Protocol Title:

QUINOLONE PROPHYLAXIS FOR THE PREVENTION OF BK VIRUS INFECTION IN KIDNEY TRANSPLANTATION: A PILOT STUDY

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1. THE NEED FOR A TRIAL

1.1 WHAT IS THE PROBLEM TO BE ADDRESSED?

Kidney transplantation is the treatment of choice for end-stage renal disease as it prolongs survival, improves quality of life and is less costly when compared to dialysis. Acute rejection, once the major obstacle to successful transplantation, has now been reduced to historically low levels (10% in 2005). This major advancement in care, however, has been replaced by a new threat, BK virus infection. BK virus is a polyomavirus that occurs worldwide with a prevalence of 60 to 80% in the general population. In kidney transplant recipients, immunosuppression leads to reactivation of the virus. BK virus replication progresses through specific stages appearing first in the urine (BK viruria), then in the blood (BK viremia) and finally in the kidney transplant as a nephritis (BK virus nephropathy). BK virus nephropathy has a poor prognosis with an average transplant failure rate of 46% but reaching as high as 100% in some series.

Except for one study published in 1980, there are no randomized controlled trials (RCT) evaluating strategies to prevent or treat BK viruria, viremia or nephropathy. Based on uncontrolled data, experts and guidelines have recommended that screening be adopted to detect BK viruria or viremia followed by a reduction in immunosuppression. Intuitively a good idea, this approach is problematic since many patients don’t actually clear the virus with this strategy. Immunosuppression reduction can lead to acute and chronic rejection and most importantly there is only one study evaluating long-term outcomes with this screening strategy.

We propose to conduct an RCT to determine if we can prevent BK virus infection from occurring rather than trying to treat the virus once replication has been an established. Prophylactic strategies such as this are familiar to kidney transplant patients and physicians as they have become standard of care for the prevention of cytomegalovirus and pneumocystis infection. Quinolone antibiotics are safe, commonly used medications that also have antiviral properties against BK virus. We hypothesize that the administration of a quinolone, if given early post-transplantation, will prevent BK viral replication in the urine and thus prevent systemic BK virus infection.

1.2 WHAT ARE THE PRINCIPAL RESEARCH QUESTIONS TO BE ADDRESSED?

Primary question: For our definitive trial, we will ascertain whether a 3-month course of the quinolone levofloxacin decreases the occurrence of doubling creatinine, transplant failure or death compared to placebo in kidney transplant recipients. Before proceeding with a large multicentre study to address this question, we will assess the efficacy, safety and feasibility of a 3-month course of levofloxacin in a pilot study. Under efficacy, this pilot will determine whether levofloxacin can decrease the incidence of BK viruria and peak urine BK viral load. Under safety, this pilot will determine the incidence of adverse events with levofloxacin. Under feasibility, this pilot will determine the number of kidney transplant patients randomized over an eight month enrolment period, adherence to the levofloxacin and frequency of patient drop-out and loss to follow-up.

1.3 WHY IS A TRIAL NEEDED NOW?

1.3.1 BK VIRUS INFECTION IS A SIGNIFICANT PROBLEM IN KIDNEY TRANSPLANTATION

Despite progress in short-term results, long-term kidney transplant survival has not improved over the past decade. In fact, the graft half-life (time required for 50% of transplants to fail) for deceased donor kidney transplants has actually fallen from 11.4 years in 1995 to 10.5 years in 2002. This unsettling trend in graft survival has occurred at a time when acute rejection, a major risk factor for transplant failure, has fallen dramatically. Although newer, more potent immunosuppressive drugs are responsible for this improvement in rejection, many experts suggest that the emergence of BK virus infection is the direct result of these new medications and is a contributing factor in the failure to improve long-term outcomes. In response to the growing problem of BK infection, the FDA issued a warning on July 14, 2009 that will affect labelling of
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... immunosuppressants in transplantation. The warning stated: “Based on this new safety information, FDA is requiring, under the authorities granted under the Food and Drug Administration Amendments Act of 2007, that manufacturers of these immunosuppressants update their prescribing information to include stronger warnings about the risk of BK virus-associated nephropathy.”

Prospective studies have shown that BK viral replication occurs commonly in kidney transplant recipients. BK viruria has been reported in 19 to 74% of patients and BK viremia occurred in 7 to 36% with an average of 17% Hirsch et al found that decoy cell shedding occurred in 30% of patients a median of 16 weeks post-transplant. This preceded the development of BK viremia in 13% a median of 23 weeks post-transplant. Bressollette-Bodin and colleagues demonstrated viruria in 57% and viremia in 29% of patients. BK viremia started within the first 3 months post-transplant in 80% of patients. Brennan et al. analyzed the incidence of BK viruria and viremia in 200 patients. Their findings were similar to Hirsch, with viruria occurring in 35% and BK viremia in 11.5%. The median time to viruria and viremia was 40.5 and 60 days respectively with 85% of viremia occurring in the first 3 months. BK viremia never occurred without viruria.

The incidence of BK virus nephropathy varies across centres due to differences in immunosuppression, diagnostic definitions and the use of different screening protocols. In a review of 11 centres, the incidence of biopsy proven BK virus nephropathy ranged from 1.1% to 10.3%. Across these same centers, the mean incidence of graft loss due to BK virus nephropathy was 46.2% but varied from 0-100%. Despite widespread knowledge of this condition and screening at many centres, there has not been a reduction in BK virus infection. New data from the United States shows that BK virus infection requiring treatment continues to increase each year, even in the most recently available era of 2006. Compared to the reference year of 2003, patients transplanted in 2004 had an 81% (P<0.001) increased risk of needing treatment for BK virus and this risk had increased fourfold by 2006 (adjusted HR 4.0, 95% CI 3.34-4.79).

1.3.2 THERE IS NO EFFECTIVE TREATMENT FOR ESTABLISHED BK VIRUS INFECTION

Only one randomized trial (published in 1980) has been reported (see section 1.4) and thus treatment recommendations are based on anecdotal reports and small case series. In the absence of rigorous data, experts and guidelines have recommended a reduction in immunosuppression as the primary therapy for BK virus infection. Schold et al have shown in a national database analysis (n=36,101) that treatment for BK virus infection was associated with a nearly twofold increased risk for graft loss (adjusted HR 1.90, 95% CI 1.44-2.51). In this study, immunosuppression reduction was the reported treatment in 84% of cases. The three-year graft survival was significantly lower in patients requiring treatment for BK virus infection compared those who did not (79% vs 90%; P<0.001) (see Figure below). In fact, BK virus infection now has a greater impact on graft survival than acute rejection.

Importantly, among patients without a history of acute rejection, treatment for BK virus (most often immunosuppression reduction) was associated with a twofold increased risk of rejection in the subsequent 6 months (adjusted OR 2.01, 95% CI 1.38-2.93). Thus, some of the risk of graft loss associated with BK virus may actually be due to the treatment (i.e. immunosuppression reduction) of established infection. This is consistent with other reports showing that 10 to 30% of BK infected patients develop acute rejection with a high rate of transplant failure. In addition, those without rapid transplant failure are often left with poor kidney function and are at increased risk for metabolic and cardiac complications as well as...
eventual graft loss. In a large BK virus nephropathy series, immunosuppression reduction resulted in rejection in 26%, transplant failure in 30% and one third had a creatinine >265 umol/L. BK virus nephropathy causes transplant failure by triggering an extensive inflammatory infiltrate which leads to eventual fibrosis and loss of function. In addition, the reduction in immunosuppression that is used to treat BK virus infection can lead to acute cellular and antibody-mediated rejection as well as interstitial fibrosis and tubular atrophy (previously referred to as chronic allograft nephropathy or chronic rejection). Therefore, patients initially diagnosed with BK virus infection might have a terminal biopsy that has a histological pattern that is indistinguishable from acute rejection or chronic allograft nephropathy without any evidence of residual infection. Ramos et al found that 35% of patients with BK virus nephropathy lost their transplant because of acute rejection or acute and chronic rejection together. In a recent analysis, BK virus was the leading cause of graft loss due to interstitial fibrosis and tubular atrophy. In that analysis, investigators subdivided graft loss into 21 separate categories and showed that overall, BK virus was the fourth leading cause of graft loss. In a kidney-pancreas series, BK virus infection was the leading cause of kidney allograft failure accounting for over 50% of all graft loss.

Due to the poor outcome with current therapies, another RCT is being conducted by the Canadian renal transplant community (PI, Dr. Tibbles) addressing a new treatment strategy for established BK virus infection. Our preventive strategy is complementary with this proposal in a manner analogous to CMV infection. With CMV, patients receive prophylactic therapy for a certain period of time (e.g. 3 months) but if they do develop CMV infection then a distinct treatment protocol is initiated. Similar prophylaxis and treatment strategies for BK virus infection are needed. This protocol has no overlap with our current proposal as Dr. Tibbles plans to treat patients with established disease while we are planning a strategy that will prevent BK virus infection altogether.

1.3.3 SCREENING FOR BK VIRUS IS RECOMMENDED BUT OF UNPROVEN BENEFIT

The poor prognosis of established BK virus nephropathy has led to strategies aimed at earlier intervention during the viruric or viremic stage of infection. Current opinion based recommendations advocate screening for viruria or viremia followed by a reduction in immunosuppression in an effort to prevent BK virus nephropathy. The widespread adoption of these recommendations is problematic because screening methods and schedules have not been standardized, testing is expensive and not routinely available, and more importantly, screening has not been evaluated in a randomized controlled trial or a rigorous diagnostic screening cohort. We have summarized the studies that have evaluated screening followed by immunosuppression reduction (Table 1, Appendix II). The nine publications were all uncontrolled case series precluding definitive conclusions regarding the utility of screening and reduction of immunosuppressive medication. Clearance of viremia ranged from 35 to 100% with an average clearance of 70%. Clearance of viruria ranged from 21 to 89% with an average clearance of only 55%. This is not without consequence as experts suggest that even low grade viruria or viremia promotes the development of allograft nephropathy. Follow up in these studies was limited; only one study reported 5-year outcomes with most providing 1-year data. Despite the short follow-up there was evidence that immunosuppression reduction may be risky as 0 to 23% of patients experienced acute rejection. In the one study that reported late outcomes, the 5-year acute rejection rate was higher in patients with viruria vs no viruria (19% vs 8%; P=0.036). More significant was the fact that patients with BK viremia lasting longer than one month (who also had immunosuppression reduced) had a significant reduction in overall patient survival compared to
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those without viremia (73% vs 92%; P=0.04). Finally, screening did not actually prevent the occurrence of BK virus nephropathy in all patients. In fact, BK virus nephropathy developed in 0 to 40% of patients despite screening. Because no control group was used in any study, it is unclear if a screening strategy followed by immunosuppression reduction actually reduced the incidence of BK virus nephropathy. Thus, while screening has some intuitive merit, it remains an unproven strategy.

1.3.4 PREVENTION OF BK VIRUS IS THE MOST DESIRABLE MANAGEMENT STRATEGY

Universal prophylaxis, rather than screening followed by treatment, is now routine for cytomegalovirus and pneumocystis infection in kidney transplantation. Given the limitations to screening and potential risk of immunosuppressant drug reduction noted above, prevention has been suggested as the optimal strategy for BK virus infection.

1.3.5 QUINOLONE ANTIBIOTICS HAVE ACTIVITY AGAINST BK VIRUS

Quinolones are among the most successful antibiotics due to their spectrum of activity, safety profile and good bioavailability. Quinolones rapidly inhibit bacterial DNA synthesis by inhibiting the activity of two members of the type 2 topoisomerase class of enzymes: topoisomerase IV and bacterial DNA gyrase. Topoisomerase IV is responsible for unlinking DNA following DNA replication. Quinolones have been evaluated for activity against BK virus. The mechanism of action, may involve the inhibition of host topoisomerases, which are utilized by BK virus for replication. Based on limited sequence homology between eukaryotic topoisomerase II and its bacterial counterparts, quinolones do have activity against host topoisomerases.

Polyomavirus T Ag also possesses several other biochemical activities including helicase activity. Recent data has shown that quinolones appear to be directly inhibitory to the helicase activity encoded by the closely related polyomavirus SV40. Portalini et al evaluated first generation quinolones nalidixic acid and oxolinic acid for their ability to inhibit BK virus replication. Inhibition of viral replication was detectable at day 4 post-infection. Using in vitro studies, Leung et al showed that levofloxacin could inhibit 8 of 8 BK virus isolates tested and ciprofloxacin could inhibit 3 of 8 isolates. Ali et al showed that levofloxacin could inhibit the cytopathic effect of the SV40 polyomavirus at a concentration of 0.06 mM while ciprofloxacin and ofloxacin required larger concentrations in the order of 1.0 to 2.0 mM. In contrast, Randhawa et al. demonstrated only modest activity of quinolones against BK virus. However, in this study only a single strain of BK virus (Gardner) was tested and activity was assessed using a PCR based assay rather than traditional viral susceptibility testing which rely on culture and cytopathic effect.

1.3.6 QUINOLONE ANTIBIOTICS CAN REDUCE BK VIRUS IN IMMUNOSUPPRESSED PATIENTS

These intriguing in vitro observations are supported by clinical reports suggesting the efficacy of quinolones in preventing BK infection in human transplant recipients. In a case series, kidney transplant recipients with active BK viremia or viruria were given a 10 day course of gatifloxacin without a reduction in immunosuppression. Investigators found that 7/10 patients responded with clearing of urinary decoy cells or >80% reduction in blood viral load. In a letter to the editor, Koukoulaki et al reported that urine BK viral load was lower while patients were receiving a course of ciprofloxacin compared to when the medication was stopped. However, because of the small sample size and lack of a control group the investigators concluded that further clinical trials were needed to determine the role of quinolones in BK virus infection. In a non-randomized study, ciprofloxacin was given prophylactically to allogeneic stem cell transplant recipients. The median peak urine viral load was 3 x 10^5 copies/ml for those on ciprofloxacin, which was significantly lower than the 2.6 x 10^9 copies/ml in cephalosporin treated patients (P=0.021). In addition, ciprofloxacin treated patients were less likely to have a ≥ 3 log increase in urine BK viral titre compared to those who did not receive ciprofloxacin. (P=0.013). All 8 patients who developed hemorrhagic cystitis had a ≥ 3 log increase in urine BK viral titre whereas none of the 32 patients without such an increase developed cystitis (P<0.001).
1.3.7 QUINOLONE ANTIBIOTICS CAN PREVENT BK VIREMIA IN KIDNEY TRANSPLANT RECIPIENTS

A recent cohort study (n=220) demonstrated a significant reduction in BK viremia with the prophylactic administration of quinolone antibiotics. Kidney transplant recipients received levofloxacin or ciprofloxacin for a minimum of 30 days starting immediately after transplantation. Quinolone treated patients had only a 4% incidence of BK viremia compared to 24.5% in those not receiving a quinolone (P=0.02). These encouraging observational data highlight the need for further study of quinolones in a prospective randomized fashion.

1.3.8 CONCLUSIONS

We conclude that 1) BK virus infection is a common problem in kidney transplantation; 2) There is no effective treatment for BK virus nephropathy and the risk of graft loss remains high; 3) Screening followed by immunosuppression reduction is recommended but only supported by uncontrolled studies in which viral clearance was incomplete, there was a risk of acute rejection, and long-term outcomes including the risk of chronic rejection were not reported; 4) Infection prophylaxis is standard practice in organ transplantation but has not been rigorously evaluated for BK virus; 5) Recent observational data suggests that quinolones can effectively prevent BK viremia in kidney transplant recipients. Given these issues, we propose a randomized controlled trial to determine if the quinolone levofloxacin can prevent BK virus infection in kidney transplantation.

1.4 SYSTEMATIC REVIEW OF THE LITERATURE

In preparation for this grant submission, we conducted a systematic review of interventions to prevent or treat BK virus infection in kidney transplant patients (manuscript published; details in Appendix III). We identified three RCTs and 36 non-randomized studies. There were no systematic reviews or meta-analyses identified by our search.

Randomized controlled trials: In an RCT published in 1980, interferon did not reduce seroconversion or viral excretion in 41 kidney transplant recipients. An RCT comparing FK778 (an analogue of the active metabolite of leflunomide) to standard care in kidney transplant recipients with BK virus nephropathy was terminated early without publication of results. In the third RCT identified, Brennan et al determined the incidence of BK virus infection in patients randomized to cyclosporine or tacrolimus. They did not randomize patients to different interventions once BK virus infection occurred. We updated our search in February 2010 and no further RCTs were identified.

Non-randomized studies: These studies examined the role of immunosuppression alone or with the combination of cidofovir, leflunomide, intravenous immunoglobulin and quinolones. The majority of these studies were small uncontrolled case series. Given the lack of relevant RCTs and the absence of methodological rigor in the observational studies, no firm conclusion can be made about the effectiveness of any of these interventions on BK virus infection.

1.5 HOW WILL THE RESULTS OF THIS TRIAL BE USED?

The results of the pilot study will provide data crucial to the planning of a large multicentre trial to determine whether levofloxacin reduces BK virus infection in kidney transplant recipients. At our Canadian Renal Transplant Study Group meetings investigators felt that BK virus infection was the most important topic that needs to be addressed in a trial. For the large multicentre trial we plan to examine whether levofloxacin decreases the occurrence of doubling creatinine, transplant failure or death. Based on data from the literature, we estimate that approximately 1100 patients will be needed to answer this question. Since this sample size would be substantially larger than most trials in kidney transplantation, we as well as members of the Canadian Renal Transplant Study Group felt it would be important to have pilot data before embarking on the larger trial. This pilot study is specifically designed to measure efficacy, safety and feasibility of a 3-month course of levofloxacin. If levofloxacin significantly reduces BK viruria and urine viral loads it will provide important justification of biologic effect in this population to progress to the larger trial. The pilot study will also carefully document all adverse events, including the development of Clostridium
difficile–associated diarrhea. Knowledge of the frequency and severity of adverse events will be
used by the trial steering committee along with members of the Canadian Renal Transplant Study
Group to determine if the potential benefits of levofloxacin outweigh the potential risks in a larger
trial of patients. Finally, this pilot study will provide important feasibility data. Knowing the
proportion of patients that can be randomized to this protocol will allow us to estimate the number
of sites required to conduct the definitive trial. Protocol adherence and follow-up measurements will
provide additional feasibility information to further refine sample size estimates for the larger study.
A randomized trial demonstrating that levofloxacin prevents BK virus infection would be a major
advance in the care of transplant recipients given the lack of proven therapies for this condition.

1.6 POTENTIAL RISKS TO THE SAFETY OF PARTICIPANTS INVOLVED IN THE TRIAL

The intervention arm will receive a quinolone antibiotic that has been approved by Health Canada.
Quinolones as a class have well-established side effect profiles, are widely used and considered safe
medications17. Our systematic review did not yield any randomized trials evaluating levofloxacin in
kidney transplantation. However, ciprofloxacin has been studied in kidney transplantation. In a
randomized trial evaluating a 6-month course of ciprofloxacin, only 2.4% of renal transplant
recipients had to stop study medication because of adverse events65. In addition, there were fewer
overall infections in the ciprofloxacin group without an increase in treatment-related infections such
as Clostridium difficile–associated diarrhea65. In another trial comparing 6-months of ciprofloxacin
to trimethoprim-sulfamethoxazole, Hibberd et al found that only 6% had to stop ciprofloxacin
because of drug-related toxicity66. These two trials suggest that a six-month course of a quinolone is
safe and well-tolerated in the kidney transplant population. In phase III clinical trials involving the
nontransplant population (n=5912), levofloxacin has been well tolerated with comparable treatment
related adverse events to the control groups67. The incidence of drug-related reactions was 6.3% and
only 4.4% of subjects had to discontinue levofloxacin because of adverse events67. Although most
trials of levofloxacin have involved short-courses of therapy, there have been longer studies in
chronic prostatitis68,69. Nickel et al randomized patients to 6-weeks of levofloxacin (500 mg daily;
our proposed dose) or placebo68. Twenty percent developed mild drug-related adverse events
compared to 17% in the placebo group and no patient withdrew because of adverse events68.
Bundrick et al randomized 377 patients to levofloxacin (500 mg daily; our proposed dose) or
levofloxacin for a total of 28 days69. In this trial, 87.3% in the levofloxacin group completed
therapy compared to 85.0% for ciprofloxacin69. These data suggest that quinolones as a class and levofloxacin in particular are safe and well
tolerated. Rare but serious side effects such as angioedema, anaphylaxis, hypoglycemia,
rhabdomyolysis, arrhythmia due to QT prolongation and tendon rupture have been reported with
quinolones17,67,70. In addition, quinolone use has been associated with changes in the intestinal flora,
bacterial resistance and Clostridium difficile–associated diarrhea17,71. Based on these potential side
effects we have made safety a main objective of this pilot study (see section 2.8). Side effects of
levofloxacin will be ascertained at each study visit using a standardized form. Detailed reports of all
adverse events will be forwarded to the Data Safety and Monitoring Committee (section 3.3).

2. THE PROPOSED TRIAL

2.1 WHAT IS THE PROPOSED TRIAL DESIGN?

This pilot study will be a multi-centre, double-blind, randomized controlled trial comparing a 3-
month course of the quinolone levofloxacin to placebo in 154 renal transplant recipients.

2.2 WHAT ARE THE PLANNED TRIAL INTERVENTIONS?

2.2.1 JUSTIFICATION FOR QUINOLONE SELECTION AND DOSING REGIMEN

While all quinolones have been evaluated for activity against BK virus18,19,54,55, we chose
levofloxacin for three main reasons. (1) Antiviral activity against BK virus: Levofloxacin has
consistent activity against BK virus documented by more than one group. Leung et al. showed that
with different drug concentrations, levofloxacin could inhibit 8 of 8 BK virus isolates tested. In
contrast, 5 of 8 BK virus isolates were resistant to ciprofloxacin. Ali et al showed that
levofloxacin could inhibit the cytopathic effect of the closely related SV40 polyomavirus at a
concentration of only 0.06 mM. In contrast, ciprofloxacin and ofloxacin required much larger
concentrations of drug in the order of 1.0 to 2.0 mM to inhibit the polyomavirus. They also found
that viral replication was inhibited by levofloxacin at a concentration of only 0.02 mM. Ciprofloxacin
and ofloxacin required a drug concentration that was five times higher to inhibit
quantitative viral replication. (2) High urinary concentration of drug: Levofloxacin is primarily
renally excreted and achieves high kidney tissue concentration. For levofloxacin, 81% of a dose
appears in the urine compared to only 36% for ciprofloxacin and 57% for ofloxacin. In addition,
the maximal urine concentration of levofloxacin is 406 mg/L, which is 1.5 times greater than
ciprofloxacin and two fold greater than norfloxacin. (3) Once daily dosing: Levofloxacin has been
shown to maintain a high urine concentration beyond 12 hours whereas the urine level of cipro fell
to only 13 mg/L at 12-24 hours post-dose. Canadian guidelines recommend that levofloxacin be
given every 24 hours, whereas ciprofloxacin, norfloxacin and ofloxacin should be given every 12
hours. Once daily dosing of levofloxacin will reduce patient burden and improve compliance.

2.2.2 TRIAL INTERVENTION

In the proposed trial, the target dose of levofloxacin will be 500 mg daily for 3 months. This dose
has been chosen based on the pharmacokinetic properties of levofloxacin noted above as well the
in vitro properties against BK virus. With normal kidney function, a single 500 mg tablet of
levofloxacin will produce a maximal urinary drug concentration of 406 mg/L. At a concentration
of 250 μg/ml, levofloxacin was shown to inhibit 3/8 BK virus isolates. At a concentration of 500
μg/ml, levofloxacin was able to inhibit the remaining 5/8 isolates. Thus, we expect that the 500 mg
tablet of levofloxacin given daily will result in sufficient urinary drug concentration to inhibit BK
virus replication. Since this is a proof of concept pilot trial there is no published data to guide the
duration of therapy. Given that 80-85% of BK virus infections begin within the first three months
post-transplantation, a 3-month course of levofloxacin should prevent the majority of infections.
While 2-4 weeks of therapy might be the most conservative approach with regards to safety, we
believe this would be insufficient to effectively prevent BK virus infection. Similarly, a prolonged
course of therapy (e.g. 6-9 months) might prevent more BK virus infections but would have the
potential for more adverse events. Thus, the 3 month treatment intervention represents a balance
between efficacy and safety appropriate for a pilot study.

Levofloxacin will be given orally once daily in the morning. The 500 mg daily dose will be
given as two 250 mg capsules to allow for dose reductions if required. At each study visit creatinine
clearance will be estimated using the Cockcroft-Gault formula and the dose of levofloxacin will
be adjusted based on Canadian guidelines (details in Appendix IV). The medication will be started
as soon as the patient is able to take oral medications but must be started within 5 days post-
transplant. A delay of up to 5 days is unlikely to result in viruria before starting study medication
as the median onset of BK viral replication is 40.5 days. The levofloxacin will be re-encapsulated
and the placebo will be identical in appearance to the study medication. We believe a placebo
control group is justified as there is no proven intervention that will prevent BK virus infection.

2.2.3 OTHER STUDY MANOEUVRES

Outside of the primary trial intervention, the only major interventions that will be controlled are
cytomegalovirus (CMV) and Pneumocystis jiroveci infection prophylaxis. All participants will
receive prophylaxis against CMV and Pneumocystis based on established guidelines. Consistent
with current clinical practice, patients with a significant unexplained and sustained increase in
serum creatinine will undergo transplant biopsy. Acute rejection will be treated using a standardized
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A set protocol will be developed outlining when study medication must be stopped (e.g. C. Difficile diarrhea, rash, tendonitis etc). Co-interventions that might influence BK infection, such as immunosuppressive medication use, will be thoroughly documented but not controlled. Although immunosuppressive strategies are somewhat variable between centres, mandating a strict immunosuppressive regimen would limit site participation and generalizability of study findings. More importantly, all immunosuppressive medications have been associated with BK virus infection and the overall burden of immunosuppression appears more important than individual agents. Quinolone use within the study: As per our eligibility criteria (section 2.5), the routine use of quinolones for bacterial prophylaxis (e.g. UTI) will not be permitted. The investigators have agreed to not use quinolones for empiric antibiotic therapy. If a quinolone is absolutely necessary, then investigators have this option for the safety of the patient. We expect this situation to be rare given the choices of antibiotics available (e.g. cephalosporins, amoxicillin/clavulanic acid, nitrofurantoin etc). A set protocol will be developed to guide the sites on non-study use of quinolones. Once cultures are available, patients will be switched to a non-quinolone regimen. If the infection is only quinolone-sensitive or the patient is intolerant/allergic to other antibiotics, then quinolone use (beyond 24-48 hours) will be permitted. During this time, study medication (placebo or levofloxacin) will be withheld. All non-study use of quinolones will be thoroughly documented.

2.3 ARRANGEMENTS FOR ALLOCATING PARTICIPANTS TO TRIAL GROUPS?

A web-based randomization system will be used in this trial. A permuted blocked randomization method stratified by centre will be used to allocate patients. An independent statistician will generate the randomization scheme. The randomization process will consist of a computer-generated random listing of the treatment allocations stratified by centre in variable permuted blocks of 2 and 4. The system will have backup in the form of a statistician and designated research pharmacist at the coordinating centre. Only the independent statistician and designated research pharmacist at the coordinating centre will have knowledge of the randomization codes to ensure concealed randomization of the patients. After screening the patient for eligibility and obtaining informed consent, the study nurse will access the trial website and provide the subject’s unique ID as well as a confirmation of consent and eligibility. The web site will provide the next available randomization number.

2.4 METHODS FOR PROTECTING AGAINST SOURCES OF BIAS?

In order to minimize selection and ascertainment biases, physicians, nurses, investigators and research staff will be blinded to the randomization schemes and treatments administered. The study medication and placebo will be identical in appearance. The trial statistician will designate another statistician to prepare the randomization schemes. Only the independent statistician and the designated research pharmacist at the coordinating centre will be aware of the treatment allocation for individual patients. The designated research pharmacist at the coordinating centre will be expressly forbidden to discuss individual treatment allocation with the study team or patient at each participating centre. In addition, the staff at the central laboratory performing the BK virus measurements will not know the patient’s treatment allocation. Given that this trial will be blinded, contamination and cointerventions should not become imbalanced between the treatment arms. We have set guidelines for the use of quinolone antibiotics outside of the study protocol and will rigorously ascertain and document their utilization. We will document the use of co-interventions that may have some impact on BK virus such as immunosuppressive drug selection, dose and levels, leflunomide, cidofovir and intravenous immunoglobulin.

2.5 WHAT ARE THE PLANNED INCLUSION/EXCLUSION CRITERIA?

Inclusion criteria: a) primary or repeat kidney transplant recipient (deceased or living donor); b) age ≥18 years. Exclusion criteria: a) unable to provide informed consent; b) greater than 5 days...
post-transplantation; c) BK virus nephropathy with a previous transplant; d) history of allergic reaction to any quinolone antibiotic; e) history of quinolone associated tendonitis or tendon rupture; f) corrected QT interval prolongation on EKG as defined by Al-Khatib; g) concomitant use of medication known to prolong the QT interval such as class IA antiarrhythmic drugs (e.g. quinidine, procainamide, disopyramide), class III antiarrhythmic drugs (e.g. amiodarone, sotalol), azole antifungals (e.g. fluconazole) or macrolide antibiotics (e.g. erythromycin); h) pregnant or breastfeeding as safety of levofloxacin not established; i) recipient of a multi-organ transplant (e.g. kidney-pancreas); k) currently enrolled in another interventional trial; l) previously enrolled in this study; m) history of rhabdomyolysis; n) significant allergic reaction to ≥ 3 classes of antibiotics as these patients may have no other option other than quinolones for routine infection.

2.6 WHAT IS THE PROPOSED DURATION OF TREATMENT PERIOD?

From time of randomization, patients will receive levofloxacin or placebo for three months.

2.7 WHAT IS THE PROPOSED FREQUENCY AND DURATION OF FOLLOW-UP?

Patients will be followed one year from the time of randomization. Study visits will take place every 4 weeks for the first 24 weeks then at 32, 40 and 52 weeks (trial flow, Appendix V). Visits will be more frequent early post-transplant in order to document viruria as close as possible to its onset. Routine post-transplant care includes clinical assessments at least weekly for 12 weeks, and at least monthly for 52 weeks, thus the proposed assessments will be feasible during regular clinical encounters. The follow-up period was extended beyond the treatment period to document BK viruria that might occur after the study medication is discontinued which has been noted in CMV infection. One year follow-up should be sufficient since only 1.0-1.3% develop viremia and only 3% develop viruria beyond 40 weeks post-transplantation.

2.8 WHAT ARE THE PROPOSED PRIMARY AND SECONDARY OUTCOME MEASURES?

Primary outcome: The primary outcome of the pilot study will be a measure of efficacy, the time to occurrence of BK viruria within the first year post-transplantation. BK viruria will be defined as ≥1000 copies/mL of BK virus DNA in the urine. This value corresponds to the lower limit of detection for our BK virus assay for urine samples. BK viruria was chosen as the outcome for the pilot study because it is the earliest clinical manifestation of BK virus replication, precedes viremia by 4 to 8 weeks and is on the causal pathway to BK nephropathy. In addition, viremia and BK nephropathy do not occur in the absence of BK viruria. The investigators also felt that the timing of BK viruria was important not just the presence or absence of viruria.

Secondary outcomes: (i) Safety: Secondary safety outcomes include: (a) incidence and type of all adverse events; (b) incidence of acute rejection; (c) incidence of microbiologically confirmed Clostridium difficile–associated diarrhea; (d) incidence of other infections (viral, bacterial and fungal) based on established guidelines; (e) incidence of quinolone resistance where a quinolone would have been a therapeutic option (e.g. E. Coli UTI); (f) effect of levofloxacin on immunosuppressive drug doses and blood levels; (g) transplant failure and mortality.

(ii) Feasibility: Secondary feasibility outcomes include: (a) number of patients transplanted during the eight month recruitment period who are randomized into the trial; (b) proportion of randomized participants who are adherent to the protocol. Participants who take at least 80% of study medication and do not report any episodes of non-adherence will be classified as adherent. Based on data from the literature and the fact that our trial involves 3 months of therapy, we will judge this outcome to be successful if at least 75% of participants are adherent; (c) the use of quinolones outside of the protocol; (d) proportion of patient drop-out and loss to follow-up.

(iii) Clinical: Secondary clinical outcomes include quantitative BK urine viral load and the time to occurrence of BK viremia (defined as ≥250 copies/mL of BK virus DNA in the plasma). These outcomes will be measured because urine viral titre is an important predictor of BK viremia and BK
viremia is an important predictor of BK nephropathy. In an analysis performed at our lab, we found that patients with a peak urine BK viral load of < 10^7 copies/ml never developed viremia. When the peak urine BK viral load reached 10^7, 10^8, 10^9 and ≥10^10 copies/ml, BK viremia was detected in 20, 33, 50 and 100% of patients respectively. Given the known link between viruria, urine viral load and viremia, demonstrating a reduction in viruria and peak urine viral load with levofloxacin will provide a strong biologic rationale for the proposed larger trial.

2.9 How will the outcome measures be measured at follow-up?

Primary outcome: BK virus infection will be determined at each study visit (every 4 weeks for the first 24 weeks then at 32, 40 and 52 weeks) by testing for BK viral DNA in urine samples. We have established methods to perform real-time quantitative PCR (rt-QPCR) for the detection of BK virus at our central laboratory at the University of Alberta. Our assay has been in clinical use for provincial renal transplant programs and has been used as a reference assay for clinical trials including one funded by the NIH. Detailed performance characteristics of the assay have been published. Briefly, during the initial development and validation, the detection limit was determined by repetitive runs using known standard plasmid DNA preparations. The rt-QPCR assay was found to detect BK virus DNA100% of the time when 1.0 to 10 log_{10} copies was used as the input. Based on the amount of sample used for the starting PCR, this corresponds to a lower limit of detection of 2.4 log_{10} (250) copies/ml for plasma and 3.0 log_{10} (1000) copies/ml for urine. Validation was performed against other viruses including JC virus, SV40, EBV and CMV, none of which cross-reacted with BK in the rt-QPCR. The intra-assay coefficient of variation was 5.7% and 5.9% in 60 replicates of 6.3 and 3.3 log_{10} copies of plasmid DNA versus 4.1% and 5.0% in 6 replicates of urine and plasma samples, respectively. The assay then underwent validation using 607 urine and 223 plasma samples prior to clinical use (details in Pang et al and Appendix VI).

Secondary outcomes: (i) Safety: Potential medication related adverse events will be ascertained at each study visit using an objective checklist that includes the most common and serious side effects of levofloxacin. In addition, patients will be informed of the potential side effects and will be instructed to contact the study coordinator if symptoms appear in between visits. As suggested by one reviewer, we will screen for musculoskeletal side effects with measurement of CPK at baseline and monthly for 3 months. We will also screen for cardiac side effects by perming an EKG at baseline and monthly for 3 months to assess QT prolongation. Study medication will be stopped if patient develops QT prolongation. The occurrence of all infections, including Clostridium difficile–associated diarrhea, will be documented by patient interview and review of the medical record. The presence of infection will be defined using specific published criteria. Culture and sensitivity reports will be obtained for all samples submitted to the site microbiology laboratory and resistance to quinolones will be documented. Immunosuppressive drug levels (if measured as part of usual care) will be recorded at each study visit. Clinical outcomes such as rejection (defined by standard criteria used in previous trials), transplant failure and death will be documented by review of the medical record. (ii) Feasibility: (a) The total number of transplants performed at each site will be recorded on a monthly basis. This data collection is routinely performed at all transplant centres and forwarded to provincial transplant agencies. The data recorded on patients who don’t consent to participate will be anonymous and will only include the reason for not participating. No other potentially identifying data will be collected. We will determine the proportion of transplanted patients randomized to the trial each month. Reasons for exclusion and participant/physician refusal will be documented. We will review this information from each site at 4 week intervals. If recruitment targets are not met, we will closely examine the reasons in an effort to improve recruitment in subsequent months. (b) At each visit, medication adherence will be evaluated with pill counts and patient self-report (questions about missing or lateness with medication similar to Butler et al), which was found to be the most accurate method of measuring compliance compared to other methods.
to an electronic monitoring device in kidney transplant recipients\textsuperscript{91}. (c) Use of quinolones outside of study protocol will be determined by patient interview and review of the medical and pharmacy records. (d) Non-attendance for study visits will be documented at each site. In an effort to locate any patient lost to follow-up, consultation with the clinical care team will be carried out to determine alternate contact information. (iii) Clinical: BK viremia will be measured using the same method described for viruria. Since viremia does not occur in the absence of viruria\textsuperscript{26,85}, only samples with viruria will be tested for viremia. Sites will be notified of any positive BK viremia samples. At this point participants will stop taking study medication since the primary outcome and main secondary clinical outcome will have occurred and clinicians will likely modify immunosuppression. Patients will continue to be followed until 1-year post-transplant. Sites will not be notified of positive BK urine samples and participants will remain in the trial on blinded study medications unless they become viremic. Investigators have agreed on this procedure since none currently modify treatment unless viremia also occurs.

2.10 WILL HEALTH SERVICE RESEARCH ISSUES BE ADDRESSED?
As this is a pilot study, health services issues will not be addressed. For the full trial, however, cost-effectiveness will be included to evaluate this new and potentially beneficial therapy.

2.11 WHAT IS THE PROPOSED SAMPLE SIZE?
The primary outcome for the pilot study will be the time to occurrence of BK viruria. Based on data from the literature, we estimate that 35% of patients in the placebo group will develop BK viruria by one-year post-transplantation\textsuperscript{26}. In order to detect an absolute reduction in BK viruria of 20% (from 35% to 15%) with a two-sided alpha error of 0.05, a beta error of 0.2 and a 5% lost to follow-up rate, we would need 154 patients in total (77 per group). Sensitivity analysis around these estimates is in Appendix VII. The minimal clinically important difference of 20% was justified based on a survey of experts from the Canadian Renal Transplant Study Group. The investigators wanted to see a substantial effect on viruria in order to justify proceeding with a larger trial examining the more clinically relevant endpoint of time to doubling serum creatinine, transplant failure or death. This sample size will also provide sufficient power to detect important changes in BK urine viral load. Brennan et al found that the median peak urine titre was 8.98 log_{10} copies/ml (range, 5 to 12.5)\textsuperscript{26}. Since this interval is roughly symmetric about the median, the mean and median are likely very similar. Thus, assuming that log_{10} (copies/ml) is normally distributed, we estimate the mean and standard deviation to be 8.98 and 1.45, respectively. A drop of one order of magnitude in copy number corresponds to a drop of 1 in log_{10} copies/ml. With 154 patients (with 146 after drop-outs), we will have 100% power to detect this change.

2.12 WHAT IS THE PLANNED RECRUITMENT RATE?
One of the goals of this pilot is to determine patient recruitment. To conduct this trial we have the support of 11 kidney transplant centres (see Appendix I for letters of support). In preparation for this grant, we performed a data audit at each site to determine the number of transplants performed in the past 2 years. As shown in Appendix VIII, the 11 sites in this trial have performed an average of 1006 kidney transplants per year over the past two years. The enrolment period for this trial is eight months. Therefore, we estimate that 670 kidney transplants will be performed during the recruitment period. Given that our estimated sample size is 154, there will be sufficient number of kidney transplants to achieve our recruitment target. Although enrolment will be competitive, we expect the following enrolment per site based on our audit: Vancouver General n=11; St. Paul’s n=14; Edmonton n=14; Winnipeg n=7; Toronto (UHN) n=23; Toronto (St Mikes) n=18; Ottawa n=11; Hamilton n=14; London n=12; Montreal n=15; Halifax n=15. This translates into the enrolment of approximately 1-3 patients per month depending on the site. This level of recruitment will be feasible given the lack of other trials currently being conducted in this particular population at participating sites. Our trial includes living and deceased donors. Living kidney transplantation is
a planned, elective procedure so candidates will be known in advance. When an operation is confirmed, the clinical team will notify research personnel so they can approach patients for enrolment prior to surgery. Deceased donor kidney transplantation is completely unpredictable and depends on organ availability and particular allocation algorithm at each site. Each centre will have research personnel on-call so that evening and weekend cases are not missed. Pre-printed order forms will be developed at each site to prompt the clinical team to review for trial eligibility. Research personnel will be notified to come in for consent and randomization. All sites in this study have considerable experience randomizing patients into trials in a similar fashion on nights and weekends.

2.13 ARE THERE LIKELY TO BE ANY PROBLEMS WITH COMPLIANCE?

Measuring adherence to levofloxacin is one of the objectives of this pilot trial. Based on low discontinuation rates in 6-month trials of the quinolone ciprofloxacin in renal transplant recipients, we don’t expect adherence to be a major issue. The intervention in this trial (two capsules) is not complex and the once daily dosing regimen should lead to fewer missed doses compared to BID or TID dosing. Nonetheless, because regimens are more complex and the number of medications taken in the post-transplant period is much greater than in the aforementioned trials, we will evaluate compliance in this pilot study.

2.14 WHAT IS THE LIKELY RATE OF LOSS TO FOLLOW-UP?

Measuring loss to follow-up is one of the objectives of this pilot trial. We believe there will be few patients lost to follow-up because renal transplant patients are a unique population closely followed and monitored in transplant programs across Canada. In our ongoing CIHR funded trial evaluating ramipril in kidney transplant recipients, no patient to date has been lost to follow-up. In published kidney transplant trials, the proportion lost to follow-up ranged from 0.33% to 1.04%.

2.15 HOW MANY CENTRES WILL BE INVOLVED?

Eleven Canadian centres (Appendix VIII) will be involved in this pilot trial. Given the identified importance of BK virus, this trial has strong support at other centres within the Canadian Renal Transplant Study Group should we proceed to the larger, multicentre trial. Recruitment data from this pilot will be used to determine the number of centres required to complete the definitive trial.

2.16 WHAT IS THE PROPOSED TYPE OF ANALYSES?

Descriptive analysis: Baseline characteristics of patients in the two treatment arms will be assessed using frequency distributions and univariate descriptive statistics including measures of central tendency and dispersion. Analyses will be performed by intention to treat. Since this is a pilot study to assess biologic efficacy, the intention to treat analysis will be supplemented by a sensitivity analysis that excludes patients who did not complete the allocated treatment plan. Primary analysis: The primary analysis will use a nonparametric log-rank test, stratified by centre, to compare the time to occurrence of BK viruria between the control and levofloxacin treatment groups. Kaplan-Meier survival curves will also be plotted to visually assess differences in incidence over time.

Handling of Missing Data: Since renal transplant patients are followed very closely as part of routine clinical care, we do not anticipate many missing values or loss-to-follow-up. It is, however, conceivable that there may be some missing values. Any missing values that occur because the patient dies or is lost to follow-up will simply be treated as censored values at the time of the last visit. Any missing values that occur in the middle of the follow-up period (i.e. from baseline to 12 months) will need special treatment. We will use multiple imputation to accommodate any such missing values as implemented using the SAS MI procedure. We emphasize that we expect to observe few, if any, missing values.

Secondary analyses: For secondary safety outcomes, the proportion of adverse events occurring in each treatment arm will be compared using an unadjusted chi-square test or Fisher’s exact test if cell sizes are small. We will evaluate overall adverse events as well as specific serious adverse
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events (e.g. Clostridium difficile–associated diarrhea). For the secondary feasibility outcome of recruitment, we will calculate the proportion (and 95% confidence interval) of all patients transplanted during the recruitment period who are randomized into the trial. In addition, we will calculate the proportion that are eligible but consent declined, eligible but not approached and not eligible. These analyses will be performed at each site and for the trial overall. For the secondary feasibility outcomes of adherence, non-study quinolone use, drop-out, and loss to follow-up, the proportion of each outcome will be compared between the arms with an unadjusted chi-square or Fisher’s exact test. To analyze the quantitative measurements of BK viruria, the peak urine BK viral titre will be compared between the two treatment arms using either a non-parametric (Wilcoxon Rank Sum) or parametric (independent t-test) procedure as appropriate. BK viremia will be analyzed as described above for BK viruria. This analysis will be interpreted with extreme caution given that our sample size is underpowered for this outcome.

2.17 What is the proposed frequency of analyses?
Since this is a pilot study we have not planned for any formal interim analyses.

2.18 Are there any planned subgroup analyses?
Since this is a pilot study we have not planned for any formal subgroup analyses.

2.19 Has any pilot study been carried out using this design?
This grant submission is for a pilot study. We have completed relevant background work (study group meetings, systematic review, site audit and established methodology for BK virus testing) in order to conduct this pilot trial (see Summary of Progress).

3. TRIAL MANAGEMENT

3.1 Arrangements for Day to Day Management of the Trial
The Coordinating Centre will be located at the Clinical Epidemiology Program (CEP) of the Ottawa Hospital Research Institute and will be under the guidance of the Study Chair, Study Coordinator, senior trial methodologist and trial statistician. The CEP has considerable experience and is currently managing over 40 multi-centred trials. The Coordinating Centre will be responsible for receiving, processing, editing, storing and analyzing all data from the sites and the central laboratory.

3.2 What will be the role of each principal applicant and co-applicant proposed?
Dr. Knoll (nominated principal applicant) will serve as Study Chair and Dr. Gill (principal applicant) will serve as Study Vice-Chair. Dr Humar (principal applicant) will serve as laboratory director; Dr. Fergusson (co-applicant) will serve as senior trial methodologist; Dr Ramsay (co-applicant) will serve as the trial statistician. Drs Knoll, Gill, Humar, Fergusson and Ramsay will sit on the study Executive Committee. Co-applicants Drs Cockfield, Cantarovich, Karpinski, Treleaven, Keown and House will serve on the Steering Committee.

3.3 Trial Steering Committee and the Data Safety and Monitoring Committee
The Steering Committee will consist of all members of the Executive Committee, all site principal investigators and research staff. The committee will have a face-to-face meeting before study initiation and teleconferences after three months of recruitment, at one year and trial completion. The committee will review and implement all aspects of this trial. Data Safety and Monitoring Committee (DSMC) will have responsibility for monitoring of adverse events and ensure the safety of patients is protected. The DSMC will receive a report of all adverse events after 20 patients have been randomized and received at least one month of study drug. This process will be repeated every 20 randomized patients for a total of 8 reports. An interim analysis will be conducted if requested by the DSMC. Should a safety issue arise that the DSMC feels compromises participant safety, they will immediately notify the Study Chair. The DSMC will work independently from the trial and serve in an advisory role to the Executive Committee and Study Chair. The DSMC will consist of
four individuals with expertise in clinical trials, biostatistics, transplant nephrology and infectious diseases. The Executive Committee will choose the Chair of the DSMC. The other DSMC members will be selected with the assistance of the DSMC Chair.