The Cairo Kidney Center Protocol for Rapamycin-based Sequential Immunosuppression in Kidney Transplant Recipients: 2-Year Outcomes

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Objective: **This study examines the outcomes of de novo kidney transplants treated by a sequential protocol, designed to target the succession of immunologic events following engraftment.**

Subjects: **A total of 113 sequential live-donor recipients were randomized into 2 arms. Patients in arm A received prednisolone, cyclosporine, and sirolimus for 3 months (phase 1), followed by replacement of cyclosporine with mycophenolate mofetil (phase 2). Those in arm B (controls) received prednisolone/cyclosporine/mycophenolate mofetil throughout the study. The primary endpoints were patient and graft survival rates at 2 years. Secondary endpoints included biopsy-proven acute rejection, early and late graft function, hypertension, and adverse reactions.**

Results: **The 2-year intent-to-treat patient and graft survival rates (95.8% vs 91.4% and 94.6% vs 90.2%) were numerically but not significantly higher in arm A. The overall incidence of biopsy-proven acute rejection was numerically lower (13.5% vs 18.9%), yet it occurred exclusively with cyclosporine C2 levels below 770 ng/mL (*P* = .28). Mean time for serum creatinine to reach 132 μmol/L was significantly longer in arm A (7.3 vs 2.9 days). Graft function at 2 years (eGFR, 70.2 vs 55.9 mL/min) and number of drugs needed to control blood pressure (mean 1.7 vs 2.25) were significantly more favorable in group A. Significant adverse effects for patients in arm A included proteinuria (36.8% vs 18.6%), hyperlipidemia (peak cholesterol > 7.75 mmol/L in 32.9% vs 23.7% of patients) and thrombocytopenia (platelet count < 100 × 10^9/L in 32.9% vs 13.5 % of patients).**

Conclusions: **The described protocol reduced the incidence of biopsy-proven acute rejection in patients after kidney transplant, particularly in those with adequate cyclosporine blood levels. Despite the significantly higher incidence of certain adverse effects (ie, delayed graft function, proteinuria, hyperlipidemia, and transient thrombocytopenia), patient and graft survival rates at 2 years were numerically, though not statistically, improved in patients in arm A. At 2-year analysis, compared with patients in the control arm (arm B), graft function significantly improved in patients in arm A, and the number of drugs needed to control blood pressure was significantly lower.**

Key words: *Mycophenolate mofetil, Cyclosporine, Graft function, Renal transplant, Live donors*

Despite considerably improved outcomes during the past 2 decades, risk-free, near-normal, long-term patient survival and desirable quality of life remain elusive for persons undergoing renal transplant. The ambition to achieve these, however, has been a driving force for better understanding of transplant immunity, new drug discovery, development of new strategies, and the implementation of new protocols, all with impressive successes as well as disappointing failures.

In this paper, we report the results of a protocol from The Cairo Kidney Center in Cairo, Egypt, that was designed and implemented in response to contemporary developments in basic science as well as earlier global and local clinical experiences. Our aims with this protocol were to rationally address the time-related sequence of lymphocyte activation; utilize the powerful immunosuppressive effect of calcineurin inhibitors while avoiding their long-term negative consequences; utilize the promising immunologic and growth-checking potential of mTOR inhibitors; and avoid the early postoperative

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This trial was performed exclusively by The Cairo Kidney Center team without technical or financial support by any other institution, firm, or organization.

Experimental and Clinical Transplantation (2007) 2: 649-657

use of mycophenolate mofetil, the gastrointestinal adverse effects of which may impose dosage reduction or even withdrawal in up to two-thirds of patients in some series, at the most critical period of graft survival.

The protocol is composed of 2 sequential phases, with a dividing line arbitrarily set at the end of the third month after transplant. It is assumed that in the first phase, the graft is most vulnerable to extensive proinflammatory cytokine release by activated lymphocytes with little or no suppressor or regulatory counteractivity. Beyond this phase, the graft becomes more stable, yet susceptible to the consequences of "inappropriate proliferation" of lymphocytes, fibroblasts, and vascular endothelial and smooth muscle cells, in addition to long-term drug toxicity. The penalty for uncontrolled proliferation extends beyond the graft to the recipient as a whole, who then is at increased risk for atherosclerosis and malignancy.

Accordingly, our aim of immunosuppression in phase 1 was to prevent acute rejection by an acknowledged powerful drug combination composed of prednisolone, cyclosporine, and sirolimus. In addition to the theoretical advantage of multiple-level signal interference (1) (Figure 1), this combination has been shown, in vitro and in vivo, to exert a significant synergistic effect in suppressing lymphocyte proliferation (2), intimal proliferation (3), and collagenosis (4). It has been clinically tested and approved based on pivotal studies (5) and subsequently used in many trials (6, 7, 8, 9). In phase 2, while maintaining the long-term immunosuppressive, antiproliferative (10), antifibrotic (11), and anti-carcinogenic potentials of sirolimus (12), cyclosporine was withdrawn to avoid its long-term consequences, which include nephrotoxicity, hypertension, accelerated atherosclerosis, and interference with tolerogenic mechanisms (13). It was replaced by mycophenolate mofetil to engage its calcineurin-inhibitor–sparing potential (14), strong antiproliferative effect (15), and fair safety profile. Based on the different modes of action and distinct levels of cell

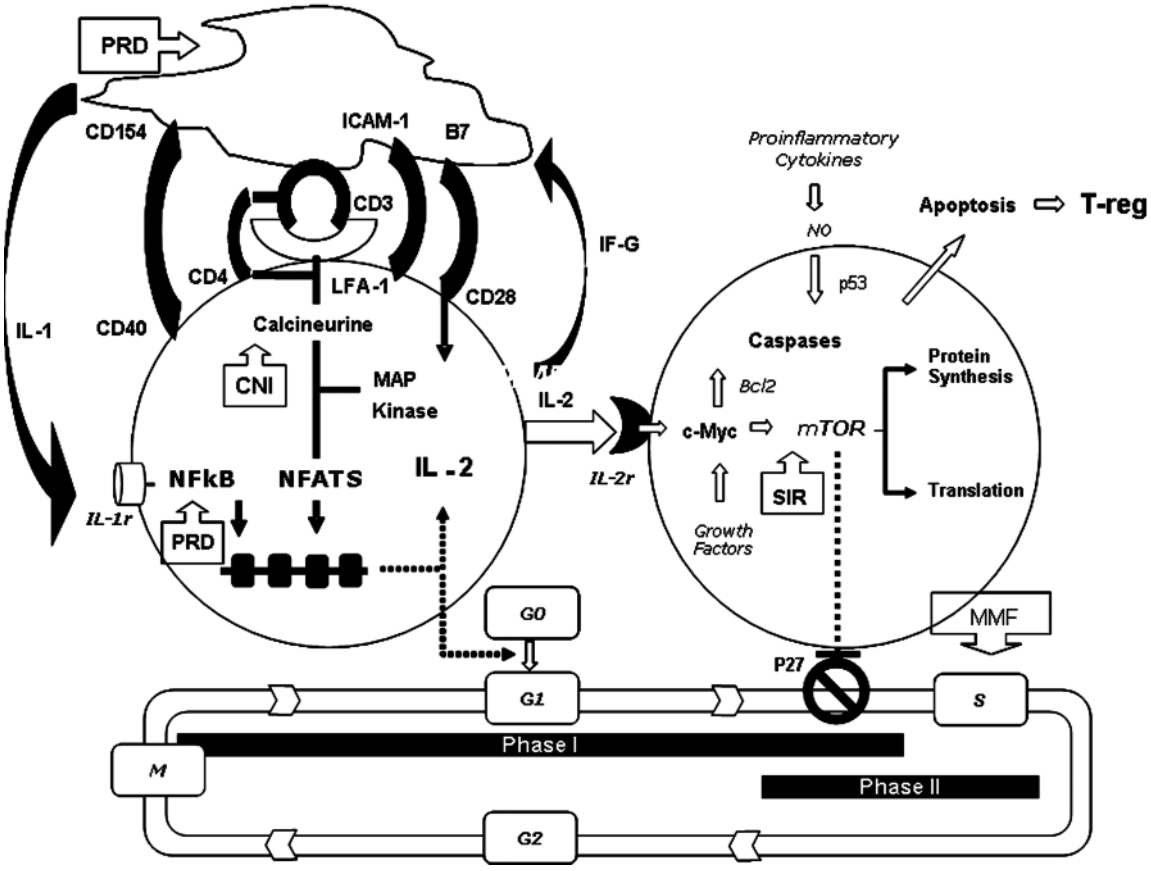

Figure 1. Molecular pathways involved in transplant rejection. Note the sites of action of corticosteroids (PRD) and calcineurin inhibitors (CNI) during the early phases of activation, sirolimus (SIR) and mycophenolate (MMF) in late phases. The latter spare c-Myc, and hence the potential of apoptosis and consequent T-regulatory (T-reg) lymphocyte differentiation. The successive phases of the sequential protocol are displayed in relation to the cell cycle as shown in the lower panel (G0, 1, 2, respective gaps; M, mitosis; S, synthesis).
Abbreviations: IL-*n*, Interleukin-*number*; IL-*n*r, IL-n receptor; CD*n*, cluster differentiation *number*; ICAM-*n*, intercellular adhesion molecule-*number*; LFA-1, lymphocyte function-associated antigen-1; IFN-γ, interferon gamma; MAP, mitogen-activated protein; NFκB, nuclear factor kappa B; NFATS, nuclear factor of activated T cells; NO, nitric oxide; mTOR, mammalian target of rapamycin; c-Myc, Bcl2, p53, respective oncoproteins.

Table 1. Treatment protocol in the study groups

		Day (-2)	Day (-1)	Day 0	0-1 month	1-3 months	3-6 months	6-12 months	12-24 months
Methyl prednisolone (mg)	arm A	-	250	500	-	-	-	-	-
	arm B	-	250	500	-	-	-	-	-
Oral PRD (mg/day)	arm A	-	-	-	50-20	20-10*	10	± 10-5†	± 5-0†
	arm B	-	-	-	50-20	20-10*	10	10-5	± 5-0†
SIR dose (mg/day)	arm A	-	-	9	2 – X‡	X‡	X‡	X‡	X‡
	arm B	-	-	-	-	-	-	-	-
SIR trough level (ng/mL)	arm A	-	-	-	5-10	5-10	10-15	10-15	10-15
	arm B	-	-	-	-	-	-	-	-
CyA dose (mg/kg/day)	arm A	6	6	6	6-X‡	X‡	-§	-	-
	arm B	6	6	8	8- X‡	X‡	X‡	X‡	X‡
CyA C2 level (ng/mL)	arm A	-	-	-	600	600	-	-	-
	arm B	-	-	-	1600	1600	1600	1200	1000
MMF dose (g/day)	arm A	-	-	-	-	-	2¶	2	2
	arm B	-	-	1	2¶	2	2	2	2

Sirolimus and cyclosporine blood levels were measured by TDx.
*Gradual dosage decrements over a given period; †Withdrawal considered as described in text; ‡X=adjusted dosage according to blood level; §CyA withdrawal over 1 week ; ¶MMF dosage build-up from 1 Gm to 2 Gm over 3-7 days depending on gastrointestinal tolerance.
Abbreviations: CyA, cyclosporine; MMF, mycophenolate mofetil; PRD, prednisolone; SIR, sirolimus.

Table 2. Baseline demographic and clinical characteristics

	Total	Percentage (%)	Arm (A)	Percentage (%)	Arm (B)	Percentage (%)	*P* value
Number	113		76		37		
Mean duration of follow-up (months)	48.4		30.3		31.4		
Demographic							
Age (years)			45±15.3		44±15.0		
Male sex	74		47	61.8	27	73.0	NS
Original disease							
ADPKD	3	2.7	3	3.9	0	0.0	NS
CGN	20	17.7	15	19.7	5	13.5	NS
Nephrosclerosis	25	22.1	18	23.7	7	18.9	NS
Diabetes	28	24.8	15	19.7	13	35.1	NS
Failed transplant	7	6.2	5	6.6	2	5.4	NS
Other	19	16.8	10	13.2	9	24.3	NS
Unknown	11	9.7	10	13.2	1	2.7	NS
Pre-emptive	30	26.5	23.0	30.3	7.0	18.9	NS
HLA mismatches /6	3		3.1±0.89		2.8 ± 1.0		NS
Pretransplant infection							
HCV	30	27	19.0	25.0	11.0	29.7	NS
CMV	100	88	68.0	89.5	32.0	86.5	NS

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; CGN, chronic glomerulonephritis; HCV / CMV, positive serological markers for hepatitis C and cytomegalovirus infection; NS, nonsignificant.

cycle restraint (1), it was expected that the favorable potentials of mycophenolate mofetil and sirolimus would be additive (16) (Figure 1).

Materials and Methods

Study design

This study was designed as a prospective, sequentially randomized, open-label trial including all eligible patients receiving kidney transplants at the Cairo Kidney Center in Cairo, Egypt, from July 2002 to July 2006. The Ethics Committee of Cairo University Medical School approved the study protocol in May 2001, and it conforms to the ethical guidelines of the 1975 Helsinki Declaration. After completing a pre-transplant assessment and obtaining legal clearance from the health authorities and formal patient and donor consents, additional written informed consent for inclusion in the study was obtained from each recipient.

In total, 113 de novo transplants were enrolled in the study. They were sequentially assigned in a 2:1 succession either to arm A (76 patients) to arm B (37 patients) as described in Table 1.

All donors (mean age, 33.6±4.2 years; age range, 25-43 years) were living; 84% were unrelated, with no blood group incompatibility, and they had a mean HLA (molecular typing, amplification refractory mutation system-polymerase chain reaction) mismatch of 3/6. There were no donor/recipient viral incompatibilities (ie, cytomegalovirus, hepatitis C virus, hepatitis B virus [markers by enzyme-linked immunosorbant assay]). A total of 26.5% of the transplants were pre-emptive. There were no statistically significant differences in any of the relevant baseline demographics or clinical characteristics of patients or donors assigned to either arm (Table 2).

The immunosuppression protocol design is shown in Table 1. Steroid withdrawal was considered by the ends of months 6 and 12 in those patients

whose grafts had been stable and showed no episodes of acute rejection. A graft biopsy specimen was obtained (Tru-cut needles, Baxter Laboratory, Morton Grove, IL) in borderline cases, stained with hematoxylin and eosin, Masson trichrome, and periodic acid-Schiff, and examined by light microscopy. In eligible patients, steroids were withdrawn gradually over 4 to 6 weeks.

All patients received prophylactic perioperative ceftazidime and postoperative cotrimoxazole and nystatin for 3 months. Blood pressure was controlled at 130/80 mm Hg by stepwise administration of amlodipine, carvedilol, ramipril, frusemide, and prazosin, in that order. A statin was added when necessary to maintain the blood total cholesterol levels at < 5.17 mmol/L (200 mg/dL) and a low-density lipoprotein cholesterol level at < 3.1 mmol/L (120 mg/dL). Fenofibrate was added only if the plasma triglyceride level exceeded 5.5 mmol/L (500 mg/dL) despite adequate diet and control of diabetes.

Acute rejection was diagnosed by graft biopsy, scored according to Banff criteria, and treated with methylprednisolone in 500-mg pulse doses daily for 6 to 8 days. Steroid-resistant rejections were treated with rabbit anti-thymocyte globulin for 14 days. Following control of the rejection, arm-A patients in phase 1 were not switched to phase 2 for at least 3 months, while those in phase 2 received additional cyclosporine in small doses and were kept on quadruple therapy for at least 3 months.

Study parameters

The primary endpoints of the study were patient and graft survival at 2 years. Secondary endpoints included biopsy-proven acute rejection, early graft function (time to achieve normal serum creatinine), 2-year estimated glomerular filtration rate (eGFR, using the Modification of Diet in Renal Disease-4 formula), blood pressure control (number of drugs required for adequate control), and adverse reactions (as shown in Table 3). Survival data and graft function were analyzed on an intent-to-treat as well as a patients-on-therapy basis, while compliance and other secondary endpoints were analyzed only on a patients-on-therapy basis. Parametric data are provided as means ± standard error and were analyzed with the *t* test. Nonparametric data were analyzed with the chi-square test. Kaplan-Meier curves were used to estimate survival data, and the log-rank test was used to compare them. The confidence limit for statistical significance was set to 95%. The StatDirect software package (CamCode, Ashwell, United Kingdom) was used to analyze these data.

Results

Protocol compliance

By the end of 2 years, owing to delayed wound healing, gross edema, delayed graft function, shortage of funds (2 patients each), interstitial pneumonia, rectal ulcerations, and difficulty in achieving therapeutic drug levels owing to the use of oral anticoagulants (1 patient each), sirolimus was withdrawn in 11 patients in arm A (14.5%) after a mean of 6.7 months (range, 0.7-15 months). Conversely, owing to significant cyclosporine adverse effects, 8 patients in arm B (21.1%) were switched to sirolimus after a mean of 4.3 months (range, 1.0-10 months).

The mean time for switching patients in arm A from phase 1 to phase 2 was 3 months, in accordance with the protocol. However, 34.2% of patients had to be switched earlier owing to the adverse effects of cyclosporine, and 9.2% were switched later either because they had developed early acute rejection or because they were intolerant to the gastrointestinal adverse effects of mycophenolate mofetil.

Rapamycin blood trough levels varied considerably, necessitating frequent dosage adjustments. The mean levels during phase 1 were 9.5 ± 2.55 and 9.3 ± 2.55 ng/mL by the end of months 1 and 3, respectively. The mean levels in phase 2 were 12.6 ± 2.85, 11.7 ± 3.35, 11.8 ± 3.15, and 11.4 ± 2.6 ng/mL by months 6, 9, 12, and 24, respectively.

The target cyclosporine C2 level was even more difficult to maintain, the means during phase 1 being 869 ± 169.0 and 811 ± 137.5 ng/mL by the ends of months 1 and 3, respectively. The mean in patients with delayed switching was 741 ± 108.6 ng/mL, and the mean in patients maintained on quadruple therapy was 423 ± 67 ng/mL.

In patients in arm A, corticosteroids were completely withdrawn from 24.4% of patients by 1 year and in 46.9% of patients at 2 years, as opposed to 13.6% and 25.0% at 1 and 2 years, respectively, in patients in arm B. The mean steroid doses were 5.4 and 6.6 mg at 1 year and 5.2 and 4.9 mg at 2 years in arms A and B, respectively. These differences were not statistically significant.

Patient survival

Three patients in each arm died during the study, yielding a 2-year cumulative patient survival rate of 95.8% and 91.4% by intent-to-treat analysis and 95.1% and 92.1% by patients-on-therapy analysis in arms A and B, respectively; this difference was not statistically significant (Figure 2A). The causes of death in arm A were pulmonary embolism in 1 patient, disseminated bronchial carcinoma in another

patient, and retroperitoneal bleeding in a patient on oral anticoagulants for a prior aortic valve replacement. Causes of death in arm B were acute myocardial infarction in 1 patient, mesenteric vascular occlusion in another patient, and invasive cytomegalovirus disease while abroad in another patient.

Graft survival

In addition to those grafts lost owing to the patient's death, 1 graft in 1 patient in each arm was lost owing to resistant vascular rejection. The cumulative 2-year graft survival rate was thus 94.6% and 90.2% by intent-to-treat analysis and 93.5% and 88.5% by patients-on-therapy analysis in arms A and B, respectively (Figure 2B). The difference was not statistically significant, both on uncensored and death-censored analyses.

Graft function

All grafts functioned immediately. The mean length of time for the serum creatinine level to drop beyond 132 μmol/L (1.5 mg/dL) was 7.3 days (range, 1-56 days) in arm A and 2.9 days (range, 1-9 days) in arm B. Delay beyond 72 hours was encountered in 44.4% of the grafts in arm A compared with 14.3% of the grafts in arm B ($P < .01$).

Acute rejection

Over the entire study, biopsy-proven acute rejection was encountered in 13.2% of patients in arm A, of

Table 3. Main adverse effects

	arm (A)		arm (B)		
Surgical Complications					*P* value
Delayed wound healing	6.60%		7.90%		NS
Local hematomas > 30 mL	9.20%	[1]	0.00%		NS
Retroperitoneal hematomas	2.60%		0.00%		NS
Lymphoceles	14.50%		10.60%		NS
Hypertension [2]	52.6%		91.8%		**< .05**
Peripheral edema	36.8%	[3]	37.8%	[3]	NS
Without or disproportionate of proteinuria	32.9%		35.1%		NS
Nephrotic edema	3.9%		2.7%		NS
Necessitating drug withdrawal	2.6%		0.0%		NS
Thrombosis	9.2%		15.8%		NS
Thrombotic microangiopathy	1.3%		0.0%		NS
Cardiovascular events	1.3%		8.1%	[4]	NS
Mean time of onset (weeks after transplant)	22		78		NS
Deep Venous Thrombosis	7.9%		13.5%		NS
Mean time of onset (weeks after transplant)	15.4		42		NS
Pulmonary embolism	2.6%	[5]	5.4%		NS
Pneumonia	11.8%		10.8%		NS
Microbial	7.9%		10.8%		NS
No organism detected	3.9%	[6]	0.0%		NS
Herpes viral infection	15.8%		21.1%		NS
Mean time to disease (weeks after transplant)	7.7		12.6		NS
Gut ulcers					
Oral ulcers	13.2%		5.4%		NS
Rectal ulcers	1.30%		0.0%		NS

	arm (A)		arm (B)		
Malignancy					*P* value
Lung	2.70%	[7]	0.0%		NS
Prostate	2.70%		0.0%		NS
Proteinuria					
Overall frequency	36.80%		18.60%		**< .05**
Mean time of onset (weeks after transplant)	34.9		78.3		NS
Mean albumin/creatinine ratio	0.36		1.85		NS
Nephrotic range	3.9%		2.7%		NS
Hematologic					
Hemoglobin <11g/dL	39.5%		18.9%		NS
Neutrophil count < 10^8/L	6.6%		5.4%		NS
Platelet count < 100^9/L	32.9%		13.5%		**< .01**
Mean time to nadir (days after transplant)	6.9		6		NS
Hepatic		[8]		[8]	
> 2-fold elevation of ALT	11.8%		10.8%		NS
Mean time to peak (days after transplant)	8.7		8.7		NS
> 2-fold elevation of AST	6.6%		2.7%		NS
> 2-fold elevation of GGT	21.1%		21.6%		NS
Peak serum bilirubin (umol/L)	23.8		28.9		NS
Mean time to peak (days post Tx)	10		9.4		NS
Posttransplant diabetes mellitus	3.60%		8.10%		NS
Hyperlipidemia					
Peak cholesterol > 7.75mmol/L	32.90%		23.70%		**< .05**
Use of statins	57.90%		60.50%		NS
Peak triglycerides > 5.5 mmol/L	13.20%		7.90%		NS

[1] Fatal in 1 patient.
[2] See Figure 4.
[3] Attributed to calcium channel blockers in arms A (n=21) and B (n=12).
[4] 2 myocardial infarctions (1 died); 1 fatal superior mesenteric artery thrombosis.
[5] Fatal in 1 case.
[6] Interstitial pneumonia attributed to sirolimus.
[7] Fatal by month 4.
[8] No correlation with HCV or CMV, anesthesia, ferritin, sirolimus, or cyclosporine blood levels.
Abbreviations: ALT, alanine transferase; AST, aspartate transaminases; GGT, gamma glutaryl transferase.

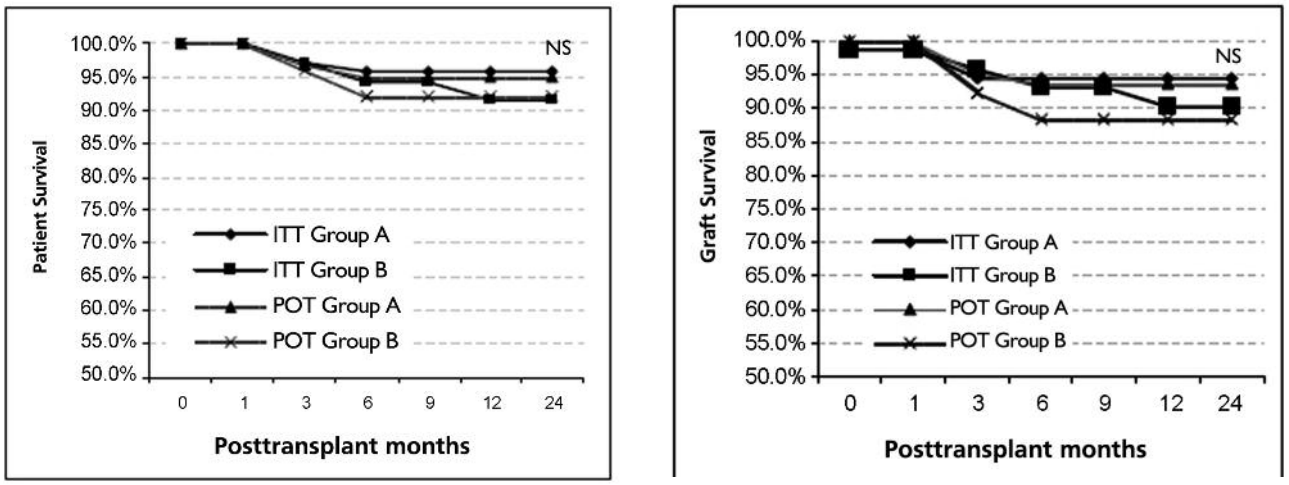

Figure 2. Intent-to-treat Kaplan-Meier curves: (A) patient survival; (B) uncensored graft survival.
Abbreviations: ITT, intent-to-treat; POT, patients-on-therapy analysis

which 10.5% occurred during phase 1. In arm B, biopsy-proven acute rejection occurred in 18.9% of patients, of which 16.2% occurred during the first 3 months. These differences were not statistically significant by either intent-to-treat or patients-on-therapy analysis.

One rejection in each arm was scored as Banff class III, and these proved resistant to steroids and thymoglobulin and ultimately led to graft loss. All others were classified as Banff class IA or IB, and responded to steroid pulses, recovering their prerejection serum creatinine levels.

The incidence of biopsy-proven acute rejection episodes was unrelated to the number of HLA mismatches, hepatitis C, or cytomegalovirus status, or the duration of prior dialysis. The incidence was not related, either, to the rapamycin trough blood level within the 5- to 10-ng/mL range set forth in the protocol. On the other hand, the mean cyclosporine C2 level in those who developed a biopsy-proven acute rejection during phase 1 was 565 ± 116.5 ng/mL compared with 896 ± 167 ng/mL in those who did not develop a rejection ($P = .028$). No biopsy-proven acute rejection occurred with C2 levels above 770 ng/mL regardless of the sirolimus trough levels. On the other hand, in patients in arm B, the mean C2 level was 1463 ± 218 ng/mL for those who developed biopsy-proven acute rejection compared with 1787 ± 294 ng/mL for those who did not; these values were statistically significantly higher than the respective values for patients in arm A ($P < .01$).

On patients-on-therapy analysis, serum creatinine levels at 24 months showed a progressive decline in patients in arm A to a mean of 96.8 μmol/L (1.1 mg/dL) compared with 126.72 μmol/L (1.44 mg/dL) in patients in arm B ($P = .001$). This reflected on the eGFR which, in patients in arm A, showed a statistically significantly progressive rise from 61.85 ± 10.45 mL/min/1.73 m² at baseline to 70.2 ± 8.0 mL/min/1.73 m² at 2 years ($P = .024$), while it showed a numerical (although not statistically significant) decline in patients in arm B from 63.77 ± 8.9 mL/min/1.73 m² to 55.86 ± 7.8 mL/min/1.73 m² ($P = .065$). The eGFR at year 2 (Figure 3) was statistically significantly higher in patients in arm A than in those in arm B ($P = .03$). Similar conclusions were made by intent-to-treat analysis as shown in Figure 3.

Adverse effects and complications

With the exception of hypertension, dipstick–detectable proteinuria, thrombocytopenia, and hyperlipidemia, the incidence of adverse effects between the 2 arms was not statistically significantly different (Table 3 and Figure 4).

Discussion

Our protocol is essentially a hybrid of acknowledged combinations of immunosuppressive drugs, previously used in the setting of renal transplant, with predictable results. The combination of sirolimus/cyclosporine/prednisolone has been associated with a strikingly low incidence of acute rejection (6, 7, 8, 17), yet its prolonged use consistently has been associated with significant nephrotoxicity (9, 17, 18) and other adverse effects attributed to a drug-drug interaction (19, 20, 21, 22, 23). On the other hand, the combination of sirolimus/mycophenolate mofetil/prednisolone has been associated with better graft function (24, 25), but its de novo use notoriously has been associated with a high rate of acute rejection

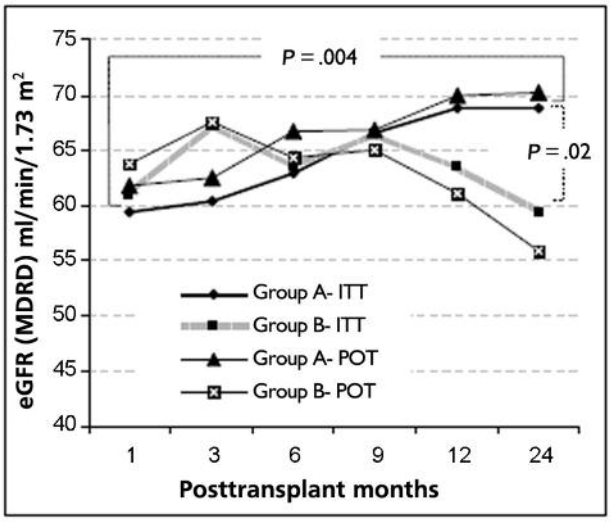

Figure 3. Estimated glomerular filtration rate (eGFR) calculated by the modification of diet in renal disease (MDRD)- formula 4, by intent-to-treat (ITT) and patients-on-therapy (POT) analysis.

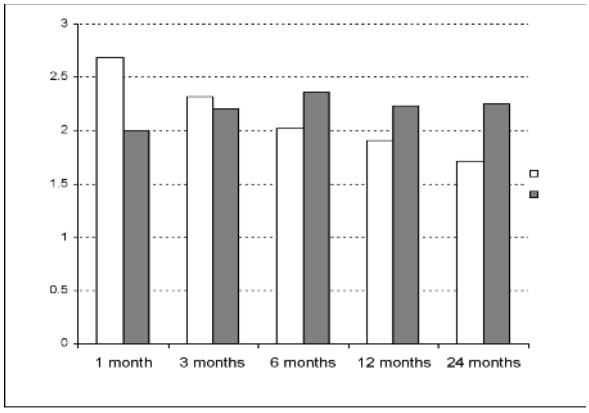

Figure 4. Number of stepwise antihypertensive drugs used to maintain blood pressure at or below 130/80 mm Hg.

that blunts or even reverses its ultimate benefit (26, 27, 28). This is often confounded by the inevitable use of suboptimal dosages of mycophenolate mofetil to overcome gastrointestinal adverse effects (29).

The basic concept in our design is to use the same combinations in a sequence that addresses evolving targets along the course of a transplant, as described above. This is translated into a biphasic protocol, using the early benefits of sirolimus/ cyclosporine/ prednisolone and the late benefits of sirolimus/ mycophenolate mofetil/prednisolone, while avoiding their respective time-related sequelae.

The protocol was randomly applied in 76 de novo transplants (arm A), and the results were compared with those in 37 controls (arm B) who were enrolled in a standard protocol composed of cyclosporine/ mycophenolate mofetil/prednisolone. Since we already had evaluated the latter, we believed that a relatively small number of control subjects would be justified to accelerate building up our experience with the new protocol, hence the 2:1 randomization. Adherence to both protocols (85.5% for arm A and 78.9% for arm B) is comparable to that reported in similar studies (29, 30).

The primary endpoint outcome was a numerical, yet not statistically significant, advantage of The Cairo Kidney Center protocol over the conventional protocol. The 2-year patient survival rate was 95.8%, and the uncensored graft survival rate was 94.6% in arm A, compared with 91.4% and 90.2%, respectively, in arm B. Even the latter compares favorably with US national averages (31), which confirms the safety and, at least, equal efficacy of The Cairo Kidney Center protocol with widely used local and international immunosuppression strategies for kidney transplant.

The Kaplan-Meier survival curves for both arms coincided during phase 1, and then progressively diverged all the way to the end of the study, yet no statistically significant difference was observed. A longer follow-up would be expected to reach statistical significance, at least based on this mathematical trend. In further support of this expectation are the numerically reduced incidence of biopsy-proven acute rejections in phase 1 (10.5% versus 16.5%) and the statistically significant progressive improvement of graft function in phase 2 (up to 70.2 ± 8.0 versus 55.86 ± 7.8 mL/min/1.73 m^2 by 2 years). Since these outcomes are superior to those reported with either combination alone, it must be assumed that sequencing was critical. Phases 1 and 2 in our protocol may be envisaged as being complementary, with the former providing initial protection for the latter to exert its long-term benefits. Additional advantages of the new protocol included better blood pressure control and a significantly higher proportion of successful steroid withdrawal.

However, there also was a downside of each phase. In phase 1, the well-known pharmacokinetic and pharmacodynamic (1) interactions between sirolimus and cyclosporine at the levels of hepatic degradation by CYP450 A3 (20, 21), intestinal net transfer (P-glycoprotein) (19, 22), and target organ concentration (23) and action (1) made adjusting patients' drug dosages fairly difficult. Even with adequate blood levels, there was a statistically significant delay in reaching normal serum creatinine levels in almost half of the patients and transient thrombocytopenia in about one-third. There also was a numerically increased incidence of anemia (39.5%), lymphoceles (14.5%), hematomas (11.8%), and earlier onset of venous thrombosis, which matches with comparable reports (32). Since the pharmacokinetic interactions were probably

avoided by frequent manipulation of the dosages to maintain appropriate blood levels, the pharmacodynamic interactions were blamed for the pathogenesis of many adverse effects. For example, delayed graft function was probably the outcome of persistent renal ischemia induced by cyclosporine (33) and impaired tubular regeneration caused by sirolimus (34). The earlier onset of venous thromboses was apparently the result of increased platelet aggregation induced by calcium influx under the influence of cyclosporine (35) in addition to the endothelial damage associated with down-regulation of vascular endothelial growth factor caused by sirolimus (36).

The negative aspects in phase 2 were mainly related to sirolimus rather than to mycophenolate mofetil, and there was no evidence of a drug-drug interaction. Proteinuria, hypercholesterolemia, and recurrent interstitial pneumonia were the main concerns. While they also were encountered in the control arm, their timing and pathogeneses were different. Proteinuria was encountered in arm A at a lower frequency (36.8%) than that reported in other sirolimus-based protocols (37). While the pathogenetic mechanisms involved are unknown, both increased glomerular leakage (38), probably caused by abnormal podocyte function (39), and impaired tubular reabsorption (40), may be involved. On the other hand, proteinuria in arm B (18.4%) was usually part of chronic allograft nephropathy.

As in many other reports, a tendency toward dyslipidemia was common in this series, with blood cholesterol levels exceeding 7.75 mmol/L (300 mg/dL) in one-third of patients. However, plasma lipid levels were readily controlled with statins. Interestingly, the need for such therapy was almost the same in both arms of this study.

Nonmicrobial pneumonia is another adverse effect of sirolimus, encountered in 3.9% of our patients and reported in other studies with varying frequencies. Little is known about its pathogenesis. However, since its severity in our patients correlated with fluid overload, it might reflect increased pulmonary endothelial permeability.

It may be worthwhile noting that our protocol has apparently offered some protection against several adverse effects that were observed more frequently in the control group. These include, beside the use of less medications to control hypertension, a numerically lower incidence of posttransplant herpes viral infection (15.8%) and diabetes mellitus (3.6%).

In the final analysis, the strengths of the sequential Cairo Kidney Center protocol seem to outweigh its weaknesses. If the relatively high toxicity profile in phase 1 could be overcome without encroaching on efficacy, the protocol should hold promising potential. While this may be achieved partly by careful patient monitoring and physician awareness, replacing the individual components with drugs with more favorable pharmacokinetics (41-45) may prove more practical.

In conclusion, the outcomes of the Cairo Kidney Center protocol concur with the theoretical concept behind a sequential immunosuppression policy in transplant. The Cairo Kidney Center protocol could overcome the main disadvantages of other protocols using the same ingredients at different times following transplant. It provides a valid framework for further trials and potential improvements in choosing drugs within the boundaries of tested classes.

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