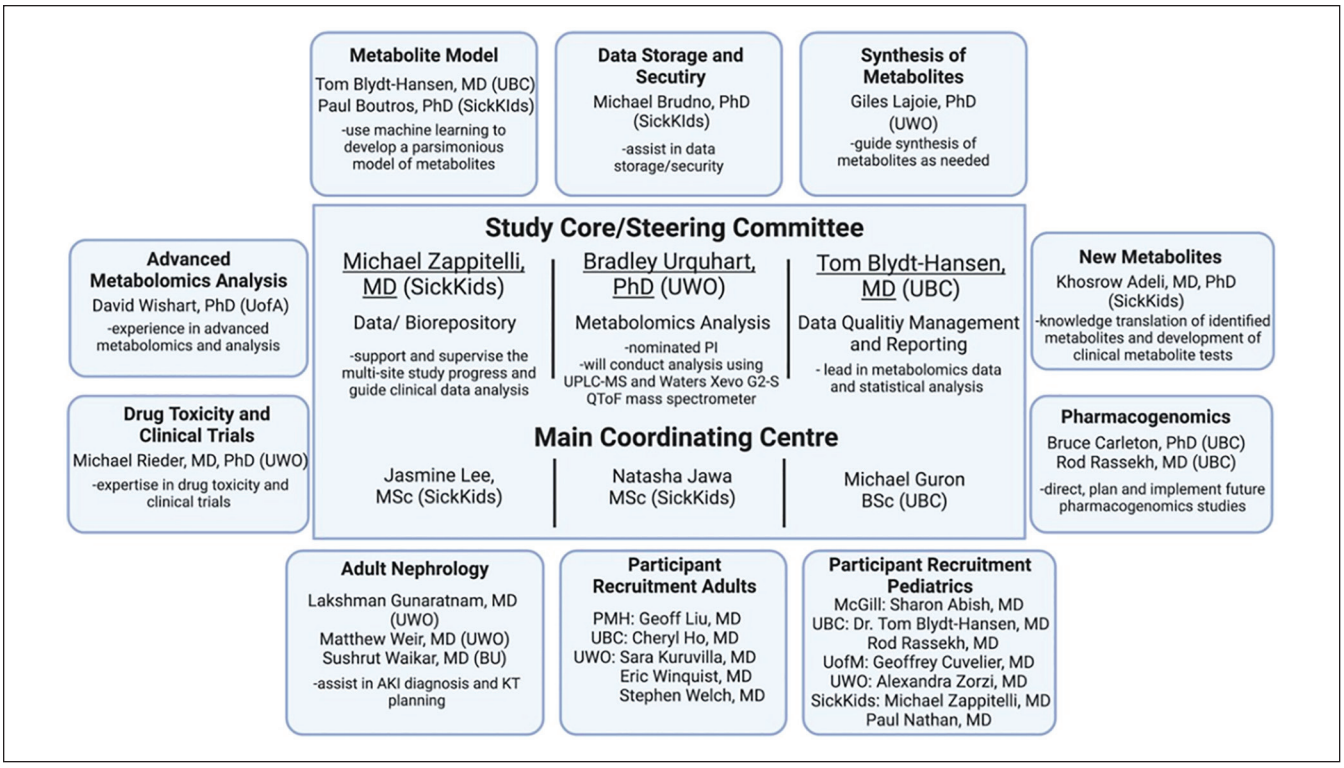

Figure 1. Steering committee, participating centers, and collaborators.
Note. The figure demonstrates the individuals involved in each section of the project and their locations. UBC, University of British Columbia; UWO, University of Western Ontario; UofA, University of Alberta; UofM, University of Manitoba; PMH, Princess Margaret Hospital; BU, Boston University. This figure was created with BioRender.com.

hypertension, and sometimes need for dialysis. Proximal tubule injury from cisplatin also manifests as polyuria and electrolyte wasting (potassium, magnesium, phosphate).[3,19-21] Although higher dose, very young age, and female sex are known risk factors for cisplatin-mediated tubule injury, accurate prediction of patients who will develop nephrotoxicity remains elusive.[22-24]

Cisplatin AKI mechanisms. The majority of cisplatin is eliminated by the kidneys. Proximal tubule cells are a major site of cisplatin accumulation and injury, mediated by several transport proteins (Figure 2).[25,26] Organic cation transporter 2 (OCT2) and copper transporter 1 (CTR1) on the basolateral membrane mediate cisplatin uptake from blood into proximal tubule cells.[27-30] Cisplatin is metabolized to reactive thiol metabolites [GSH-Pt, Cys-Gly-Pt, NAC-1] which are substrates of organic anion transporters (OAT1 and OAT3).[25,31,32] Cisplatin and its metabolites induce necrosis of proximal tubule cells by causing pro-inflammatory cytokine release, endoplasmic reticulum stress, mitochondrial damage, and generation of reactive oxygen species.[33]

In addition, the multidrug and toxin extrusion protein (MATE1) and multidrug resistance associated protein (MRP2) mediate efflux of cisplatin and its metabolites into the tubule lumen.[26,34] Modulation of these transporters plays

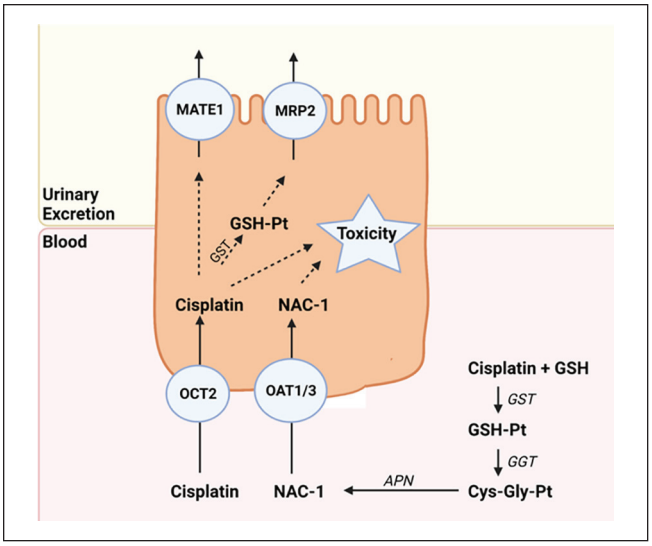

Figure 2. Schematic of proposed proximal tubule cisplatin toxicity.
Note. GST = glutathione S-transferase; GGT = γ glutamyl transpeptidase; APN = aminopeptidase.[24,25,27,33] This figure was created with BioRender.com.

a critical role in cisplatin AKI, by determining tubule exposure to cisplatin. These transporters have endogenous small

Table 1. Cisplatin AKI Prevalence and Timing During Cisplatin Treatment in the ABLE Cohort.

	n with AKI (%)			
AKI definition	Early CisP cycle	Later CisP cycle	Early or later	Early & later
KDIGO (SCr)	48 (30%)	23 (16%)	59 (37%)	12 (8%)
NCI (electrolyte)	106 (67%)	100 (70%)	134 (84%)	72 (143%)
Both KDIGO and NCI	32 (20%)	16 (11%)	41 (26%)	7 (5%)
Post-CisP day of AKI	Median (minimum, maximum)			
KDIGO (SCr)	2 (1 to 5)	2 (1 to 4)		
NCI (electrolyte)	1.5 (1 to 3)	1 (1 to 2)		

Note. ABLE study (159 children). Kidney Disease: Improving Global Outcomes (KDIGO): SCr-definition; National Cancer Institute (NCI): electrolyte-based definition. AKI = acute kidney injury; ABLE = Applying Biomarkers to Minimize Long-Term Effects; SCr = serum creatinine.

molecule substrates whose concentrations can impact cisplatin transport and toxicity. For example, the metabolite trimethylamine *N*-oxide (TMAO) is a substrate of OCT2, while indoxyl sulfate, *p*-cresyl sulfate, phenyl sulfate, and hippuric acid are OAT transporter substrates.[35-39] In animal models, cisplatin AKI can be detected based on altered urinary TMAO, indoxyl sulfate, and hippuric acid concentrations.[24,40-45] Interindividual variability in cisplatin transporter activity may underlie toxicity and can be characterized by examining serum and urine metabolite patterns of transporter substrates. Interindividual differences in levels of metabolites that are substrates for cisplatin transporters may help identify individualized risk of cisplatin AKI.[46-50]

Proximal tubule mitochondria are a primary target of cisplatin nephrotoxicity and cisplatin has been shown to alter metabolites associated with oxidant stress (eg, glutathione) and mitochondrial dysfunction (eg, fatty acids) in response to injury.[51,52] Metabolites associated with oxidant stress or mitochondrial dysfunction may be metabolic markers of early nephrotoxicity.

Need for predictive cisplatin AKI biomarkers. In ABLE, onset of AKI with elevated SCr occurred in children within 3 days following cisplatin infusion (Table 1), consistent with timing of AKI evolution in other injury settings.[15,16] By this time, intervention to mitigate SCr rise due to tubule injury, the most common reason for clinically significant AKI, is limited to supportive care, due to established tissue damage and impaired kidney function.[16,53]

There is international consensus that earlier, more specific AKI diagnosis is needed to identify early harm mitigating treatments (eg, conservative treatments including altering cisplatin dosing or timing, active nephrotoxic medication avoidance, or hydration protocols; experimental treatments including pharmacotherapy from animal studies described in the references).[16,18,30,54-62] Cisplatin injury occurs through a known mechanism, providing a unique opportunity to identify predictive markers. Although cisplatin nephrotoxicity has not been studied using metabolomic techniques in humans, pre-clinical studies identified metabolites that were detectable before SCr rise (Table 2).[41]

Metabolomic approaches have been applied in humans to determine pretreatment risk of drug toxicity for other chemotherapies like capecitabine and improved prediction of drug efficacy or toxicity in several other diseases.[64] Likewise, ACCENT is designed to identify metabolites involved in cisplatin AKI that allow diagnosis before SCr rise.

Methods

ACCENT Hypotheses and Study Aims

Hypothesis 1: Urine and serum metabolite profiles will be identified that *predict the risk* of cisplatin AKI.
Hypothesis 2: Urine and serum metabolite profiles will be identified, including markers of oxidant stress and mitochondrial dysfunction that *diagnose* cisplatin AKI prior to the reference standard clinical evidence of AKI (SCr rise and/or electrolyte abnormalities).

To address the two hypotheses, the following aims will be completed:

1. *Identify* patterns of metabolites and specific metabolites prior to and shortly after cisplatin treatment that predict AKI risk and identify the onset of AKI early (discovery cohort).
2. To *independently validate* the findings and develop a precision medicine algorithm using metabolites to predict patients at high risk for developing cisplatin AKI (validation cohort).

Study Design and Setting

This is a prospective, multicenter, observational cohort study in 300 adults and 300 children treated with cisplatin. This study includes 6 centers (3 pediatric; 2 combined pediatric and adult; 1 adult). Each participating center is led by the site investigator, a clinician supervising study recruitment (Table 3).

Table 2. Non-Exhaustive Summary of Known Metabolites Related to Cisplatin Nephrotoxicity.

Target	Metabolite	Pathophysiology	Study design
OCT2	TMAO, dopamine, tryptophan	OCT2 is involved in cisplatin uptake.[30,63] Metabolites found to be upregulated with nephrotoxicity.[24,39,40]	Transporter knockout mice study.
OAT1, OAT3	Uraemic toxins: hippuric acid, indoxyl sulfate	OAT-1/OAT3 is responsible for the cellular uptake of these organic anions which have been shown to concentration dependently stimulate cellular free radical production.[35–37]	Renal proximal tubular cell line, opossum kidney (OK) cells, was transformed with human OAT1. Analyzed uptake of organic anions in transformed cells.[37]
	p-cresyl sulfate (PCS)	The serum levels of PCS are ~30 times higher than in healthy subjects as renal dysfunction progresses, OAT1/OAT3 are involved in the uptake of PCS.[38]	Uptake of PCS was investigated using rat renal cortical slices and human proximal tubular cells.[38]
	kynurenine, pantothenic acid, cyclic nucleotides, phenyl sulfate	OAT1 mediates transport of these metabolites which are toxins associated with renal failure and uremia.[36]	Untargeted metabolomics on the plasma and urine from OAT1 knockout mice used to identify metabolites. A pharmacophore model based on several identified Oat1 substrates was used to screen the National Cancer Institute database and candidate compounds interacting with Oat1 were validated in an in vitro assay.[36]
MATE1, MRP2	glutathione, bilirubin	Transgenic expression of human MRP2 gene in knockout mice reduces cisplatin accumulation. Kidneys from naive Mrp2-null mice had elevated glutathione S-transferase mRNA levels, which could increase the formation of cisplatin-glutathione conjugates that may be metabolized to toxic thiol intermediates.[26]	Transgenic expression of Mrp-transporter in MRP2 null mice.
Amino acid transport	alanine, valine, leucine, methionine	Metabolites found on analysis of urine after cisplatin induced acute renal failure. Aminoaciduria may be explained by the effects of cisplatin on amino acid transporters.[41]	Metabolomic study of cisplatin-induced nephrotoxicity.

Note. Experiments conducted with animal models..

Table 3. Participating Sites of the Cisplatin Metabolomics Study.

Participating site (hospital)	Site investigator	Site coordinator	City
Pediatric cohort (N = 141 over 3 years)			
Hospital for Sick Children	Dr Michael Zappitelli/Dr Paul Nathan	Grace Tran	Toronto
McGill University	Dr Sharon Abish	Dominique Lafreniere	Montreal
University of British Columbia	Dr Tom Blydt-Hansen/Dr Rod Rassekh	Ritu Ratan	Vancouver
University of Manitoba	Dr Geoffrey Cuvelier	Krista Meuller Rebekah Hiebert	Winnipeg
University of Western Ontario	Dr Alexandra Zorzi	Barbara Murray Awatif Abuzgaia	London
Adult cohort (N = 300 over 2 years)			
Princess Margaret	Dr Geoff Liu	Devalben Patel Khaleeq Khan	Toronto
University of British Columbia	Dr Cheryl Ho	Aria Shokoohi	Vancouver
University of Western Ontario	Dr Sara Kuruvilla Dr Eric Winquist Dr Stephen Welch	Kathie Baer Robin Sachdeva	London

Site coordinators will conduct study visits and be trained via teleconference by the main coordinating center before initiating study activities.

Following patient recruitment, untargeted metabolomics using liquid chromatography mass spectrometry (LC-MS) will be performed on serum and urine from 200 adults and

Table 4. Inclusion and Exclusion Criteria for Nephrotoxic Medications.

	Child cohort	Adult cohort
Inclusion criteria	• Greater than 3 months of age • Less than 18 years of age • Initiating cisplatin treatment at pediatric participating sites. • Consent/assent to participate in the study	• 18 years of age or older • Initiating cisplatin treatment (≥70 mg/m^2) for head/neck or lung cancers at one of the adult participating sites
Exclusion criteria	• Baseline diagnosis of CKD • Previous use of nephrotoxic medications in the 2 weeks leading up to cisplatin treatment (see "Prohibited Nephrotoxic Medications List") • Previous use of cisplatin • Radiotherapy (total body irradiation or abdominal radiation only) in the 1 month prior to the study • Previous hematopoietic stem cell transplant • Any chronic or acute health condition that the investigator feels would render the patient inappropriate for this study, including but not limited to significant uncontrolled cardiorespiratory, hepatic, infectious, or renal disease at the discretion of the investigator	

Prohibited Nephrotoxic Medications List:
- Acyclovir
- Aminoglycosides (including gentamycin, tobramycin, and amikacin)
  - Not inhaled tobramycin, no creams or ointments
- Amphotericin B
- Caspofungin
- Carboplatin
- Cidofovir
- Previous Cisplatin
- Cyclophosphamide
- Cyclosporine
- Nonsteroidal anti-inflammatory drugs (NSAIDs)
- Ifosfamide
- Methotrexate
- Trimethoprim
- Tacrolimus
- Vancomycin

Note. CKD = chronic kidney disease.

200 children to identify metabolite profiles associated with prediction or early detection of cisplatin AKI (discovery cohort). Based on the identified metabolites, a targeted fully quantitative, LC-MS metabolomic analysis of serum and urine for the remaining 100 adults and 100 children will follow for independent validation (validation cohort).

AKI definition. AKI will be primarily defined by the Kidney Disease: Improving Global Outcomes (KDIGO) definition: SCr rise by ≥26.5 μmol/L (0.3 mg/dL) within 48 hours or to ≥1.5 times baseline, or oliguria.[54] However, this definition, developed for critical care patients, does not consider specific features of cisplatin AKI including electrolyte wasting. The two AKI phenotypes (SCr rise, electrolyte disturbance) may represent different injury types; a recent review concluded that the cisplatin AKI definition should include electrolyte criteria.[15] The National Cancer Institute (NCI) has published an electrolyte disturbance definition based on which we propose a new definition of AKI which comprises electrolyte disturbances.[65] Therefore, we will classify cisplatin AKI by

a. KDIGO definition (SCr-based, primary),
b. Modified NCI electrolyte disturbance definition (electrolyte-based, ≥Grade 1 NCI-AKI, defined as $Mg^{2}+$, PO_4^{3-} or K^+ below age-appropriate values), or
c. Both SCr and electrolyte criteria.

Study Participants and Recruitment

Children and adults will be recruited to address known differences in drug response with age.[66] Previous regular use of any nephrotoxic medications included on the provided "Prohibited Nephrotoxic Medications List" in the 2 weeks prior to first cycle of cisplatin treatment will render them ineligible to participate. These medications and other inclusion and exclusion criteria are outlined in Table 4. The screening log describing our patient selection approach is shown in Appendix. To minimize effects of instrument drift and other confounders, all 600 patients will be recruited, and AKI phenotype determined *before* metabolomics analysis. After AKI classification, participants will be randomly split into discovery and validation cohorts for analysis.

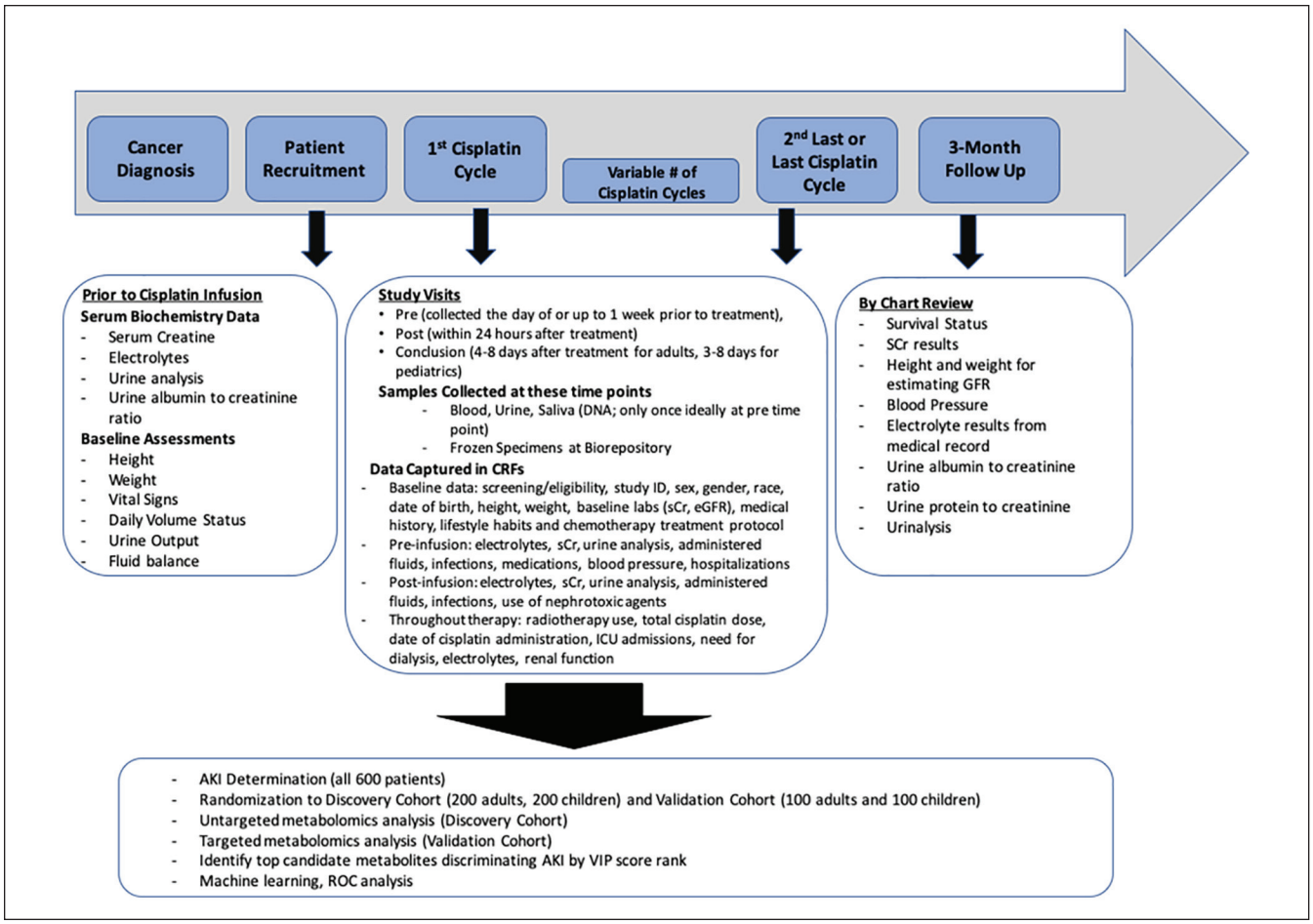

Figure 3. Overview of experimental design.
Note. DNA is captured only once at the first cisplatin cycle. Both blood and urine are collected 6 times during the study (3 collection time points for each of 2 cisplatin cycles).

Child cohort. ABLE was a 12-center cohort study, in which inclusion criteria, study procedures, and data collected closely resemble the present study. Consent was previously provided by the 159 ABLE participants (80 males, 79 females) to use specimens for biomarker discovery. The remaining children will be recruited over 3 years to reach a target of 300. Collection time points for blood and urine occur during two cisplatin infusions for both studies. However, ABLE participants could be recruited prior to their second cisplatin cycle; in ACCENT, only cisplatin-naive patients will be recruited. In total, 91 of the 159 ABLE study participants were cisplatin naive.[4] Five ABLE sites will return to participate in ACCENT.

Adult cohort. A total of 300 adults will be recruited over 2 years. A pilot and feasibility study initiated at Western University (Urquhart) has collected data and samples on 30 patients to date.

Ethics Approval and Consent to Participate

This study is being conducted in accordance with the World Medical Association Declaration of Helsinki. Written informed consent (or assent) will be obtained from all participants, after cancer diagnosis and before cisplatin treatment. Ethics approval was granted to participating centers in one of three ways: (1) SickKids provided overarching ethics approval for pediatric centers (REB Number: 1000071380). Once the application was approved at SickKids, submissions were made to ethics bodies at other pediatric sites. (2) We took advantage of the streamlined review process offered by the Ontario Cancer Research Ethics Board (OCREB) for our Ontario adult sites (CTO Project ID: 3269). OCREB services Ontario hospitals/cancer centers conducting oncology trials. Upon OCREB approval, participating centers may submit their abbreviated center-specific application for expedited approval. (3) The British Columbia (BC) adult site received ethics approval through their center.

Sample and Data Collection

Figure 3 summarizes the study protocol and data/biospecimen collection. The two cisplatin cycles anchoring study procedures are the first cycle (when participants are cisplatin-naive) and a later cycle (last or second-last cycle).

Standard clinical monitoring during cisplatin. Standard of care assessments include serum biochemistry assays to screen for AKI and electrolyte disturbances. Routine monitoring of AKI, acidosis, and hypophosphatemia may sometimes include urinary electrolyte monitoring. Other routinely performed clinical assessments include baseline assessment of height, weight, vital signs, daily volume status, urine output, and fluid balance. This study will record these standard of care values.

Data will be captured in case report forms (CRFs). CRFs were designed by the steering committee and refined following feedback from site investigators and coordinators. Most data described below will be obtained from retrospective and prospective chart review when feasible.

- Baseline data includes screening/eligibility, Study ID, sex, gender, race, date of birth, height, weight, baseline labs (SCr, eGFR), medical history, lifestyle habits, and chemotherapy treatment protocol.
- Identical sets of data will be collected during two cisplatin cycles, including
  - Pre-Infusion: laboratory results just before cancer therapy (electrolytes, SCr, urine analysis), administered fluids, infections leading up to treatment, medications, blood pressure (BP), hospitalizations
  - Post-Infusion: laboratory results after cancer therapy (electrolytes, SCr, urine analysis), administered fluids, infections shortly after infusion, use of nephrotoxic agents
- Throughout cisplatin therapy: use of concomitant radiotherapy, total cisplatin dose, date of cisplatin administration, any hospital or ICU admissions, need for dialysis, and laboratory results (monthly kidney function and electrolytes).
- Three-month follow-up: to record 3-month post-cancer treatment mortality and CKD status. Namely, survival status, SCr results, height, and weight for estimating GFR, BP, electrolyte results from medical records, urine albumin to creatinine and urine protein to creatinine ratios and urinalysis.

Samples will be collected at study visits during 2 cisplatin infusion cycles (Figure 3). The following will be collected:

- *Blood*. Wherever possible, research blood collection will occur simultaneous to collection for routine clinical care (children: 3 mL; adults: 10 mL).
- *Urine*. An additional volume of urine (30 mL) will be collected, from an existing urinary catheter, by midstream collection in a urine collection cup, or using diaper- embedded cotton balls.
- *Saliva*. Saliva (5 mL, in Oragene Saliva DNA kits) will be collected once (ideally before initial cisplatin cycle) for future genetic research. Swab will be accepted for those having difficulty producing sputum.
- Blood and urine will be collected at the following time points; which were selected based on feasibility discussions with each site, and a desire to have specimens from key time points useful for predicting nephrotoxicity:
  - Before the first cisplatin dose on the same day as the infusion ("pre"), or up to 1 week prior.
  - ~24 hours after cisplatin ("post"). This sample should be collected as close to 24 hours following the cisplatin dose as possible and within 16-24 hours from cisplatin infusion end.
  - 4-8 days later for adults, 3-8 days later for pediatrics ("conclusion" sample).

We will use blood and urine from each time point for SCr and electrolyte measurements to define AKI by the KDIGO and electrolyte NCI definitions. SCr and electrolyte values performed in routine care will also be recorded to enrich our dataset for AKI ascertainment. If a participant is missing the "pre" or "post" sample, no further samples will be collected or included in the metabolomics analysis. However, baseline and 3-month follow-up data will still be collected and compared with those included in metabolomics analysis to evaluate for bias.

Biospecimen Processing / Storage

- *Blood*: centrifuged at 4°C (2000g × 10 minutes), aliquoted into 1 mL cryovials supplied in the study kits and serum temporarily stored (up to 3 months) at −80°C at each site.
- *Urine*: poured into two 15 mL conical tubes, unprocessed and temporarily stored (up to 3 months) at −80°C at each site.
- *Saliva*: stored temporarily at room temperature at study sites (up to 3 months).
- 3-4 times per year, specimens will be shipped on dry ice (DNA samples at room temperature) to the biorepository (at SickKids) where they will be stored at −80°C until time of testing. Upon receipt of urine specimens, urine will be thawed, centrifuged at 4°C (2000g × 10 minutes) and aliquoted into 1 mL cryovials for storage. Samples will be stored for future hypothesis generation and validation studies.

Data management. Once enrolled, participants will be assigned a unique study code and entered into an online research electronic data capture system (REDCap; a secure, Web-based data management system housed at SickKids), informing the main coordinating center in real-time and populating a specimen capture form. Collected samples will be de-identified using a barcode with a unique study identification (ID) code. Bar codes will be scanned by each site into

the REDCap database to facilitate real-time specimen tracking (collection, processing, and freezing times) by the main coordinating center and to enable quick responses to issues. The first online specimen log triggers electronic reminders for future collections. This strategy, coupled with frequent communication between the main coordinating center and study sites, resulted in >95% sample collection success in ABLE. Other data collected are completed on paper case report forms (CRFs) before being scanned and uploaded to the REDCap platform. Data will be entered by the database manager into the central data repository with regular, integrated data checks and site queries to capture missing data and correct errors. This will be accompanied by monthly coordinator calls and routine engagement by the main coordinating center to address any issues. These rigorous data quality control measures resulted in a 0.04% missing data rate in the ABLE study. Data security and integrity are crucial, given the large clinical and metabolite data that will be received. The database will be shared with the UBC statistical analysis core for final database management and analysis. The mass spectrometry metabolomics data from the Urquhart lab will be encrypted and backed up daily to a UWO network server for immediate storage, followed by transfer/storage (with clinical data) with the support of the High-Performance Computing for Healthcare (HPC4Health) facility; a secure cloud providing high performance computational resources (Figure 1).

Sample Size Considerations

Metabolomics studies rarely have traditional sample size calculations because the data are highly dimensional and correlated.[67] Previous studies have used synthetic data to estimate sample sizes for metabolomics studies based on false discovery rates (FDR).[68] Assuming an FDR of 0.05, 200 metabolites, and assuming 20% of metabolites will differ between groups, around 20 subjects are required per group.[68] Based on 46% AKI incidence in the national cisplatin cohort throughout therapy, 400 subjects (200 children, 200 adults) will yield >20 subjects in each AKI phenotype (KDIGO, NCI). The sample size determined for this study aligns with previously published drug metabolomic studies and cisplatin AKI incidence.[69-73]

Metabolomics analysis. Metabolomics analyses of serum and urine for the discovery cohort will be done using a Waters Xevo G2-S quadrupole time-of-flight (QToF) mass spectrometer. An untargeted metabolomics approach will be used to analyze serum and urine samples from the discovery cohort at the "pre" and "post" time points to identify candidate metabolite indicators of cisplatin AKI. To minimize instrument drift, sample order will be randomized such that "pre" and "post" samples along with the site of collection will be injected in random order. The "pre" samples will be used to identify biomarkers of AKI risk, and the "post" samples for early AKI diagnosis. Cisplatin nephrotoxicity pathways and previous research have provided promising candidate metabolites to consider (Table 2).[24,40-43] Candidate metabolites identified from the discovery cohort will be subjected to targeted, quantitative metabolomics on the validation cohort (both serum and urine). The fully quantitative metabolite assays will be performed on an AB Sciex QTRAP 5500 mass spectrometer using kits developed by Dr Wishart from The Metabolomics Innovation Centre.

Data Analysis

Discovery cohort. An overview of the data analysis process is outlined in Figure 4. Data from untargeted metabolomics will be processed using the isotopologue parameter optimization (IPO) and XCMS packages in R. IPO will be performed on pooled sample injections to optimize the parameters used for XCMS-facilitated data processing (Figure 4). Serum and urine metabolite peak areas will be normalized to internal standard peak areas, with additional normalization to creatinine in urine specimens. We will use EZInfo 2.0 (Umetrics, Umeå, Sweden) to perform multivariate analyses. Principal component analysis (PCA) will be used to visualize general data trends, and orthogonal partial least squares discriminant analysis (OPLS-DA) will be used to determine the metabolites with the greatest contribution to the variation/separation between groups of interest (Figure 4). Variable Importance in Projection (VIP) scores will be used to rank metabolites by their discriminating importance and metabolites with VIP score >1 (standard for untargeted metabolomics studies) will be identified as candidates.[74-77] Candidate metabolites will be subjected to multivariate analysis to exclude possible confounding with other clinical/patient characteristics. Subsequently, a nested case-control approach will be used for quantitative metabolomics analysis to measure the absolute concentration of candidate metabolites on 120 repeated samples (60 adults, 60 children) from the discovery cohort (n = 20 no AKI, n = 20 KDIGO AKI, n = 20 NCI AKI). Absolute quantification of candidate metabolites is an essential step in transitioning candidate biomarkers to robust tests that can be compared between testing sites.[78-81] PCA, OPLS-DA, and VIP ranking of the fully quantified metabolites will be repeated to ensure the most reliable candidate metabolites move forward to validation testing (Aim 2). We anticipate metabolite profiles for KDIGO AKI (SCr based) vs NCI AKI (electrolyte based) phenotypes will differ. The primary analysis will compare KDIGO AKI vs non-AKI metabolite profiles. Secondary analyses will compare metabolite profiles of (1) males vs females, (2) NCI (electrolyte) AKI vs non-AKI, and (3) KDIGO and NCI AKI vs no AKI.

Validation cohort. This analysis will use candidate metabolites measured in the discovery cohort to validate findings. This step allows the independent validation of the findings from the

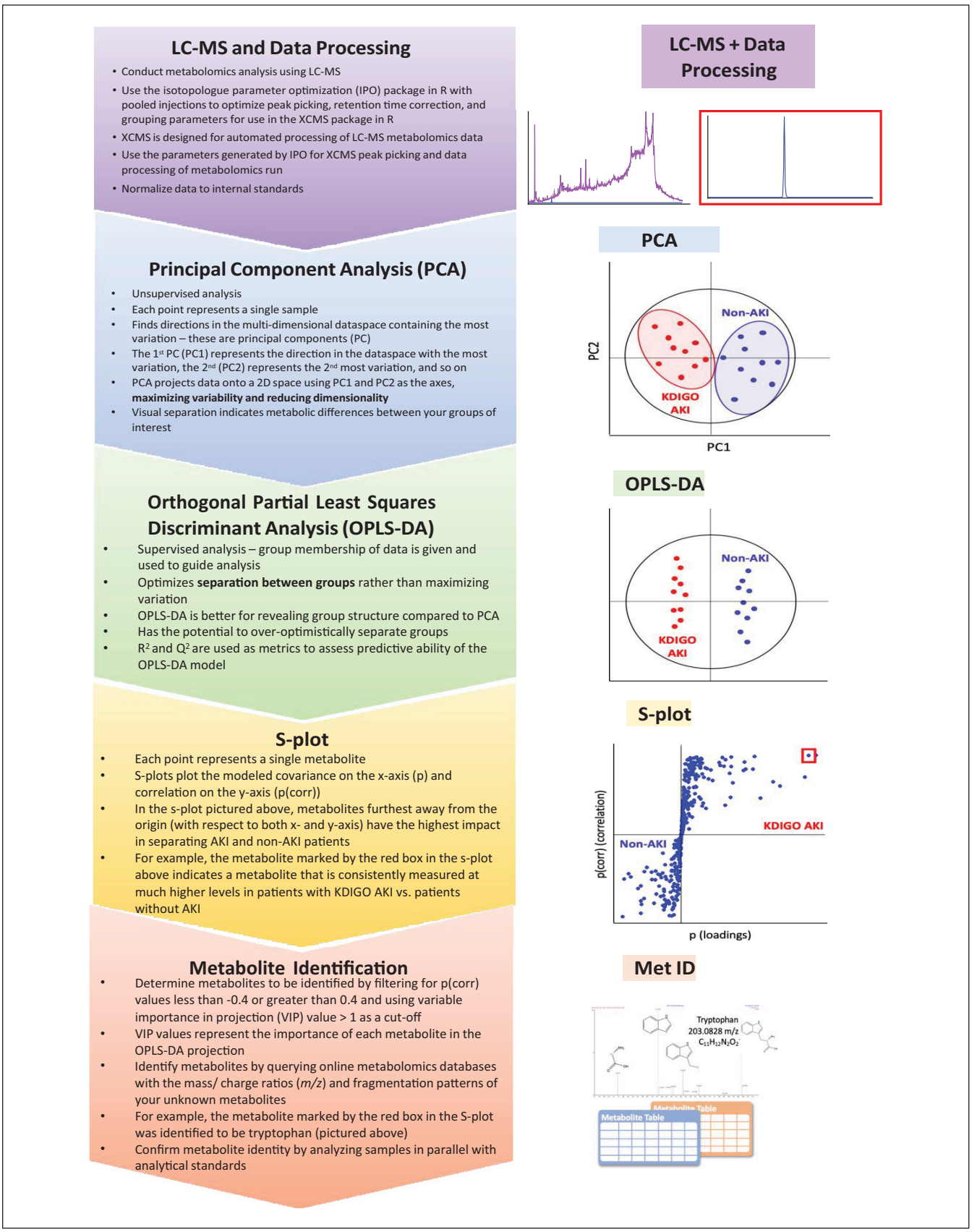

Figure 4. Sequential overview of the data analysis process.
Note. Encompasses data collection, data processing, and multivariate analysis.

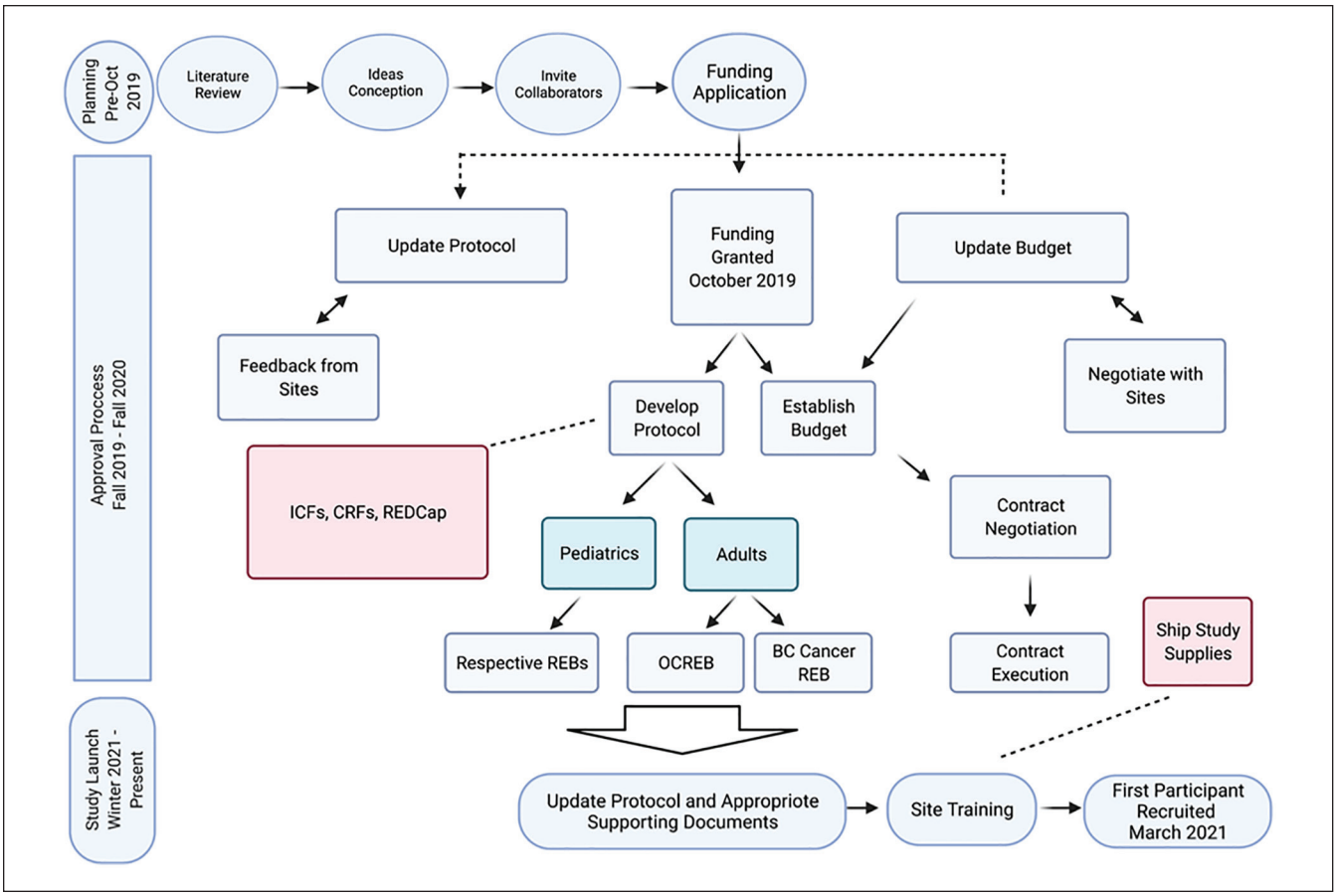

Figure 5. Timeline of progress to date.
Note. Description of progress as of March 2021. This figure was created with BioRender.com.

discovery cohort and higher-level bioinformatics to refine a targeted predictive algorithm. First and second last or last cisplatin infusions will be analyzed from the validation cohort samples. This approach allows the validation of within-subject metabolite variability and determines whether metabolite patterns change after cisplatin exposure. It is likely the analysis will identify many metabolites implicated in cisplatin AKI, some of which will be co-linear in their predictive utility. Therefore, metabolite concentrations will be used to perform machine learning to limit the number of metabolites to a level that maximizes prediction and is feasible for implementation. Based on previous experience, the combination of hyperparameter optimization and feature selection typically results in biomarkers composed of 5 to 15 metabolites in the final model.[50,71-73] Metabolite levels with stratification of clinical data from patients (eg, age, cisplatin dose, sex, gender, ethnicity) will be used to create signatures that best predict AKI. The primary figures of merit for optimization will be area under the operating characteristic curve (AUROC) and feature-number, with sensitivity, specificity, positive predictive value, and negative predictive value as secondary metrics. By focusing on a parsimonious model with the least number of metabolites given statistically indistinguishable maximal AUC it is possible to maximize the ease of translation of the model. Both the final signature and the set of individual univariably significant metabolites will be characterized with MetaboAnalyst to identify pathways implicated by metabolites that contribute most to AKI discriminating algorithms and will help identify injury mechanisms and intervention strategies. The metabolite model will be explicitly compared with standard AKI prediction with clinical variables alone (net reclassification/integrated discrimination improvement), comparing the difference in AUCs using the accelerated bootstrap.[82-84] Subgroup analyses will be performed (sex, age, cisplatin dose, gender, ethnicity) and cisplatin prediction using the identified metabolites will be compared between the first vs last or second last cisplatin infusion. Generalization error will be assessed via cross-validation and the final locked model only will be assessed in the validation cohort.

Progress. Summary of progress to date shown in Figure 5.

Discussion

ACCENT is a unique study addressing several knowledge gaps in cisplatin AKI in adults and children. This study has

several strengths. First, it includes multiple Canadian cancer treatment centers, with positive implications for generalizability and future knowledge translation. Study protocols were developed based on experience from the ABLE study, balancing multicenter feasibility with validity and rigor. Our consideration of metabolite profile and AKI phenotype (KDIGO vs electrolyte-based) demonstrates meticulous attention to disease phenotype allowing for increased accuracy and clinical relevance when identifying biomarkers of cisplatin AKI. This study will generate a large, valuable biorepository of urine, serum, DNA, and clinical data with the most detailed and accurate phenotypic description of acute cisplatin nephrotoxicity available. The robust data analysis strategy ensures the most reliable candidate metabolites are identified and our approach in the validation cohort independently validates these findings. The repeated time points assessment also allows for the validation of within-subject metabolite variability and determines whether metabolite patterns change after cisplatin exposure.

A goal is to identify the most predictive metabolites, which would be amenable to high throughput clinical testing, like other metabolite panels routinely measured (eg, metabolic diseases, newborn screening). Clinical trials of AKI mitigation strategies are urgently needed but currently impractical due to delayed AKI diagnosis. With the discovery of cisplatin AKI metabolites, and by working with industry and biochemistry labs, a rapid and sensitive test can be developed. An AKI metabolite biomarker panel may be translated for clinical use to pinpoint the highest risk patients and trigger conservative AKI mitigation strategies like avoidance of other nephrotoxic medications, increasing fluid administration, vigilant monitoring, and active dosage reassessment for future cisplatin infusions. These types of conservative interventions could be tested, via biomarker (predictive metabolite panel)-driven or guided conservative process of care intervention trials. In the future, predictive metabolite panels may be used to risk-stratify patients for randomization into therapeutic intervention trials.[16,18,54,55] We recognize conservative mitigation strategies triggered by identification of AKI biomarkers pose a potential risk of undertreated cancer and would need to consider this when designing future intervention trials. The interdisciplinary nature of our expert team is a strength by increasing potential for future research, rapid knowledge dissemination, and knowledge translation.

Our study has some limitations. Although a relatively large study, we may be unable to adjust for all clinical variables of interest when evaluating prediction of AKI. The multicenter nature, while a strength, also presents opportunities for variability in data collection despite our best efforts. There is risk of selection bias, as acutely ill patients may not be recruited in time. We will monitor for this regularly to ensure capture of a clinically representative population and will modify our recruitment strategies if needed. The difference in enrollment period between pediatric and adult patients may impact sample stability due to different storage times. This is unavoidable as cisplatin is used less frequently in the pediatric population and slower recruitment is predicted; however, the sample storage method should be sufficient to ensure sample stability for the majority of metabolites. It is possible we do not find metabolites that predict or aid in early diagnosis of AKI using our planned method. If no new metabolites are observed during the untargeted LC-MS analysis, we will use commercially available targeted LC-MS metabolomics kits. If still unsuccessful, we will use a complementary technique such as nuclear magnetic resonance (NMR). If too many metabolites are identified, modern shrinkage-based machine learning can be used to create parsimonious models that optimize the AUROC while minimizing the number of features. A similar model is used for newborn screening for inborn errors of metabolism. This test is performed on every newborn with 30 to 40 metabolites rapidly screened as an effective diagnostic test. Similarly, it has been shown that a panel of 7 metabolites predicts CKD better than SCr alone (AUC 0.937 vs 0.745), and that classifiers for non-graft rejection kidney injury based on hundreds of metabolites lose very little performance when only the top 20 metabolites are used.[50,73] This is also relevant when AKI after cisplatin infusion occurs *before* metabolite biomarkers are obtained. In these cases, the metabolite panel will not result in earlier AKI diagnosis but may predict other relevant outcomes like AKI severity or CKD. This study focuses on acute cisplatin injury, and not capturing the important potential associations with CKD; we have mitigated this through the 3-month chart review, providing valuable hypothesis-generating data to plan future metabolomics studies to predict long-term kidney injury. Although our inclusion of a discovery and validation cohort is a strength, we have chosen to select these cohorts by random sampling. It may be argued that choosing specific study sites to provide patients for the discovery cohort, and other sites, for the validation cohort (ie, select by site) may be more realistic and generalizable. However, this method may lack feasibility as metabolomics analyses will only be performed *after* all participants have been recruited. If we see that a site-based approach to discovery/validation is feasible, we may choose this method instead. Any study attempting to establish new AKI biomarkers faces the issue that the reference standard (ie, SCr rise) is flawed, as described above. Our work in the ABLE study confirmed that incorporating electrolyte abnormalities into the AKI definition may be important for more accurately phenotyping cisplatin-mediated AKI. Our study is novel as it attempts to improve our reference standard by incorporating electrolyte abnormalities into our reference standard definition.

Future directions may include developing a precision medicine algorithm where knowledge users can input patient factors, metabolite concentrations, and pharmacogenetic variants to determine risk. Given that urine and serum will be stored, it may be possible to measure protein biomarkers and compare them against the metabolite measurements already

planned. Although our study focuses on cisplatin AKI, our unique methodology may serve as a blueprint for other nephrotoxic therapies like gentamicin and ifosfamide.

Conclusion

To our knowledge, this is the largest study to follow both children and adults treated with cisplatin. Our study is unique, innovative, translational, and aims to reimagine the current model for AKI diagnoses; we will identify novel metabolomic biomarkers for earlier detection and prediction of AKI. AKI is associated with CKD and cardiovascular-related outcomes, which can severely compromise quality of life. Thus, early detection and prediction may have important public health implications.

Appendix

Inclusion Criteria

Patients who meet the following criteria are eligible for enrollment as study participants:

Adult	Pediatric	Yes	No
Initiating treatment with cisplatin (≥70 mg/m2) for head/neck or lung cancer at one of the adult participating sites; 18 years of age or older	Initiating treatment with cisplatin for any cancer diagnosis at one of the pediatric participating sites; greater than 3 months of age; less than 18 years of age		
Consent and/or assent to participate in the study			

Exclusion Criteria

Patients who meet *any* of these criteria are *not* eligible for enrollment as study participants:

	Yes	No
Diagnosis of chronic kidney disease (CKD) at baseline		
Previous use of any nephrotoxic drugs included on the provided "Prohibited Nephrotoxic Medications" list in the 2 weeks prior to the first cycle of cisplatin treatment		
Previous use of cisplatin		
Previous radiotherapy (total body irradiation or abdominal radiation) in the last month		
Previous hematopoietic stem cell transplant		
Any chronic or acute health condition that the investigator feels would render the patient inappropriate for this study, including but not limited to significant uncontrolled cardiorespiratory, hepatic, infectious, and kidney disease at the discretion of the investigator		

Eligibility

Did the participant meet the eligibility requirements for this study? **Yes** **No**

- **If Yes**, informed written consent obtained **Yes** **No**
- **If No**, do not continue; participant must be excluded.
- >complete ***Participant Refusal Log***

Additional Consents

	Yes	No
Consent obtained for genetic testing		
Consent obtained for future contact		
Consent obtained for longitudinal data collection		
	Open	**Closed**
Consent for future research of leftover blood and urine samples		

Ethics Approval and Consent to Participate

This study is being conducted in accordance with the World Medical Association Declaration of Helsinki. Written informed consent (or assent) will be obtained from all participants, after cancer diagnosis and before cisplatin treatment. Ethics approval was granted to participating centers in one of three ways: (1) SickKids provided overarching ethics approval for pediatric centers (REB Number: 1000071380). Once the application was approved at SickKids, submissions were made to ethics bodies at other pediatric sites. (2) We took advantage of the streamlined review process offered by the Ontario Cancer Research Ethics Board (OCREB) for our Ontario adult sites (CTO Project ID: 3269). OCREB services Ontario hospitals/cancer centers conducting oncology trials. Upon OCREB approval, participating centers may submit their abbreviated center-specific application for expedited approval. (3) The BC adult site received ethics approval through their center.

Consent for Publication

Consent for publication was obtained from all authors.

Availability of Data and Materials

Not applicable

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Declaration of Conflicting Interests

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