COMBINATION OF LEFLUNOMIDE AND EVEROLIMUS FOR TREATMENT OF BK VIRUS NEPHROPATHY

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Abstract:

BK nephropathy (BKN) is a common cause of graft dysfunction following kidney transplantation. Minimization of immunosuppressive therapy remains the first line of therapy but this may lead to rejection and graft loss. In some cases, despite lowering immunosuppression, BK infection can persist, leading to chronic damage and kidney failure. Currently there is no specific anti-BK viral therapy. Recent in-vitro experiments have demonstrated a reduction in BK viral replication when infected cells are treated with the combination of Leflunomide and Everolimus. This study aims to explore the effect of this drugs combination on viral clearance and graft function in patients with persistent disease despite reduction in immunosuppression. We treated 3 patients with combination Leflunomide and Everolimus. Data on medical history, biochemical parameters and viral loads were collected. Significant improvement in viral loads was observed in 2 cases with resolution of viraemia in another (Table 1). Two recipients had preserved allograft function. The remaining graft was lost due to combination of obstruction and BKN. No adverse reactions such as bone marrow toxicity were observed. Combination of Leflunomide and Everolimus is safe and should be considered as a rescue therapy in treatment of BKN, especially in those who fail to clear this infection despite reduction of immunosuppressive therapy.
The natural history and virology of BK virus has been recently described by Jamboti (1). BKV infection in renal transplant recipients may be linked to the use of newer and more potent immunosuppressive agents. Detection of BK viremia within the first year following transplantation has reached an equivalent rate to that of acute rejection while on newer immunosuppressive therapy (2-4). Hirsch reported an occurrence of BK viremia in 13% of transplant recipients with nephropathy in 8% (5). Despite the lack of a randomized controlled trial, treatment has been directed towards reduction of immunosuppressive medication to prevent graft loss. This needs to be balanced with the risk of rejection. In some cases, despite reduction of immunosuppression, infection with polyomavirus can persist and leads to graft loss.

Recent in-vitro experiments have shown a reduction in BK viral proliferation with the combination of Leflunomide and Sirolimus, an inhibitor of mammalian target of rapamycin (mTORi) (6).
Liacini et al proposed the hypothesis of intracellular protein kinase pathways inhibition activated by BK virus as a potential effective therapeutic target rather than reduction of immunosuppression. They observed that infected renal epithelial cells lines and human primary tubular epithelial cells expressed an increased phosphorylation of 3'-phosphoinositide-dependent kinase-1 (PDK-1), the protein kinase Akt (Akt), mammalian target of rapamycin (mTOR), and 70 kDa ribosomal protein S6 kinase (p70S6K). Sirolimus targets p70S6K phosphorylation and was able to reduce BK virus large T antigen expression in a dose-dependent manner, while Leflunomide, a tyrosine kinase inhibitor decreased PDK1 and Akt phosphorylation and inhibited BK virus genome replication and early gene expression. The resultant outcome is inhibition of viral replication, large T antigen expression, PDK1, Akt, mammalian target of rapamycin, and p70S6K phosphorylation. This is summarized in Figure 1.

Based on this observation, we described our experience in treating 3 renal allograft patients who failed to respond to standard immunosuppression reduction for treatment of BKN. The current local protocol for immunosuppression reduction was initially 25-50% reduction in dose of mycophenolate mofetil, followed by lowering Tacrolimus dose to target level of 4-6. Leflunomide was substituted for mycophenolate mofetil if the prior reductions in immunosuppression failed to lower BK viral load. In the patients who did not have an improvement in renal function and a fall in BK viral load over the following month/s Everolimus was substituted for Tacrolimus.

Results:
This case study included 3 patients with 2:1 M:F ratio and median age of 59 (range 55-67). All patients received deceased donor kidneys, with 1 patient receiving combined kidney pancreas transplant. Diagnosis of BKN was based on biopsy findings, early in the course of transplantation at 4 months (P1) and 5 months (P3) post transplantation. Both P1 and P3 had a monthly plasma and urine BKV screening according to local hospital protocol. The biopsies were performed 3 months after 50% reduction in dose of MMF in both cases due to worsening of allograft function. P2 had BKN detected after 48 months. Renal biopsies for all patients were performed in the setting of rising serum BK viral load and increased serum creatinine to establish a diagnosis of BK nephropathy and exclude the possibility of rejection after several reductions in immunosuppressive therapy.

Leflunomide was started after 3 months as a replacement of MMF due to failure to respond to reduction of immunosuppression (MMF). Everolimus was introduced at different times for all patients ranging from 1 to 5 months following commencement of Leflunomide. Dose was titrated to aim for level of 5mg/L. The highest BK viral titre was for P1 at 40 million copies/mL followed by P3 at 9.4 million copies/mL then P2 at 6 million copies/mL. All biopsies showed stage B3 BKN.

A significant improvement in BK viral load was observed in 2 cases with resolution of viraemia in another (Table 1). Patient 1 (P1) has an undetected BK viral load at 16 months, while patient 2 (P2) has a low BK viral titre after 9 months of combination therapy. Two recipients had preservation of allograft function, although function remained worse than baseline creatinine (P1, P2). P1 received an additional 2 doses of 1g/kg IVIG. The remaining one graft was lost due to multiple factors including combination of obstruction and BKN in a pre-existing nephrosclerosis which was
evidenced on implantation renal biopsy. No adverse reactions such as bone marrow toxicity were observed. One patient (P3) has elevated ALP level which correlates to moderate renal impairment while on renal replacement therapy.

Discussion

Leflunomide is an immunomodulatory therapy used in treatment of Rheumatoid arthritis. The active metabolite Teriflunamide has antiviral activity against polyomavirus both *in-vitro* and in experimental animals (7). The use of Leflunomide as a substitute for mycophenalate mofetil was explored in 17 renal transplant patients showing either a clearance or significant reduction of BK viral load in both serum and urine. This result occurred when Teriflunamide levels > 40 μg per milliliter (8). There was limited report of graft survival. Another study by the same group showed reduction of viremia when Teriflunamide drug levels > 35 μg/ml using combination of Leflunomide and Cidofovir (9). A lower incidence of graft loss of 15% was reported. However, wide variation of Teriflunamide drug levels was observed with recipients receiving daily leflunomide doses between 20 to 60 mg. More recently, Leca et al demonstrated no difference in clearance of viremia or graft loss when comparing two groups with higher drug level (>40 μg/ml) vs lower drug level (<40μg/ml) (10). Adverse events were common in all patients, especially with higher drug level. Given the findings of the latter study, it was decided to prescribe Leflunomide 10-20mg for our patients without measurement of levels.

The use of mTORi for treatment of BKN has gained much popularity in the recent years. However, its use is currently reserved as a rescue therapy when reduction of immunosuppression failed to clear polyomavirus infection. *In-vitro* study showed that mTORi is indeed effective in reducing BK-
dependent T cell expansion in a dose dependent manner (11). The synergistic effect of mTORi and leflunomide proposed by Liaicini et al was demonstrated in a single case study for treatment of Kapoki’s sarcoma in renal transplant patient due to human herpes virus 8 infection (6, 12). Leflunomide works by tyrosine kinase inhibition to reduce PDK-1, Akt phosphorylation and inhibition of viral genome replication and early gene expression. mTORi targets p70S6K phosphorylation and a reduction of BK virus large T antigen expression in a dose-dependent manner. The expected outcome would be viral inhibition and eventual viral clearance with preservation of graft function.

IVIG has been described as an adjunct therapy for BK nephropathy. Previous study by Wadei et al failed to demonstrate improvement in graft survival when IVIG was used to treat BKN (13). However, recent literatures have suggested IVIG as most potent therapy for BKN (14,15). While the mechanism of action remains unclear it may include an immunomodulatory effect (16) and direct potent neutralizing anti-BK viral activity (17, 18).

In this case study, P1 received 2 doses of IVIG at the time of Leflunomide commencement and this may have contributed to the fall in BK viral load. P2 did not receive IVIG due to previous anaphylactic reaction. IVIG was not administered in P3 given multiple factors contributed to graft loss as previously described. The RCT on IVIG NCT02659891 is in progress and should provide additional information regarding the usefulness of IVIG in treatment of BK nephropathy.
All 3 patients in this report received combination therapy with Everolimus/Leflunomide only after progressive dose reductions in Mycophenolate mofetil and tacrolimus failed to lower BK viral load. Both patients P1 and P3 cleared the virus from the blood and P2 has had a progressive decline in BK viral load over the past year.

P3 had significant confounding factors with a high risk of allograft loss. However, we have included P3 in our case series to report our cumulative experience with combination therapy (Everolimus/Leflunomide). While on combination therapy P3 demonstrated and significant reduction in viral load and finally cleared the BK virus. Unfortunately, the kidney transplant ultimately failed and P3 returned to haemodialysis

Our experience with the combination of these two medications was positive with preservation of allograft function in 2/3 of patient and suppression of viremia in all 3 patients. Despite a short follow-up period of 10-22 months, it has proven to be safe with no adverse bone marrow and liver toxicity observed. Therefore, this regimen should be considered in patients with BKN who have failed to respond to reduction in immunosuppression.

Conclusion

BKN is one of the leading causes of allograft dysfunction in the modern era of immunosuppression. Reduction of immunosuppressive is the mainstay of therapy despite lack of randomized controlled trial. Infection with polyomavirus may persist and lead to graft loss regardless of immunosuppression reduction. Based on our observation, combination of Leflunomide and
Everolimus is safe and should be considered as a rescue therapy for the treatment of BKN, especially in those who fail to clear infection despite reduction of immunosuppressive therapy.

References:


18. Puliyanda D, Radha RK, Amet N et al. IVIG contains antibodies reactive with polyoma BK virus and may represent a therapeutic option for BK nephropathy. *abstract* *Am J Transplant.* 2003; 3 (suppl): 393
Table 1- Baseline characteristics, medications timeline, follow-up and outcomes

<table>
<thead>
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<th>Patient</th>
<th>1 (P1)</th>
<th>2 (P2)</th>
<th>3 (P3)</th>
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<tbody>
<tr>
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<td>F</td>
<td>M</td>
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<tr>
<td>Age (years)</td>
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<td>Pred, MMF, Tac</td>
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