Rationale and Design of The Genetic Contribution to Drug Induced Renal Injury Study (DIRECT)

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Rationale and Design of The Genetic Contribution to Drug Induced Renal Injury Study (DIRECT).

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Abstract

Background: Nephrotoxicity from drugs accounts for 18-27% of cases of acute kidney injury. Determining a genetic predisposition may potentially be important in minimizing risk. The aims of this study are:

1. To determine if a genetic predisposition exists for the development of drug induced kidney disease (DIKD), using genome-wide association and whole genome sequencing studies.
2. To describe the frequency, course, risk factors, resolution and outcomes of DIKD cases.
3. To investigate the role of ethnic/racial variability in the genetics of DIKD.
4. To explore the use of different tools establishing causality of DIKD.

Methods/Design: 800 patients will be enrolled worldwide and blood samples for DNA collected. Data on the patient risk factors, vital signs, laboratory parameters, drug exposure, and DIKD course will be recorded. A panel of nephrologists will adjudicate all cases. Genome wide association studies will be conducted using population controls matched on biogeographic ancestry to determine if there is a genetic predisposition to DIKD. The primary endpoint is identification of specific drug-related polymorphisms associated with DIKD. Secondary endpoints include frequency of DIKD by causal drug and drug combinations; DIKD genetic variability; exploration of causality assessment tools; risk factor identification; description of the course of DIKD; including mortality and dialysis dependency at hospital discharge, 28 and 90 days post-event.

Discussion: The DIRECT study will be the first observational cohort study investigating genetic determinants of DIKD. If the trial is positive, its findings will potentially translate

into safer patient outcomes, by genotypic individualization of therapy and minimization of harm.

Key Words: Pharmacogenomics, Nephrotoxicity, AKI, Antimicrobials, Calcineurin inhibitors, NSAIDs

Background

Drug induced kidney disease (DIKD) is a common cause of acute kidney injury (AKI) in ambulatory and hospitalized patients. The phenotype of DIKD is variable as injury can occur in different structures of the kidney including the vascular endothelium, glomeruli, tubules and the interstitium. Additionally, DIKD can manifest as acute and/or chronic alteration of kidney function with onset varying from hours to weeks. It is often asymptomatic and the diagnosis based on a biomarker change such as an increased serum creatinine (Scr) or urinary findings including proteinuria and hematuria consistent with glomerular injury. Common drug culprits include antimicrobials, calcineurin inhibitors and chemotherapeutic agents. Risk factors for DIKD have been reported for individual drugs; they can be patient-specific (e.g. age, chronic kidney disease (CKD)), disease-related (e.g. sepsis, volume depletion) and process of care-related (e.g. drug dose and duration). Most cases of DIKD resolve with drug discontinuation or dose reduction. However, it is recognized that recurrent or prolonged cases of AKI may lead to the development or progression of CKD. Effective strategies to predict DIKD may help to reduce the risk of recurrent injuries.

The pharmacogenomics of DIKD remains to be established. Similar to adverse drug reactions affecting other organs, it is most likely that there will be interplay between a number of different genes and environmental factors. The genes involved may affect diverse cellular pathways, including drug metabolism and transport, apoptosis, immune responses and cellular repair and regeneration. Several drugs interact with organic anion transporters (OAT) and may cause kidney injury due to intracellular accumulation caused by alterations in OAT function.[1] The genotype of two transporters, OCT2 and

multi-drug and toxin extrusion protein (MATE), contribute to the susceptibility of cisplatin nephrotoxicity.[2] Similar mechanisms could operate for other drugs. The immune response in drug induced acute interstitial nephritis (AIN) has not been well studied. Genetic polymorphisms in human leukocyte antigens (HLA) have been documented in antibiotic associated hepatotoxicity and may similarly have a role in drug induced AIN.[3,4]

Genomic approaches such as genome-wide association studies (GWAS) and whole genome sequencing enable the detection of rare, serious adverse effects provided that a well-defined, reliable phenotype is established. Given recent success in identifying genes associated with other adverse drug reactions, including liver,[5] skin[6] and muscle injury,[7] a similar study to identify genetic factors relevant to the risk of DIKD is timely. This would be helpful to personalize drug treatment and to inform drug development processes where nephrotoxicity limits the generation of new drugs. The DIRECT study is an observational genomic study of patients who have developed DIKD to determine the genetic predictors of DIKD occurring in adult and pediatric patients from an international consortium of investigators.

Study Objectives

The primary objective of the study is to identify common polymorphisms in subjects with DIKD compared to population based controls using genome wide association analysis. Secondary objectives include (1) to investigate the role of ethnic/racial variability in the genetics of DIKD associated with specific, high volume drugs, (2) to describe the course, clinical risk factors, resolution and outcomes of DIKD and (3) to explore the utility of different causality assessment tools when adjudicating cases of DIKD.

Study Design

This study is an international, multi-center observational cohort study of patients who have developed DIKD as defined by phenotype standardization.[8] Drug induced kidney disease is a spectrum of injury that often goes unrecognized. Phenotype standardization allows for definition of the injury with the aims to improve identification across a variety of clinical settings.[8] A panel of nephrologists, adult and pediatric, and pharmacists convened to standardize the phenotype of DIKD for the purpose of inclusion into this study and the phenotype is summarized in Table 1.[8] The DIRECT study will primarily focus on the AKI and glomerular phenotypes since it was felt that tubular disorders and nephrolithiasis would be more difficult to link to genetic polymorphisms. Additionally, the study will assess multi-drug injury, allowing up to 3 causal drugs, since clinical spectrum of DIKD often includes more than one causal agent.

For the primary endpoint, population based controls matched on ancestry will be utilized from the Population Reference Sample (POPRES) database.[9] Population based controls have been effectively used in previous genome wide association studies to examine the genetic basis of serious, rare adverse events like drug induced liver injury or skin hypersensitivity.[6,10,11] Drug exposed controls allow for greater account of possible co-variates in the development of the adverse reaction. However, it is often impractical to perform prospective studies following drug-exposed patients for the development of nephrotoxicity given the low occurrence of this adverse event and the resources required to study multiple drugs.

At inclusion into the study, subjects will have already experienced kidney injury. Study time points will be based on historical drug exposure and course of injury, including baseline assessment, hospital admission (for inpatients), pre-drug exposure, start of drug exposure, DIKD day, peak serum creatinine (Scr), drug discontinuation or dosage adjustment, nadir Scr, hospital discharge (for inpatients), 28 and 90 days post injury (Tables 2 and 3). These time points will enhance causality assessment and inform on outcomes. Specifically, the time points were chosen for the following reasons: (1) to establish temporal association between the drug exposure and the injury (2) to determine the maximal severity of injury and (3) to measure outcomes of recovery including complete and partial resolution and non-recovery. Determination of AKI recovery has been variably reported in the literature. For example, resolution has been reported by the nadir Scr time point or at hospital discharge as well as day 28 and 90. We will employ definitions proposed by the KDIGO guidelines, determination of acute kidney disease at day 28 and chronic kidney disease at day 90 post-injury. Studies have demonstrated that non-recovery from AKI is associated with increased mortality.[12,13]

The following medications or medication classes will be included in the study:

1. Antivirals
2. Anti-retrovirals
3. Aminoglycosides
4. Amphotericin
5. Cephalosporins
6. Chemotherapeutic agents

7. Colistin
8. Calcineurin inhibitors
9. Hydralazine
10. Non-steroidal anti-inflammatory drugs
11. Pamidronate
12. Penicillins
13. Propylthiouracil
14. Proton pump inhibitors
15. Quinolones
16. Rifampin
17. Sulfamethoxazole/trimethoprim
18. Vancomycin
19. *Additional medications to be added as identified*

Study Population

Previously identified or new cases of DIKD will be recruited as conducting prospective studies on drug exposed patients to follow them for the development of nephrotoxicity would be impractical given the low incidence of reactions reported for some drugs. This current approach allows for the enrollment of greater number of cases that have experienced toxicity from a spectrum of drugs.

Subjects who have developed DIKD will be identified by investigators and recruited from hospitals or ambulatory care clinics through two main approaches (Figure 1):

1. *Recall and review of medical records of discharged patients who had DIKD:* Patients who developed DIKD previously will be identified through recall, or a review of kidney biopsy logs, or previous nephrology consults. They will be contacted for participation and informed consent will be obtained.
2. *Concurrent identification of patients under active treatment:* Subjects in hospital or in ambulatory care clinics, who developed DIKD as defined by the primary and secondary criteria in Table 2, will be recruited for the study. They will be identified through screening electronic medical records (EMR) when available. In the absence of an EMR, nephrologists will identify potential subjects from their consult service or from referral from a colleague. If deemed appropriate by their primary physicians, identified cases will be approached for participation and consent.

Inclusion criteria include all patients aged ≥2 years who are exposed to a candidate drug for at least 24 hours and develop the AKI or glomerular injury as defined by the primary criteria for these phenotypes (Table 2).[14]

Exclusion criteria are: history of or current kidney transplant recipient; history of or current stem cell transplant recipient; CKD Stage 5; patient receiving more than 3 causal drugs as determined by the investigators; incomplete patient information on the time course of drug exposure.

Patients will be screened electronically for inclusion into the study using electronic screening in a web-based database, www.obriendata.org/direct (Last Accessed: June 2016). Electronic screening will ensure phenotype criteria from Table 2 are met in relation to the time frame for drug exposure (Appendix 1). Reasons for screen and

consent failure are recorded. Electronic screening allows for tracking the screened population and most common reasons for screen failure. Additionally, this strategy allows for determination of consenting rates.

This study protocol was approved by the UC San Diego Human Research Protection Program (reference #121651). Participating centers will obtain ethics approval through their local ethics committees. All patients will be asked to give their written informed consent to participate in the study. Surrogate consent will be requested if a subject lacks the capacity to provide consent. Participating centers will follow their local ethics committee regulations for consenting procedures. This study was registered as clinical trial number NCT02159209 at www.ClinicalTrials.gov (Last Accessed: June 2016).

Data Collection

Data will be entered into a web-based database, www.obriendata.org/direct (Last Accessed June 2016). Data validity and integrity is ensured by electronic rules such as maximum/minimum value checking. Alerts are issued on data in question and users are required to verify accuracy of data. At baseline, demographics including age, gender, height, weight, self-reported race/ethnicity and medical history will be obtained from the medical record, including co-morbidities, reason for hospital admission or clinic appointment, nephrology consultation notes, renal biopsy findings and previous surgeries or procedures. The following data will be collected at all time points (Appendix 1):

1. Physical Examination: The presence of heart abnormalities, peripheral or pulmonary edema, ascites, jaundice, indwelling bladder catheter as well as any active infections will be recorded.
2. AKI Risk Factors: Risk factors for AKI will be recorded, including: exposure to contrast agents, surgical procedures, need for blood transfusion, nephrotoxic exposures, hypotension (systolic blood pressure < 90 or mean arterial pressure < 65), hyperglycemia (blood sugar > 110 mg/dL), intravascular fluid losses (burns, hypovolemia, hemorrhage, paracentesis or diuresis), sepsis, cardiac failure and liver disease.
3. Vital Signs: Height, weight, blood pressure, temperature, respiratory rate, fluid balance, and intake/output will be recorded. If the patient is in the intensive care unit, we will record the following (if available): central venous pressure, cardiac output, fraction of inspired oxygen, arterial blood gases and Glasgow Coma Scale.
4. Laboratory: Standard of care comprehensive metabolic panel, complete blood counts and coagulation studies will be recorded. Each participating site will process its own standard-of-care labs. Interventions will be determined by the attending physicians and not influenced by the study personnel.
5. Urinary Studies: Standard of care urinalysis, urine microscopy, cytology and urine chemistry studies will be recorded.

After the development of AKI, the following information will be collected:

1. Renal Replacement Therapies: The reason for initiating dialysis, type of dialysis, start and stop dates of dialysis and dialysis discontinuation will be recorded.

2. Survival status: The subject's survival status will be established by review of the EMR and telephone contact with patient at hospital discharge, days 28 and 90.
3. Scr: Standard of care laboratory assessment of Scr will be recorded from the EMR at hospital discharge, days 28 and 90.

Adjudication

Cases must pass adjudication by a panel of adult and pediatric nephrologists to be included the final analysis. Kidney injury is often multifactorial and adjudication is required to determine the underlying causes.[15] An adjudication process for evaluating DIKD has not been previously developed. Prior published processes for adjudication of drug induced liver injury and skin hypersensitivity were used as a framework for designing adjudication of DIKD.[16,17] Two independent nephrologists will review each blinded case, presented as a summary of completed data (Figure 2), to ascertain causality for DIKD and assess the contribution of risk factors for the development of kidney injury. Specifically, the adjudicator will make a determination of DIKD; evaluate the relative contribution of each causal drug and the relative contribution of recorded AKI risk factors (Appendix 1). Considering adjudication processes for DIKD have not been previously published, this study will investigate the reliability and validity of this adjudication tool.

Biological Samples

Each subject will provide 15-50 mL of urine for biomarkers and 15 mL of blood for DNA and biomarkers. DNA will be isolated from a whole blood sample and stored at the UCSD O'Brien Core Laboratory for genetic analysis. Centers will retain a 5 mL blood sample for DNA at their site for back up if the original sample sent to the O'Brien Center was lost or destroyed.

DNA Preparation and Genotyping

Genomic DNA will be prepared from blood leukocytes at UCSD's Institute for Genomic Medicine facility and samples will be genotyped at the Broad Institute, using the Illumina Human Core plus Exome (or similar GWAS) array.

Protocol Definitions:

Since DIKD includes different mechanisms and sites of injury, the clinical presentation can be categorized into 4 major phenotypes including: AKI, tubular dysfunction, glomerular disorders and nephrolithiasis (Table 2).

AKI: is a process that causes an abrupt reduction in kidney function and is defined by meeting any of the following criteria[18]:

i. An absolute increase in Scr (≥ 0.3 mg/dl or ≥ 26.4 μmol/l) (within 48 hour time window) from the reference Scr value
ii. A percentage increase in Scr of ≥50% (1.5-fold from reference) within 7 days
iii. A reduction in urine output (documented oliguria of < 0.5 ml/kg/hr for >6 hours) despite adequate fluid resuscitation when applicable.
iv. An absolute decrease in Scr of (≥ 0.3 mg/dl or ≥ 26.4 μmol/l) (within 48 hour time window) from the reference Scr.

v. A relative decrease in Scr of ≥50% (1.5-fold from reference) within 7 days.

Sub-acute DIKD: is a process that causes a slower reduction in kidney function and is defined by meeting any of the following criteria:

i. A percentage increase in Scr of ≥50% (1.5-fold from reference) occurring between 7 and 90 days after the initiation of the drug or within 2 weeks of drug discontinuation.

ii. A relative decrease in Scr of ≥50% (1.5-fold from reference) within 90 days of a change in drug dosing or discontinuation.

CKD: Prior evidence of markers of kidney damage for ≥3 months (microalbuminuria or proteinuria or abnormalities in imaging tests) or the presence of glomerular filtration rate (GFR) <60 mL/min/1.73 m2 for ≥3 months calculated with MDRD (Modification of Diet in Renal Disease) equation.[19,20] Chronic kidney disease will be staged from stage 1 to 5 based on the calculated CKD-EPI or Ckid (pediatrics) GFR.[21,22]

Reference Scr to determine timing of AKI: The following criteria will be used in order of preference depending on available values

a) Lowest Scr immediately prior to index event. Must meet following criteria
    a. Precede drug exposure
    b. Within 90 days of index event
    c. Closest value to index event
    d. Lowest value prior to drug exposure
    e. If no Scr within 90 days of index use the hospital admission creatinine value

b) For declining Scr criteria with no prior reference level: will use the lowest value post drug reduction or stoppage as reference
c) For AKI phenotype will have two reference Scr values:
    a. Reference 1:
        i. Lowest value within 90 days of initiation of primary drug
    b. Reference 2:
        i. Lowest value closest to initiation of drug

Baseline Scr to determine CKD status: Creatinine values > 90 days from index event

a) Lowest values within 90 days to 12 months to establish eGFR stage based on CKD-EPI or Ckid (pediatrics)
b) Historical evidence of CKD based on standard criteria: proteinuria, biopsy, ultrasound findings
c) Imaging studies consistent with CKD
d) For chronic drug exposure need values prior to drug initiation

New-onset AKI: Evidence of AKI without prior evidence of kidney damage and calculated MDRD GFR is ≥90 mL/min/1.73m^2.

AKI-on-CKD: Evidence of AKI with criteria of kidney damage as defined above, occurring in a patient with CKD criteria, will be considered as AKI-on-CKD.

Definition of end of trial

The end of trial is the date of the last follow up of the last patient.

Withdrawal of patients/subjects

Patients may withdraw or may be withdrawn from the study for any of the following reasons:

- Patient decides not to continue with the study
- Administrative decision by the investigator
- Significant protocol deviation
- Patient is unable to provide adequate blood sample for DNA
- Case does not pass adjudication by adjudication committee

Assessment of Safety

Since this is an observational cohort study, the main risks include that of blood sampling and loss of confidentiality. Measures will be taken to minimize those risks. All adverse and serious adverse events will be reported to the appropriate ethics committees.

Genetic Data management and quality control

Data management of the large amount of genotype data and quality control (QC) will be performed using the software PLINK.[23] Initial data cleaning will include multi-step standard procedures.[24] In short, QC steps include removal of samples and single nucleotide polymorphisms (SNPs) with low genotyping quality, genetic assessment of gender and ancestry to flag inconsistencies with self-report, and assessment of cryptic relatedness of subjects, and will result in a filtered dataset of high quality.

Procedure for Accounting of Missing Data

To maximize information present in our data and allow for a potential comparison of our result across multiple studies genotyped on other platforms, we will impute genotypes of SNPs not present on our array. Imputation will be performed using IMPUTE2, a method found to be especially useful in the context of samples including mixed ancestries.[25] Reference data will include phased haplotypes from the 1000 Genomes Project.[26]

Based on the distribution of our study sites we anticipate the inclusion of several major ethnic/racial groups (Europeans/European Americans, African-Americans, Asians, and Hispanics) as well as admixed individuals from different ancestries. It is well established that allele frequencies across the genome can vary among subjects of different ancestral groups, and allelic association studies including subjects of different biogeographic ancestry are at high risk of this artifact. In addition, locus heterogeneity can lead to false negative results due to variation in genetic backgrounds. We will take advantage of the large amount of genotypic information available and control for potential population stratification in a two-step process.[27] First we will identify major groups of subjects with similar biogeographic ancestry utilizing approaches such as the program STRUCTURE.[28] The inclusion of population reference samples compiled by our group[29] will increase the power of these approaches. Analyses are then conducted separately on these more homogeneous ancestry groups, including principal components (PC's) derived from the program EIGENSOFT v3.0[30] to control for additional population stratification.

Statistical Analysis

In this observational cohort study, descriptive statistics will be calculated for demographic and baseline characteristics including:

- Demographics
- Baseline characteristics
- Past medical history
- Initial health status measures
- Composition of sample and patient location (e.g. surgical vs. medical ICU)
- Concurrent care (e.g., medications, interventions including surgery, and invasive procedures)
- Drug exposure (dose, frequency, route, timeline)
- Renal function estimates (Scr, GFR)

Genome Wide Association Study

We will perform GWAS to examine the association of genetic variants with the risk to development of DIKD. Mapping genetic determinants of DIKD requires a multilevel approach, including an understanding of interactions between environmental stressors (i.e. AKI risk factors) and individual constitutional factors.

Logistic regression models will be used to test for associations between SNP's and case/control status under the assumption of an additive genetic model. Initially we will adjust each phenotype for the typical covariates of age, gender, indices of ancestry, and study cohort. Additional covariates predicted to be of high importance include AKI risk factors such as volume status, concomitant nephrotoxins, comorbidities such as diabetes or hypertension, etc. To assess significance thresholds and correct for

multiple comparisons, we will use conventional methods such as Bonferroni correction for a genome-wide approach (i.e., $p<5x10^{-8}$), permutation tests to derive an empirical level of significance, and false-discovery rate analysis. PLINK and R code will be used to conduct these analyses.

The highly polymorphic HLA system has been shown to be especially important in adverse drug reactions. In order to derive classical HLA alleles, we will take advantage of the dense SNP coverage in the major histocompatibility complex (MHC) region of the Illumina Human Core plus Exome array. Specific methods as developed by Zheng and colleagues take advantage of the extended haplotype structure within the MHC to reliably predict HLA alleles based on genotypes from these arrays.[31]

Sample Size

A total sample of approximately 800 patients will be enrolled via 40 clinical centers worldwide. In previous studies examining the genetic predisposition to drug induced liver injury (DILI) and serious skin reactions, the associations between certain polymorphisms in HLA and afore mentioned injuries were highly significant.[5,6] In a study of DILI caused by flucloxacillin, possession of the HLA-B5701* allele was associated with an odds ratio (OR) of 80.6 (95% CI 22.8-284.9) for this adverse effect.[5] The HLA-A*3101 allele, prevalence of 2 to 5% in Northern Europeans, was found to be a risk factor for carbamazepine induced hypersensitivity syndrome (OR,12.41; 95% CI, 1.27 to 121.03), maculopapular exanthema (OR, 8.33; 95% CI, 3.59 to 19.36), and Stevens Johnson-toxic epidermal necrosis (OR, 25.93; 95% CI, 4.93 to 116.18).[6]

We conducted power calculations for our proposed case-control association study based on a range of realistic assumptions for trait heritability: assuming an

additive model, type I error rate of $5x10^{-8}$ for the GWAS, perfect linkage disequilibrium between marker and trait allele for common alleles (MAF 5% - 20%) and a disease prevalence of 20%. We conservatively base our calculations below on N=6,000 POPRES controls, which are treated as unselected population controls.[32] However, we note that at the time of analysis we will take advantage of additional available control subjects, which will increase the power of the study. Figure 3 shows the number of subjects (cases plus controls) required to achieve 80% power to detect a locus with a specific genotype relative risk (GRR), considering a range of number of cases and 6000 controls, respectively, for a MAF of 20% (panel A) and a MAF of 5% (panel B), which is typical for the HLA alleles. These calculations are considering a joint analysis of all subjects together, subsets of cases within a particular phenotype (N=50-600) as well as medication specific analyses within a drug class or specific drug (N=50-600). Assuming an incidence of 20% of an adverse reaction to a specific medication, in a sample of N=600 cases and 6000 population controls, we would have 80% power to detect a locus with a small GRR of 1.6 in the case of a common SNP with MAF= 20% (panel A). Power is reduced for rare alleles, and we will have power to detect a locus with a GRR of 1.98 in the case of a rare allele with a MAF = 5% (panel B).

Discussion

DIRECT is the first cohort study designed to evaluate whether there is a genetic basis for drug-induced nephrotoxicity. Other networks have been developed in DILI and serious skin injury but large networks for DIKD have not been previously established. Many genetic studies on DIKD to date have focused on single drugs or classes of drugs. DIRECT is the first study to establish an international network of centers

enrolling cases of DIKD from a broad range of drugs. Data from analysis of the genome will provide preliminary information on significant genetic polymorphisms associated with DIKD from each drug. This will enhance our understanding of mechanisms of toxicity for each drug. DIRECT will provide preliminary information on target genes to further validate the utility of genetic screening in addition to current clinical testing for prediction of risk.

Strengths of the DIRECT study include the broad enrollment of patients of different ages from various clinical scenarios, standardization of the DIKD phenotype, detailed information on the course of injury and clinical risk factors and the causality assessment process.

With the inclusion of hospitalized and ambulatory care patients with DIKD from various countries, DIRECT will inform on the spectrum of DIKD, variation in drug utilization and practice patterns in the different age groups and countries. We anticipate variation in co-morbidities and causal drugs in pediatric compared to adult patients. Additionally, drug utilization and practice patterns vary internationally. We anticipate genetic susceptibility will vary by race and ethnicity. DIRECT will capture a global snapshot from countries in North and South Americas, Europe and Asia.

The development of standardized phenotypes for DIKD was critical to studying genetic susceptibility. All cases must meet standard criteria for enrollment. Using inclusion criteria of stage 2 AKI will improve specificity of cases and enhance causality, thereby increasing the likelihood of finding genetic susceptibility. Moreover, phenotype standardization will assist clinicians, researchers, industry and regulatory bodies in designing future studies of DIKD.

The design of this study captures real life clinical scenarios including complex patients with multiple co-morbidities, risk factors and multi-drug injury. Acute kidney injury is multifactorial and requires assessment of the contribution of competing risk factors to injury. Detailed information on comorbidities, concurrent risk factors, the timing and extent of drug exposures will enable the complete description of the spectrum of injury in DIKD, risk factors for DIKD by causal drug and outcomes of injury.

The development of an adjudication process for nephrotoxicity is novel and will provide insight into causality assessment and attribution of risk. Clinical adjudication has been previously employed in AKI biomarker studies, where adjudicators are presented with information on a patient's Scr values and asked to make a judgement of whether AKI was present or absent.[33] However, adjudication of DIKD requires additional consideration of all potential contributing factors to AKI. Adjudication is a complex process requiring evaluation of causality using published criteria including: (1) strength of association, (2) temporality, (3) consistency of the adverse event in different subjects, (4) specificity of the drug for the adverse event, (5) biological gradient for the effect (dose response relationship), (6) plausibility, (7) coherence, (8) experimental evidence that can alter the adverse event and (9) analogy between drugs of the same class.[34] The gold standard for adjudication is expert consensus. Two independent nephrologists will adjudicate each case with a third acting as a tiebreaker. Published causality assessment tools will be utilized and compared to one another for DIKD. These tools can help reduce disagreement between adjudicators and classify the relationship likelihood. A limitation of these general causality assessment tools is lack of assessment of competing risk factors. Acute kidney injury is a syndrome with multiple

etiologies and many contributing risk factors. Assessment of these risk factors improves the specificity of cases and ultimately strengthens causal association. However, the method for attributing risk to each risk factor has not been previously delineated which may lead to variability in these assessments. Adjudicator intra- and inter-rater reliability will inform on the effectiveness of tools for causality assessment. We developed case report forms for adjudication, which were pilot tested by two adjudicators and refined for clarity (Appendix 1). We created a web based adjudication platform where adjudicators could access their randomly assigned cases. All adjudicators were trained to complete the adjudication electronically in a blinded process. The data from adjudication will capture the complexity of causality assessment in DIKD. The adjudication pass rates for each of the causal drugs in DIKD will inform on the complexity of cases and the consideration for sample size determination in the design of future studies.

Limitations of this study include the lack of drug-matched controls for GWAS as well as the lack of validated causality assessment tools for nephrotoxicity. We acknowledge that drug-matched controls would enhance the ability to use drug exposure data in clinical risk profiling. However, obtaining such controls requires time and resources and given the low incidence and the severity of nephrotoxicity, we opted to utilize population-based controls as this has been successfully done in other studies of serious, rare adverse events.[6,10,11] Additionally, the OR ranged from 8.33-80.6 for DILI and serious skin injury which suggests the DIRECT cohort size is adequate to detect DIKD associated polymorphisms with OR above 2. Validated causality assessment tools in DIKD are lacking. The Naranjo and Liverpool tools have not been validated in DIKD but do perform well for determining causality of general adverse drug

events. By utilizing these tools in addition to the adjudication process, we hope to refine the causality assessment of DIKD leading to the development of validated tools.

Information obtained from causality assessment, attribution of risk from concurrent risk factors coupled with genetic determinants of injury may be used to develop predictive risk scores for DIKD. The results of the DIRECT study may translate into safer patient outcomes through individualization of therapy based on the patient's clinical risk factors and genotype.

Trial Status

This study has closed to recruitment.

Competing interests

The authors have received funding from the International Serious Adverse Events Consortium to conduct this study.

Authors Contributions

L. Awdishu and R. Mehta are the Co-Principal Investigators who designed the study, chaired the development of the phenotype definition, led the coordinating center and prepared the manuscript. A. Davenport, P. Murray, E. Macedo, J. Cerda, R. Chakaravarthi, A. Holden and S. Goldstein were involved in phenotype definition and manuscript preparation. C.M. Nievergelt was involved in developing the statistical analysis plan and manuscript preparation. S. Ramachandran Rao was involved in study design and manuscript preparation.

Figures and Legend

Figure 1: Screening Approach for DIRECT Study

This figure demonstrates the two main approaches to screening subjects who have developed drug induced kidney injury. The first approach is direct recall of cases in which the investigators have consulted on or provided clinical care to. The second approach utilizes electronic surveillance of cases that have recently developed drug-induced injury and are currently being hospitalized or receiving care at an outpatient clinic.

Figure 2: Patient Summary Report

This figure captures the patient summary report that is presented to adjudicators at the time of adjudication. The data is extracted from various case report forms and presented in a visual format to summarize the patient's medical history, time course for drug exposure, trend in Scr, vital signs, concurrent AKI risk factors at each time point and drug dosing history.

Figure 3: Sample Size Estimation for DIRECT Study

This figure demonstrates the power estimates for individual drugs, drug classes and phenotypes for common alleles (Minor Allele Frequency (MAF) = 20% (Panel A) and 5% (Panel B)).

Appendix 1:

The appendix contains all case report forms utilized in the DIRECT study. These forms were used to develop the web-based database.

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Table 1: Primary and Secondary Criteria for Individual Phenotypes

Phenotype	Acute kidney Injury	Glomerular Disorder	Nephrolithiasis	Tubular Dysfunction
Characteristics	• ATN[1] • AIN • Osmotic nephrosis	• Hematuria, • Proteinuria	• Crystalluria • Nephrolithiasis • Ultrasound findings of stone with or without obstruction	• Renal tubular acidosis • Fanconi syndrome • SIADH[2] • Diabetes Insipidus • Phosphate wasting
Primary Criteria	• Rise in Scr that presents as or progresses to Stage 2 (KDIGO) 2-2.9 x reference Scr or higher • If child has baseline Scr < 0.5 mg/dL, must double Scr to get to at least 0.5 mg/dL or above • For the sub acute phenotype the rise in Scr to stage 2 may occur over a period > 7 days but less than 90 days OR • Decline by at least 50% from peak Scr over 7 days in relationship to	• Biopsy proven drug induced glomerular disease (within 4 weeks of stopping drug) AND *Proteinuria as defined by:* • 24 hr collection > 1 gram protein • UPC or UACR > 0.8 • Urinalysis 2+ protein100-300 mg/dL albumin • Children: 100 mg/m2/day or 4 mg/m2/hr Hematuria	• Must be new onset following drug exposure with no prior history of nephrolithiasis • No evidence of congenital etiology for nephrolithiasis • If obstructive, rise in Scr that presents as or progresses to Stage 2 (KDIGO) or higher • If non obstructive, then: • Urinalysis with crystals • Ultrasound with stone	Tubular: *Hypo-phosphatemia* OR *Glycosuria* • Urinalysis with 3+ glucose without diabetes OR Hyperchloremic metabolic acidosis AND Hypokalemia or hyperkalemia *Diabetes Insipidus:* • Hypernatremia > 155 mEq/L on multiple occasions • Polyuria > 3L/day

	change in drug dosing adjustment or discontinuation within 2 weeks • For the sub acute phenotype the decline in Scr may occur between 7-90 days of drug discontinuation or dose adjustment.	• > 50 rbc /HPF		
Secondary criteria	• Oliguric <500ml/day or <0.5ml/kg/hr for12 hrs (KDIGO Stage2) • Non-oliguric >500 ml/day, > 1mL/kg/hr for 24 hours (pediatrics) • Urinalysis findings: granular and muddy casts consistent with ATN, urinary eosinophils, proteinuria • FeNa > 1% • Negative ultrasound findings • Positive	• Culture negative leukocyturia • > 50 wbc/HPF • Casts • RBC; Granular, • Absence of secondary disorder that can cause GN: DM, lupus, post infectious, hepatitis etc. • Microangiopathic changes in blood • Smear, LDH; haptoglobin • Nephritic, nephrotic, mixed	• Urine electrolytes • Stone work up	*Phosphaturia* • FePO4 > 5% • Urinary PO4 excretion > 100 mg/day *Hypomagnesemia* • Serum magnesium < 1.2 mg/dL *Hypouricemia* • Serum uric acid < 2 mg/dL *Tubular Proteinuria* • 24 hr collection < 1 gram protein • UPC < 0.8 • Urinalysis < 2+ protein *Diabetes Insipidus* • Serum osmolality > 300 mosm/kg

	gallium scan for AIN • Clinical symptoms for AIN: fever, rash, joint pains			• Urine osmolality < 100mOsm/kg • Urine sodium < 10 mEq/L

[1]Hemodynamic changes may contribute to ATN, however, in the absence of any specific features are not considered individual criteria for the AKI phenotype.

[2]SIADH does not reflect direct tubular damage but rather the impact of a drug on ADH secretion and subsequent impaired water handling.

AIN = acute interstitial nephritis, ATN = acute tubular necrosis, DM = diabetes mellitus, FeNa= fractional excretion of sodium, FePO4 = fractional excretion of phosphorus, GN = glomerulonephritis, HPF = high-powered field, LDH = lactate dehydrogenase, RBC= red blood cell, SIADH= syndrome of inappropriate antidiuretic hormone, UPC = urine protein to creatinine ratio, UACR= urine albumin to creatinine ratio, WBC = white blood cell.[8]

Table 2: Schedule of Assessments – Hospitalized Subjects

Variable	Hospital Day 1	Pre-drug Exposure	Day of Drug exposure	Day of DIKI	Peak Scr or peak severity of injury	Drug DC or dosage adjustment	Nadir Scr or resolution of event***	Hospital DC	Status at Day 28 & 90
Demographics	X								
Primary criterion**	X	X	X	X	X	X	X		
Etiology of DIKI				X	X				
Duration of DIKI							X	X	
Risk Factors for DIKI	X	X	X	X	X	X	X	X	
Drug dosing & concentrations			X	X	X	X	X	X	
Concomitant Drugs	X	X	X	X	X	X	X	X	
History and Physical Exam	X	X	X	X	X	X	X	X	
Hemodynamics and Fluid Balance	X	X	X	X	X	X	X	X	
Other organ involvement	X	X	X	X	X	X	X	X	
SOFA Score #	X	X	X	X	X	X	X	X	
Sepsis Score #	X		X	X	X	X	X	X	

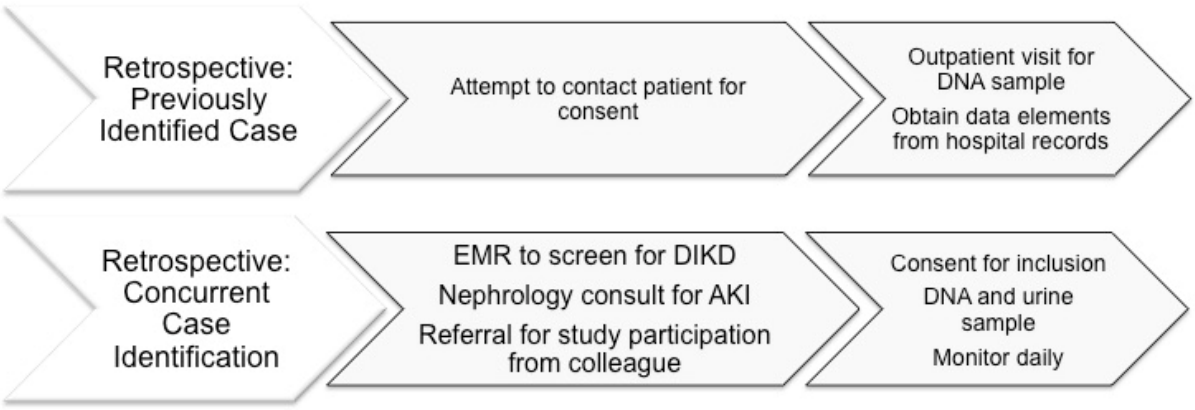
Lab and imaging data	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	
Assessment of Renal Function	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Blood for DNA and biomarkers*				**X**					
Urine for Biomarkers*				**X**					
Kidney Biopsy if done								**X**	
Dialysis Requirements				**X**	**X**	**X**	**X**	**X**	**X**
Hospital LOS								**X**	
Survival Status								**X**	**X**

DIKD = drug induced kidney injury, Scr = serum creatinine, DC = discontinuation, SOFA = sequential organ failure assessment, LOS = length of stay. # Computed. *If feasible, otherwise can be at any time point when consent is obtained. **Capture reference and other elements. ***If no resolution, data at Day 14.

Table 3: Schedule of Assessments – Ambulatory Subjects

Variable	Pre-drug Exposure	Drug exposure	Day of DIKI	Peak Scr or Peak severity of injury	Drug DC or dosage adjustment	Nadir Scr or Resolution of event
Demographics		X				
Primary criterion**	X		X	X	X	X
Etiology of DIKI			X	X		
Duration of DIKI						X
Drug dosing history & concentrations		X	X	X	X	X
History and Physical Exam	X		X	X	X	X
Laboratory and Imaging Assessment	X	X	X	X	X	X
Assessment of Renal Function	X	X	X	X	X	X
Concomitant Drugs	X	X	X	X	X	X
Blood for DNA*						X
Dialysis Requirements			X	X	X	X
Survival Status						X

DIKD = drug induced kidney injury, Scr = serum creatinine, DC = discontinuation. # Computed. *If feasible, otherwise can be at any time point when consent is obtained. **Capture reference and other elements



Genomics of Drug Induced Renal Injury : Patient Summary at a Glance

Patient Information

Patient ID:	SA0009
Patient Type:	Inpatient
Gender:	Male
Age:	23
Race:	Hispanic or Latino
Weight (kg):	117.9
Height (cm):	177.8
BSA:	2.41
BMI:	37.3
Phenotype:	AKI (↑SCr)
RefSCr1(mg/dl):	0.87 (04/17/2013)
RefSCr2(mg/dl):	1.33 (04/16/2013)

Medical History

Reason for Hospital Admission or Clinic Visit:

Patient has h/o plaque psoriasis with flare lesions covering 75% BSA. Patient returns to ED after blood cultures drawn in ED grew 3/4 bottles GPC which look like staph. Nephrology consult thinks AKI is probably multifactorial, but could have been contributed to by the vancomycin in the setting of nonsteroidal use and also underlying sepsis.

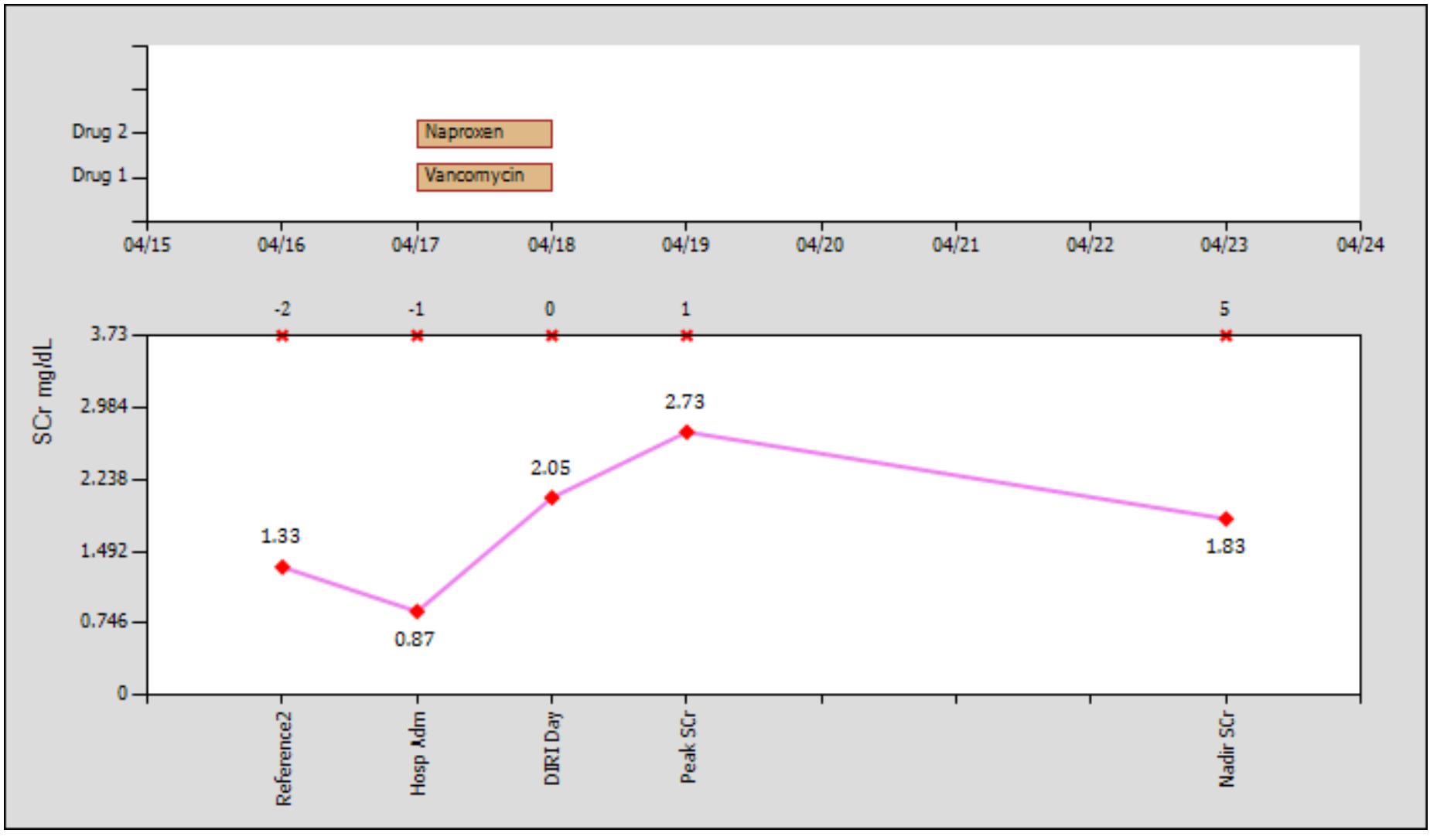
Known Medical History:

Autoimmune disease, Current Smoker

Kidney Biopsy

No kidney biopsy.

Serum Creatinine and Drug Time Course:



Clinic Data:

	Hosp Adm	Pre-Exposure	Exposure Start	Exposure Stop	DIRI Day	Peak SCr	Nadir SCr	Hosp Disch
CRF06_VitalsLabs								
Date	2013-04-17	2013-04-17	2013-04-17	2013-04-17	2013-04-18	2013-04-19	2013-04-23	2013-04-23
Days from DIRI	-1	-1	-1	-1	0	1	5	5
Temperature	36.8	36.8	36.8	36.8	36.3	36.7	36.6	36.6
SBP/DBP	121/70	121/70	121/70	121/70	140/71	136/85	147/81	147/81
Heart Rate	115	115	115	115	84	78	88	88
SCr (mg/dL)	0.87	0.87	0.87	0.87	2.05	2.73	1.83	1.83
On Dialysis	Yes	No	No	No	No	No	No	No
GFR	121.7	121.7	121.7	121.7	44.4	31.4	50.9	50.9
BUN (mg/dL)	7	7	7	7	11	16	14	14
Intake (ml)	1590/24hr	1590/24hr	1590/24hr	1590/24hr	2210/24hr	2313/24hr	1080/15hr	1080/15hr
Output (ml)	2000/24hr	2000/24hr	2000/24hr	2000/24hr	2491/24hr	2345/24hr	2100/15hr	2100/15hr
Urine Output (ml)	2000/24hr	2000/24hr	2000/24hr	2000/24hr	2490/24hr	2345/24hr	2100/15hr	2100/15hr
CRF07_UAUC								
UA Protein					Negative			
UA RBC					NA			
UA WBC					NA			
UA Eosinophils					Absent			
CRF04_PhysicalExam								
Edema	None	None	None	None	Not Recorded	Not Recorded	None	None
Lung Rales	Absent	Absent	Absent	Absent	Present	Absent	Absent	Absent
Heart	Arrythmias	Arrythmias	Arrythmias	Arrythmias	No Abnormalities	No Abnormalities	No Abnormalities	No Abnormalities
Ascites	Not Recorded	Not Recorded	Not Recorded	Not Recorded	Not Recorded	Not Recorded	Not Recorded	Not Recorded

Jaundice	Not Recorded	Not Recorded	Not Recorded	Not Recorded	Not Recorded	Not Recorded	Not Recorded	Not Recorded
Foley Catheter	Yes	No	No	No	No	No	No	No
Fluid Overload	X-Ray Not Done	X-Ray Not Done	X-Ray Not Done	X-Ray Not Done	X-Ray Not Done	No	X-Ray Not Done	X-Ray Not Done
Surgical Status	None	None	None	None	None	None	None	None
Source of Infection	Known	Known	Known	Known	Known	Known	Known	Known
Cultures Done?	Yes	Yes	Yes	Yes	Yes	No	No	No
CRF05_RiskFactors								
Contrast Agents	Yes (1)	Yes (1)	Yes (1)	Yes (1)	No	No	No	No
Nephrotoxic Agents	Yes (2)	Yes (2)	Yes (2)	Yes (2)	Yes (2)	Yes (1)	Yes (1)	Yes (1)
Surgeries requiring anesthesia	No	No	No	No	No	No	No	No
MAP < 65	No	No	No	No	No	No	No	No
Addtl Risk Factors	Yes (2)	Yes (2)	Yes (2)	Yes (2)	Yes (1)	Yes (1)	No	No
RBC Transfusion	No	No	No	No	No	No	No	No
Hyperglycemia	No	No	No	No	No	No	No	Yes

Drug Dosing:

	Episode 1	Episode 2	Episode 3	Episode 4	Episode 5	Episode 6	Episode 7	Episode 8	Episode 9	Episode 10
Drug 1: Vancomycin (V01)										
Start Time	04-17 02:55	04-17 10:45								
End Time	04-17 02:55	04-18 05:11								
Dose	1750 mg	1000 mg								
Frequency (hr)	24	6								
Route	IV	IV								
Concentration		46.2 mcg/mL								
Conc Time		04-18 05:46								
Drug 2: Naproxen (N02)										
Start Time	04-17 09:48									
End Time	04-18 17:41									
Dose	500 mg									
Frequency (hr)	12									
Route	PO									
Concentration										
Conc Time