RESEARCH PAPER | Medical Science | Volume : 6 | Issue : 4 | April 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50


# Successful therapy with tacrolimus and mycophenolate mofetil in Chinese renal allograft recipients with C4d-positive chronic rejection

**KEYWORDS**: kidney transplantation,chronic rejection,C4d, conversion,tacrolimus,mycophenolate mofetil

**Ji Shu-Ming**
National Clinical Research Center of Kidney Diseases, Jinling Hospital, Nanjing University School of Medicine, Nanjing, China, 210016

**Chen Jin-Song**
National Clinical Research Center of Kidney Diseases, Jinling Hospital, Nanjing University School of Medicine, Nanjing, China, 210016

**Wen Ji-Qiu**
National Clinical Research Center of Kidney Diseases, Jinling Hospital, Nanjing University School of Medicine, Nanjing, China, 210016

**Cheng Dong-Rui**
National Clinical Research Center of Kidney Diseases, Jinling Hospital, Nanjing University School of Medicine, Nanjing, China, 210016

**ABSTRACT**

*Objective To investigate the efficacy of combination with tacrolimus (TAC) and mycophenolate mofetil (MMF) rescue therapy for C4d-positive chronic rejection of renal transplants. Methods Thirty six of these patients were prospectively enrolled in the present analysis. Male 15, female 21, mean age 34.6±12.9 years. Criteria for inclusion were C4d-positive chronic rejection. Basal immunosuppression was changed in 36 recipients, including conversion from cyclosporine A to TAC (at initial dose of 0.15mg /Kg per day, and the dose was subsequently adjusted to maintain FK506 whole blood through levels between 5-10μg/L. Results After 3-year of follow-up, protienuria after FK506 conversion had reduced from (3.7±1.2) g/24hr. to (0.9±0.4 g/24hr), P <0.001. Serum creatinine(SCr) levels after TAC conversion had reduced from (3.1 ±1.2)mg/dl to (1.8 ±0.6) mg/dl, P <0.001. Among the thirty-six patients with improvement in the rate of decline of renal function, twenty-three patients(63.9%) had their regression lines become positive and eight patients(22.2%) had their regression lines become less negative. Five patients(13.8%) had increased rate of decline in renal function with their regression lines becoming more negative. By 3-year follow-up, pathological changes had been significantly improved in accordance with the 97' Banff classification: renal tubular atrophy (52.8% vs 44,4%), interstitial fibrosis (50.0% vs 30.6%) and arterial hyaline degeneration (52.8% vs 25.0%). Transplant renal pathological suggests chronic damage index (CADI) are: 8.3 ± 2.6 and 3.0 ± 0.7. Repeated renal biopsy revealed that 22 cases (61.1%) became C4d negative in renal tissue and no case become positive, and 14 case (38.9%) showed steady C4d positive accompany with hepatitis C (renal graft biopsy showed membranous nephropathy) after the switch TAC. Classification according to the Banff 07 criteria: 22 cases of C4d0,5 cases of C4d1, the 5 cases of C4d2, 4 cases of C4d3. During the follow-up of 3 years, C4d turn negative 22 cases (61.1%), and weakened C4d 5 cases (13.9%), C4d for 4 cases (11.1%) were positive. After the treatment with TAC, with the disappearance of C4d deposition in allograft, HLA-II class antibody levels decreased from 57 ±9% (35-87.5%) to 6.1 ± 1.2% (3.9-7.3%). No deaths and no new acute rejection during the 3-year follow-up. 2 cases concurrent with BK virus nephropathy, the other 3 cases of initial SCr > 4.0 mg/dl, eventually leading to renal allograft loss return dialysis. Graft survival rate was significantly increased in TAC treatment (93.5%) follow-up 3-year. Conclusion The optimal treatment for alloantibody mediated C4d-positive chronic rejection remains undefined. Our findings suggest that combining TAC-MMF treatment represents a powerful immunosuppressive regimen that limits both T-cell and B-cell responses. In such a combination protocol, not only effective control of antidonor antibody production but also improvement of long-term graft survival may be achieved.*

## INTRODUCTION

Chronic rejection (CR) is a histopathological diagnosis used to denote features of glomerular basement membrane duplication (chronic transplant glomerulopathy) and/or arterial intimal fibrosis with intimal mononuclear cell infiltration within the renal allograft〔1〕. It remains the most common cause of graft dysfunction and graft loss in patients following renal transplantation〔2〕. Protocol-biopsy studies revealed that some degree of chronic rejection is already present at 1 year in 80–100% of the patients and moderate to severe forms are seen in 67% of the patients after 5 years〔3〕. Recently, with the routine use of biopsy staining for the split fraction of the complement C4d, it has become evident the role of antibody-mediated damage in chronic rejection〔4〕. For antibody-mediated rejection(AMR), the efficacy of particular treatments such as plasmapheresis,immunoadsorption(IA),rituximab and bortezomib,high-dose intravenous immunoglobulin and/or tacrolimus (TAC) and mycophenolate mofetil(MMF) rescue therapy has been suggested〔5,6,7,8,9,10〕. However, the capability of such strategies to prevent or reverse C4d-positive chronic rejection is not yet established. The aims of this study were to investigate the efficacy of combination with TAC and MMF rescue therapy for C4d-positive chronic rejection of renal transplants in China.

## PATIENTS AND METHODS

### Study patients

Between January 2005 and May 2010, thirty six of these patients were prospectively enrolled in the present analysis. Male 15, female 21, mean age 34.6±12.9 years. Criteria for inclusion were〔11〕: ① Progressive rise in serum creatinine over 6 to 12 months with proteinuria; ② Typical pathological features ("chronic transplant glomerulopathy" and transplant arteriopathy), with less specific features of interstitial fibrosis and tubular atrophy; ③ Widespread C4d deposits by immunofluorescence in peritubular capillaries;and ④ Demonstration of previously undetectable donor specific antibody in recipient sera at the time of biopsy.

### Conversion to tacrolimus

Basal immunosuppression was changed in all 36 recipients, including conversion from cyclosporine A(CsA) to TAC (at initial

Volume : 6 | Issue : 4 | April 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

dose of 0.15mg /Kg per day, and the dose was subsequently adjusted to maintain TAC whole blood through levels between 5-10μg/L detected using a cellular enzyme linked immunosorbant assay, ELISA.) and, in the case of mizoribine(MZR)- or sirolimus(SRL)-based treatment, conversion to MMF (initial dose: 1.5 g/day orally), MMF was administered orally,adjusted by the 12 h blood concentration area under the curve (AUC0-12h), and was maintained at 35-45 mg.h/L. Prednisolone were continued according to the posttransplant immunosuppressive protocol in our center 〔12〕. The study was approved by the committee of ethics at Jinling Hospital. All patients gave their written informed consent.

**Renal allograft pathology**

Renal tissue was obtained by percutaneous biopsy. Paraffin-embedded tissue sections (2–3 mm) were stained with hematoxylin and eosin and by the periodic acid-Schiff method. Histologic lesions were classified and scored according to the 2001 Banff Meeting 〔4,13〕. All of them were repeated biopsy before and after conversion.

**C4d Staining**

C4d staining was performed on frozen tissue using an indirect immunofluorescence technique with a primary affinity-purified monoclonal antibody (mouse anti-human; dilution, 1:50; 1.5-hour incubation at room temperature; Quidel SanDiego, Calif, USA) and an FITC labeled affinity-purified secondary rabbit anti-mouse immunoglobulin G antibody (1:20; 40-minute incubation at room temperature; DAKO, Denmark). A positive staining was defined as reported in 2001 Banff Meeting 〔13〕.
Panel Reactive Antibodies (PRA)

Flow-PRA were performed pretransplantation. HLA class I and HLA class II antigens were detected upon flow cytometry analysis using the method described by Pei et al 〔14〕.Sera with >10% flow-PRA class I or II reactivity were considered anti-HLA antibody-positive.

**Efficacy evaluation**

All patients were followed for a minimum of 36 months. The rate of decline of renal function before and after conversion was represented by regression lines (slope) of the reciprocal of serum creatinine versus time 〔15〕. Efficacy evaluation include improvement rate: Slope of the serum creatinine<-5%; Stabilize:slope of the serum creatinine <-5% to 5%; Deterioration:slope of the serum creatinine >5%.

**Statistical analysis**

Statistical analysis was performed using a piecewise regression technique with Student's t test assess differences in means and chi-square to detect differences in proportions.

## RESULTS

**Clinical efficiency after conversion**

All of them were on tripletherapy (CsA plus MMF(n=14) or SRL(n=8),MZR(n=6) plus prednisolone and SRL plus MMF and prednisolone (n=8) before conversion. Flow PRA class I antibodies ≥10% in 2 cases, 19.3% and 28.5%, respectively, the other was < 10%; Flow PRA - class II antibodies≥10%, 56±9%(36—88.5%) before conversion. 21 cases has a history of acute rejection.

After 3-year of follow-up, protienuria after TAC conversion had reduced from (3.7±1.2) g/24hr. to (0.9±0.4 g/24hr), P <0.001. Ending pain and swelling in renal allograft were rarely seen. SCr levels after TAC conversion had reduced from (3.1 ±1.2)mg/dl to (1.8 ±0.6) mg/dl, P <0.001. The changes of 1/SCr level and time slope between pro-conversion and post-conversion are shown in Figure 1. Among the thirty-six patients with improvement in the rate of decline of renal function, Twenty-three patients(63.9%) had their regression lines become positive and eight patients(22.2%) had their regression lines become less negative. Five patients(13.8%) had increased rate of decline in renal function with their regression lines becoming more negative.

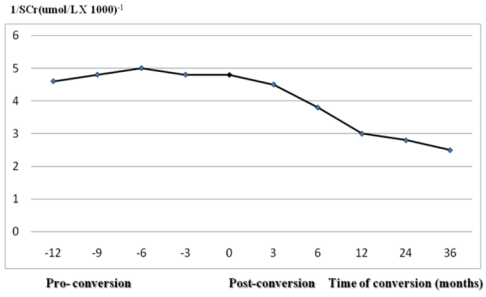


**Figure 1.** Two groups of patients using linear regression analysis method to calculate the serum creatinine reciprocal (1 / average serum creatinine (umol/LX1000) - 1) slope with time. Post-conversion vs. pro-conversion * P < 0.05.

**Histopathological changes of graft after conversion**

There were both with mild to moderate interstitial infiltration of inflammatory cells, interstitial fibrosis, tubular atrophy,mesangial matrix proliferation, glomerular sclerosis,intimal hyperplasia between two groups before the conversion (P = NS). TAC conversive treatment by 3-year follow-up, pathological changes had been significantly improved in accordance with the 97' Banff classification: renal tubular atrophy (52.8% vs 44,4%), interstitial fibrosis (50.0% vs 30.6%) and arterial hyaline degeneration (52.8% vs 25.0%). Transplant renal pathological suggests chronic damage index (CADI) are: 8.3 ± 2.6 and 3.0 ± 0.7. Repeated biopsy prompted that the degree of allograft pathological changes was significantly reduced (Table 1.).

**Table 1. Renal allograft histological changes after repeat renal biopsy**

| | Before conversion ( n=36,%) | After conversion ( n=36,%) | P values |
|---|---|---|---|
| Glomerulitis | 1( 2.8) | 0(0) | |
| Tubulitis | 2( 5.5) | 0(0) | |
| Interstitial infiltration | 4(11.1) | 1( 2.8) | <0.001 |
| Vasculitis. | 1( 2.8) | 0(0) | |

Volume : 6 | Issue : 4 | April 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

| | | | |
|---|---|---|---|
| Glomerular sclerosis | 12(33.3) | 19(52.8) | <0.001 |
| Renal tubular atrophy | 19(52.8) | 16(44.4) | <0.05 |
| Interstitial fibrosis | 18(50.0) | 11(30.6) | <0.05 |
| Arterial hyaline degeneration | 19(52.8) | 9(25.0) | <0.05 |
| CADI | 8.3±2.6 | 3.0±0.7 | <0.001 |

CADI: chronic damage index (chronic allograft damage index), including interstitial inflammatory cell infiltration, interstitial fibrosis,tubular atrophy, mesangial matrix proliferation, glomerular sclerosis, endometrial hyperplasia.

**Evolution of C4d deposition in allograft**

Thirty six cases of repeated biopsy specimens were stained by immunofluorescence, C4d positive 36 cases (100.0%) in the all of allogaft. Evolution of C4d deposition in allografts during the 3-year followe-up is shown in Table 2. Repeated renal biopsy revealed that 22 cases (61.1%) became C4d negative in renal tissue (Figure 2) and 14 case (38.9%) showed steady C4d positive accompany with hepatitis C (renal graft biopsy showed membranous nephropathy) after the switch TAC. Classification according to the Banff 07 criteria: 22 cases of C4d0,5 cases of C4d1, the 5 cases of C4d2, 4 cases of C4d3 Table 3.

**Table 2. Evolution of the allograft tissue C4d deposition before and after conversion**

| | Before conversion ( n=36,%) | After conversion ( n=36,%) | P values |
|---|---|---|---|
| Before conversion | | | |
| C4d positive | 36(100.0) | | |
| After conversion | | | |
| C4d from positive to negative | 0 | 22(61.1) | <0.001 |
| C4d C4d positive steady | 26(72.2) | 14(38.9 ) | <0. 001 |
| C4d from negative to positive | 10(27.8) | 0 | <0. 001 |

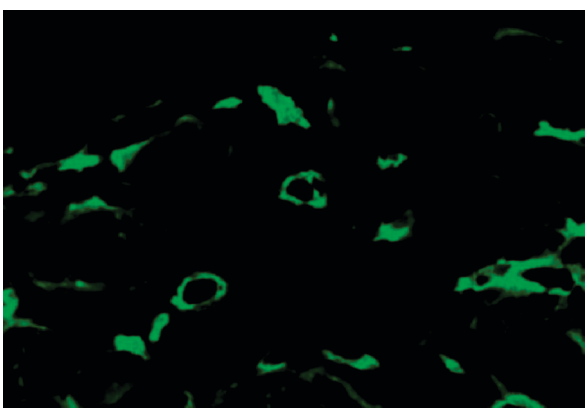

**Figure 2. PCT-C4d deposition in allograft before and after the conversion**

(indirect immunofluorescence, X200). A. PCT-C4d deposition in allograft before conversion B. Repeated renal biopsy after the conversion: PTC - C4d deposition disappear

**Table 3. The graft C4d deposition change within allograft**

| The degree of C4d deposition C4d deposition | Before conversion (n=36,%) | After conversio n (n=36,%) | P values |
|---|---|---|---|
| C4d 0 | 12(33.3) | 22(61.1) | <0.001 |
| C4d 1 | 1( 2.8) | 5(13.9) | <0.001 |
| C4d 2 | 8(22.2) | 5(13.9) | <0.05 |
| C4d 3 | 18(50.0) | 4(11.1) | <0.001 |

* P < 0.001, **P = NS, C4d deposition after conversion vs. before conversion

**C4d deposition in allograft and PRA levels**

During the follow-up of 3 years, C4d turn negative 22 cases (61.1%), and weakened C4d 5 cases (13.9%), C4d for 4 cases (11.1%) were positive. After the treatment with TAC, with the disappearance of C4d deposition in allograft, HLA-II class antibody levels decreased from 57 ±9% (35-87.5%) to 6.1 ± 1.2% (3.9-7.3%).

Volume : 6 | Issue : 4 | April 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

**Patient and graft survival**

No deaths and no new acute rejection during the 3-year follow-up. 2 cases concurrent with BK virus nephropathy, the other 3 cases of initial serum creatinine > 4.0 mg/dl, eventually leading to renal allograft loss return dialysis. Graft survival rate was significantly increased in TAC treatment (93.5%) follow-up 3-year.

## DISCUSSION

In this article, we report on 36 Chinese renal allograft recipients in whom C4d-positive chronic rejection was later confirmed. All of them were treated with the combination of TAC and MMF without immunoadsorption or plasmapheresis or rituximab and bortezomib. C4d-positive chronic rejection were controlled among the thirty-six patients with improvement in the rate of decline of renal function, twenty-three patients(63.9%) had their regression lines become positive and eight patients(22.2%) had their regression lines become less negative. Five patients(13.8%) had increased rate of decline in renal function with their regression lines becoming more negative. No deaths and no new acute rejection during the 3-year follow-up. Graft survival rate was significantly increased in TAC treatment (93.5%) follow-up 3-year. Our results suggest that combined TAC and MMF treatment is a potentially safe and economical method with acceptable efficacy for most Chinese renal allograft recipients who have C4d-positive chronic rejection.

Over the last decade, newer immunosuppressive agents such as tacrolimus, mycophenolate mofetil, and sirolimus have become available but their efficacy in controlling the process of chronic rejection remains to be determined [16,17] .Preliminary clinical studies have suggested that addition of mycophenolate mofetil with reduction of cyclosporine dosages, or conversion to tacrolimus [18] , might be effective therapeutic strategies. Based on our previous experience with the treatment of acute humoral renal allograft rejection [19,20] , we have evaluated tacrolimus-mycophenolate mofetil "rescue" in 36 consecutively identified allograft recipients with chronic humoral rejection. This was defined as chronic renal dysfunction associated with detectable serum levels of HLA-I and II alloantibodies in recipients and characteristic renal biopsy findings of chronic rejection with C4d deposits in peritubular capillaries. We report successful control of the C4d-positive chronic rejection with this regimen, a finding that may be important for the treatment and prevention of chronic allograft rejection in humans.

The use simply of the combination of TAC and MMF in the treatment of C4d-positive chronic rejection had not been studied. Actually, some investigations showed that TAC–MMF played a critical role in the rescue of AHR[21]. It is TAC–MMF that suppresses the production of new DSA. MMF inhibits in vitro antibody production by B cells and has in vivo been shown to reduce humoral responses in renal transplant recipients[22]. The 36 renal transplant recipients reported herein presented with classic clinical and pathological features of chronic rejection. TAC after the conversive treatment by 3-year follow-up, pathological changes had been significantly improved in accordance with the 97' Banff classification: renal tubular atrophy (52.8% vs 44,4%), interstitial fibrosis (50.0% vs 30.6%) and arterial hyaline degeneration (52.8% vs 25.0%). Transplant renal pathological suggests chronic damage index (CADI) are: 8.3 ± 2.6 and 3.0 ± 0.7. Repeated biopsy prompted that the degree of allograft pathological changes was significantly reduced. The detection of HLA-I or II antibodies in recipients and C4d deposits in peritubular capillaries of the biopsy specimens strongly implicate humoral mechanisms in the pathogenesis of the rejection process, a condition that we have recently termed "chronic humoral rejection" .Rescue therapy with tacrolimus and mycophenolate mofetil was associated with a sustained decrease in antidonor antibodies. During the follow-up of 3 years, C4d turn negative 22 cases (61.1%), and weakened C4d 5 cases (13.9%), C4d for 4 cases (11.1%) were positive. After the treatment with FK506, with the disappearance of C4d deposition in allograft, HLA-II class antibody levels decreased from 57 ±9% (35-87.5%) to 6.1 ± 1.2% (3.9-7.3%). Our study indicated that combining these two drugs results in a powerful immunosuppressive regimen that limits both T cell and B cell responses. TAC was effective to prevent not only T cell–mediated cellular rejection, but also antibody-mediated humoral rejection. Theruvath et al [23]demonstrated that rescue therapy with TAC and MMF was associated with a sustained decrease in antidonor antibodies, which was evident after 12-month period of therapy. Previous studies reported that MMF inhibits in vitro antibody production and in vivo humoral responses. When used in combination with tacrolimus,MMF can limit B-cell responses in renal recipients with AHR. Interestingly, TAC but not cyclosporine appears to interfere with the metabolism of MMF, which may increase the biological effects of this drug on alloantibody production[24].

**Conclusion** Combining TAC-MMF treatment represents a powerful immunosuppressive regimen that limits both T-cell and B-cell responses. In such a combination protocol, not only effective control of antidonor antibody production but also improvement of long-term graft survival may be achieved.

## REFFERENCE

1. Bosmans JL, Ysebaert DK, Verpooten GA.Chronic allograft nephropathy: what have we learned from protocol biopsies? Transplantation. 2008; 85(Suppl 7):S38-41

2. Haas M. Chronic allograft nephropathy or interstitial fibrosis and tubular atrophy: what is in a name? Curr Opin Nephrol Hypertens. 2014;23(3):245-250.

3. Nankivell BJ, Borrows RJ, Fung CL,et al. The natural history of chronic allograft nephropathy..N Engl J Med. 2003;349(24):2326-2333.

4. Colvin RB. Pathology of Chronic Humoral Rejection. Contrib Nephrol. 2009;162:75-86

5. Péfaur J, Díaz P, Panace R,et al. Early and late humoral rejection: a clinicopathologic entity in two times Transplant Proc. 2008;40(9):3229-3236

6. Singh N, Pirsch J, Samaniego M.et al.Antibody-mediated rejection: treatment alternatives and outcomes.Transplant Rev (Orlando). 2009;23(1):34-46

7. Bohmig GA, Regele H, Exner M, at el.C4d-positive acute humoral renal allograft rejection:effective treatment by immunoadsorption. J Am Soc Nephrol 2001;12: 2482–2489

8. Ji SM, Liu ZH,Chen JS, at el. Rescue Therapy by Immunoadsorption in Combination With Tacrolimus and Mycophenolate Mofetil for C4d-Positive Acute Humoral Renal Allograft Rejection. Transplantation Proceedings 2006;38:3459–346

9. Celik A,Saglam F,Cavdar C,et al. Successful therapy with rituximab of refractory acute humoral renal transplant rejection: a case report.Transplant Proc. 2008;40(1):302-304.

10. Markus Wahrmann, Michael Haidinger, Gu¨nther F. Ko¨rmo¨czi,et al. Effect of the Proteasome Inhibitor Bortezomib on Humoral Immunity in Two Presensitized Renal Transplant Candidates. Transplantation 2010;89(11):1385-1390

11.David-Neto E,Prado E,Beutel A,et al.C4d-Positive Chronic Rejection: A Frequent Entity With a Poor Outcome. Transplantation 2007;84(11):1391-1398

12.Ji SM,Li LS,Sha GZ,et al. Conversion from cyclosporine to tacrolimus for chronic allograft nephropathy. Transplant Proc. 2007;39(5):1402-1405

13. Solez K,ColVin RB,Racusen LC,et al. Banff '05 Meeting Report: Differential diagnosis of chronic allograft injury and elimination of chronic allograft nephropathy ('CAN'). A J of Transplantation 2007;7: 518–526

14. Pei R, Wang G, Tarsitani C, et al: Simultaneous HLA Class I and Class II antibodies screening with flow cytometry. Hum Immunol 1998;59:313

15.Yeung S, Lee W, Tong KL, et al. Effect of mycophenolate mofetil on progression of chronic allograft nephropathy. Transplantation Proceedings 2003;35:176–178

16. H Ekberg, C Bernasconi, J Nöldeke, et al. Cyclosporine, tacrolimus and sirolimus retain their distinct toxicity profiles despite low dose in the Symphony study. Nephrol Dial Transplant 2010:25(6): 2004-2010.

Volume : 6 | Issue : 4 | April 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

17. Pascual M, Saidman S, Tolkoff-Rubin N et al. Plasma exchange and tacrolimus-mycophenolate rescue for acute humoral rejection in kidney transplantation. Transplantation 1998; 66: 1460–1464

18. Ji Shu-Ming, Chen Jin-Song, Ni Xue-Feng, et al.Conversion from cyclosporine to tacrolimus in renal allograft recipients with chronic allograft nephropathy: Assessment of efficacy by repeated biopsies. Indian Journal of Applied Research. 2015; 5(1):482-487

19. M. Liu, S.M. Ji, Zh. Tang,et al.C4d-Positive Acute Humoral Renal Allograft Rejection: Rescue Therapy by Immunoadsorption in Combination With Tacrolimus and Mycophenolate Mofetil. Transplantation Proceedings, 2004;36, 2101–2103

20. Liu Min, Ji Shuming, Tang Zheng,et al.Novel rescue therapy for C4d-positive acute humoral renal allograft rejection. Clin Transplant 2005;19: 51–55

21. Pascual M, Saidman S, Tolkoff-Rubin N et al. Plasma exchange and tacrolimus-mycophenolate rescue for acute humoral rejection in kidney transplantation. Transplantation 1998; 66: 1460–1464

22. Kimball JA, Pescovitz MD, Book BK, Norman DJ. Reduced human IgG anti-ATGAM antibody formation in renal transplant recipients receiving mycophenolate mofetil.Transplantation 1995; 60: 1379–1383

23. Theruvath TP, Saidman SL, Mauiyyedi S, et al. Control of antidonor production with tacrolimus and mycophenolate mofetil in renal allograft recipients with chronic rejection. Transplantation 2001; 72(1): 77–83

24. Zucker K, Rosen A, Nichols A, et al. A definitive effect of administration of tacrolimus on the pharmacokinetics of mycophenolate mofetil in renal transplant patients. Transplantation 1999; 67: S544.