Natural History of Infection Caused by BK Virus (and other Opportunistic Viral Pathogens) in Renal and Renal-Pancreas Transplant Recipients

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STATEMENT OF COMPLIANCE

Each investigator must adhere to the protocol as detailed in this document. Each investigator will be responsible for enrolling only those study participants who have met protocol eligibility criteria. This study will be conducted in compliance with the protocol and in accordance with Good Clinical Practices (GC) as required by the following:

- International Conference on Harmonization Good Clinical Practice E6 (ICH-E6 GCP) and the following applicable regulatory requirements:

- U.S. Code of Federal Regulators applicable to clinical studies (45 CFR 46 and 21 CFR 11 including parts 50 and 56 concerning informed consent and IRB regulations).

- NIH Terms of Award

All key personnel (all individuals responsible for the design and conduct of this study) have completed Human Subjects Protection Training.

PROTOCOL SIGNATURE PAGE

By my signature below, I, _____, agree to conduct this protocol **"Natural History of Infection Caused by BK Virus (and other Opportunistic Viral Pathogens) in Renal and Renal-Pancreas Transplant Recipients"** and provide the necessary assurances that this study will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines. I understand that no deviations from the protocol may be made without permission of the sponsor. I also understand that information in this protocol is proprietary and therefore, I agree to maintain the confidentiality of the protocol and data generated from the protocol until permission to publicize results is granted by the NIH.

SITE PRINCIPAL INVESTIGATOR:

_____ _____
Signature Date

_____
Title

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LIST OF ABBREVIATIONS

ABBREVIATION	TERM
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
BKC	BK Cystitis
BKV	BK Virus
BKVN	BK Virus-Associated Nephropathy
BLQ	Below the Limit of Quantitation
BMI	Body Mass Index
BP	Blood Pressure
CDC	Centers for Disease Control
Cº	Celsius/Centigrade
C_{max}	Maximum Concentration
CFR	Code of Federal Regulations
CL_T	Total Clearance
CMV	Cytomegalovirus
CrCl	Creatinine Clearance
CRF	Case Report Form
DMID	Division of Microbiology and Infectious Diseases
DNA	Deoxyribonucleic Acid
EBV	Epstein Barr Virus
eCRF	Electronic Case Report Form
FDA	U.S. Food and Drug Administration
FK506	Tacrolimus
FWA	Federal wide Assurance
GCP	Good Clinical Practice
GI	Gastrointestinal
GFR	Glomerular Filtration Rate
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HHV-6	Human herpesvirus 6
HSCT	Hematopoietic Stem Cell Transplant
ICF	Informed Consent Form
ICU	Intensive Care Unit

IEC	Institutional Ethics Committee
IRB	Institutional Review Board
IS	Immunosuppressant
IRB	Institutional Review Board
IVIS	Intravenous Immunosuppressant
kg	Kilograms
mg	Milligrams
ml	Milliliters
min	Minute
MMF	Mycophenolate Mofetil
MOP	Manual of Procedures
N	Number (typically refers to subjects)
NIAID	National Institute of Allergy and Infectious Diseases Number
NIH	National Institutes of Health
PCR	Polymerase Chain Reaction
PI	Principal Investigator
pl•BKV DNAPI	Plasma levels of BKV DNA
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
QA	Quality Assurance
QC	Quality Control
RBC	Red Blood Cells
SCT	Stem Cell Transplant
SOP	Standard Operating Procedure
SOT	Solid Organ Transplant
T	Temperature
T_{max}	Maximum Time
T_{max}	Maximum Time
µg	Microgram
ur•BKV DNA	Urine levels of BKV DNA
µL	Microliter
ur•BKV DNA	Urine levels of BKV DNA
UTI	Infection
WBC	White Blood Cells
WHO	World Health Organization

PROTOCOL SUMMARY

Title:	Natural History of Infection Caused by BK Virus (and other Opportunistic Viral Pathogens) in Renal and Renal-Pancreas Transplant Recipients
Phase:	Not Applicable (Natural History Study)
Study Population:	Male and female renal or renal-pancreas transplant recipients at risk for BK virus infection
Sample Size:	Approximately 450 subjects will be enrolled for screening for BK viremia (15% over-enrollment allowed, to a maximum of 518 screened subjects) to allow identification of approximately 40 (to a maximum of 60) subjects with viremia.
Number of Sites:	Approximately 6
Study Duration:	3 years
Subject Participation Duration:	Maximum of 24 months

Objectives:

Primary Objectives:

- To establish the natural history of BK viremia in a multicenter population of renal and renal/pancreas transplant patients, to include incidence, time to onset, duration, and quantitation of viral load;
- To assess associations between BK viremia and decline in GFR in renal transplant recipients.

Secondary Objectives:

- To define the donor and recipient-related risk factors for BK viremia and BKVN in a multicenter population of renal and renal-pancreas transplant patients;
- To define the frequency of clinical outcomes that are potentially related to BKV infection.

Tertiary (Exploratory) Objectives:

- To compare quantitative measurements of BKV DNA by PCR in plasma and (when appropriate) in urine, in order to determine biologic significance;
- To assess plasma for evidence of other opportunistic viral pathogens, including CMV, EBV, HHV-6, and adenovirus;
- To assess urine for evidence of other opportunistic viral pathogens, including CMV and adenovirus;

- To compare quantitative BKV PCR results from each site (local or reference laboratory) with matched results from the Central Unit laboratory;
- To explore the potential of biomarkers of tubular injury for predicting increased risk of BKVN;

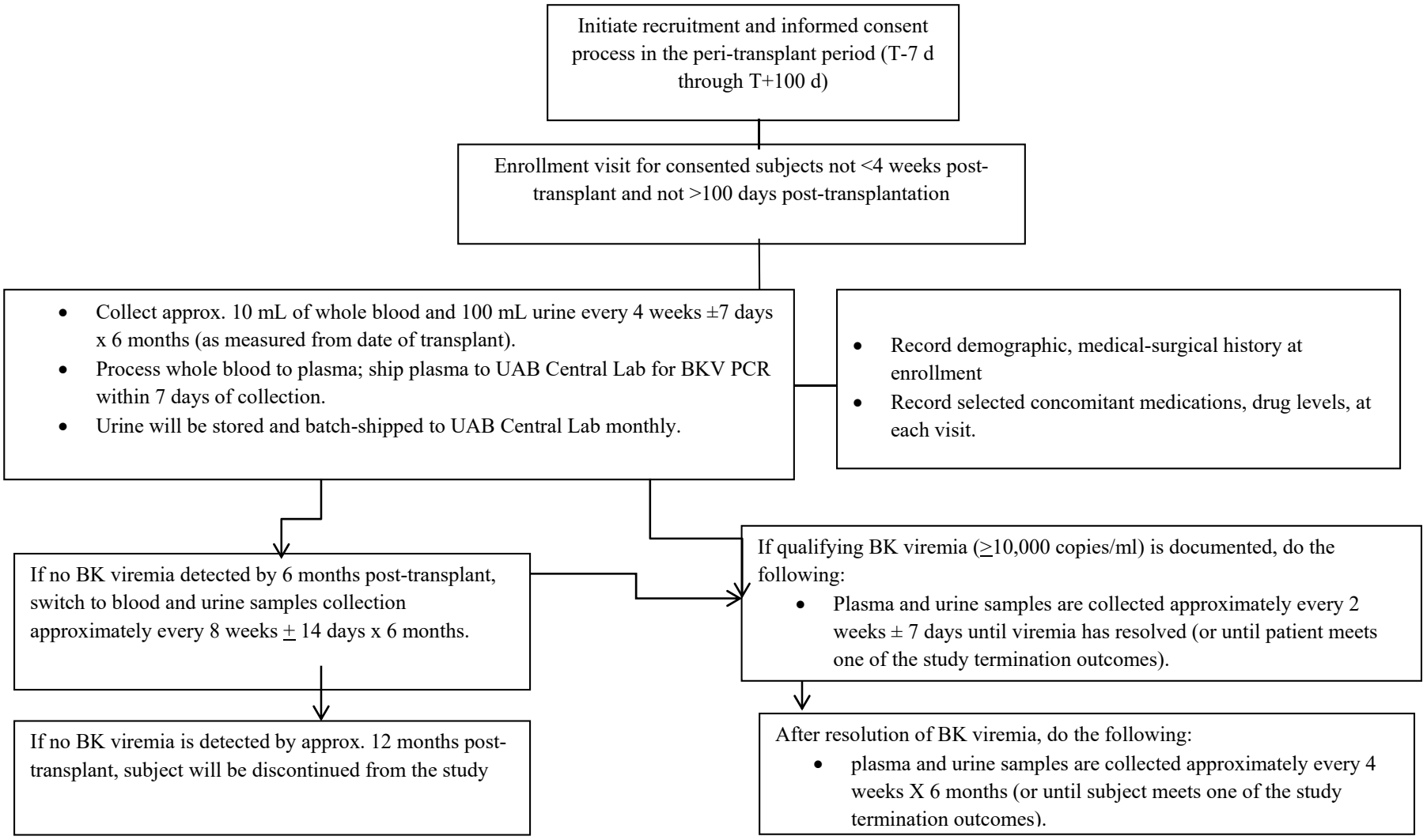
Description of Study Design:

The primary objective is to define the natural history of BK viremia. In order to understand the natural history of infection, we will measure the time (days post-transplant) to the development of BK viremia and its correlation with progression to end-organ disease (BKVN or BK hemorrhagic cystitis). Data from this prospective monitoring will allow for the identification of the types of high-risk patients who might benefit from future studies of therapeutic interventions for BKV infection (when effective therapy becomes available). This will be accomplished by serial quantitative BK DNA measurements in blood (plasma), assayed by polymerase chain reaction (PCR). Phases of the study can be summarized as follows:

- Subject identification and informed consent. Informed consent should be obtained early in the peri-transplant period (between 7 days pre-transplantation [T-7d] and 100 days post- transplantation [T+100 d]).
- Prospective screening for BK viremia. Enrollment and initiation of specimen collection should begin no sooner than 4 weeks after transplantation. Enrollment cannot occur later than 100 days after transplantation. Subjects will be screened (collection of blood and urine for laboratory studies, including BK PCR) approximately monthly for 6 months (timed from the date of transplantation), then every other month for 6 months. For those subjects who remain BKV-negative, screening will terminate approximately 12 months from the date of transplantation.
- Intensive sampling of subjects identified with BK viremia. Subjects identified with qualifying viremia will be sampled approximately every two weeks until resolution of viremia (defined as PCR negative, or <1000 copies/ml).
  Note: Patients who did not participate in the prospective screening phase but are identified with BK viremia by standard-of-care testing may be enrolled, as long as they are still within 100 days of transplantation.
- Post-viremia follow-up. Following resolution of viremia, subjects will be sampled (collection of blood and urine) approximately monthly for 6 months (unless another termination event occurs sooner).

SCHEMATIC OF STUDY DESIGN

DMID Protocol # 11-0071 Active Screening/Natural History Phase



1. KEY ROLES

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2. BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1. Background Information

2.1.1. BK Virus (BKV) and BKV-Associated Nephropathy (BKVN)

BK virus (BKV) is a small (45 to 55 nm) non-enveloped polyoma virus, having a circular double-stranded DNA (dsDNA) genome with approximately 5,000 base pairs. BKV was first isolated in 1970 from a renal transplant patient (initials "BK") with ureteral stenosis. Seroepidemiologic studies have demonstrated that BKV infection is virtually universal and that asymptomatic primary infection occurs in early childhood. In a healthy Swiss cohort aged 20-59 years, the seroprevalence for BKV was 82%.[1] Following primary infection, BKV appears to preferentially establish latent infection in the genitourinary tract. Four distinct genotypes of BKV have been identified which have varying geographic distributions and may not all be equally pathogenic in the setting of organ transplantation. In addition, recent studies suggest that the 4 genotypes may also be serologically distinct.

BKV rarely causes disease in immunocompetent adults, but in the setting of immunosuppression (such as renal transplantation) BKV can reactivate and cause asymptomatic viruria or symptomatic disease. The donor kidney also appears to be an important source of BKV infection in the graft recipient. Studies have shown that the higher the BKV antibody titer in the donor, the greater the incidence of BKV disease in the renal transplant recipient.[2] BKV nephropathy (BKVN) and ureteral stenosis are the most commonly described clinical manifestations of BKV infection in renal transplant recipients.[3] Hemorrhagic cystitis due to BKV, another common manifestation, is observed most often in hematopoietic stem cell transplant (HSCT) patients, but occurs in renal transplant patients as well.

Studies of the natural history of BKV infection have been greatly facilitated by the development of reliable PCR assays for BKV DNA.[4] Approximately 30 to 60% of all renal transplant recipients will have detectable levels of BKV DNA in their urine.[5-7] Approximately 10 to 15% of all renal transplant recipients will have BKV viremia (preceded in most cases by viruria).[5, 8] Among the patients who progress to BKVN, virtually all will have BKV viremia. BK viremia is most frequently detected in the first six months following renal transplantation.[9,10] Predictors of viremia include early onset of BKV viruria, rapid increase in viruria, and high peak titers of BKV DNA in urine.[5,11] Progression to BKVN often occurs without clinical signs or symptoms and is manifest only by a progressive increase in serum creatinine. Histopathologic examination of the renal allograft has traditionally been the "gold standard" for the definitive diagnosis of BKVN. However, recent studies have demonstrated that BKV DNA can be readily detected in blood using polymerase chain reaction (PCR) methodology in virtually all patients with histologically proven BKVN. Indeed, BKV DNA can often be detected in blood or urine for several months prior to the development of renal dysfunction, presenting an opportunity for therapeutic intervention. Although BKVN was first reported in 1978, dramatic increases in the incidence of the syndrome were noted after the

introduction of third-generation immunosuppressive drugs in the mid-1990s. Although the incidence of BKVN has not been precisely defined, transplant centers have reported rates of BKVN ranging from 1 to 10%.[12-14] One prospective study reported an 8% incidence of BKVN in renal transplant patients who received one or more of the newer immunosuppressive agents, such as tacrolimus, mycophenolate mofetil, and sirolimus.[15] Two lines of evidence suggest that development of BKVN may relate to the intensity of anti-rejection immunosuppressive therapy. First, BKVN has been seen much more commonly in the era of more potent immunosuppressive agents; most patients with documented BKVN have received one or more of these newer immunosuppressive drugs. Second, some patients with BKVN appear to clear virus from the blood and urine after reduction of the intensity of immunosuppressive therapy.[5] These observations provide only indirect evidence, however, and the natural history of BKV remains incompletely described.

BK polyomavirus infection is mediated by direct infection of allograft renal tubular epithelial cells (TECs). Molecular analysis has demonstrated that there is substantial inflammatory injury associated with a profibrotic milieu.[16] Monitoring for infection is performed by detecting viral copies in urine and blood. However, virus is frequently shed in the urine in the absence of invasion leaving the urinary monitoring as a suboptimal screening mechanism in terms of management of recipients that are infected. A testable hypothesis is that tubular injury, in the context of viral infection, indicates invasive disease. Detection of this tubular injury may provide an opportunity to manage kidney graft recipients at an earlier stage before invasive BK disease. Coupled with urine PCR, detection of renal tubular injury in recipient urine is a simple technique. Urine markers of injury have already been identified in other causes of acute kidney injury and are commercially available. These include cystatin C, renin, clusterin, Kim-1, b2-microglobulin, osteopontin, retinol binding protein, and markers of cell apoptosis.

Currently, no antiviral drug is approved for treatment of BKV infection.[17] Anecdotal reports have claimed benefit from cidofovir, leflunomide, quinolone antibiotics, and intravenous immunoglobulin. However, the current standard of care is early screening for BKV viremia and reduction in immunosuppressive therapy in an effort to improve graft survival, although it has not been studied in a controlled fashion.[12,18,19] At some institutions, modulation of immunosuppressive therapy has dramatically reduced the rate of allograft loss due to BKVN.[14] Reduction in the immunosuppressive regimen is not without consequence and can result in graft loss from immune-mediated organ rejection.[20] Despite reduction in immunosuppression, in some studies 30 to 70% of patients with BKVN have progressive deterioration in renal function, eventually resulting in allograft loss. Declining renal function typically occurs over months and is thought to result from a combination of persistent viral infection, organ rejection (induced by reduction in immunosuppression), and renal fibrosis.

This study is being performed to identify the natural history of BK viremia in renal transplant patients with the intent to identify high-risk groups that may benefit in the future from therapy with

safer and more effective antiviral medications.

2.2. Scientific Rationale

Renal or renal-pancreas transplant patients are at high risk of significant consequences from BKV infection, but have limited treatment options. No antiviral therapies of proven clinical value are currently available to treat BKV infection. The natural history of infection in this population is incompletely understood. Thus, BKV-infected renal or renal-pancreas transplant patients have been selected for inclusion in this study as they are an at-risk patient population with an unmet medical need.

2.2.1. Renal (or Renal-Pancreas Transplant) Patients

Approximately 30 – 60% of all renal or renal-pancreas transplant patients will have detectable levels of BKV DNA in their urine in the post-transplant period. Approximately 10 – 15% of renal transplant patients will have BK viremia. Among the patients who progress to BKVN, virtually all will have BK viremia. Predictors of viremia include early onset of BK viruria, rapid increase in quantitative viruria, and high peak titers of BKV DNA in urine. In an effort to capture these subjects early in the course of the evolving BK infection and avoid subjects who have already developed evidence of BKVN, this protocol will emphasize the initiation of screening in the early post-transplant period. In addition, since BKVN tends to occur most frequently within the first six months after transplantation, screening will be more frequent early in the post-transplant period.

2.3. Potential Risks and Benefits

2.3.1 Potential Risks

There are no potential risks to participants, other than the minimal risk associated with routine phlebotomy. Most data and specimens can be timed for collection at the participants' standard-of-care clinic visits. There are no potential benefits for an individual volunteering to take part in this natural history study, other than contributing to a better understanding of an infectious disease for which he/she is at risk. The participant will be allowing the research to collect data and residual blood samples that can be used to research BK infection and provide potentially useful data for monitoring and treatment of future transplant patients.

2.3.2. Potential Benefits

There may not be any direct benefit for individual subjects enrolled in this study. Some study specimens will be batch-shipped and processed, so results will not always be available to inform the subjects' clinical management. Indirectly, the data from this study will teach the transplant community when interventions are most likely to be successful.

3. OBJECTIVES

3.1. Study Objectives

3.1.1. Primary Objectives

- To establish the natural history of BK viremia in a multicenter population of renal and renal/pancreas transplant patients, to include incidence, time to onset, duration, and quantitation of viral load;
- To assess associations between BK viremia and decline in GFR in renal transplant recipients.

3.1.2. Secondary Objectives

- To define the donor and recipient-related risk factors for BK viremia and BKVN in a multicenter population of renal and renal-pancreas transplant patients;
- To define the frequency of clinical outcomes that are potentially related to BKV infection.

3.1.3. Tertiary (Exploratory) Objectives

- To compare quantitative measurements of BKV DNA by PCR in plasma and, when appropriate, in urine, in order to determine biologic significance;
- To assess plasma for evidence of other opportunistic viral pathogens, including CMV, EBV, HHV-6, and adenovirus;
- To assess urine for evidence of other opportunistic viral pathogens, including CMV and adenovirus;
- To compare quantitative BKV PCR results from each site (local or reference laboratory) with matched results from the Central Unit laboratory;
- To explore the potential of biomarkers of tubular injury for predicting increased risk of BKVN.
- Relationship of development of BKVN with immunosuppressive therapy

3.2. Study Outcome Measures

3.2.1. Primary Endpoints

- Incidence of BV viremia in the study population of screened renal or renal/pancreas transplant subjects.

3.2.2. Secondary endpoints

- Timing of onset of BK viremia (number of days post-transplant)

- Duration of BK viremia
- Incidence of BKVN in the study population
- Dynamics of quantitative measurements of BK viremia over time
- Frequency of donor and recipient-related risk factors for BV viremia, including:
    a. Donor-derived factors:
        i. Deceased donor – Standard criteria donor (SCD), Donor after cardiac death (DCD), Expanded criteria donor (ECD)
        ii. Living donor – related donor or unrelated donor
        iii. ABO incompatibility
        iv. Total HLA mismatches
        v. HLA C7 status
        vi. Race/ethnicity
        vii. Sex
    b. Recipient-derived factors:
        i. Delayed graft function (DGF), defined as use of dialysis within 7 days of the transplant
        ii. Cold ischemia time (CIT)
        iii. Warm ischemia time (WIT)
        iv. Use of stents
        v. HLA C7 status
        vi. Type/intensity of immunosuppression, including corticosteroids
        vii. Acute rejection
        viii. Age
        ix. Race/ethnicity
        x. Sex

- Frequency of clinical outcomes that are potentially related to BKV infection, including:
    a. subject death,
    b. loss of graft function as defined by GFR <10 mL/min/1.73 m^2
    c. development of urinary outlet obstruction
    d. development of gross hematuria
    e. development of lymphocele
    f. acute rejection
    g. initiation of dialysis
    h. re-transplantation
    i. renal biopsies

3.2.3. Exploratory Endpoints

- Frequency of BK viruria (incidence, time of onset, duration, viral load quantitation by PCR) in subjects with and without BK viremia

- Frequency of CMV, EBV, HHV-6, and adenovirus viremia at multiple time points in subjects with and without BK viremia
- Frequency of CMV and adenovirus viruria at multiple time points in subjects with and without BK viremia
- Results of BK PCR assays performed on matched specimens at the participating sites (local or reference laboratories) and at the UAB Central Laboratory
- Results of urinary biomarkers of tubular injury measured at multiple time points in subjects with and without viremia, including any subjects with BKVN. Potential urinary biomarkers include cystatin C, renin, clusterin, Kim-1, b2-microglobulin, osteopontin, retinol binding protein, and markers of cell apoptosis. Urine biomarkers will not be resulted in real-time.

4. STUDY DESIGN

The primary objective is to define the natural history of BK viremia in renal or renal-pancreas transplant recipients. In order to understand the natural history of infection, we will measure the time to the development of BK viremia and its correlation with progression to end-organ disease (BKVN or BK hemorrhagic cystitis). Data from this intensive natural history monitoring period will allow for the identification of the types of high-risk patients who might benefit from future studies of therapeutic interventions for BKV infection, when effective therapy becomes available. This will be accomplished by serial quantitative BK DNA measurements by PCR in blood (plasma). It is anticipated that most samples (blood and urine) will be collected during routine standard-of-care clinic visits and will not require extra study visits. Phases of the study can be summarized as follows:

- Subject identification and informed consent. Informed consent should be obtained in the peri-transplant period (between 7 days pre-transplantation [T-7d] and 100 days post-transplantation [T+100 d]).
- Prospective screening for BK viremia. Enrollment and initiation of specimen collection should begin no sooner than 4 weeks after transplantation. Enrollment cannot occur later than 100 days after transplantation. Subjects will be screened (collection of blood and urine for laboratory studies, including BK PCR) approximately monthly for 6 months (timed from the date of transplantation), then every other month for 6 months. For subjects who remain BKV-negative, screening will terminate approximately 12 months from the date of transplantation.
- Intensive sampling of subjects identified with BK viremia. Subjects identified with qualifying viremia will be sampled approximately every two weeks until resolution of viremia (defined as PCR negative, or <1000 copies).
  Note: Patients who did not participate in the prospective screening phase but are identified with BK viremia by standard-of-care testing may be enrolled, as long as they are still within 90 days of transplantation.
- Post-viremia follow-up. Following resolution of viremia, subjects will be sampled (collection of blood and urine) approximately monthly for 6 months (unless another termination event occurs sooner).

4.1. Informed Consent and Enrollment

In an effort to capture subjects early in the course of the evolving BK infection and to avoid subjects who have already developed evidence of BKV-induced nephropathy (BKVN), this protocol will emphasize the natural history of early-onset BK viremia. Obtaining informed consent early in the peri-transplant period is encouraged, although enrollment and study-related specimen collections will not begin until ≥4 weeks post-transplant. The consent form will secure the subject's informed consent to participate in the screening phase as well as the intensive monitoring phase (if qualifying viremia is subsequently identified).

Consented subjects can be enrolled as early as 4 weeks and as late as 100 days following renal/renal-pancreas transplant surgery. The 4-week delay prior to enrollment and initiation of specimen collection will allow post-transplantation stabilization of renal function, immunosuppressive dosing, and other clinical parameters.

4.2. Prospective Screening Phase

Approximately 450 subjects will be screened for BK viremia (15% over-enrollment allowed, to a maximum of 518 screened subjects). During the screening phase, subjects will submit blood (plasma) for quantitative measurement of BKV DNA approximately every 4 weeks, ± 7 days, for 6 months (timed from the date of transplantation) and then approximately every 8 weeks, ± 14 days for 6 months. Also, urine specimens will be collected at each visit and stored for subsequent testing. During the screening phase, the specified intervals between sample collections should be followed as closely as possible, but may vary with subject availability. If qualifying BK viremia is not detected, surveillance will terminate approximately 12 months after the date of transplantation (or when another study termination endpoint is reached, whichever occurs sooner).

NOTE: BKV PCR results of $\geq$10,000 from the study central laboratory, the site laboratory, or a reference laboratory are acceptable to qualify the patient for inclusion in the Intensive Monitoring Phase of the study.

NOTE: Non-study patients who are determined to have a qualifying blood BK viral load ($\geq$10,000 copies/ml) during the course of routine clinical care (i.e., patients not consented and not identified by prospective screening/surveillance) will also be eligible for enrollment directly into the intensive monitoring surveillance phase after providing consent as long as they remain within the window of $\leq$ 100 days following transplantation.

Subjects will remain in the screening/surveillance phase: 1) for 12 months post-transplantation; 2) until qualifying BK viremia is documented; 3) until renal graft failure, re-transplantation, and/or initiation of chronic dialysis occurs; 4) death; 5) until subject decides to terminate participation; 6) until the investigator decides that the subject should terminate participation; or 7) until the study closes (whichever occurs first). Reason for termination of participation in the study will be documented.

4.3. Intensive Sampling Phase

Approximately 40 viremic subjects (up to a maximum of 60) will be asked to participate in more intensive sampling. Those subjects in whom BK viremia is identified (by PCR result demonstrating BK DNA $\geq$10,000 copies/ml) will be shifted to a more intensive sampling schedule. A review of the informed consent will be initiated by the research staff to confirm the subject's continued willingness and ability to participate in the intensive monitoring phase until resolution of viremia or until another discontinuation event occurs.

Blood and urine specimen collection will occur every 2 weeks, ± 7 days, until the BK viremia has resolved (or another study termination endpoint is reached). Resolution of BK viremia is defined as PCR becoming negative (or viral load <1000 copies/ml). Start and stop dates for the Intensive Sampling Phase will be defined by the date that the qualifying (initial positive and subsequent negative) blood samples were drawn.

4.4. Post-Viremia Follow-Up

Following resolution of viremia, subjects will return to the previous sampling schedule (approximately every 4 weeks, ± 14 days) for an additional 6 months (or until another study termination endpoint is reached, whichever occurs sooner). The first follow-up visit should occur approximately 4 weeks after the BK viral load was documented to be negative.

Subjects will remain in the post-viremia follow-up phase: 1). for 6 months following resolution of the BK viremia; 2). until another study termination endpoint is reached; 3). until death; 4). until they decided to terminate participation; 5). until the investigator decides that the subject should terminate participation; or 6). until the study closes (whichever occurs first). Reason for termination of study participation will be documented.

4.5. Specimens for Biomarker Studies

Detection of tubular injury by measurement of biomarkers may provide an opportunity to manage renal graft recipients at an earlier stage before invasive BK disease. Coupled with urine PCR, detection of renal tubular injury in recipient urine is a simple technique. Urine markers of injury have already been identified in other causes of acute kidney injury and are commercially available. These include cystatin C, renin, clusterin, Kim-1, b2-microglobulin, osteopontin, retinol binding protein, and markers of cell apoptosis. For biomarker studies, approximately 100 mL of urine will be collected, aliquoted and stored at -70C on each subjects at each scheduled study visit (see MOP for details). Urine will also be collected at the time of any renal biopsy, if performed. We will retrospectively analyze expression of these urine markers of injury using urine specimens collected at the time of detection of BK virus (in urine and/or blood) and at serial time-points. Using appropriate control specimens, values will be compared for: 1) Subjects who are BK viremic vs. non-viremic; 2) Subjects who subsequently develop BKVN vs. subjects with stable GFR vs. subjects with declining GFR attributed to a cause other than BKVN.

4.6. Documenting Special Events

Specific clinical events and outcomes that may be related to BKV infection will be recorded, including:

- subject death
- loss of graft function as defined by GFR <10 mL/min/1.73 m^2
- development of urinary outlet obstruction
- development of gross hematuria

- development of lymphocele
- acute rejection
- initiation of dialysis
- re-transplantation
- renal biopsies – Should renal biopsy be performed per standard-of-care during the course of the study, results will be submitted using information from the pathology report.

All data will be recorded on source documents, then entered on eCRFs and submitted electronically.

4.7. Adverse Events

No protocol-defined interventions are included in this study; thus, adverse event recording will not be required unless the event is related to a study-mandated procedure (e.g., phlebotomy).

4.8. Clinical Laboratory Data

Specimens for laboratory studies will be collected as detailed in Section 7.2.

4.9. Study Visits

The study visits for the screening phase will be every 4 weeks ± 7 days for 6 months (timed from date of transplantation) and then every 8 weeks ±14 days. The study visits during the intensive monitoring period are every 2 weeks ± 7days until resolution of BK viremia and then every 4 weeks ± 7 days for 6 months.

Because the first year following kidney transplantation is challenging for patients, all post-enrollment study visits and visit windows are approximations and may fluctuate with study subject availability.

Events occurring at each study visit are listed in Appendix A.

5. STUDY POPULATION

5.1. Study Population Selection

All adult renal or renal-pancreas transplant patients who meet protocol inclusion/exclusion criteria are eligible for participation in this study.

In an effort to capture subjects early in the course of the evolving BK infection and avoid subjects who have already developed evidence of BKVN, this protocol will emphasize consent in the early peri-transplant period. Patients may be consented while hospitalized pre-transplantation (as early as 7 days prior to transplantation) or at an outpatient follow-up visit (as late as 100 days post-transplantation). The study enrollment visit and initial collection of protocol-defined specimens will take place between 4 weeks and 100 days following renal or renal/pancreas transplantation.

5.2. Inclusion of Women, Children and Minorities

All adult renal or renal/pancreas transplant patients meeting protocol inclusion/exclusion criteria will be potentially eligible for participation in this study. Subjects will not be excluded on the basis of gender or ethnicity. Since there is no known gender or ethnic predilection for BKV infection, it is expected that the percentage of women and minorities included in the study will reflect the local demographics of the renal or renal-pancreas transplant population at the participating sites.

5.3. Inclusion/Exclusion Criteria

5.3.1. Inclusion Criteria

- Renal or renal-pancreas transplant patients who are within the informed consent window (as early as 7 days prior to transplantation or as late as 100 days post-transplantation).
- Age $\geq$ 18 years;
- Willing and able to understand and provide written informed consent.

5.3.2. Exclusion Criteria

- Evidence of proven or suspected BKVN by clinical or pathologic diagnostic criteria;
- Initiation of chronic dialysis post-transplantation due to graft failure.

6.0. SCHEDULE OF EVENTS

6.1. Enrollment Visit (Day 1)

Subjects who meet criteria for the eligibility criteria and have provided informed consent should be scheduled for an Enrollment Visit. The enrollment visit should be scheduled no sooner than 4 weeks after the transplant, but not more than 100 days after the transplant. At the initial Enrollment Visit, the following assessments should be performed:

1. Confirm that informed consent has been obtained and that the subject still wishes to participate;
2. Review the inclusion-exclusion criteria to assure that the patient remains eligible for study participation. Subjects who are not willing or eligible to participate in the clinical trial should be logged as screen failures and reported on the Failed Screening page located on the Central Unit website.
3. Record donor and recipient demographic and risk factor data, to include:
    a. Donor-derived factors:
        i. Deceased donor – Standard criteria donor (SCD), Donor after cardiac death (DCD), Expanded criteria donor (ECD)
        ii. Living donor – related donor or unrelated donor
        iii. ABO incompatibility
        iv. Total HLA mismatches
        v. HLA C7 status
        vi. Age
        vii. Race/ethnicity
        viii. Sex
    b. Recipient-derived factors:
        i. Delayed graft function (DGF), defined as use of dialysis within 7 days of the transplant
        ii. Cold ischemia time (CIT)
        iii. Warm ischemia time (WIT)
        iv. Use of stents
        v. HLA C7 status
        vi. Type/intensity of immunosuppression, including corticosteroids
        vii. Acute rejection
        viii. Age
        ix. Race/ethnicity
        x. Sex

4. Document medical and surgical history
5. Document use of selected concomitant medications (specifically, antiviral agents, immunomodulators, and immunosuppressive medications – see the listing provided in

the MOP). Record all immunosuppressive drugs administered since transplantation, including peri-operative induction therapy with biological agents;
6. Document blood concentrations of immunosuppressive drugs measured for clinical reasons;
7. Obtain blood for serum creatinine determination and estimated GFR (if these studies were performed for routine standard-of-care within 7 days prior to enrollment, then the results of those assays can be recorded for study purposes);
8. Collect 10 ml of blood for study-specified PCR assays (BK and other viruses), to be performed at the UAB Central Laboratory. Blood will collected, separated to plasma, then stored and shipped per instructions in the Manual of Procedures;
9. If blood BK PCR was performed for routine standard-of-care in a local or reference laboratory, record the results of that assay as well. (Specimen for PCR will still be submitted to the UAB Central Laboratory);
10. Optional urinalysis - if UA was performed for routine standard-of-care with 7 days of enrollment, then the results of those assays can be recorded;
11. Obtain a urine sample (approximately 100 ml) for study-specified PCR assays (BK and other viruses) and measurements of urinary biomarkers to be performed at the UAB Central Laboratory. Urine will be collected, stored, and shipped per instructions in the Manual of Procedures.
12. Notify the UAB Central Unit if the subject has consented to any other research study.

6.2. Screening Study Visits (approximately every 4 weeks +/- 7 days for 6 months and then approximately every 8 weeks +/- 14 days for 6 months)

At each subsequent protocol-defined screening visit, the following events will occur:

1. Document interval medical history to specifically include:
    a. subject death,
    b. loss of graft function as defined by GFR <10 mL/min/1.73 m^2
    c. development of urinary outlet obstruction
    d. development of gross hematuria
    e. development of lymphocele
    f. acute rejection
    g. initiation of dialysis
    h. re-transplantation
    i. renal biopsies – Should renal biopsy be performed per standard-of-care during the course of the study, record the results from the pathology report.

2. Document use of selected concomitant medications (specifically, antiviral agents, immunomodulators, and immunosuppressive medications – see MOP for listing);
3. Record blood concentrations of immunosuppressive drugs obtained for clinical care purposes;

4. Obtain blood for serum creatinine determination and estimated GFR (if these studies were performed for routine standard-of-care within 7 days prior to the study visit, then the results of those assays can be recorded for study purposes);
5. Obtain 10 ml of blood for study-specified PCR assays (BK and other viruses), to be performed at the UAB Central Laboratory. Blood will collected, separated to plasma, then stored and shipped per instructions in the Manual of Procedures;
   If blood BK PCR was performed for routine standard-of-care at a local or reference laboratory, record the results of that assay in the research record;
6. Optional urinalysis - if UA was performed for routine standard-of-care within 7 days of enrollment, then the results of those assays can be recorded);
7. Obtain urine (approximately 100 ml) for subsequent study-specified PCR assays (BK and other viruses) and measurements of urinary biomarkers to be performed at the UAB Central Laboratory. Urine will be collected, stored, and shipped per instructions in the Manual of Procedures.
8. Notify the UAB Central Unit if the subject has consented to any other research study.

6.3. Intensive Monitoring Phase for BK Viremic Subjects: (approximately every 2 weeks +/- 7 days until viremia has resolved (PCR negative or <1000 copies/ml), then approximately every 4 weeks +/-14 days for 6 months).

At each protocol-defined study visit, the following events will occur:

1. Document interval medical history to specifically include:
   a. subject death,
   b. loss of graft function as defined by GFR <10 mL/min/1.73 m^2
   c. development of urinary outlet obstruction
   d. development of gross hematuria
   e. development of lymphocele
   f. acute rejection
   g. initiation of dialysis
   h. re-transplantation
   i. renal biopsies – Should renal biopsy be performed per standard-of-care during the course of the study, record the results from the pathology report.

2. Document use of selected concomitant medications (specifically, antiviral agents, immunomodulators, and immunosuppressive medications – see MOP for listing);
3. Record blood concentrations of immunosuppressive drugs obtained for clinical care purposes;
4. Obtain blood for determination of creatinine and GFR. If results of serum creatinine and GFR obtained for clinical care purposes (within 7 days) are available, those results may be used instead;

5. Obtain 10 ml of blood for study-specified PCR assays (BK and other viruses), to be performed at the UAB Central Laboratory. Blood will collected, separated to plasma, then stored and shipped per instructions in the Manual of Procedures;
6. If blood BK PCR was performed for routine standard-of-care at a local or reference laboratory, record the results of that assay in the research record;
7. Optional urinalysis – if results of urinalysis obtained for clinical care purposes (within 7 days) are available, those results may be recorded;
8. Obtain urine (approximately 100 ml) for subsequent study-specified PCR assays (BK and other viruses) and measurements of urinary biomarkers to be performed at the UAB Central Laboratory. Urine will be collected, stored, and shipped per instructions in the Manual of Procedures.
9. Notify the UAB Central Unit if the subject has consented to any other research study.

6.4. Subject Early Termination

Subjects will be terminated from the study for any of the following reasons:

- Death;
- Initiation of chronic dialysis for loss of graft function;
- Re-transplantation;
- Inability or unwillingness to comply with study requirements.

6.5. Management of Subject Withdrawals

Study subjects may withdraw voluntarily from participation in the study at any time. If a study subject withdraws or is discontinued from the study at any time prior to completion of the study, the reason for this decision will be recorded on the study termination page of the eCRF.

6.6. Termination of Study

The UAB Central Unit (in consultation with DMID) and NIH/NIAID have the right to terminate this study at any time. Reasons for terminating the study may include, but are not limited to, the following:

- Study subject enrollment is unsatisfactory;
- Data recording is inaccurate or incomplete;
- Investigators do not adhere to the protocol or applicable regulatory guidelines in conducting the study.

7.0. STUDY PROCEDURES

The scheduled study procedures and evaluations are summarized in Appendix A.

7.1. Demographics, Baseline Medical-Surgical History, Clinical Outcomes

At the time of enrollment, baseline demographics and medical-surgical history (cardiovascular, respiratory, musculoskeletal, gastrointestinal, lymphatic, urinary, nervous, endocrine, skin, reproductive, EENT) will be obtained from the medical charts or from subject interviews. Transplant-specific history (HLA match, organ source, surgical date and select donor and recipient risk factors) will be recorded.

At subsequent visits, changes in the medical and surgical history will be documented (including selected concomitant medications, immunosuppressive drug levels and pathology results from renal biopsies conducted for clinical care). Clinical outcomes will be recorded including subject death, loss of graft function, acute graft rejection or initiation of dialysis.

7.2. Laboratory – Specimen Collection

7.2.1. At Each Screening Visit:

Blood:
- Blood (10 ml) for viral PCR submitted as plasma to UAB Central Laboratory).
- Serum creatinine and GFR performed at local laboratory (or results of assay performed for clinical standard-of-care will be recorded)

Urine:
- specimen (approximately 100 ml) for biomarkers and viral PCR (UAB Central laboratory)
- optional urinalysis (dipstick and microscopic, to include: pH, bilirubin, blood, nitrite, protein, specific gravity, glucose, ketones, RBC/HPF, WBC/HPF, urobilinogen, leukocyte esterase) performed at local laboratory (or results of assay performed for clinical standard-of-care can be recorded)

7.2.2. At Each Intensive Follow-up Visit for Viremic Subjects:

Blood:
- Blood (10 ml) for viral PCR submitted as plasma to UAB Central Laboratory.
- Serum creatinine and estimated GFR – performed at local laboratory (or results of assay performed for clinical standard-of-care will be recorded)

Urine:

- Optional urinalysis (dipstick and microscopic, to include: pH, bilirubin, blood, nitrite, protein, specific gravity, glucose, ketones, RBC/HPF, WBC/HPF, urobilinogen, leukocyte esterase) performed at local laboratory (or results of assay performed for clinical standard-of-care can be recorded)
- specimen (approximately 100 ml) for biomarkers and viral PCR (UAB Central laboratory)

7.3. Reporting of Concomitant Medications

Only selected concomitant medications will be recorded at each subject visit. Only the following will be reported:

- immunosuppressive drugs (including peri-transplant induction therapy),
- immunomodulators,
- antiviral drugs.

A list of the selected medications is found in the Manual of Procedures. The generic name of each medication, the dosage, the route, and the start/stop dates of administration will be recorded in the appropriate sections of the eCRF. Failure to document use of selected concomitant medications will be considered a protocol deviation.

7.4. Dosing of Immunosuppressive Drugs

Immunosuppressive (IS) drug selection and doses will be determined by the site physicians/investigator in accordance with best clinical practice and local treatment standards. Modification of immunosuppressive drug dosing in response to documented BK viremia (or other indications) may be performed at the discretion of the treating physicians/investigator. All doses and dose changes should be documented in the concomitant immunosuppressive medications section of the eCRF. Failure to document concomitant medication information will be reported as a protocol deviation during screening/surveillance and intensive monitoring periods.

Measurements of immunosuppressive drug levels are not a protocol requirement. However, IS levels that are obtained for standard-of-care medical management during the study will be recorded on the eCRF.

7.5. Measurement of Viral DNA

BKV DNA levels will be measured at the UAB Central Lab. Plasma specimens will be shipped to the UAB Central Laboratory once weekly (Monday through Thursday – see Manual of Procedures

for details) for **real-time** viral load determinations. The BKV DNA in plasma will be assayed using a quantitative PCR assay. Assays will be performed approximately once weekly and results will be shared with the site investigators as soon as they are available.

NOTE: BK PCR assays performed on specimens collected during the post-viremia follow-up period will not be run in real time; real-time results will not be available to the investigator. These can be batch-shipped at the conclusion of the follow-up period.

Retrospective analysis of urine for BKV DNA and tubular injury biomarkers will be performed in viremic subjects where correlations between viremia and viruria can be made. PCR of urine for BKV DNA **will not be** performed in real time. Urine specimens for PCR and for biomarker assays will be collected, frozen, and batch-shipped to the UAB Central Laboratory (see Manual of Procedures for details).

Plasma and urine assays for other non-BK viral pathogens will be performed retrospectively. These assays **will not be** performed in real time and results **will not be** provided to the site principal investigators in real time.

Any blood BKV DNA result obtained at the local (or reference) laboratory for standard-of-care purposes will be reported on the eCRF.

7.6. Specimen Preparation, Handling and Shipping

Guidance for specimen processing, shipping and storage will be found in the MOP.

8. STATISTICAL CONSIDERATIONS

8.1. Study Hypothesis

Elucidation of the natural history of BK viremia in renal transplant patients will identify those groups at high risk for developing BKVN who may potentially benefit from therapeutic interventions (when these become available). In particular, it is of interest to compare the change in the glomerular filtration rate (GFR) a year after transplant between the BKV viremic and non-viremic subjects. We hypothesize that BKV viremia will have a negative effect on renal function as measured by GFR.

8.2. Sample Size Considerations

8.2.1. Sample Size

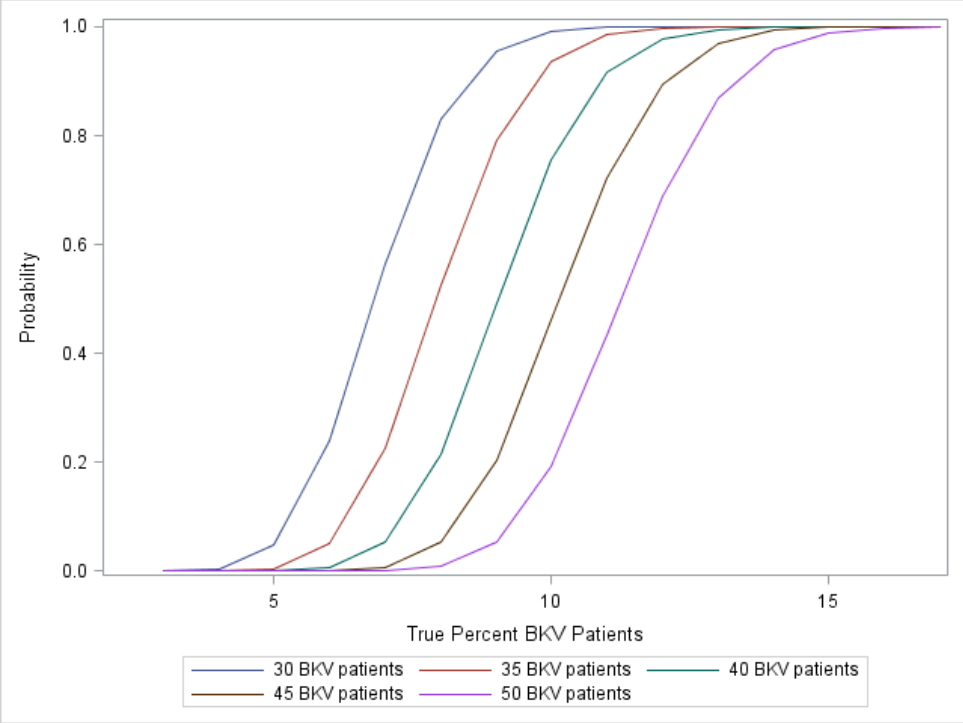
We propose to enroll approximately 450 (up to a maximum of 518) patients in this study. The sample size justification is based on observing a target number of BKV viremia patients from the patients enrolled in the screening group. Table 1 below shows varying values of proportion of patients with BKV viremia. Figure 1 summarizes the probability of observing K number of BKV patients (K=30, 35, 40, 45, and 50) assuming varying values of true proportion of BKV viremia patients (3% to 17%) out of 450 enrolled patients. To interpret this graph, assume that the true proportion of BKV viremia patients is 10%. Then the probabilities of observing at least 30, 35, 40, 45, and 50 BKV viremia patients out of the 450 enrolled are about 0.99, 0.94, 0.76, 0.46, and 0.19, respectively.

In this study, we would like to target approximately 40 BKV viremia patients (up to a maximum of 60). From Figure 1, enrolling 450 subjects give us at least 90% probability of identifying at least 40 BKV viremia patients, if the true percent of BKV viremia patients is at least 11%.

Table 1

Location	Study Type	Sample size (N)	% with BKV viremia	Reference
USA[3]	Prospective	200	11.5%	Brennan 2005
Spain[5]	Prospective	156	12.2%	Munoz 2005
USA[23]	Prospective	66	16.6%	Petrov 2009
Czech Republic[24]	Prospective	120	6%	Girmanova 2011
USA[25]	Retrospective	134	16%	Koleilat 2011

Figure 1: Probability of Observing a Given Number of BKV Subjects or More as a function of True Percent with BKV viremia



We are also interested in comparing the change in GFR between those with and without BKV viremia. In this power analysis, we will only be concerned about the change from baseline (time of enrollment) to Month 12 (time post-transplant). Assuming the standard deviation of the change in GFR is assumed to be the same for those with and without BKV viremia and equals 10.9 ml/min/1.73 m2 (Ref: Srinivas TR, Flechner SM, Poggio ED, et al. Glomerular filtration rate slopes have significantly improved among renal transplants in the United States. Transplantation 2010; 90:1499-505.). With 40 BKV viremia subjects and 410 non-BKV viremia subjects, we will detect a mean difference in the change in GFR of at least 5.9 ml/min/1.73 m2 with 90% power between these two groups of subjects at 5% level of significance based on a t-test. In the final analysis, we will be using all available GFR data for each subject which should increase the power as more information is used. This will enable us to still use data from subjects who dropped out before the end of the study.

8.2.2. Estimated Annual Accrual

We anticipate enrolling approximately 250 subjects per year into prospective screening

8.3. Demographic Analysis

Demographics and clinical characteristics will be summarized using means and standard deviations for continuous variables and counts and proportions for categorical variables. ANOVA

(or its nonparametric analog Krusall-Wallis) for continuous variables and Fisher's exact test for categorical variables will be utilized to compare these characteristics. Demographic characteristics will be summarized in tabular format.

8.4. Final Analysis Plan

All statistical tests will be performed at 5% significance level and confidence interval at 95% level. There will be no adjustment on the levels for multiple testing. There will also be no imputation methods that will be used for missing data. Any viral load <100 (i.e., undetectable) will be replaced by viral load value of 10. Quantitative viral loads will be analyzed based on the log based 10 transformation.

8.4.1. Primary Outcome Analysis

Incidence of BK viremia will be estimated using a 95% confidence interval for single proportion. Logistic regression will be used to investigate the risk factors for BKV viremia and BKVN. Factors will include:

- Donor-derived factors:
    1. Deceased donor – Standard criteria donor (SCD), Donor after cardiac death (DCD), Expanded criteria donor (ECD)
    2. Living donor – related donor or unrelated donor
    3. ABO incompatibility
    4. Total HLA mismatches
    5. HLA C7 status
    6. Race/ethnicity
    7. Sex
- Recipient-derived factors:
    1. Delayed graft function (DGF), defined as use of dialysis within 7 days of the transplant
    2. Cold ischemia time (CIT)
    3. Warm ischemia time (WIT)
    4. Use of stents
    5. HLA C7 status
    6. Type/intensity of immunosuppression, including corticosteroids
    7. Acute rejection
    8. Age
    9. Race/ethnicity
    10. Sex

To compare the change in GFR over time between those with and without BKV viremia, we use all available GFR data from each subject. We will use mixed model for repeated measures to analyze the GFR data over time. This analysis method will allow us to use any of the available GFR data

even if they are not complete. We will determine the appropriate covariance structure to use as indicated by the Akaike Information Criterion.

8.4.2. Secondary Endpoints

The distribution of the time to onset and duration of BKV viremia will be estimated using survival analysis methods such as Kaplan-Meier estimator for a single group. This will provide estimates of the median time to onset or median duration of BKV viremia. To compare different groups and/or determine risk factors, we will use Cox proportional hazard regression model if the proportional hazards assumption is found to be reasonable. Otherwise, we will consider using parametric survival regression models (in particular, accelerated failure time model). Parametric regression models are useful especially in cases where sample sizes are small or when the percent censored is high. This will also be the tool of preference if there will be left-censored subjects, i.e., subjects who at enrollment show evidence of BK viremia. Cox model cannot handle left-censored data.

As with the BKV viremia, incidence of BKVN will be estimated using a 95% confidence interval for single proportion. We will utilize logistic regression to compare groups, or adjust for covariates or determine risk factor.

To investigate the change in the quantitative BK viremia over time and how it is associated with important factors such as the use of immunosuppressive therapy and recipient's demographic and clinical characteristics, we will again use mixed regression modeling for repeated measures.

8.4.3. Exploratory Endpoints

To investigate the association between the incidence between BK viremia and BK viruria, we will utilize the Fisher's exact test or the large-sample chi-squared test for association whichever is applicable. Time to onset and duration of BK viremia and BK viruria will be analyzed using survival analyses.

Logistic regression will be utilized to determine if other viruses such as CMV, adenovirus, etc. are risk factors for BK viremia. This tool will also be used to determine if selected urinary biomarkers of tubular injury are predictors of BKVN.

To assess the relationship of the time to development of BKVN with the use of immunosuppressive therapy, we will use survival regression analysis with the length of use of immunosuppressive weighted by dose as a predictor variable of interest.

Finally, to examine the reproducibility of the quantitative PCR assays at the participating sites and PCR assays at UAB, we compute the differences in the results and construct a 95% confidence interval for the mean difference. We will also create a Bland-Altman plot which will display the differences and their 95% confidence intervals as a function of the mean readings of the two PCR results.

9. SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/ DOCUMENTS

Documentation of source data is necessary for the reconstruction, evaluation, and validation of clinical findings, observations, and other activities during a clinical trial. Source documentation serves to substantiate the integrity of trial data, confirms observations that are recorded, and confirm the existence of study participants. This standard also serves to ensure data quality by creating audit trails and enabling verification that data are present, complete, and accurate. DMID studies will be monitored using these standards.

Sites that are participating in this trial should consult the MOP, Central Unit website, and DMID/NIAID Source Document Standards (most current version) for specific instructions and forms.

Local, state, institution, IRB/independent ethics committee (IEC) policies and procedures may be different from those stated in this standard. The site should refer to local, state, institution, IRB/IEC policies and procedures and follow them if they are more stringent than the DMID Standards.

According to the ICH E6 Guidelines 4.9, “The investigator should ensure the accuracy, completeness, legibility, and timeliness of the data reported to the sponsor in the CRFs and in all required reports. Data reported on the CRFs, which are derived from source documents, should be consistent with the source documents or discrepancies should be explained.”

10. QUALITY CONTROL AND QUALITY ASSURANCE

The study site will implement a quality management plan. The quality management procedures are described herein, as well as in the Manual of Procedures and the study site quality management plan (QMP). Per the QMP, data will be evaluated for compliance with the protocol and accuracy in relation to source documents. The study will be conducted in accordance with procedures identified in the protocol. Items to be reviewed include, but are not limited to: eligibility (including informed consent), any required AE reporting, study/clinical endpoints, follow-up visits, regulatory documents, missed calls, and review of clinical records. Data that will be reviewed, who is responsible for implementation, and the schedule for internal reviews will be specified or referenced in the quality management plan.

The UAB Statistical and Data Coordinating Center will implement quality control procedures beginning with the data entry system, and will generate data quality control checks that will be run on the database within 24 hours of new data entered into the system. Full documentation of these checks will be provided to the UAB Central Unit so that any resulting queries can easily be understood and transmitted to the respective site for resolution within a short period of time. These processes are validated by a second programmer and also tested with faulty test data.

11. ETHICS AND PROTECTION OF HUMAN SUBJECTS

11.1. Ethical Standard

This trial will be conducted in compliance with the protocol, International Conference on Harmonization E6 Good Clinical Practice (ICH E6 GCP), and the applicable regulatory requirements, including:

- U.S. Code of Federal Regulations applicable to clinical studies (45 CFR 46 and 21 CFR 11 including parts 50 and 56 concerning informed consent and IRB regulations).
- Completion of Human Subjects Protection Training. Refer to http://grants.nih.gov/grants/guide/notice-files/NOT-OD-01-061.html; http://cme.cancer.gov/clinicaltrials/learning/humanparticipant-protections.asp

11.2. Institutional Review Board

Reviewing IRBs must be registered with the OHRP to conduct FDA-regulated studies. In the United States and in other countries, only institutions holding a current US Federalwide Assurance (FWA) issued by OHRP will be eligible to participate in this protocol.

This protocol, informed consent documents, relevant supporting information, and all types of volunteer recruitment or advertisement information will be submitted to the Institutional Review Board (IRB) for review and must be approved before the study is initiated. Any amendments to the protocol must also be approved by the IRB prior to implementing changes in the study.

The investigator is responsible for keeping the IRB apprised of the progress of the study and of any changes made to the protocol as deemed appropriate, but in any case at least once per year. The investigator must also keep the IRB informed of any significant AEs.

All IRB approved documents as well as relevant study correspondence should be copied and sent to the UAB Central Unit.

11.3. Informed Consent Process

The process of obtaining informed consent must be documented in the medical records, clinic chart, and/or research chart. The consent form must be signed and dated by the study participant/study participant's legal guardian before participation in the study. Only in the case of an established legal guardian may the consent be signed by someone other than the subject. A copy of the signed consent form must be provided to the study participant/study participant's legal guardian. Signed consent forms must remain in each study participants study file and must be available for verification by study monitors at any time.

The investigational nature and research objectives of this trial, the procedure, and its attendant risks and discomforts will be carefully explained to the study participant/study participant's legal guardian. A signed informed consent document will be obtained from each study participant/study participant's legal guardian prior to entry into this study. At any time during participation in the protocol, if new information becomes available relating to risks, AEs, or toxicities, this information will be provided orally or in writing to all enrolled or prospective study participant/study participants' legal guardian. Documentation will be provided to the IRB and, if necessary, the informed consent will be amended to reflect any relevant information.

An investigator shall seek such consent only under circumstances that provide the prospective subject or the representative sufficient opportunity to consider whether or not to participate and that minimize the possibility of coercion or undue influence. The information that is given to the subject or the representative shall be in language understandable to the subject or the representative.

Subjects/subject's legal guardian will sign the informed consent document prior to any procedures being done specifically for the study. Subjects/subject's legal guardian should have the opportunity to discuss the study with their family, friends or personal physician, or think about it prior to agreeing to participate. Subjects/subject's legal guardian may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the subjects /subject's legal guardian for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

If an investigator anticipates enrolling non-English subjects, he/she must provide an approved non-English consent form. The non-English consent form must be submitted to the Central Unit with an accompanying signed attestation that the non-English consent matches in content and meaning the IRB approved English consent. If a subject presents him/herself for enrollment, the PI must identify personnel who will be conduct the consent procedures, discussion and communicate other information to the study subject. Assurance must also be documented that the person proficient in the non-English language is available at all subject visits and at any time interaction is needed with the subject. The Principle Investigator or designee will document the process.

If a subject is unable to read or if a legally acceptable representative is unable to read, an impartial witness should be present during the entire informed consent discussion. After the written informed consent form and any other written information to be provided to subjects, is read and explained to the subject or the subject's legally acceptable representative, and after the subject or the subject's legally acceptable representative has orally consented to the subject's participation in the trial and, if capable of doing so, has signed and personally dated the informed consent form, the witness should sign and personally date the consent form. By signing the consent form, the witness attests that the information in the consent form and any other written information was accurately explained to, and apparently understood by, the subject or the subject's legally acceptable representative,

and that informed consent was freely given by the subject or the subject's legally acceptable representative. The Principle Investigator and or designee will document the consenting process.

11.4. Exclusion of Women, Minorities, and Children (Special Populations)

All adult renal or renal-pancreas transplant patients with BKV viremia meeting protocol inclusion/exclusion criteria will be potentially eligible for participation in this study. Subjects will not be excluded on the basis of gender or ethnicity. Since there is no known gender or ethnic predilection for BKV infection, it is expected that the percentage of women and minorities included in the study will reflect the local demographics of the renal or renal-pancreas transplant population at the participating sites. Children will not be enrolled.

11.5 Subject Confidentiality

The information obtained during the conduct of this clinical study is confidential, and disclosure to third parties other than those noted below is prohibited. The results of the research study may be published, but study participant's names or identities will not be revealed. Records will remain confidential. To maintain confidentiality, the principal investigators at each site will keep records in locked cabinets and the results of tests will be coded to prevent association with volunteers' names. Data entered into computerized files will be accessible only by authorized personnel directly involved with the study and will be encoded. However, subject specific information will be available to the clinical monitors, to the FDA and to health authorities where provided by law.

The study investigator is obliged to provide the NIAID/DMID and UAB Central Unit with complete test results and all data developed in this study. The NIAID/DMID or UAB Central Unit may disclose this information to appropriate regulatory authorities as deemed necessary by the NIAID/DMID or UAB Central Unit

Subject-specific information may be provided to other appropriate medical personnel only with the study participant's parent/legal guardian permission. To ensure compliance with current ICH E6 Guidelines, data generated by this study must be available for inspection upon request by representatives of national and local health authorities, the NIAID/DMID, UAB Central Unit, and the IRB/IEC for each study site.

11.6. Recruitment and Compensation

Study participants will be recruited using IRB-approved approaches. The investigators will make every effort to recruit adult male and female volunteers from all ethnic backgrounds.

Consented study participants/legal guardians may receive nominal payments to offset the cost of travel, parking, and meals for study visits in accordance with local site policies and local IRB policies, procedures, and approval.

11.7 Future Use of Stored Specimens

Some of the specimens obtained from study participants during this study (urine and plasma) will be stored indefinitely in the UAB Central Laboratory and may be used in future studies of viral infections in renal or renal-pancreas transplant patients. These specimens will be labeled with a code number and not with the study participant's name. At the time of consent for study participation, study participants will have the opportunity to either agree or decline to have their specimens used in future research. The study participant will indicate his/her preference by initialing the appropriate line or checking the appropriate box of the Consent Form in the section entitled, "Future Use of Specimens". Non-protocol designated, future testing of samples will be performed only on samples from study participants who have consented for future testing of samples. These specimens will only be utilized to better understand the natural history of studies of viral infections in renal or renal-pancreas transplant patients or improve diagnosis.

A repository for residual samples will be established according to OHRP guidelines ensuring that codes or other personally identifying links will not be distributed to future researchers.

The specimens will be stored indefinitely in the UAB Central Laboratory. Specimens from study participants will be labeled and coded without study participant's identifiers. If the study participant has indicated in the signed consent form that he/she does not agree to allow the future use of specimens for future studies of viral infections in renal or renal-pancreas transplant patients, then his/her specimens will be destroyed at the completion of this study.

12. DATA HANDLING AND RECORD KEEPING

The investigator is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported.

Electronic case report forms (eCRFs) will be developed by the UAB Statistical and Data Coordinating Center. The eCRFs will be provided electronically by the UAB Central Unit. Original data will be recorded on source documents (e.g., medical records, research progress notes, or Source Document Worksheets documenting research related procedures). The DMID 11-0071 Data Management Plan provides a description of how the system will function. Source Document Worksheets that mirror each data field on the eCRF will be available for use by sites as a tool to record and maintain data for each study participant enrolled in the study when other source documents are not used to collect original data. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. When making a change or correction, cross out the original entry with a single line and initial and date the change. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL. Specific guidance to investigators and study staff on making corrections to source documents and eCRFs will be provided in the MOP for this study.

Data recorded on the eCRF that differ from source documents must be explained on the Comments eCRF and in the subject's source documents.12.1 Data Management Responsibilities.

All eCRFs must be reviewed by the investigator's research team, under the supervision of the investigator, who will ensure that they are accurate and complete. All data must be supported by source documents, which will remain available for review by regulatory personnel and monitors. Adverse events must be graded, assessed for intensity and causality, and reviewed by the site investigator or designee.

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site PI. During the study, the investigator must maintain complete and accurate documentation for the study.
The UAB Central Unit and Statistical and Data Coordinating will be responsible for data management, quality review, analysis of the study data, and writing of the clinical study report. These tasks are detailed in the DMID 11-0070 DMP.

12.1 Data Capture Methods

Clinical and laboratory data will be entered into a 21 CRF Part 11 compliant electronic Data Entry System (eDES) provided by the UAB Statistical and Data Coordinating Center. The data system includes password protection and internal quality checks, such as automated range checks, to identify data that appear to be inconsistent, incomplete, or inaccurate.

12.2 Types of Data

Data for this study will include clinical laboratory and virologic results, and clinical and outcome measures.

12.3 Timing/Reports

There are no planned interim analyses or safety reviews for this trial. There also are no stopping rules.

12.4 Study Records Retention

Records and documents pertaining to the conduct of this study, including source documents and consent forms, must be retained by the investigator for at least 2 years following completion of the study. No study records shall be destroyed without prior authorization from the UAB Central Unit. These documents should be retained for a longer period, however, if required by local IRB or the site research office.

12.5 Protocol Deviations

Each investigator must adhere to the protocol as detailed in this document and agree that any changes to the protocol must be approved by the UAB Central Unit and NIAID/DMID prior to seeking approval from the IRB/IEC. Each investigator will be responsible for enrolling only those study participants who have met protocol eligibility criteria.

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or Manual of Procedures requirements. The noncompliance may be either on the part of the study participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6 GCP sections:

4.5 Compliance with Protocol, Sections 4.5.1, 4.5.2, and 4.5.3

5.1 Quality Assurance and Quality Control, Section 5.1.1

5.20 Noncompliance, Sections 5.20.1 and 5.20.2.

It is the responsibility of the site to use continuous vigilance to identify and report deviations within 1 business day of identification of the protocol deviation that increases subject risk. Deviations that

do not increase subject risk can be reported within 5 business days of knowledge of the event. All deviations must be promptly reported to the UAB Central Unit. UAB will report all deviations to DMID in accordance with DMID's instructions.

All deviations from the protocol must be addressed in the source documents. A completed copy of the DMID protocol deviation form must be maintained in the regulatory file (Project Notebook or designated location) as well as in the subject's source documents. A log of protocol deviation will be maintained in the Project Notebook. Protocol deviations must be sent to the local IRB per the IRB's guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB/IEC requirements.

13. PUBLICATION POLICY

Following completion of this study, the investigators are expected to publish the results in a scientific journal. All research reports and other publications resulting from the work completed in this protocol shall:

- Acknowledge the support of the National Institutes of Health whenever publicizing the results from this clinical trial in any media.
- Be submitted to the Project Director in the form of advance copies for review and comment prior to the publication to ensure appropriate coordination of the research results.
- Be furnished in a list of publications resulting from the research as part of the annual progress report submitted to the principal investigator.

The International Committee of Medical Journal Editors (ICMJE) member journals has adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies.

Unless exempted, this trial will be registered prior to enrollment of study subjects. It is the responsibility of the study's PI (i.e., Dr. Whitley) to register the non-exempted trials and post results in compliance with Public Law 110-85, the Food and Drug Administration Amendments Act of 2007 (FDAAA).

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APPENDIX A-1: Table of Study Procedures - Screening Phase

Procedures	[A] 4 weeks to 100 days Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 8	Month 10	Month 12
[1] Review informed consent	X									
Assess eligibility criteria-inclusion/exclusion	X									
[1a] Record medical/ surgical history	X									
[2] Record donor and recipient risk factor data	X									
[2a] Record Specific Interval History Data		X	X	X	X	X	X	X	X	X
[3] Record IS drug trough levels	X	X	X	X	X	X	X	X	X	X
[4] Obtain ≥10 mL of blood for viral load by PCR	X	X	X	X	X	X	X	X	X	X
[4] Obtain approximately 100 mL of urine for PCR and for biomarkers	X	X	X	X	X	X	X	X	X	X
[5] Record selected concomitant medications	X	X	X	X	X	X	X	X	X	X
[6] Serum Creatinine, estimated GFR (local lab)	X	X	X	X	X	X	X	X	X	X
[7] Urinalysis - optional (local lab)	X	X	X	X	X	X	X	X	X	X
[8] Study Termination										X

LEGEND

[A] The post-transplant specimen collection and study procedures should not be implemented until 4 weeks (out to 100 days) post-transplant

[1] The informed consent may be obtained during the peri-transplant admission (as early as T-7 days) out to T+100 days[1a] The medical history includes assessment of the following: cardiovascular, respiratory, musculoskeletal, gastrointestinal, lymphatic, urinary, nervous, endocrine, skin, reproductive and EENT Surgical history includes organ source, and date of surgery .

[2] Record donor/recipient risk factors data: donor= [deceased, living, ABO incompatibility, total HLA mismatches, HLA C7, age, race/ethnicity, sex] recipient= [delayed graft function(DGF), cold ischemia time (CIT), warm ischemia time (WIT), use of stents, HLA C7 status, type/intensity of immunosuppression, acute rejection, race/ethnicity, age, sex]

[2a] Subject death, loss of graft function as defined by GFR <10 mL/min/1.73 m^2, development of urinary outlet obstruction, development of gross hematuria, development of lymphocele acute rejection, initiation of dialysis, re-transplantation, renal biopsies – Should renal biopsy be performed per standard-of

[3] Record lab results for immunosuppressive drug trough levels, if performed for standard of care

[4] Collect and process blood and urine samples according to instruction outlined in Manual of Procedure

[5] Record selected concomitant medication generic name dosage, start/stop date, and route of administration, failure to record, report as a protocol deviation.

[6] Record results of serum creatinine and estimated, if obtained for standard of care.

[7] Optional - Record urinalysis results performed at local lab for dipstick and microscopic for pH, bilirubin, blood, nitrate, protein, specific gravity, glucose, ketones, RBC/HPF, WBC/HPF, urobilinogen, leukocyte esterase

[8] Screening and study participation will terminate for subjects who remain BKV negative at 12 months post-transplantation.

APPENDIX A-2: Table of Study Procedures for BK Viremia Intensive Sampling Phase

Procedures	BK Viremia diagnosis	BK viremia monitoring	Post BK Viremia Follow-Up					
	Baseline	[A] Every 2 weeks +/- 7 days until resolved	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
[1] Review informed consent process	X							
[1a] Assess BK Viremia criteria	X	X						
[2]Record Specific Interval History Data		X	X	X	X	X	X	X
[3] Record IS trough levels		X	X	X	X	X	X	X
[4] Obtain ≥10 mL of blood for viral load by PCR		X	X	X	X	X	X	X
[4] Obtain approximately 100 mL of urine for PCR and for biomarkers		X	X	X	X	X	X	X
[5] Record selected concomitant medications		X	X	X	X	X	X	X
[6] Serum Creatinine, estimated GFR (local lab)		X	X	X	X	X	X	X
[7] Urinalysis - optional (local lab)		X	X	X	X	X	X	X
Study Termination								X

LEGEND:

[A] When diagnosed with BK Viremia, perform procedures every 2 weeks until viremia has resolved.

[1] Review informed consent document with subject if consented during the surveillance period.

[1a] The BK viremia criteria includes Central Unit Lab and Local plasma PCR BK viral load results, and dates of collection and dates of results

[2] Subject death, loss of graft function as defined by GFR <10 mL/min/1.73 m^2, development of urinary outlet obstruction, development of gross hematuria, development of lymphocele acute rejection, initiation of dialysis, re-transplantation, renal biopsies – Should renal biopsy be performed per standard-of care.

[3] Record lab results for immunosuppressive drug trough levels, if performed for standard of care

[4] Collect and process blood and urine samples according to instruction outlined in Manual of Procedure

[5] Record selected concomitant medication generic name dosage, start/stop date, and route of administration.[6] Record results of serum creatinine and estimated, if obtained for standard of care.

[7] Record urinalysis results performed at local lab, within 7 days of visit, for dipstick and microscopic for pH, bilirubin, blood, nitrate, protein, specific gravity, glucose, ketones, RBC/HPF, WBC/HPF, urobilinogen, leukocyte esterase