Clinical Transplantation

Renal function with cyclosporine C_2 monitoring, enteric-coated mycophenolate sodium and basiliximab: a 12-month randomized trial in renal transplant recipients

Abstract: Background: Cyclosporine exposure, as estimated by the area under the curve (AUC), predicts outcomes in renal transplantation. Cyclosporine concentration at two h post-dose (C_2) has been shown to be the most reliable, single-point surrogate marker for AUC. The objective of this study was to measure renal function beyond month 2 post-transplant using two different C_2 maintenance targets in combination with enteric-coated mycophenolate sodium (EC-MPS), corticosteroids, and basiliximab induction.
Methods: In this open-label, multicenter trial, renal transplant recipients entered one of two randomized groups at day 61 post-transplant: group A (higher-C_2 range) or group B (lower-C_2 range).
Results: Patients (164) were recruited, and 141 patients were entered the randomized groups (group A, n = 66; group B, n = 75). At 12 months, the mean calculated creatinine clearance was significantly greater in group B than in group A (79.2 vs. 71.0 mL/min, $p < 0.05$). Biopsy-proven acute rejection occurred in 14.7% patients in group B and in 24.2% patients in group A (n.s.). During the 12-month trial, 17.7% patients discontinued EC-MPS because of adverse events. Group B (44.0%) had fewer serious adverse events when compared with group A (62.1%; $p = 0.04$). Overall patient and graft survival were 99.4% and 95.7% respectively. Among 99 high-risk patients (i.e., African-American race, previous transplant, PRA >35% or >4 HLA mismatches), mean creatinine clearance at 12 months was 65.6 mL/min and biopsy-proven rejection occurred in 20.2% patients.
Conclusions: Low cyclosporine C_2 levels are associated with improved renal function compared with higher C_2 levels when used in conjunction with EC-MPS, steroids and basiliximab induction. EC-MPS with low cyclosporine C_2 levels, corticosteroids and basiliximab provides excellent renal function with good efficacy even in high-risk patients.

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Over the last decade, the incidence of acute rejection has markedly declined because of the introduction of new immunosuppressive agents but there has been little improvement in long-term graft survival rates (1). The most common causes of late renal allograft loss are chronic allograft nephropathy and death with a functioning graft (2). Previous immunosuppressive strategies have had the primary objective of reducing acute rejection. In contrast, newer immunosuppressive combinations are attempting to reduce chronic allograft nephropathy by discontinuing the use of calcineurin inhibitors (CNIs). Avoidance or withdrawal of CNIs is often associated with an increase in allograft rejection or with other adverse outcomes (3–6). The most promising strategy appears to be a CNI-based maintenance regimen in combination with other immunosuppressive agents to reduce the incidence of chronic allograft nephropathy and to minimize the undesirable side effects of these agents.

Blood cyclosporine (CsA) concentration at two h post-dose (C_2 monitoring) correlates better with exposure to CsA than trough (C_0) monitoring in renal (7), liver (8, 9) and heart (10) transplant patients by acting as a surrogate marker for the area under the curve (AUC). Compared with trough measurements, C_2 levels have a higher correlation coefficient with the AUC (10–13) and are therefore more reflective of overall CsA exposure. Targets for C_2 have been published previously (14, 15), but C_2 targets in combination with mycophenolic acid remain to be defined.

Mycophenolic mofetil (MMF) is used routinely now as part of triple immunosuppression to reduce rejection (16, 17) and to improve graft survival (18). The enteric-coated formulation of mycophenolate sodium (EC-MPS; *myfortic*®, Novartis Pharmaceuticals Corporation, East Hanover, NJ, USA) provides equivalent efficacy to MMF when used in combination with cyclosporine microemulsion (CsA-ME) and has been approved for use by the FDA in this regimen (19, 20).

The study was designed to test the hypothesis that lower CsA C_2 levels would provide equivalent efficacy and safety as higher CsA C_2 levels when used in combination with EC-MPS, corticosteroids and basiliximab induction therapy. Using creatinine clearance as an outcome measure of long-term function, the primary endpoint of the study was renal function at 12 months post-transplant in *de novo* renal transplant patients randomized to one of two C_2 target ranges at day 61 post-transplant. Biopsy-proven acute rejection (BPAR), graft and patient survival, and safety and tolerability of the regimen were secondary end-points. Patients were followed for one yr post-transplantation and over half the patients were followed for an additional year in the extension study.

Materials and methods

Study conduct

Patients were recruited at 14 U.S. transplant centers following approval of the study protocol from the institutional review boards at each center. Written informed consent was obtained from all study participants. The study was conducted in compliance with good clinical practice and the Declaration of Helsinki guidelines.

Study design

The study was an open-label, randomized, 12-month prospective trial of *de novo* renal transplant recipients. All patients were given the same immunosuppressive regimen, composed of CsA-ME, EC-MPS, corticosteroids, and basiliximab induction. Patients were managed based on identical C_2 target ranges for the first two months post-transplant after which the patients were allocated on day 61 to one of two non-overlapping C_2 target ranges

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Table 1. CsA-ME C_2 targets during the core 12-month study and the extension study

	Core study						Extension study
	Days 1–28	Days 29–60	Group	Month 3	Months 4–6	Months 7–12	Months 13–24
Target C_2 level (range, ng/mL)	1700 (1600–1800)	1500 (1400–1600)	Group A: higher-C_2	1300 (1200–1400)	1100 (1000–1200)	900 (800–1000)	700 (600–800)
			Group B: lower-C_2	1100 (1000–1200)	900 (800–1000)	700 (600–800)	700 (600–800)

(Table 1). Randomization to these two C_2 target groups took place on the first day of EC-MPS administration (within 48 h post-transplant) using centrally generated treatment allocation cards carrying randomization numbers on the outside with concealed information about maintenance group allocation. Investigators selected the next numbered card in sequence. Investigators remained blinded until the end of the second month post-transplant. Patients completing the core 12-month study were eligible for inclusion in an open-label one-yr extension study during which the lower-C_2 target range was adopted for all participants.

Study population

Male or female patients 18–70 yr of age receiving a primary or secondary kidney transplant from a deceased, living-unrelated or living-related donor were eligible for inclusion in the study. Patients were excluded if they received a multi-organ transplant or a kidney from a deceased donor over 60 yr of age; if cold ischemia time exceeded 24 h; or if the most recent measurement of panel reactive antibodies (PRA) was >20%. Other exclusion criteria included thrombocytopenia (defined as <75 000/mm^3) with neutropenia (<1500/mm^3), and/or leukopenia (<2500/mm^3), and/or hemoglobin <6 g/dL at baseline.

Immunosuppression

All patients received CsA-ME (Neoral®; Novartis Pharmaceuticals Corporation), EC-MPS (myfortic®; Novartis Pharmaceuticals Corporation), and corticosteroids with basiliximab induction (Simulect®; Novartis AG, Basel, Switzerland).

EC-MPS was initiated within 48 h of transplantation. The standard dose of EC-MPS was 1440 mg/d given in two divided doses. Patients considered by the investigator to be at high-risk for graft rejection (e.g., African-American) could receive doses up to 2160 mg/d. Corticosteroids were administered as per center practice. Basiliximab was administered on days 0 and 4 post-transplant at a dose of 20 mg either by bolus injection or by 30-min venous infusion. CsA-ME was initiated within 24–48 h of transplantation either orally or via nasogastric tube at a dose of 10 mg/kg/d in two divided doses. The dose was then adjusted to achieve a C_2 target level of 1700 ng/mL (range: 1600–1800) by day 5 post-transplant. Identical C_2 targets were employed in all patients until the end of month 2 after which patients entered into either group A (higher-C_2) or group B (lower-C_2) treatment arms (Table 1). C_2 measurements were undertaken at local laboratories using a monoclonal immunoassay or high-performance liquid chromatography performed on whole blood samples taken two h after the morning CsA-ME dose. Sampling time at 2 h ± 15 min post-dosing was considered acceptable. During the open-label extension study (months 13–24 post-transplant), CsA-ME dose was adjusted to the same C_2 target in all patients (600–800 ng/mL) while the EC-MPS dosage remained unchanged and corticosteroids were continued as per center practice.

In cases of suspected acute rejection, the protocol stipulated that a renal biopsy must be performed prior to or within 24 h of initiating anti-rejection therapy. If C_2 levels were below target in patients experiencing rejection, the CsA-ME dose was increased to achieve the C_2 target level if possible. Anti-rejection treatment with antithymocyte globulin or OKT®3 was implemented according to local center practice and Banff classification (21).

Study end-points

The primary end-point of the study was creatinine clearance, as estimated by the Cockcroft–Gault formula (22), in groups A and B at 12 months post-transplantation. Secondary efficacy end-points included the incidence of BPAR and treated acute rejection, graft and patient survival, and discontinuation of either EC-MPS or CsA-ME at two, six, and 12 months post-transplant. In addition, infections, adverse events, serious adverse events, and hematologic abnormalities were assessed.

Evaluation

Study visits were scheduled at screening (i.e., within seven d prior to transplant), baseline or day 1 post-transplant, days 3, 5, and 8, weeks 2 and 4, and months 3, 6, 9, and 12. At each visit, vital signs, body weight, and any adverse events were recorded and laboratory tests (including serum creatinine, hematology, and chemistries) were performed. Serious adverse events were defined as those which were fatal or life-threatening, required or prolonged hospitalization, significantly or permanently disabled or incapacitated the patient, or were regarded as an "important medical event." C_2 levels were recorded at all study visits, at additional time points for drug monitoring, and for all rejection episodes. During the open-label extension study, serum creatinine, hematology, and any adverse events were recorded at quarterly intervals. EC-MPS compliance was also checked at each study visit based on pill count with patients taking >80% of tablets considered to be compliant.

Statistical methods

Efficacy analyses were performed on the intent-to-treat (ITT) population, which comprised all patients who underwent transplantation, received at least one dose of study medication (EC-MPS), and provided at least one efficacy recording. A *post-hoc* analysis was performed to analyze the efficacy outcomes among a subset of patients defined as being at high-risk for rejection because of the presence of one or more of the following risk factors: African-American race, previous transplant, PRA >35% (i.e., patients who were recruited into the trial despite the exclusion criterion for recent PRA >20%) or ≥4 HLA mismatches.

Results

Patient population

One hundred and sixty-four patients were recruited into the study and received at least one dose of study medication. During the first two months post-transplant, 23 patients discontinued study medication because of adverse events (n = 10), unsatisfactory therapeutic effect (n = 6), withdrawal of consent (n = 3), graft loss (n = 2), or an abnormal laboratory value (n = 2), leaving 141 patients to enter either group A (higher-C_2 target, n = 66) or group B (lower-C_2 target, n = 75) on day 61. Demographics and baseline characteristics were similar between the two groups (Table 2) except that group B had a significantly higher

proportion of patients with a PRA > 20% (8.0% vs. 0%, p = 0.03). Delayed graft function (DGF), defined as a requirement for dialysis within the first week post-transplantation, occurred in 26 patients (15.9%) with a similar incidence in group A (n = 7, 10.6%) and group B (n = 10, 13.3%).

After entering the randomized groups, an additional 29 patients discontinued study medication before month 12 (11 in group A, 18 in group B). Of these patients, 19 discontinued because of adverse events (seven in group A, 12 in group B), seven discontinued because of unsatisfactory therapeutic effect (three in group A, four in group B), two lost their grafts (one in each group) and one discontinued because of administrative problems (group A). Thus, 112 patients completed the 12-month core study, of which 102 electively entered into the extension phase (post-transplant months 13–24); reasons for non-entry into the extension were not recorded. One hundred patients completed the extension phase with a mean follow-up of 555 ± 104 d (one patient was lost to follow-up and one patient withdrew consent).

Immunosuppression

Mean CsA dose declined from 8.3 mg/kg/d at day 3 to 5.6 mg/kg/d at the end of month 2 (Fig. 1B). Although the mean C_2 level was within target range from week 2 and remained within the target for each treatment group (Fig. 1C), only a minority of patients were within the C_2 target range at any single timepoint during the first two months post-transplant (7% at day 5 and 20% at month 2), with more patients above than below the target from week 4 onwards. During months 3–12, more patients in group A were within target range (26–40%) than in group B (18–37%).

The mean daily dose of EC-MPS was 1453 mg during the first two months post-transplant (Fig. 1A). This daily dose remained largely unchanged throughout the study with a mean of 1381 mg/d at month 12 and median of 1440 mg at each study visit. Mean EC-MPS dose was 1347 ± 306 mg/d in group A and 1414 ± 381 mg/d in group B (n.s.). Cumulative corticosteroid dose, including anti-rejection treatment, was higher in group A during post-transplant months 3–12, although the differences did not reach statistical significance. During months 3–12, 69% of patients in group B received prednisone when compared with 80% in group A. All but two patients received both doses of basiliximab as per protocol; the other two patients received only the initial dose. During the extension phase, mean dose of EC-MPS was largely unchanged compared with

Variable	All patients (n = 164)	Group B (n = 75)	Group A (n = 66)
Age (yr)			
Mean ± SD	47.5 ± 12.1	49.4 ± 11.6	46.9 ± 11.6
Median (range)	50 (20–75)	52 (20–69)	49 (22–75)
Male sex (%)	100 (61)	48 (64)	42 (64)
Race (%)			
Caucasian	100 (61.0)	45 (60.0)	46 (69.7)
Black	43 (26.2)	20 (26.7)	13 (19.7)
Oriental	7 (4.3)	2 (2.7)	3 (4.5)
Other	14 (8.5)	8 (10.7)	4 (6.1)
Retransplants (%)	6 (3.7)	3 (4.0)	2 (3.0)
Cause of end-stage renal disease (%)			
Glomerulnephritis/glomerular disease	22 (13.4)	13 (17.3)	8 (12.1)
Polycystic disease	15 (9.1)	11 (14.7)	3 (4.5)
Hypertension/nephrosclerosis	42 (25.6)	17 (22.7)	16 (24.2)
Diabetes mellitus	39 (23.8)	20 (26.7)	17 (25.8)
Other/unknown	46 (28.0)	14 (18.7)	22 (33.3)
Peak PRA >20% (%)	6 (3.7)	6 (8.0)[a]	0
Human leukocyte antigen mismatch (%)			
0	19 (11.6)	7 (9.3)	12 (18.2)
1	9 (5.5)	5 (6.7)	3 (4.5)
2	22 (13.4)	10 (13.3)	11 (16.7)
3	43 (26.2)	21 (28.0)	17 (25.8)
≥4	71 (43.3)	32 (42.7)	23 (34.8)
Donor age (yr)	37.7 ± 12.9	39.3 ± 12.3	35.4 ± 14.3
Type of donor (%)			
Cadaveric heart beating	69 (42.1)	27 (36.0)	32 (48.5)
Cadaveric non-heart beating	4 (2.4)	3 (4.0)	1 (1.5)
Living-related	62 (37.8)	31 (41.3)	21 (31.8)
Living-unrelated	29 (17.7)	14 (18.7)	12 (18.2)
Cytomegalovirus status (%)			
D+/R−	24 (14.6)	11 (14.7)	12 (18.2)
D+/R+	62 (37.8)	28 (37.3)	22 (33.3)
Epstein–Barr virus status			
D+/R−	6 (3.7)	2 (2.7)	3 (4.5)
D+/R+	44 (26.8)	19 (25.3)	19 (28.8)

Table 2. Patient and donor demographics and baseline characteristics

Group A, higher-C_2 targets; group B, lower-C_2 targets. Continuous variables are shown as mean ± SD. Numbers in brackets represent percentage of patients. All differences were non-significant unless stated otherwise.
[a]$p = 0.03$ vs. higher-C_2 group.

the core study phase (mean 1320 ± 380 mg/d). At the end of the extension, mean CsA-ME dose was 2.6 mg/kg/d and mean C_2 level was 655 ± 233 ng/mL.

Efficacy

The primary efficacy end-point, mean calculated creatinine clearance at 12 months, was significantly greater in group B than in group A (79.2 vs. 71.0 mL/min, $p < 0.05$; Fig. 2), as would be expected as group B had lower C_2 targets. These values represented a mean increase of 9.6 and 6.6 mL/min, respectively, compared with calculated creatinine clearances at the end of month 2. At 12 months post-transplant, mean serum creatinine was 1.5 mg/dL (132 μmol/L) in group B and 1.6 mg/dL (141 μmol/L) in group A. Mean

creatinine clearance at the end of the extension phase was 75.6 ± 24.2 mL/min; for patients originally randomized to group A, the mean creatinine clearance was 71.5 mL/min, while for patients randomized to group B, it was 79.4 mL/min.

Patient and graft survival at 12 months were 99.4% and 95.7%, respectively. One patient died from sudden cardiac death in group B. There were five graft losses during the first two months post-transplant from infection, renal vein thrombosis, renal infarction, CNI-related toxicity, and hyperacute rejection. One graft in group A was lost because of renal artery thrombosis and one graft in group B never functioned. During the extension phase, there were no deaths or graft losses.

BPAR occurred in 33 of 164 patients (20.1%) in this study with 11 patients (14.7%) in group B and 16 patients (24.2%) in group A and one patient in
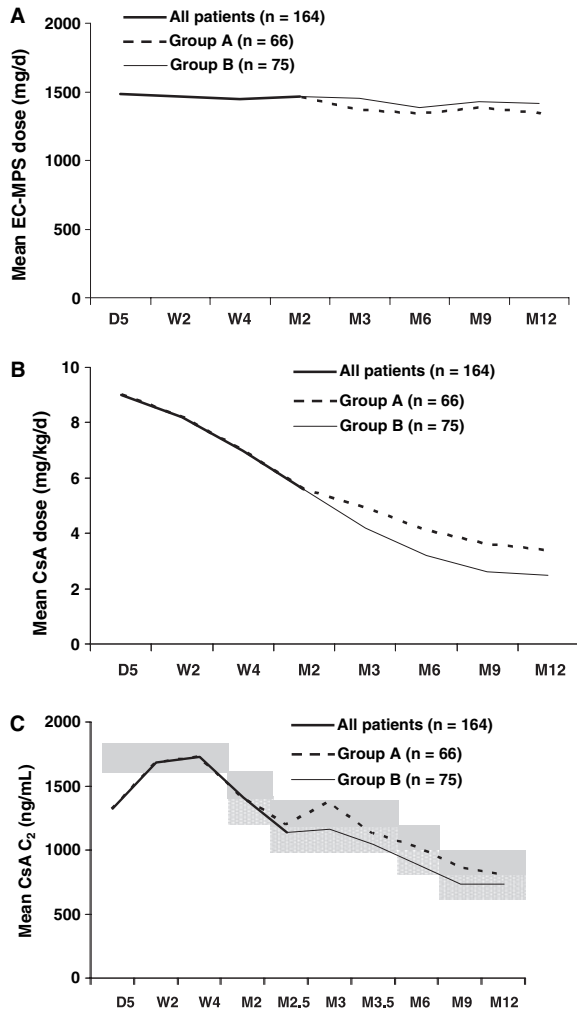
Fig. 1. (A) Mean dose of EC-MPS (B) mean dose of CsA and (C) mean CsA C_2 level for all patients to month 2 and by treatment group during months 3–12. Shaded bars indicate CsA C_2 target range.
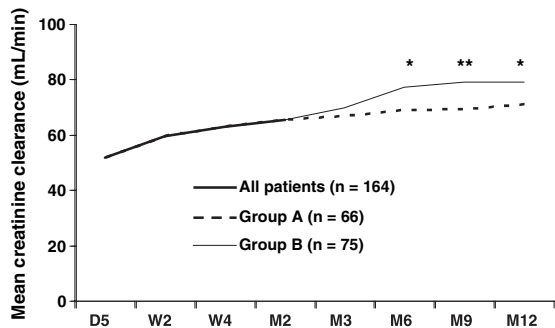

Fig. 2. Calculated creatinine clearance (Cockcroft–Gault formula) (22) for all patients to month 2 and by treatment group for months 3–12. *p < 0.05; **p < 0.03.

each group having two rejection episodes (Table 3); the other six episodes occurred in patients who discontinued prior to entering randomized groups on day 61 (Table 3). In most

patients who experienced BPAR, the most severe grade was mild based on Banff 1997 criteria (21) (20 grade IA, eight grade IB, one grade IIA, three grade IIB, and one grade III) with no difference in severity between the treatment groups. Acute rejection was treated in 32 patients (11 patients in group B, 15 patients in group A, and six patients who discontinued before day 61); no treatment was reported for one case of borderline acute rejection. When BPAR was analyzed according to the time it occurred, nine patients experienced BPAR during month 1 and 3 patients experienced BPAR during month 2. Thirteen patients (19.7%) in group A and nine patients (12.0%) in group B experienced BPAR during months 3–12. One patient experienced BPAR during months 0–2, and again during months 3–12 in group B. Mean C_2 level in these patients was 1205 ± 411 ng/mL during month 1, compared to 1485 ± 388 ng/mL in those patients who remained rejection-free in the same period. Only one rejection episode occurred during the extension study so that the total incidence of BPAR at a mean follow-up of 555 d post-transplant was 20.7%.

Safety

Table 4 summarizes adverse events reported in patients regardless of the relation to study drug. All patients experienced at least one adverse event of which gastrointestinal (GI) disorders and infections were the most common. No significant differences were observed between group A and group B except that group A had significantly more urinary tract infections than group B, and group B had significantly more nausea than group A, which may be related to the higher mean EC-MPS dose in group B (although not statistically significant). The incidence of post-transplant diabetes mellitus was 2.4% (n = 4). Neutropenia occurred in 3.0% of patients (n = 5) and thrombocytopenia in 4.3% of patients (n = 7). Other than non-melanoma skin cancers, only two malignancies were reported, both of which were thyroid neoplasms. Serious adverse events occurred in 87 patients regardless of relation to study drug during the 12-month study including 14 cases of GI disorders. Group B had significantly fewer serious adverse events (44.0%) than group A (62.1% p = 0.04) during months 3–12. Renal failure occurred in three patients during months 0–2, and four patients each in groups A and B (causes were not recorded).

The most frequent reasons for discontinuation were GI effects (n = 6), toxicity related to treatment (n = 4), infection (n = 4), hirsutism (n = 3),

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Variable	All patients (n = 164)	Group A (n = 66)	Group B (n = 75)
Creatinine clearance (mL/min)	67.2	71.0[a]	79.2
Biopsy-proven acute rejection (%)	33 (20.1)	16 (24.2)	11 (14.7)
1 episode	31 (18.9)	15 (22.7)	10 (13.3)
2 episodes	2 (1.2)	1 (1.5)	1 (1.3)
Severity of biopsy-proven acute rejection (%)[b]			
IA	20/33 (60.6)	10/16 (62.5)	8/11 (72.7)
IB	8/33 (24.2)	3/16 (18.8)	2/11 (18.2)
IIA	1/33 (3.0)	1/16 (6.3)	0/11
IIB	3/33 (9.1)	2/16 (12.5)	1/11 (9.1)
III	1/33 (3.0)	0/16	0/11
Treated acute rejection (%)	32 (19.5)	15 (22.7)	11 (14.7)
Death (%)	1 (0.6)	0	1 (1.3)
Graft loss (%)	7 (4.3)	1 (1.5)	1 (1.3)
Biopsy-proven acute rejection, death or graft loss (%)	39 (23.8)	16 (24.2)	13 (17.3)

Table 3. Efficacy outcomes for all patients and by treatment group at month 12 (intent-to-treat population)

Group A, higher-C_2 targets; Group B, lower-C_2 targets. Numbers indicate number of patients; numbers in parentheses indicate percentage of patients. Twenty-three patients discontinued study medication before entering the randomized groups. All differences were non-significant unless stated otherwise.
[a]$p < 0.05$ vs. lower-C_2 group.
[b]Banff 1997 classification (21).

	All patients (n = 164)	Group A (n = 66)	Group B (n = 75)
Adverse events regardless of relation to study drug (%)			
Any adverse event	164 (100)	66 (100)	75 (100)
Blood and lymphatic	70 (42.7)	32 (48.5)	33 (44.0)
Anemia	47 (28.7)	20 (30.3)	23 (30.7)
Leukopenia	25 (15.2)	14 (21.2)	11 (14.7)
Endocrine disorders	30 (18.3)	13 (19.7)	15 (20.0)
Hirsutism	18 (11.0)	7 (10.6)	11 (14.7)
Gastrointestinal disorders	144 (87.8)	57 (86.4)	69 (92.0)
Nausea	77 (47.0)	27 (40.0)	44 (58.7)[a]
Constipation	63 (38.4)	25 (37.9)	30 (40.0)
Diarrhea	51 (31.1)	21 (31.8)	26 (34.7)
Vomiting	51 (31.1)	22 (33.3)	20 (26.7)
Dyspepsia	33 (20.1)	10 (15.2)	18 (24.0)
Abdominal pain	28 (17.1)	8 (12.1)	15 (20.0)
Abdominal distension	19 (11.6)	5 (7.6)	11 (14.7)
Infections	114 (69.5)	51 (77.3)	53 (70.7)
Urinary tract infection	32 (19.5)	21 (31.8)	9 (12.0)[b]
Oral candidiasis	23 (14.0)	9 (13.6)	13 (17.3)
Upper respiratory tract infection	19 (11.6)	6 (9.1)	13 (17.3)
Candidiasis	17 (10.4)	8 (12.1)	9 (12.0)
Adverse events with suspected relation to EC-MPS (%)			
Any adverse event	84 (51.2)	36 (54.5)	39 (52.0)
Blood and lymphatic	31 (18.9)	15 (22.7)	15 (20.0)
Leukopenia	21 (12.8)	12 (18.2)	9 (12.0)
Gastrointestinal disorders	41 (25.0)	19 (27.3)	20 (26.7)
Diarrhea	19 (11.6)	4 (6.1)	5 (6.7)
Infections	17 (10.4)	8 (12.1)	5 (6.7)

Table 4. Adverse events occurring in ≥10% of patients regardless of relation to study drug or with suspected relation to study drug, for all patients and by treatment group during months 0–12 (safety population)

Group A, higher-C_2 targets; Group B, lower-C_2 targets. Numbers in parentheses represent percentage of patients. Twenty-three patients discontinued study medication before entering the randomized groups. All differences were non-significant unless stated otherwise.
[a]$p = 0.043$ vs. higher-C_2 group.
[b]$p = 0.007$ vs. higher-C_2 group.

and leukopenia (n = 2). Over the 12-month period, 79 patients (48.2%) required a reduction in EC-MPS dose because of adverse events.

Adverse events suspected to be related to EC-MPS occurred in 84 patients (51.2%) with 41 patients (25.0%) having GI disorders (Table 4).

Anemia, leukopenia, neutropenia, and thrombocytopenia were reported in six patients (3.7%), 21 patients (12.8%), four patients (2.4%) and two patients (1.2%), respectively, during the first year post-transplant. No significant differences were observed between groups A and B. During the extension phase of this study, no apparent differences were observed in the type, the incidence or the severity of adverse events compared to those reported in the core study. Twenty-eight serious adverse events were reported in the extension phase, of which five were GI in nature. Seventeen patients (17%) experienced adverse events during the extension phase including eight patients with infections (8%), three patients with GI disorders (3%), three patients with anemia (3%), one patient with leukopenia and one patient with neutropenia (1% each). No patients discontinued study medication as a result of adverse events during the extension phase.

High-risk subpopulation

To determine whether favorable outcomes could be achieved in high-risk patients with this immuno-suppressive regimen, a *post-hoc* analysis was performed. Ninety-nine patients (60%) were considered high-risk recipients (see Statistical methods for details). Of these, 71 had >4 HLA mismatches, 43 patients were African-American, six patients were retransplants, and one patient had PRA >35%. Of these, 33 were in group A and 48 were in group B; the remaining 18 patients discontinued before entering the randomized groups, because of adverse events (7), unsatisfactory therapeutic response (4), abnormal laboratory values (2), graft loss (2), death (1), or withdrawal from the study (2). The mean C_2 level was similar in the high-risk population when compared with the total population throughout the study, both overall and for each treatment group. The median dose of EC-MPS in this group was 1440 mg/d, as in the total population, and the mean dose at 12 months was 1428 mg/d, similar to that of the entire cohort. Doses of >1440 mg/d of EC-MPS were used in 24 high-risk patients (24%), mainly during the first six months post-transplant. Steroid doses were comparable in this high-risk cohort and the total population. The incidence of BPAR was the same as in the overall population (n = 20, 20.2%) and there were six graft losses (6.1%) and no deaths. Mean calculated creatinine clearance in the high-risk group at 12 months was 65.6 mL/min. Six of the seven graft losses during the study occurred in patients in the high-risk group.

Discussion

In this randomized, prospective study, we demonstrate that reduced CsA C_2 targets with EC-MPS improves renal function during the first year post-transplant without compromising efficacy. Creatinine clearance was significantly better in the cohort with lower C_2 targets (79 mL/min) compared with the group with higher targets (71 mL/min), and remained stable throughout the study. The difference in mean creatinine clearance (8 mL/min) between the two groups was sufficiently sizeable to be considered clinically significant.

The incidence of rejection in this study was similar to current rates (1) with the majority of rejection episodes being mild and only one graft loss was attributed to acute rejection. Moreover, these results were achieved in a population in which 60% of patients were considered at high risk for rejection. Unexpectedly, there were numerically more episodes of BPAR in the higher-C_2 cohort, a finding that was particularly striking as more patients in group B (48 vs. 33) were high-risk patients, had higher PRA (8% vs. 0%), had more DGF, had more African-Americans and re-transplants and had less prednisone exposure but the differences were not statistically significant. One possible explanation for this increased incidence of BPAR in group A is selection bias; higher C_2 targets are more apt to cause renal dysfunction requiring more renal transplant biopsies. As protocol biopsies were not performed as part of this trial and group B had more high-risk patients, this is the most likely explanation for the differences seen in the incidence of BPAR between the two cohorts.

Despite declining rejection rates in recent years (1), the absence of an improvement in graft survival is in part because of a rise in the proportion of high-risk recipients and donors but newer therapeutic regimens often fail to target this growing population. Our own findings support this point as six of the seven graft losses in this study occurred in the high-risk subgroup. Analysis of the high-risk patients in this study showed that a regimen of EC-MPS with CsA-ME, steroids and basiliximab induction has a favorable therapeutic effect as demonstrated by low rates of rejection and graft loss as compared to historical cohorts. The improved creatinine clearance in the lower-C_2 patients in the overall population was also observed in group B high-risk patients and creatinine levels in the lower-C_2 patients in the overall population were also similar to group B high-risk patient creatinine levels. Only a quarter of the

high-risk patients received EC-MPS above the standard dose of 1440 mg/d at the time of transplant, but the rate of rejection in this group was very similar to that of the total patient cohort.

The safety and tolerability of EC-MPS was similar to that seen in other trials of MPA therapy with CsA-ME and steroids (7, 23, 24), and discontinuations because of adverse events were low. Although a high incidence of GI adverse events was reported (80–90%), GI events suspected to be related to EC-MPS were only seen in a quarter of the patients. Hematological abnormalities suspected to be related to EC-MPS were uncommon. Adverse events were numerically less frequent in group B although the differences were small. Significantly fewer serious adverse events occurred in the lower-C_2 regimen compared to the higher-C_2 group. The regimen was also safe and well-tolerated in the high-risk subpopulation over the 12-month trial; the mean EC-MPS dose was 96.2% of the recommended dose in this group, indicating good tolerability.

During the maintenance phase of this study, the majority of patients were not within the designated C_2 target range, as was observed previously in the MO2ART study (23). Mean C_2 levels in the higher-C_2 group tended to be at the lower end or below the minimum value of the target range. One possible explanation for this observation is that many investigators were unfamiliar with C_2 monitoring at the time of the study and may have been reluctant to dose CsA-ME based solely on C_2 targets. Additionally, clinicians may have felt the higher-C_2 target level to be too high (15). Other considerations include patient variability in performing a C_2 level correctly and a narrow coefficient of variation. These observations in C_2 monitoring are reflected in the patients that had rejection: in those with rejection, month 1 post-transplant C_2 levels were lower than the month 1 post-transplant C_2 levels of those patients without BPAR. In the MO2ART study (23), in which patients were also randomized to one of two C_2 ranges during the maintenance phase, there were no significant differences in renal function between patients assigned to the higher-C_2 or lower-C_2 arms. However, there was considerable overlap in C_2 levels between treatment groups and when the data were re-analyzed according to actual C_2 levels achieved, the improvement in glomerular filtration rate between months 3 and 12 was significantly greater in patients with the lowest C_2 levels.

Although this is a prospective, randomized trial, one obvious weakness is the lack of a control group. Nonetheless, patients treated with EC-MPS and low C_2 targets had good efficacy and tolerability of this regimen despite a high proportion of high-risk patients. Although follow-up for the majority of patients was 555 d, a longer follow-up period is needed to determine if good long-term renal function continues. In addition, another weakness of this study is that the etiology of the few cases of renal failure was not recorded.

In conclusion, the combination of EC-MPS with low CsA C_2 levels, corticosteroids and basiliximab induction provides good efficacy with excellent renal function and a low number of drug-related discontinuations. These results were achieved despite a substantial proportion of high-risk patients in the study population.

Acknowledgements

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Appendix

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