Tacrolimus and Sirolimus Once Daily Monotherapy Regimen as a Safe and Effective Long-Term Maintenance Immunosuppressive Therapy in Pediatric Liver Transplantation

S. M. Dehghani[1],
I. Shahramian[2]*,
M. Ataollahi[1], A. Bazi[3],
H. Foruzan[1], S. Gholami[1],
M. Goli[1]

[1]*Shiraz Organ Transplantation Center, Nemazee Hospital, Shiraz University of Medical Sciences, Shiraz, Iran*
[2]*Pediatric Gastroenterology and Hepatology Research Center, Zabol University of Medical Sciences, Zabol, Iran*
[3]*Faculty of Allied Medical Sciences, Zabol University of Medical Sciences, Zabol, Iran*

ABSTRACT

Background: Long-term efficiency of attenuated immunosuppressive therapies is not well characterized in pediatric liver transplantation (LT).

Objective: To assess the efficiency of tacrolimus once daily (TAC-OD) and sirolimus once daily (SLR-OD) immunosuppression in pediatric LT.

Methods: We retrospectively evaluated 59 children who underwent LT in our center during 2002 to 2016. Those including children who underwent planned decrease in immunosuppressant dose (stable clinical conditions after 2 years of LT), and those who underwent unplanned decrease in immunosuppressant dose (because of complications such as post-transplant lymphoproliferative disorder [PTLD] and renal failure).

Results: 25 of 59 children underwent planned decrease in immunosuppressant dosage (mean±SD duration of 4.5±1.8, range: 3–11 years); 34 had unplanned decrease (mean±SD of 1.3±0.6, range: 0.5–2.6 years). 19 of 25 children with planned conversion received TAC-OD; 6 received SLR-OD (22 with 1 mg/day dose, and 3 with 1 mg every two days). Of 34 children with unplanned conversion, 27 received TAC-OD, 7 SLR-OD (25 children with 1 mg/day, 7 with 1 mg every two days, 1 with 0.5 mg/day TAC, and 1 with 0.5 mg TAC every two days). We found no adverse events including acute or chronic graft rejection, renal insufficiency, infections, PTLDs, or cardiovascular thrombotic events after initiation of the modified immunosuppression in none of the groups.

Conclusion: TAC-OD or SLR-OD monotherapies are safe and effective for long-term management of LT children with either stable clinical conditions or those with LT complications.

KEYWORDS: Liver transplantation; Calcineurin inhibitors; mTOR inhibitors; Immunosuppression; FK506

INTRODUCTION

Liver transplantation (LT) is the standard therapy for end-stage liver diseases. Long-term survival in LT patients necessitates suitable administration of immunosuppressive to achieve acceptable survival [1, 2].

Traditionally, calcineurin inhibitors (CNIs)—cyclosporine or tacrolimus (TAC)—have been used as immunosuppressant agents in solid organ transplantation. TAC has been used as the main maintenance immunosuppressive therapy in the majority of organ transplant centers. Using CNIs, including TAC, has significantly attenuated the risk of acute graft rejection [3].

*Correspondence: Iraj Shahramian, Pediatric Ward, Amir-Al-Momenin Hospital, Zabol, Sistan and Balouchestan Province, Iran
ORCID: 0000-0002-8476-8492
Tel/Fax: +98-54-3223-9031
E-mail: ir_buper@yahoo.com

Table 1: The underlying etiologies for two groups of studied children

Cause	Planned immunosuppressive (n=25), n (%)	Unplanned immunosuppressive (n=34), n (%)
Biliary atresia	8 (32)	6 (18)
Tyrosinemia	5 (20)	6 (18)
Neonatal hepatitis	3 (12)	—
Familial progressive intrahepatic cholestasis	2 (8)	6 (18)
Crigler-Najjar syndrome	2 (8)	5 (15)
Cryptogenic cirrhosis	2 (8)	1 (3)
Fulminant hepatitis	1 (4)	—
Bailer	1 (4)	2 (6)
Familial hypercholesterolemia	1 (4)	3 (9)
Wilson's disease	—	3 (9)
Alpha-1 antitrypsin deficiency	—	1 (3)
Hepatocellular carcinoma	—	1 (3)
Total	25 (100)	34 (100)

CNIs administration has reduced the occurrence of life-threatening infections, metabolic complications, drug cytotoxicity, and development of neoplasms in transplant recipients. Nevertheless, using CNIs is hindered due to the associated risk of acute and chronic nephrotoxicity [4, 5].

A common strategy to avoid CNIs adverse effects is conversion of TAC twice daily (TAC-TD) approach to TAC once daily (TAC-OD) post-transplantation. TAC-OD strategy can further promote the compliance rate toward long-term immunosuppression therapy [6]. Another approach in reducing CNIs exposure is to recruit substitutes such as mechanistic target of rapamycin (mTOR) inhibitors—everolimus and sirolimus (SLR). SLR was first introduced in 1999 and has been used in many solid organ transplantations thereafter [7-9]. SLR triggers biological functions such as antiproliferative effects toward lymphocytes, fibroblasts, and neoplastic cells. In comparison to TAC, SLR is mainly known as a non-nephrotoxic agent with lower rate of side-effects such as hypertension and diabetes [7].

There is limited knowledge on the long-term effects of TAC and SLR low-dose immunosuppressive therapies in children who underwent LT. We conducted this study to assess the long-term effects of TAC-OD and SLR-OD monotherapy on children who underwent LT in Namazi Hospital, Shiraz.

PATIENTS AND METHODS

Population

The study population included children (<18 years) who received LT in Namazi Hospital, Shiraz, Iran. There were two groups of the patients based on the planned or unplanned dose decline in immunosuppressive therapy. The study was performed from 2002 to 2016. Informed written consent was obtained from the parents. Our study followed ethical guidelines set in the Helsinki Declaration.

Conversion to OD Immunosuppressive Therapy

The inclusion criteria for minimizing immunosuppressant drugs included non-immune liver diseases, no history of other organ transplantation, liver enzymes less than two times the upper limit of normal value, survival of >2 years after LT, no increase in immunosuppression dose in the precedent year, and no episode of rejection during the past year. Children with autoimmune hepatic disorders, and those with poor adherence to the immunosuppressive therapy were excluded. Patients met the above criteria for immunosuppressant minimization

Table 2: Pre-transplantation complications in the studied children

Complications	Planned immunosuppressive (n=25), n (%)	Unplanned immunosuppressive (n=34), n (%)
Jaundice	19 (76)	27 (79)
Hepatosplenomegaly	12 (48)	6 (18)
Ascites	10 (40)	4 (12)
Paresthesia	10 (40)	13 (38)
FTT	10 (40)	3 (9)
Encephalopathy	6 (24)	3 (9)
Gastrointestinal bleeding	4 (16)	4 (12)
Hepatomegaly	4 (16)	3 (9)
Coagulopathy	3 (12)	1 (3)
Infection	3 (12)	2 (6)
Spontaneous bacterial peritonitis	2 (8)	—
Hepatorenal syndrome	1 (4)	—

were placed on TAC-OD or SLR-OD with close monitoring of liver enzymes and drug levels. In those in whom liver enzymes elevated >2 times the upper limit of normal values, an abdominal ultrasonography with Doppler assessment of graft vessels was performed for the diagnosis of obstruction of hepatic biliary ducts. In those with normal sonography results, biopsy of liver was obtained to evaluate biopsy proven rejection. Graft rejection was decided based on histological examination and according to Banff criteria. These patients were then treated with short-term pulse-therapy with corticosteroids and increased immunosuppressant dose. Long-term successful minimization was defined as patients who have been maintained on once daily immunosuppressant monotherapy without rejection episodes for over three years.

Statistical Analysis

SPSS® for Windows® ver 19 was used for data analysis. Normality of data was assessed with one-sample Kolmogorov-Smirnov test. χ^2 and Student's t test for independent samples were used for statistical inference.

RESULTS

Overall, there were 59 liver transplanted children (<18 years old) who had been under immunosuppressive regimen of TAC-OD or SLR-OD (or once every two days). Of these, 25 had long-term successful minimization of immunosuppressant. In 34 patients, minimization of immunosuppressants was done unpredicted because of medication side-effects such as renal failure and post-transplant lymphoproliferative disorder (PTLD).

From the 25 children with stable clinical conditions during the first two years of LT, 18 (72%) were boys. Boys constituted 59% (18/34) of the group of children with unplanned immunosuppressive minimization. Living LT was performed in 17/25 (68%) and 11/34 (32%) of these groups, respectively.

Biliary atresia was the most common reason (32%) for LT in patients with successful planned immunosuppressant minimization. On the other hand, biliary atresia, familial progressive intrahepatic cholestasis, and tyrosinemia constituted the main reasons (each with 17.6%) in the second group of children (Table 1). The most prevalent complications before LT were cholestasis-related symptoms including jaundice and hepatosplenomegaly, as well as paresthesia and ascites in the both groups (Table 2).

Table 3 indicates post-transplantation complications in our patients. Overall, infections had constituted the most commonly encountered post-transplantation complications (22/59,

Table 3: Post-transplantation complications in the studied children

Complications*	Planned immunosuppressive (n=25), n (%)	Unplanned immunosuppressive (n=34), n (%)
Infections	10 (40)	12 (35)
PTLD†	5 (20)	8 (24)
Acute graft rejection	4 (16)	9 (26)
Biliary complications	2 (8)	3 (9)
Portal vein thrombosis	2 (8)	3 (9)
Seizure	2 (8)	3 (9)
Hepatic arterial thrombosis	1 (4)	—
Ascites	1 (4)	1 (3)
Renal complication	—	2 (6)
Bone-marrow suppression	—	1 (3)

*Complications occurred and resolved before initiation of the modified immunosuppressive therapy

†Post-lymphoproliferative disorder

37%); 13 (22%) patients developed PTLD. These were successfully treated with either immunosuppressant minimizing or rituximab and chemotherapy. Acute graft rejection was observed in 13 of 59 (22%) patients. All the cases were responsive to short-term pulse-therapy (n=11), as well as anti-thymocyte globulin (ATG) therapy (n=2). Two patients showed elevated creatinine levels following LT. One of them had urinary reflux to the ureter in the right kidney with complete deterioration of renal cortex. Creatinine levels were normalized after initiation of TAC-OD. Bone-marrow suppression was detected in one patient. All of these complications had occurred and resolved before initiation of immunosuppressant monotherapy.

In all the patients, laboratory tests showed normal range for selected variables after initiation of TAC-OD and SLR-OD (Table 4).

Table 4: Pre- and post-transplantation laboratory findings of the studied children. Values are mean±SD.

Parameter	Planned immunosuppressive (n=25)		Unplanned immunosuppressive (n=34)	
	Pre-transplant	Post-transplant	Pre-transplant	Post-transplant
WBC (10^3/µL)	8.3±4.2	6.8±2.5	8.4±3.9	7.6±3.4
Hemoglobin (g/dL)	10.5±1.8	13.8±1.5	11.1±2.4	13.4±1.7
Platelet (10^6/µL)	206±150	228±66	207±97	213±67
AST (IU/mL)	238±212	33±13	136±159	33±16
ALT (IU/mL)	161±183	25±13	84±80	26±19
ALP (IU/mL)	1187±684	704±401	1359±968	599±274
TB (mg/dL)	8.5±10.6	0.7±0.4	9.8±12.2	1.0±1.0
DB (mg/dL)	3.3±5.1	0.2±0.2	2.2±4.0	0.3±0.2
Albumin (mg/dL)	4.0±0.6	4.2±0.4	4.0±0.6	4.4±0.4
Na (mg/dL)	141±4	138±2	140±4	139±3
K (mg/dL)	4.2±0.4	4.1±0.3	4.3±0.4	4.2±0.4
INR	1.6±1.4	1.1±0.2	1.6±1.1	1.1±0.1
Cr (mg/dL)	0.5±0.3	0.7±0.2	0.4±0.2	0.6±0.2
PELD score	13.5±6.3	—	16.3±10	—
CHILD score	6.3±1.5	—	6.9±2.4	—

Table 5: Characteristic of the studied children under planned or unplanned tacrolimus once-daily or sirolimus once-daily

Parameter	Planned immunosuppressive (n=25)		Unplanned immunosuppressive (n=34)	
	Mean±SD	Range	Mean±SD	Range
Age (yrs)	12.2±5	5–26	10.5±5	2–21
Monotherapy duration (yrs)	4.5±1.8	3–11	1.3±0.6	0.5–2.6
Age at liver transplantation (yrs)	4.5±3.5	0.5–15	5.5±4.3	1–17
Donor age (yrs)	25.8±4	16–32	26±13	2–48
Immunosuppressant drugs (mg/dL)				
Tacrolimus	4.34±2.74*	1.3–10.3	4.1±1.7†	1.5–7.9
Sirolimus	6.84±4.63**	1.2–14	3.1±1.3††	1.8–5.3

*n=19; **n=6; †n=27; ††n=7

From the 25 children underwent planned immunosuppressant minimization, 19 received TAC-OD; six received SLR-OD (mean±SD duration of 4.5±1.8 years). Of these, 22 children received 1 mg/day TAC or SLR. One patient received 1 mg TAC therapy every two days; another, 1 mg SLR therapy every two days. Form 34 children with unplanned immunosuppressant minimization, 27 received TAC-OD; 7, SLR-OD (mean±SD duration of 1.3±0.6). From the patients under TAC therapy, 22 received 1 mg/daily; one, 0.5 mg/day; one, 0.5 mg every two days; and three, 1 mg every two days. From seven children under SLR therapy, three received 1 mg/day; and four, 1 mg every two days (Table 5). We found no adverse events including acute or chronic graft rejection, renal insufficiency, infections, PTLDs, or cardiovascular thrombotic events after immunosuppression conversion.

DISCUSSION

In the present study, we retrospectively assessed 59 children who were treated with TAC-OD and SLR-OD. Of these, 25 children received these drugs for at least three years (mean±SD of 4.5±1.8 years); 34 children received the drugs for a mean±SD of 1.3±0.6 years. Our results indicated that all 59 children who received either TAC-OD or SLR-OD, had normal range for main laboratory findings post-LT. None of the patients showed signs of acute or chronic graft rejection in the course of monotherapy with these drugs. It seems that these two drugs render excellent prognostic value for LT children with either stable clinical condition with planned long-term (as we observed for those received 4.4-year therapy) or complicated unplanned short-term (as we observed for those received 1.3-year therapy).

In a study on 50 children who underwent LT and had stable clinical condition, changing TAC-BD to TAC-OD regimen was safe and effective [10]. Only one patient who experienced chronic rejection following three years of TAC dose manipulation, recovered following pulse-therapy with 10 mg/kg/day corticosteroids [10]. In another study in adult LT patients under TAC-BD and TAC-OD, rejection was observed in 15.2% and 0% of patients, respectively [11]. Similar results have been reproduced regarding TAC-BD and TAC-OD by Weiler, *et al* [12], and Song, *et al* [13], in adult LT patients. Data from a large European study indicated a better prognosis regarding both graft and patient survival in LT patients who received TAC-OD [14]. In a short-period of three months following administration of TAC-OD regime, all the grafts preserved at the end of three months with no increase in complications rates and liver enzyme [15]. Long-term treatment with mTOR inhibitors was associated with no increase in rejection rates in adult [16, 17] and pediatric [18] LT patients. In another study, Jimenez, *et al*, reported acute graft rejection in 2/10 children under SLR maintenance therapy [19]. Nevertheless, we did not encounter any rejections in

our patients under SLR or TAC therapy. Generally, both TAC-OD and SLR-OD regimens seem to be safe modalities in long term with the least risk of graft rejection in pediatrics.

Renal toxicity is a main concern in TAC-based immunosuppressive therapies in LT patients. In our survey, we did not identify any evidence of renal insufficiency during TAC-OD or SLR-OD protocols. Nevertheless, creatinine level increased significantly at the end of the three months (1.1±0.4 mg/dL) in comparison to the baseline pre-transplant levels (0.8±0.3 mg/dL) in a report by Charco, *et al* [15]. In parallel to our results, renal function preserved following three years of TAC-OD administration in 50 LT children who had stable clinical conditions at the baseline [10]. Occurrence of renal insufficiency may necessitate withdrawing TAC-OD protocol in LT patients, as reported in 21.8% of patients in a study by Gastaca, *et al* [20]. Accordingly, renal function can also be preserved with a reduction in the dose of drug in TAC-OD approach [10]. In comparison to TAC, which is known to have high nephrotoxicity, SLR-based therapies are considered safe strategies regarding renal heath [16]. Initiation of SLR-based therapy can restore renal dysfunction in considerable ratio of children and adults with TAC-induced compromised renal function [5, 18]. Renal failure is a major challenge in TAC-based immunosuppressive therapy during post-LT period. Accordingly, starting a SLR-based therapy seems to provide an acceptable long-term modality for preserving renal function in patients.

Development of PTLDs is another potential dilemma in organ transplant recipients, including LT. Following initiation of either TAC-OD and SLR-OD therapeutic regimes, we found no new cases of PTLD in our patients. In a previous report of ours, 40 (6.2%) and 13 (1.1%) incidents of PTLDs have been reported in pediatric and adult LT patients during 2004–2015 [21]. In a recent study, we described a negative association between TAC serum level and development of post-transplant PTLD in pediatrics [21]. Overall, our findings indicated that either of TAC-OD or SLR-OD immunosuppressive regimens are not linked to increased risk of PTLDs in one- and three-year follow up. However, a strict conclusion on this requires monitoring LT patients for longer periods.

As immunosuppressive drugs impede the functionality of the immune system, particularly T lymphocytes, organ transplant recipients are at risk of various infectious diseases [22]. A recent study shows that a higher membrane expression of inhibitory receptors, Programmed Death 1 (PD-1) and T-cell Ig- and mucin-domain molecule 3 (Tim-3), in T-lymphocytes of LT recipients at pre-operation period is associated with higher infectious episodes in post-transplantation period [22]. These researchers further show that T lymphocytes with high expression of PD-1 and Tim-3 have lower capacity for production of INF-γ [22]. Nevertheless, a promising feature of TAC can be its differential impacts on the function of innate and adaptive immunities [23]. In fact, it has been demonstrated that TAC can target cellular activities of T-lymphocytes (i.e., cytokine production and cell-mediated immunity); however, TAC exerts no significant impact on the activities of the innate immunity (myeloid-derived cell and macrophages) [23]. Upon optimization of the dose of TAC, this feature may role as a corn stone to achieve both the least risk of rejection rate by inducing immune tolerance and high levels of protection against pathogens post-transplantation. Viral infectious episodes (i.e., cytomegalovirus) post-transplantation can signify the risk of chronic graft rejection [24].

A practical procedure toward optimization of immunosuppressive therapy can be adjusting the drug dose in the post-transplantation period based on mathematical calculations (parabolic personalized dosing [PPD]) [1, 25]. This approach has been applied for TAC monotherapy regimen by Zarrinpar, *et al* [25]. On the other hand, personalized dose optimization may also require to consider some inter-individual variations such as genetic polymorphism affecting TAC metabolism (i.e., CYP 3A5 [26, 27], IL-18 [28], or other possible and unknown genetic modifiers). Furthermore, age and sex of recipients should also be

considered in dose adjustment [26].

As with other studies on efficiency and safety of TAC-OD regime [10, 11], our results may not be generalizable to those patients with post-transplantation unstable clinical conditions.

In conclusion, prognosis of TAC-OD and SLR-OD monotherapy immunosuppressive approaches are excellent and should be regarded a possibility for deterring or even omitting immunosuppression therapy in organ transplant recipients. This was achieved without any signs of acute or chronic graft rejections or other adverse complications in our experience. Nevertheless, it is a conundrum to determine a suitable dose of immunosuppressant for each patient, hindering total omission of immunosuppression in LT children.

CONFLICTS OF INTEREST: None declared.

REFERENCES

1. Rubin A, Berenguer M. Liver transplantation: Personalized immunosuppression in patients with hepatitis c virus infection and hepatocellular carcinoma. *Gastroenterol Hepatol* 2013;**36**:48-57.
2. Penninga L, Wettergren A, Chan AW, et al. Calcineurin inhibitor minimisation versus continuation of calcineurin inhibitor treatment for liver transplant recipients. *The Cochrane Library* 2012;CD008852. doi: 10.1002/14651858.CD008852.pub2.
3. Gur O, Gumustekin M, Murat N, Gidener S. The role of cytochrome p450 3a5 enzyme on the metabolism of tacrolimus in rats. *Journal of Basic and Clinical Health Sciences* 2017;**1**:53-6.
4. Khurana A, Brennan DC. *Current concepts of immunosuppression and side effects. Pathology of solid organ transplantation.* Springer, **2009**: 11-30.
5. Casas-Melley AT, Falkenstein KP, Flynn LM, *et al*. Improvement in renal function and rejection control in pediatric liver transplant recipients with the introduction of sirolimus. *Pediatr Transplant* 2004;**8**:362-6.
6. Ekong U, Bhagat H, Alonso E. Once daily calcineurin inhibitor monotherapy in pediatric liver transplantation. *Am J Transplant* 2010;**10**:883-8.
7. Trotter JF. Sirolimus in liver transplantation. *Transplant Proc* 2003;**35**:193S-200S.
8. Moini M, Schilsky ML, Tichy EM. Review on immunosuppression in liver transplantation. *World journal of hepatology* 2015;**7**:1355.
9. Massoud O, Wiesner RH. The use of sirolimus should be restricted in liver transplantation. *J Hepatol* 2012;**56**:288-90.
10. Quintero J, Juamperez J, Ortega J, *et al*. Conversion from twice-daily to once-daily tacrolimus formulation in pediatric liver transplant recipients - a long-term prospective study. *Transpl Int* 2018;**31**:38-44.
11. Stephen S, Markkassery R, Sainudheen BE, *et al*. A comparative study of once daily versus twice daily tacrolimus in liver transplantation. *J Young Pharm* 2017;**9**:605-9.
12. Weiler N, Thrun I, Eberlin M, *et al*. Tacrolimus effects and side effects after liver transplantation: Is there a difference between immediate and extended release? *Transplant Proc* 2013;**45**:2321-5.
13. Song GW, Lee SG, Hwang S, *et al*. A pilot study of the pharmacokinetics of the modified-release once-daily tacrolimus formulation administered to living-donor liver transplant recipients. *Exp Clin Transplant* 2016;**14**:412-8.
14. Adam R, Karam V, Delvart V, *et al*. Improved survival in liver transplant recipients receiving prolonged-release tacrolimus in the european liver transplant registry. *Am J Transplant* 2015;**15**:1267-82.
15. Charco R, Caralt M, Llado L, *et al*. A prospective, multicenter study of once-daily extended-release tacrolimus in de novo liver transplant recipients. *Transplant Proc* 2011;**43**:718-23.
16. Weiler N, Bilge N, Troetschler S, *et al*. Conversion from sirolimus to everolimus in long-term liver graft recipients. *J Clin Pharmacol* 2017;**57**:837-45.
17. Castroagudin JF, Molina E, Tome S, *et al*. Safety of an immunosuppressant protocol based on sirolimus in liver transplant recipients with malignancies or high risk of tumor recurrence. *Transplant Proc* 2009;**41**:1003-4.
18. Hendrickson R, Daniel J, Andrews W, *et al*. Indications and efficacy of converting from tacrolimus (tac) to sirolimus (slr)-based immunosuppression in pediatric patients who underwent liver transplantation for unresectable *hepatoblastoma Transplantation* 2014;**98**:721-.
19. Jimenez-Rivera C, Avitzur Y, Fecteau AH, *et al*. Sirolimus for pediatric liver transplant recipients with post-transplant lymphoproliferative disease and hepatoblastoma. *Pediatr Transplant* 2004;**8**:243-8.
20. Gastaca M, Valdivieso A, Bustamante J, *et al*. Favorable longterm outcomes of liver transplant recipients treated de novo with once-daily tacrolimus: Results of a single-center cohort. *Liver Transpl* 2016;**22**:1391-400.
21. Eshraghian A, Imanieh MH, Dehghani SM, *et al*. Post-transplant lymphoproliferative disorder after liver transplantation: Incidence, long-term survival and impact of serum tacrolimus level. *World J Gastroenterol* 2017;**23**:1224-32.

22. Mysore KR, Ghobrial RM, Kannanganat S, *et al*. Longitudinal assessment of t cell inhibitory receptors in liver transplant recipients and their association with posttransplant infections. *Am J Transplant* 2018;**18**:351-63.

23. Shao K, Lu Y, Wang J, *et al*. Different effects of tacrolimus on innate and adaptive immune cells in the allograft transplantation. Scand. *J Immunol* 2016;**83**:119-27.

24. Choudhary NS, Saraf N, Saigal S, *et al*. Revisiting chronic rejection following living donor liver transplantation in the tacrolimus era: A single center experience. *Clin Transplant* 2018;32.

25. Zarrinpar A, Lee DK, Silva A, *et al*. Individualizing liver transplant immunosuppression using a phenotypic personalized medicine platform. *Sci Transl Med* 2016;**8**:333ra49. doi: 10.1126/scitranslmed.aac5954

26. Calvo PL, Serpe L, Brunati A, *et al*. Donor cyp3a5 genotype influences tacrolimus disposition on the first day after paediatric liver transplantation. *Br J Clin Pharmacol* 2017;**83**:1252-62.

27. Kato H, Usui M, Muraki Y, *et al*. Long-term influence of cyp3a5 gene polymorphism on pharmacokinetics of tacrolimus and patient outcome after living donor liver transplantation. *Transplant Proc* 2016;**48**:1087-94.

28. Zhang XQ, Xu JD, Fan JW, *et al*. Influence of il-18 and il-10 polymorphisms on tacrolimus elimination in chinese lung transplant patients. *Dis Markers* 2017; doi: 10.1155/2017/7834035.