43. Sepp-Lorenzino L, Ruddy M. Challenges and opportunities for local and systemic delivery of siRNA and antisense oligonucleotides. *Clin Pharmacol Ther* 2008; 84: 628–632
44. Amabile N, Guerin AP, Leroyer A *et al.* Circulating endothelial microparticles are associated with vascular dysfunction in patients with end-stage renal failure. *J Am Soc Nephrol* 2005; 16: 3381–3388
45. Faure V, Dou L, Sabatier F *et al.* Elevation of circulating endothelial microparticles in patients with chronic renal failure. *J Thromb Haemost* 2006; 4: 566–573
46. Levey AS, Bosch JP, Lewis JB *et al.* A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. *Ann Int Med* 1999; 130: 461–470
47. Thery C, Amigorena S, Raposo G *et al.* Isolation and characterization of exosomes from cell culture supernatants and biological fluids. *Curr Protoc Cell Biol* 2006; Chapter 3:Unit 3 22
48. Escola JM, Kleijmeer MJ, Stoorvogel W *et al.* Selective enrichment of tetraspan proteins on the internal vesicles of multivesicular endosomes and on exosomes secreted by human B-lymphocytes. *J Biol Chem* 1998; 273: 20121–20127
49. Lawrie CH, Gal S, Dunlop HM *et al.* Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. *Br J Haemat* 2008; 141: 672–675
50. Wang J, Chen J, Chang P *et al.* MicroRNAs in plasma of pancreatic ductal adenocarcinoma patients as novel blood-based biomarkers of disease. *Cancer Prev Res (Phila)* 2009; 2: 807–813
51. Tanaka M, Oikawa K, Takanashi M *et al.* Down-regulation of miR-92 in human plasma is a novel marker for acute leukemia patients. *PLoS One* 2009; 4: e5532
52. Weickmann JL, Olson EM, Glitz DG. Immunologieal assay of pancreatic ribonuclease in serum as an indicator of pancreatic cancer. *Cancer Res* 1984; 44: 1682–1687
53. Karpetsky T, Humphrey R, Levy C. Influence of renal insufficiency on levels of serum ribonuclease in patients with multiple myeloma. *J Natl Cancer Inst* 1977; 58: 875–880
54. Humphrey RL, Karpetsky TP, Neuwelt EA *et al.* Levels of serum ribonuclease as an Indicator of renal insufficiency in patients with leukemia. *Cancer Res* 1977; 37: 2015–2022
55. Connolly J, Herriott R, Gupta S. Ribonuclease in normal and uremie human blood. *Br J Exp Pathol* 1966; 43: 402–408
56. Rabinovitch M, Liverman B, Fausto N. Plasma ribonuclease activity in human uremia. *J Lab Clin Med* 1959; 187: 525–528
57. Rabinovitch M, Dohi S. Increase in serum ribonuclease activity after bilateral nephrectomy. *Am J Physiol* 1956; 187: 525–528
58. Arroyo JD, Chevillet JR, Kroh EM *et al.* Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. *Proc Natl Acad Sci U S A* 2011; 108: 5003–5008
59. Vickers KC, Palmisano BT, Shoucri BM *et al.* MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. *Nat Cell Biol* 2011; 13: 423–433

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Short Communication

FTY720 combined with tacrolimus in *de novo* renal transplantation: 1-year, multicenter, open-label randomized study

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Abstract

Background. FTY720 (fingolimod), a novel immunomodulator, has demonstrated potential for prevention of acute rejection in combination with cyclosporine.

Methods. This study evaluated FTY720 2.5 mg versus mycophenolate mofetil (MMF) in a combination regimen with standard tacrolimus and corticosteroids in *de novo* renal transplant recipients for the composite efficacy within 6 months of transplantation.

Results. Incidence of treated biopsy-proven acute rejection was 22.9% with FTY720 and 18.5% with MMF. Increased incidence of macular oedema, transient decrease in heart rate and low rate of infections were seen in the FTY720 arm.

Conclusion. FTY720 combined with tacrolimus and steroids did not show a significant therapeutic advantage over MMF for the prevention of acute rejection in *de novo* renal transplant recipients.

Keywords: FTY720; mycophenolate mofetil; renal transplantation; sphingosine 1-phosphate receptor; tacrolimus

Introduction

FTY720 (fingolimod) is a novel immunomodulator and representative of sphingosine 1-phosphate receptor

modulators that was developed to address the need for new immunosuppressive drugs that provide adequate efficacy while minimizing drug-related toxicity [1–3]. Phase II studies have demonstrated optimal efficacy and safety of FTY720 up to the doses of 5 mg [2, 4].

This Phase III study compared FTY720 versus mycophenolate mofetil (MMF) in a combination regimen with standard tacrolimus in renal transplant recipients. Recruitment into this study was suspended based on the results of other simultaneous Phase III studies in renal transplantation wherein FTY720 did not show any benefit over the standard of care. Later, this study was discontinued as a part of sponsor decision to terminate the FTY720 clinical development program in transplantation. Despite the under-expanded development, FTY720 (0.5 mg) has been recently approved by the Food and Drug Administration as the primary oral treatment for multiple sclerosis (http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm?fu-seaction=Search.DrugDetails). However, the authors felt that sharing the details of this study would be of value to the transplant community.

Materials and methods

Study design, patients and treatment

This was a 1-year, Phase III multicenter (14 centers across Europe and USA) randomized study followed by a 2-year on or off treatment.

Patients (18–65 years) who received a primary cadaveric or primary non-human leucocyte antigen matched living donor renal transplant and enrolled between April 2004 and April 2005 were randomized (1:1) to receive a pre-transplant priming dose of 5.0 mg FTY720 or 1.0 g MMF. After transplantation, FTY720 and MMF pre-treated patients received a single dose of 2.5 mg FTY720 or 2.0 g MMF divided into two daily doses, both in combination with tacrolimus 0.05 mg/kg/day (target whole blood trough levels, 1 month: 10–15 ng/mL; months 1–12: 5–12 ng/mL) and steroids according to each center's standard practice (prednisone tapered not $\leq$5 mg), respectively.

The study conformed to the ethics and principles of the Declaration of Helsinki, Good Clinical Practice guidelines and local regulatory requirements, including institutional review board approval (ClinicalTrials.gov Identifier: NCT00239876). All patients gave written informed consent.

Assessments and statistical analysis

Primary efficacy outcome was the composite of treated biopsy-proven acute rejection (tBPAR), graft loss, death or premature study discontinuation within 6 months post-transplantation. Safety assessments were done during all or planned visits. Two protocol amendments for evaluating macular edema and bronchodilator reversibility testing were made within 1 year from the start of study.

Since the study was prematurely discontinued, confirmatory statistical analysis for non-inferiority was not done and only descriptive comparisons were made. P-values were generated for composite endpoint and values <0.05 were considered statistically significant.

Results

Of the 103 patients (predominantly male and Caucasian) randomized to FTY720 ($n = 49$) and MMF ($n = 54$), 39 completed the study up to Month 12 and 4 patients entered the extension phase. After a median follow-up of 339 (0–547) days, the premature discontinuations were higher in the FTY720 versus the MMF arm (37 versus 29) mainly due to the decision to stop the study (16 versus 21) and due to adverse events (AEs) (11 versus 2).

FTY720-based immunosuppressive regimen versus MMF was associated with numerically greater incidence of tBPAR (22.9 versus18.5%, P = 0.476; Table 1). Banff Types IIA and IA were the most commonly reported biopsy-proven acute rejection (BPAR) grade for both treatments (Table 2). Neither of the treatment arms reported a significant cluster of cases at either extreme of the Banff scale, although accurate comparison between treatments is difficult due to the differing median exposure [at >450 days all of the FTY720-treated patients discontinued with the study and very few (5.6%) treated with MMF continued]. The Kaplan–Meier estimates for composite endpoint and illustrates consistently poorer results in the FTY720 versus MMF arm throughout the study, as rejections occurred earlier after transplantation in the FTY720 arm (Figure 1). Nevertheless, the proportion of patients discontinuing within 6 months post-transplantation were comparable for the treatment groups (12.5 versus11.1%).

Respective overall incidence of AEs/serious adverse events (SAEs)/drug-related AEs (100/65.3/44.9 versus 98.1/66.7/46.3%) were similar with FTY720 and MMF. Infections AEs/SAEs were more frequent with MMF versus FTY720 [70.4/33.3 versus 51.0/14.3%, respectively (Table 3)]. Gastrointestinal AEs affected more patients on MMF (72.2 versus 61.2%), predominantly diarrhoea—a

Table 1. Incidence of primary efficacy endpoints (intention to treat population)[a]

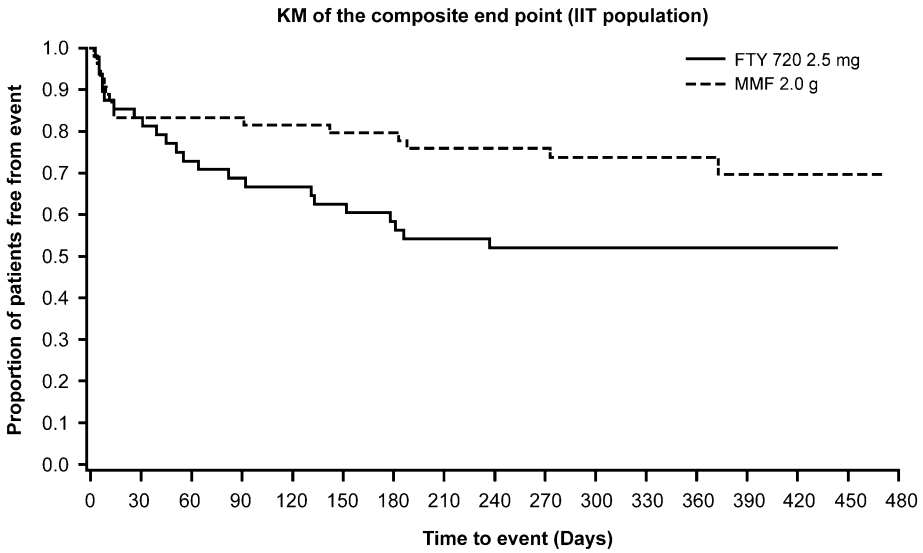
Efficacy endpoints	FTY720 2.5 mg ($n = 48$), *n* (%)	MMF 2.0 g ($n = 54$), *n* (%)	P-value
Any BPAR	13 (27.1)	10 (18.5)	0.348
Graft loss or death	3 (6.3)	3 (5.6)	1.000
Graft loss[b]	1 (2.1)	3 (5.6)	0.620
Death[c]	2 (4.2)	0	0.220
tBPAR	11 (22.9)	10 (18.5)	0.476
Patients discontinuing with 6 months	6 (12.5%)	6 (11.1%)	1.000

[a]Any BPAR refers to all patients who experienced BPAR irrespective of the other events they experienced; all renal allograft biopsies were read and graded by the 1997 Banff classification [5]. In addition, one FTY720-treated patient died on Day 37 after treatment discontinuation due to lymphoproliferative disorder, which was suspected to be related to treatment—a phenomenon observed with other immunosuppressive drugs (azathioprine, ciclosporine; CsA, tacrolimus, MMF, rapamycin, leflunomide) and considered to be a manifestation of systemic immunosuppression [2].
[b]Three patients on MMF experienced graft loss (one each renal artery thrombosis, chronic rejection and hemorrhagic renal infarction), while one patient on FTY720 experienced technical graft loss during surgery and was not part of the ITT population since the patient was not transplanted.
[c]One cardiac failure (Day 229) and one due to chronic liver disease (Day 85) were not suspected to be related to treatment.

Table 2. Banff scores of acute rejection episodes detected by biopsy (intention to treat population)[a]

tBPAR/severity	FTY720 2.5 mg ($n = 48$), *n* (%)	MMF 2.0 g ($n = 54$), *n* (%)
No grading	1 (2.1)	0
Banff Type IA	4 (8.3)	3 (5.6)
Banff Type IB	0	1 (1.9)
Banff Type IIA	5 (10.4)	3 (5.6)
Banff Type IIB	0	2 (3.7)
Banff Type III	0	1 (1.9)
Antibody-mediated rejection	1 (2.1)	0

[a]Incidence refers to the numbers of patients having the event at least once.



Day		≥28	≥60	≥90	≥120	≥150	≥180	≥270	≥360	≥450
FTY720	Continuing, N (%)	46 (95.8)	44 (91.7)	41 (85.4)	40 (83.3)	37 (77.1)	33 (68.8)	24 (50.0)	13 (27.1)	48 (100)
	Discontinuing, N (%)	2 (4.2)	4 (8.3)	7 (14.6)	8 (16.7)	11 (22.9)	15 (31.3)	24 (50.0)	35 (72.9)	0
MMF	Continuing, N (%)	52 (96.3)	52 (96.3)	51 (94.4)	51 (94.4)	49 (90.7)	49 (90.7)	38 (70.4)	22 (40.7)	3 (5.6)
	Discontinuing, N (%)	2 (3.7)	2 (3.7)	3(5.6)	3 (5.6)	5 (9.3)	5 (9.3)	16 (29.6)	32 (59.3)	51 (94.4)

Fig. 1. Kaplan–Meier (KM) plot (ITT population) of composite efficacy endpoint. Although the study was terminated early, the KM analysis for composite endpoint is presented in line with the protocol. Study was prematurely discontinued after 17 months following initiation.

Table 3. Type of infections and serious infections (safety population)

	Type of infection (AEs/SAEs)	
	FTY720 2.5 mg ($n = 49$), n (%)	MMF 2.0 g ($n = 54$), n (%)
Any infection	25 (51.0)/7 (14.3)	38 (70.4)/18 (33.3)
Bacterial	16 (32.7)/2 (4.1)	32 (59.3)/12 (22.2)
Viral	5 (10.2)/3 (6.1)	7 (13.0)/6 (11.1)
Fungal	1 (2.0)/0	0/3 (5.6)
Unknown[a]	11 (22.4)/2 (4.1)	18 (33.3)/1 (1.9)

[a]Infections either recorded on the Adverse Effect Case Report Form or not cultured.

common AE with MMF treatment. Discontinuations due to AEs were higher in the FTY720 versus the MMF arm (22.9 versus 3.7%).

Sixteen patients (32.7%) in the FTY720 arm and nine (16.7%) patients in the MMF arm reported ophthalmic AEs. Macular edema was reported in six patients in the FTY720 arm (12.2%) and five in the MMF arm (9.3%). However, patients with risk factors (diabetes, diabetic retinopathy, retinal vascular disease, past ocular surgery and uveitis) for macular edema were higher in the FTY720 versus the MMF arm (57.1 versus 50.0%). Follow-up evaluations were not available in any of these cases. Three (6.1%) patients on FTY720 and none on MMF had blurring of vision.

With both treatments, a transient decrease in heart rate was reported as an AE (FTY720: $n = 8$, 16.3%; MMF: $n = 1$, 1.9%) and events were not clinically manifested as SAEs. Systolic and diastolic blood pressure was not affected.

Decrease in lymphocytes on Day 1 with both treatments recovered on Day 7 in the MMF treatment arm but remained <30% of the baseline value in the FTY720 arm—as would be expected from its known mechanism of action [6]. No serious or severe pulmonary/respiratory events were reported. Mean creatinine clearance (Cockcroft–Gault) at end-of-treatment [FTY720: 273.5 (27–439) days, $n = 46$; MMF: 322 (67–469) days, $n = 52$] was 62 mL/min in the FTY720 arm and 64 mL/min in the MMF arm. Renal/graft function (serum creatinine and creatinine clearance) was preserved in the patients who were treated for BPAR while continuing study drug (Supplementary table 1). One patient in each treatment arm had malignancy as AE—prostatic carcinoma (MMF) and T-cell lymphoma (FTY720).

Discussion

The confounding effect of study discontinuation precludes firm conclusions about the efficacy and safety of FTY720 in renal transplant recipients; however, the incidence of tBPAR was higher in the FTY720 arm. FTY720-treated patients experienced fewer infections and gastrointestinal complications, whereas MMF-treated patients experienced fewer eye, respiratory and thoracic disorders. Increased incidences of macular edema and a transient decrease in heart rate of renal transplant patients treated with 2.5 mg FTY720 observed in this study has been reported in other transplant studies [7–9].

In conclusion, FTY720 combined with tacrolimus in *de novo* renal transplant recipients demonstrated no significant

therapeutic advantage and no significant advantage in AEs or SAEs compared to MMF-based standard treatment regimen. Renal function post-transplantation was comparable in both arms and FTY720-treated patients and FTY720 was associated with lower infection rates than MMF-treated patients.

Supplementary data

Supplementary table 1 is available online at http://ndt.oxfordjournals.org.

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Conflict of interest statement. A.J.H., D.A. and Y.V. have no competing interest. E.S.W. received research grant, honoraria and travel grants from Novartis. P.P. is an employee of Novartis.

References

1. Halloran PF. Immunosuppressive drugs for kidney transplantation. *N Engl J Med* 2004; 351: 2715–2729
2. Mulgaonkar S, Tedesco H, Oppenheimer F *et al.* FTY720/cyclosporine regimens in de novo renal transplantation: a 1-year dose-finding study. *Am J Transplant* 2006; 6: 1848–1857
3. Ferguson R. FTY720 immunomodulation: optimism for improved transplant regimens. *Transplant Proc* 2004; 36: 549S–553S
4. Tedesco-Silva H, Mourad G, Kahan BD *et al.* FTY720, a novel immunomodulator: efficacy and safety results from the first phase 2A study in de novo renal transplantation. *Transplantation* 2005; 79: 1553–1560
5. Racusen LC, Solez K, Colvin RB *et al.* The Banff 97 working classification of renal allograft pathology. *Kidney Int* 1999; 55: 713–723
6. Brinkmann V, Cyster JG, Hla T. FTY720: sphingosine 1-phosphate receptor-1 in the control of lymphocyte egress and endothelial barrier function. *Am J Transplant* 2004; 4: 1019–1025
7. Tedesco-Silva H, Szakaly P, Shoker A *et al.* FTY720 versus mycophenolate mofetil in de novo renal transplantation: six-month results of a double-blind study. *Transplantation* 2007; 84: 885–892
8. Salvadori M, Budde K, Charpentier B *et al.* FTY720 versus MMF with cyclosporine in de novo renal transplantation: a 1-year, randomized controlled trial in Europe and Australasia. *Am J Transplant* 2006; 6: 2912–2921
9. Tedesco-Silva H, Pescovitz MD, Cibrik D *et al.* Randomized controlled trial of FTY720 versus MMF in de novo renal transplantation. *Transplantation* 2006; 82: 1689–1697

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Preliminary Communication

Apolipoprotein L1 nephropathy risk variants associate with HDL subfraction concentration in African Americans

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Abstract

Background. Coding variants in the apolipoprotein L1 gene (*APOL1*) are strongly associated with non-diabetic nephropathy in African Americans. ApoL1 proteins associate with high-density lipoprotein (HDL) particles in the circulation. Plasma HDL particle subclass concentrations were compared in 73 African Americans based on *APOL1* genotypes to detect differences potentially contributing to renal disease.

Methods. HDL subclass concentrations were measured using nuclear magnetic resonance spectroscopy in African American first-degree relatives of patients with non-diabetic end-stage renal disease. Participants had estimated glomerular filtration rates (GFRs) >