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APPROVAL PACKAGE FOR:

APPLICATION NUMBER

50-791

Microbiology Review(s)

MICROBIOLOGY REVIEW
DIVISION OF SPECIAL PATHOGEN AND IMMUNOLOGIC DRUG PRODUCTS (HFD-590)

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SPONSOR: Novartis Pharmaceuticals Corporation
East Hanover, N.J. 07936

SUBMISSION REVIEWED: Original

DRUG CATEGORY: Immunosuppressive agent

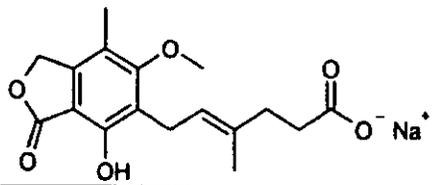
INDICATION: Prophylaxis of organ rejection in patients receiving allogeneic renal transplant.

DOSAGE FORM: Tablets for oral administration (delayed release) 180 mg and 360 mg.

PRODUCT NAMES:

- a. **PROPRIETARY:** Myfortic®.
- b. **NONPROPRIETARY:** Mycophenolate sodium, ERL 080.
- c. **CHEMICAL:** (E)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoic acid sodium salt.

STRUCTURAL FORMULA:



Molecular weight: 342.2
Empirical Formula: C₁₇H₁₉O₆Na

SUPPORTING DOCUMENTS: IND 57,005.

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1. EXECUTIVE SUMMARY:

The sponsor seeks approval of Myfortic® (ERL 080) as prophylaxis for the prevention of organ rejection in individuals receiving allogeneic renal transplant. ERL 080 is formulated as a delayed release, enteric-coated tablet which delivers mycophenolic acid (MPA).

The *in vitro* studies show that ERL 080, like mycophenolate mofetil (MMF) inhibits cell cycle progression by inhibiting DNA synthesis. ERL 080 dose dependently inhibits lymphoproliferation of human and mouse mononuclear cells. The *in vivo* studies suggest that ERL 080 was effective at decreasing leukocyte graft infiltration in rat heterotopic heart transplant recipients and prolonged the survival of rat heart and liver allografts. In some studies, ERL 080 combined with cyclosporine A (CysA) prolonged the survival of rat heterotopic renal graft. The activity of the metabolite was not measured.

2. BACKGROUND:

The subject of this NDA is ERL 080 delayed release enteric coated tablet for the prophylaxis of organ rejection in patients' receiving allogeneic renal transplant. The sponsor has recommended that the drug should be administered either as a 180 or 360 mg dose in combination with CysA. ERL 080 tablets do not release mycophenolate sodium (MPS) under stomach acid conditions (pH < 5), but is highly soluble in neutral conditions as in the intestines.

ERL 080 and MMF are therapeutically equivalent. MMF is the morpholinoethyl ester prodrug of MPA; it is rapidly converted to MPA by ester hydrolysis in the liver (Sweeney *et al.*, 1972).

The sponsor claims that ERL 080 is easily and rapidly absorbed via the gastrointestinal tract. The half life of MPA is reported to be 11.7 ± 3.2 hours. It is converted to the metabolite, mycophenolic acid glucuronide (MPAG) in the liver which is largely excreted into the bile. Both MPAG and MPA are found in the plasma of MPA treated rodents.

3. TRANSPLANTATION IMMUNOBIOLOGY OVERVIEW

Transplantation is a process that transfers cells, tissues, or organs from one site to another. Advances in transplant therapy have not improved long-term survival and the incidence of chronic rejection remains virtually unchanged. The degree of immune response to a graft varies with the type of graft. Co-stimulatory blockers and T-cell depletion may help to improve graft survival. However, graft rejection appears inevitable in some cases. In acute allograft rejection, T cell activation and antibody production occurs in response to foreign graft. The acute rejection phase that follow, may take place within 7-10 days after transplantation. Chronic rejection episode may take months or years to develop following an acute rejection; it involves localized inflammatory reaction called delayed-type-hypersensitivity (DTH) and is often difficult to manage with immunosuppressive agents.

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Allogeneic transplantation involves continuous immunosuppressive therapy to suppress antibody production and cell-mediated immunity. Immunosuppressive agents such as steroids, MMF, CysA, tacrolimus and monoclonal antibody administered separately or in combination show efficacy in improving transplant tolerance. However, in the face of many treatment options, there remains the need for safe and effective immunosuppressive therapy for allograft survival.

4. SUMMARY:

The immunosuppressive activity of ERL 080 was measured *in vitro* and *in vivo* and compared with other immunosuppressive agents including mycophenolate acid.

4.1. Mechanism of Action:

4.1.1. Effect on cell cycle:

The effect of MPA on cell cycle and expression of different surface receptors was measured using human peripheral blood lymphocyte. In addition, the activity on cell cycle progression was also investigated (Heinschink *et al.*, 2000). Peripheral blood from human volunteers was collected and centrifuged in the presence of EDTA over a Ficoll-Paque gradient. Harvested blood cells from identical blood groups were washed in phosphate buffered saline (PBS) and re-suspended in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS) and appropriate antimicrobial agents.

The cells (1.0×10^6) were incubated with 2.0 μg of phytohaemagglutinin (PHA) and increasing concentration of MPA (0-320 ng/ml) at 37 °C for 72 hours. Controls were incubated without the presence of PHA. Following incubation, the samples were prepared for fluorescence activated cell scanner (FACS) analysis to monitor cell cycle progression. Cell cycle differentiation between G0-G1, S-phase and G2-M was monitored by the use of FACS cell cycle software called "Mode-fit." Differential staining with anti-CD3, anti-CD19, and the measurement of lymphocyte activation markers (HLA-DR) and CD25 (interleukin-2-receptor) were also carried out by FACS.

The result of the study in Table 1 shows that PHA stimulation of lymphocytes led to an increase in the number of cells that were at S-phase of the cell cycle, from 0.6 to 24% ($P < 0.001$). DNA synthesis was reduced with the addition of increasing concentration MPA (22.5 to 1.9%). The incubation of lymphocytes with PHA, or PHA + MPA did not appear to have an effect on the levels of HLA-DR cell markers (Table 1). However, the mean fluorescence intensity increased with the addition of PHA and decreased with increasing concentration of MPA.

The incubation of lymphocytes with PHA and MPA led to a decrease in the mean fluorescence intensity of CD25 T cell markers. A slight reduction of T- and B-cell activation markers was observed with increasing concentration of MPA. In summary, MPA appear to affects DNA synthesis, and as such, may interfere with lymphoproliferation. ERL 080 was not used as a comparator in this study.

Table 1

Effect of mycophenolic acid (MPA) on PHA-stimulated cell cycle and expression of HLA-DR and CD25 (= IL-2 receptor) on peripheral human lymphocytes*

	Controls	PHA	PHA + MPA 0.1 µmol/l	PHA + MPA 0.5 µmol/l	PHA + MPA 1 µmol/l
Cell count (million/ml)	1.0±0.17	1.3±0.18°	1.1±0.16 ⁺	0.78±0.13*	0.78±0.1*
Cell cycle					
DNA G0-G1 (%)	94.6±7.9	76.2±9.1	76.8±8.5	81.3±4.9	95.5±3.2 ⁺
DNA S (%)	0.6±0.7	24.5±8.0°	22.5±5.6	15.5±3.1	1.9±0.7*
DNA G2-M (%)	2.4±1.2	3.7±2.6	3.0±2.0	3.0±1.7	1.3±0.6
B-lymphocytes activation markers					
HLA-DR + (%)	93.9±3.3	94.3±6.8	93.2±6.0	90.2±6.3	89.0±7.6
HLA-DR + (MFI)	768±375	2502±947°	2653±990	1589±388 ⁺	1481±420 ⁺
CD25 + (%)	38.8±14.9	89.7±10.3°	87.0±9.9	59.1±11.7 ⁺	63.1±12.9 ⁺
CD25 + (MFI)	37.2±12.0	730±405°	732±288	157±76.7 ⁺	123±46.6*
T-lymphocytes activation markers					
HLA-DR + (%)	6.2±2.9	35.1±15.2°	32.5±13.7	29.5±15.9	27.0±10.9
HLA-DR + (MFI)	57.2±19.4	72.9±26.3	73.1±28.7	68.6±22.8	77.1±23.4
CD25 + (%)	7.3±1.7	80.4±10.5°	75.5±8.9	47.5±13.5*	46.5±9.2*
CD25 + (MFI)	164±94.5	1475±637°	891±448 ⁺	459±12*	284±55.1*

* Significance in comparison to the controls (°P < 0.001) and to PHA (*P < 0.001, ⁺P < 0.05).

4.1.2. Effect on inosine 5'-monophosphate dehydrogenase:

The effect of MPA on inosine 5'-monophosphate dehydrogenase (IMP-DH) activity on human lymphocytes was investigated (Griesmacher *et al.*, 1997). Briefly, lymphocytes were isolated from peripheral blood mononuclear cells and adjusted to yield $2.5 \times 10^8 \pm 1 \times 10^6$ cells/L. Assays were carried out by incubating various concentrations of MPA (0-20 µmol/L) with cell lysates for 30 min at 37°C. Cell lysates with equal amounts of water, human serum albumin (HSA) and, or human plasma were used as controls. IMP-DH activities of control experiments are expressed in Table 1. The data shows the catalytic rate of the enzyme (expressed as nmol/10⁶ cells per hour or nmol/mg/protein per hour) in water, HSA, or plasma. The result of the study shows that IMP-DH activities measured in lymphocytes, without the presence of MPA, was decreased by 50% in HSA and 30% in plasma when compared to water.

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Table 2: Comparison of IMP-DH activities in lymphocytes:

Cell lysate (30 μ L) preincubated with 30 μ L of:	Lymphocytes	
	nmol/ 10^6 cells per hour	nmol/mg protein per hour
Water	2.7 \pm 0.5	267 \pm 36
200 g/L HSA	1.3 \pm 0.2*	129 \pm 19*
Plasma	1.9 \pm 0.6	178 \pm 29

* Statistically significant differences ($P < 0.05$): water vs HSA.

In the presence of 2.5 μ M (0.8 μ g/ml) of MPA, IMP-DH activity was reduced by 59 % in the presence of water. At 10 and 20 μ M (3.2 μ g/ml and 6.4 μ g/ml) of MPA, enzyme activity was reduced by approximately 85 % in water, and 70% in both HSA and plasma (Table 3). Seventy percent enzyme inhibition was observed at 10 and 20 μ M (3.2 μ g/ml and 6.4 μ g/ml) of MPA. In the presence of increasing concentration of MPS, dose dependent inhibition was observed, however, complete enzyme inhibition was not observed under the experimental conditions tested.

Table 3: MPA-induced inhibition of IMP-DH activity measured in lymphocytes in the presence of either water, HAS, or plasma.

Cell lysate (30 μ L) preincubated with 30 μ L of:	MPA, μ mol/L				
	0	2.5	5.0	10.0	20.0
Water					
IMP-DH, % of control	100	41 \pm 5	30 \pm 8	15 \pm 6	14 \pm 3
200 g/L HSA					
IMP-DH, % of control	100	68 \pm 11	49 \pm 13*	27 \pm 4*	22 \pm 5*
Plasma					
IMP-DH, % of control	100	71 \pm 16	46 \pm 11*	31 \pm 8*	28 \pm 4*

* Statistically significant differences ($P < 0.05$): water vs HSA or water vs plasma.

4.2. Activity *in vitro*:

4.2.1. Effect on lymphoproliferation:

The immunosuppressive activity of ERL 080, MMF and CysA was measured by two-way mixed lymphocyte reaction (MLR) using human peripheral blood mononuclear cells (PBMC) and mouse splenocytes (Report # 98-02542). Murine splenocytes from BALB/C and CBA/2 mice were co-cultured in equal proportion in the presence of appropriate concentrations of various immunosuppressants for 4 days. The activities of the immunosuppressants were measured by the incorporation of 3 H-thymidine. The time of 3 H-thymidine addition and the duration of incubation was not specified.

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MLR using human PBMC from two donors were co-cultured for three days in the presence or absence of immunosuppressants. The activation of PMBC was induced by the addition of 1 µg of phytohaemagglutinin (PHA). However, the exact time of PHA addition and incubation period was not stated. The growth factor independent T cell lymphoma Jurkat cells (grown in RPMI medium) were used as a cytotoxicity control in the evaluation of the immunosuppressants tested. The result in Table 4 shows that ERL 080, like MMF was equally effective at inhibiting mouse and human mononuclear cells. In summary, ERL 080 appears to inhibit the proliferative responses to antigenic stimulation in mixed lymphocyte reactions in human and mouse lymphocytes.

Table 4: Quantitative comparison of immunosuppressants in mouse mixed lymphocyte reaction.

	Mouse MLR (IC ₅₀ µM)	Human MLR (IC ₅₀ µM)	PHA (IC ₅₀ µM)	Jurkat (IC ₅₀ µM)
<i>Immunosuppressants</i>				
ERL 080 Na	0.018 ± 0.009	0.025 ± 0.008	0.074 ± 0.017	0.077 ± 0.043
MMF (Sigma)	0.015 ± 0.004	0.014 ± 0.006	0.075 ± 0.006	0.047 ± 0.043
MMF (Cell Cept)	0.019 ± 0.006	0.022 ± 0.009	0.115 ± 0.057	0.17 ± 0.018
CysA	0.0073 ± 0.001	0.0017 ± 0.001	0.122 ± 0.09	> 1.0

4.2.2 Effect on B cell responses to T-independent antigens:

The effect of ERL 080, MMF, MPA and CysA on mouse B cell responses to T-independent (TI) antigens was investigated using spleen cells from C57BL/6 *nu/nu* and C57BL/6 mice (Report # -98-02542). Splenocytes were prepared in IMDM-ALT medium containing 10% FCS, in the presence or absence of different concentration of test compounds [ERL 080, MMF, MPA and CysA], TI antigens [TNP-LPS (trinitrophenyl lipopolysaccharide)] (Type 1), or DAGG-Ficoll [(B-2, 4-dinitrophenyl-b-Ala-Gly-Gly-AECM-Ficoll) (Type 2)] and incubated for 4 to 5 days. Following incubation, cells were washed, pooled and diluted 4-5 fold in phosphate buffered saline (PBS). The responses to TI antigens were assessed by enzyme linked immunosorbent assay (ELISA) and the mean of 4 experiments are depicted in Table 5. Surprisingly, ERL 080 demonstrated a two-fold higher IC₅₀ value compared to MMF. ERL 080, MMF, and MPA appear to inhibit B cell responses to TNP-LPS and DAGG-Ficoll. CysA was only effective at inhibiting the responses to DAGG-Ficoll with IC₅₀ values significantly lower than that of the other immunosuppressants tested. CysA had an IC₅₀ of >1 µm against Jurkat cells. Altogether, ERL 080, MPA and MMF appear to interfere with B cell proliferation in response to T cell independent antigens.

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Table 5: IC₅₀ values of ERL 080, MMF, MPA and CsA for the inhibition of the T cell independent B cell responses (TI).

<i>Substance</i>	Type 1	Type 2
	IC ₅₀ (μM)	IC ₅₀ (μM)
ERL 080Na	0.097 ± 0.02	0.02 ± 0.015
MPA (Sigma)	0.077 ± 0.04	0.08 ± 0.017
MMF (CellCept ^r)	0.043 ± 0.005	0.03 ± 0.026
CsA	> 1	0.0047 ± 0.0015

4.3. Activity *in vivo*:**4.3.1. Effect on renal graft survival:**

The effect of ERL 080 and MMF in prolonging the survival of an orthotopic kidney transplant was evaluated in rats (Report # -2001-00138). Renal transplants were performed using male DA rats or male BN rats as donor and male Lewis as recipient. ERL 080, MMF (5, 10, 20 mg/kg/day) and CysA (2.5, 5 and 7.5 mg/kg/day) were administered orally, on a daily basis. Graft inspection, for signs of rejection, was performed 7 days following surgery. Animals without signs of acute macroscopic rejection were examined daily for changes in renal function. Experiments were terminated at 14 weeks irrespective of graft function.

Allograft histology of grafts from BN to Lewis rats in the control group displayed moderate cellular rejection 7 days following transplantation (Table 6). ERL 080 and MMF at 5 mg/kg/day was not effective at prolonging graft survival beyond 7 days. At the 10 mg/kg/day dose, long term graft survival without histological signs of rejection was achieved in two out of three cases. ERL 080 and MMF at 20 mg/kg/day were not tolerated. Animals were euthanized between 12 to 14 days following transplantation due do severe weight loss, and leukocytopenia (a reduction of the number of leukocytes). CysA (5 and 7 mg/kg/day) was more effective than ERL 080 and MMF at prolonging graft survival (≥ 98 days) in the animals tested.

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Table 6: Effect of ERL 080, MMF with or without CysA in BN to Lewis kidney transplantation.

ERL / MMF (mg/kg/d)		CsA (mg/kg/d)	Survival (day)	Histology
Control			7,7,7	Cellular, moderate
		2.5	7,7,7	Cellular, moderate-severe
		5	≥98 (n=3)	No rejection
		7.5	≥98 (n=3)	No rejection
ERL	5		7,7,7	Cellular, severe
	10		14,	Cellular, moderate
	20		≥98, ≥98	No rejection
			12*, 12*, 14*	Cellular, moderate
ERL	5	2	7,7,	Cellular, moderate-severe
			≥98	No rejection
MMF	5		7,7,7	Cellular, moderate-severe
	10		13,13,13	Cellular, moderate
	20		7,11,	Cellular, moderate
			≥43*	Cellular, marginal
MMF	2.5	1	7,7	Cellular, slight-moderate
		2	7,7,7	Cellular, moderate-severe
MMF	5	1	7,7,7	Cellular, moderate-severe
		2	7,7,17	Cellular, moderate-severe
MMF	10	2	7,12,	Cellular, moderate-severe
			≥98	No rejection

Survival with a functioning graft is indicated by ≥.

* Animal was terminated because of drug intolerance.

In the DA to Lewis treatment group, only ERL 080 (5, 10, 20 mg/kg/day) was compared with CysA (2.5, 5 mg/kg/day) at prolonging kidney transplantation. The untreated control showed signs of severe cellular rejection. In the ERL 080 treatment group, the sponsor states that a therapeutic window could not be obtained. Moreover, animals receiving 5 and 10 mg/kg/day ERL 080 showed signs of rejection. Animals who received ERL 080 at 20 mg/kg/day also showed signs of severe weight loss necessitating termination at day 13-14 following transplantation (Table 7). In an attempt to alleviate the side effect of high doses of MMF, one group of animals received MMF at 20 mg/kg/day followed by a dose reduction to 10 mg/kg/day after day 7. Animals in this group showed signs of rejection and were euthanized after 10 days following transplantation.

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Table 7: Effect of ERL 080 or MMF (Cell cept), with or without CysA in DA to Lewis kidney transplantation.

ERL (mg/kg/d)	CsA (mg/kg/d)	Survival (day)	Histology
	2.5	7,7,7	Cellular, severe
	5	≥35 ^a , ≥98, ≥98	No rejection
5		7,7,7	Cellular, severe
10		7,7,7	Cellular, severe
20		≥13 ^b , ≥14 ^b , ≥14 ^b	Cellular, no-marginal
20, 10 since day 7		10 (n=3)	Cellular, marginal-moderate
5	2.5	7,7,7	Cellular, moderate-severe
10	1.25	7,14, ≥98	Cellular, moderate No rejection
	2.5	14,14,14,16 ≥98, ≥98	Cellular, moderate No rejection
20, 10 since day 7	2.5	≥98 (n=3)	No rejection

Survival with a functioning graft is indicated by ≥.

^a Animal was terminated because of unexplained weight loss.

^b Animal was terminated because of drug intolerance.

4.3.2. Effect on heart graft survival:

The activity of ERL 080 (MPS) compared with FTY720 (a novel immuno modulator that sequesters T and B cells from peripheral tissues and blood to lymph nodes and Peyer's patches) at prolonging the survival of heterotopic heart transplants was investigated in rats (Matsumoto *et al.*, 2002). In this model DA rats were used as donor and Lewis rats as recipients. Heterotopic heart transplant was performed by removing the donor organ by ligation of all vessels except the ascending aorta and the right pulmonary artery. These vessels were then rejoined end to side to the recipient's abdominal aorta and inferior vena cava. The heart usually started beating within 1 minute of releasing the clamps. Rats were subjected to placebo, ERL 080 (3, 10 or 30 mg/kg per day) or FTY720 (0.03 or 0.1 mg/kg/day). Monotherapy was administered via osmotic pumps on the day of transplantation and continued until rejection or for up to 28 days. Body weight was recorded before surgery then weekly, and on the day of rejection. Graft function was assessed daily by palpation for ventricular contraction and those possessing a barely palpable impulse or complete cessation of ventricular motion were considered rejected.

The median survival time was 6 days for untreated placebo control rats. Severe acute rejection was also observed in the placebo group. Animals treated with 3, 10 and 30 mg/kg/day of ERL 080 had a median survival time of 6, 14.5 and more than 56 days, respectively (Figure 1). Those treated with FTY720 (0.03 and 0.1 mg/kg/day) had a median survival time of 7 and 8 days, respectively (Table 8). Side effects of weight lost, diarrhea and lymphopenia was observed at the highest ERL 080 dose

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(Table 8). Severe myocardial destruction, hemorrhage, edema, and intravascular mononuclear cell adherence were observed in animals that rejected heart grafts. Animals that received ERL 080 (3 mg/kg/day) showed borderline acute rejection and severe acute rejection. Grafts obtained from animals receiving ERL 080 at a higher dose (10 mg/kg/day) showed similar histological changes with diffuse borderline acute rejection to multi-focal moderate acute rejection. In the 30 mg/kg/day group, two of five grafts showed signs of diffuse borderline acute rejection while three showed signs of mild acute rejection to moderate acute rejection. The study shows ERL 080 to be effective in prolonging the survival of heart allograft. No approved drug was used as a comparator.

Figure 1: Survival of heterotopic rat cardiac allograft.

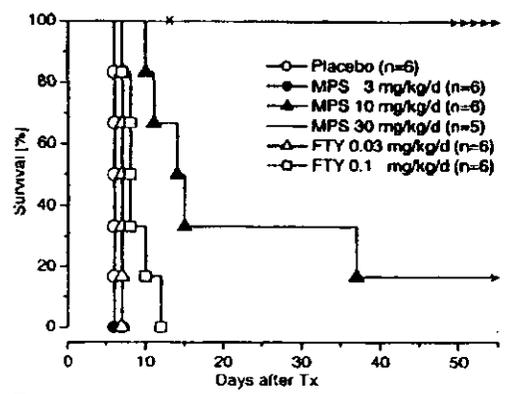


Table 8: Effects of mycophenolate sodium and FTY720 monotherapy on heart transplant.

MPS (mg/kg/day)	FTY720 (mg/kg/day)	MST ^{a,c} (days)	Baseline ^d (g)	n	1 week changes from baseline (g)	n	2 weeks changes from baseline (g)	n	4 weeks changes from baseline (g)	n	Comments
0	0	6.0	257.8±0.5	6	7.0±1.7	6					Allograft
3	0	6.0	251.2±12.8	6	9.3±1.7	6					Allograft
10	0	14.5	237.8±11.0	6	16.9±2.9	6	34.0±6.0	4	96.0, 60.0	2	Allograft
30	0	>56.0	240.2±4.3	6	-9.2±6.0	6	2.5±14.6	6	47.4±3.1	5	Allograft
0	0.03	7.0	250.2±2.8	6	7.7±0.6	6					Allograft
0	0.1	8.0	250.2±2.3	6	9.3±3.1	6					Allograft

^a Data are presented as mean±SEM.

^b Graft survival is shown as median survival time (MST).

^c Survival with beating graft is indicated by >.

^d Body weight before transplantation.

^e P<0.05 vs. placebo group (Dunnett's test).

^f P<0.05 vs. the corresponding FTY720 monotherapy group (Bonferroni's test).

^g Placebo treated isograft group published previously (17).

^h Additional group of three animals with isograft transplantations and placebo treatment.

4.3.3. Effect on liver graft survival

The effect of MMF on liver graft was investigated in rats (Huang *et al.*, 2003). There were two allogeneic models. In the first model orthotopic liver transplantation was performed using Piebald Virol Glaxo (PVG) rat livers as donors and DA as recipients. This combination spontaneously tolerated the liver (TOL). The second model involved the transplantation of PVG rat livers into Lewis recipients which resulted in the rejection of the livers (REJ). Also included was an isograft control (DA donor to DA recipient). MMF was administered subcutaneously either on the day of transplantation, and up to 4 days (0-4 days; early treatment) or delayed administration (3-7 days) following transplantation. In separate experiments recipient rats were sacrificed for tissue sample collection on days 1, 3, 5, and 7 following transplantation.

The result of the study shows that DA to DA rat transplant and allogeneic liver transplants in the TOL strain combination showed an average survival time of greater than 100 days with the exception of one animal which died on day 82 (Table 9). Histological analysis revealed no evidence of rejection in that animal. In the allogeneic model, the mean survival time of MMF treated animals was 85 days for those in the "early" treatment arm and 78 days for those in the "delayed" treatment arm. These results were not significantly different from those in the untreated TOL recipients. Although liver histology of animals that survived for less than 100 days showed signs of chronic biliary problems as opposed to acute or chronic rejection, the liver from one animal that survived for 52 days showed signs of rejection.

In the PVG to Lewis (REJ) group, receiving no treatment, a survival time of 9-16 days was observed. In the MMF treatment arm, five of six animals that received early treatment had a mean survival rate of more than 100 days. However, one animal died on day 20 with symptoms of morbidity and weight loss and no sign of rejection was observed. MMF proved less effective at prolonging graft survival when administered at 3-7 days and signs of rejection were observed in 5 of 6 recipients (Table 9). ERL 080 was not used in the study.

Table 9
Effect of mycophenolate mofetil on liver graft survival in TOL and REJ strain combinations

Donor-recipient	Treatment	n	Graft survival (d)	MST (d)	P value
DA-DA	None	6	>100×6	>100	—
PVG-DA (TOL)	None	6	82*, >100×5	>100	—
	MMF days 0-4	6	58*×2, 69*, >100×3	85	0.19
	MMF days 3-7	6	52, 70*, 72*, 83*, >100×2	78	0.08
PVG-LEW (REJ)	None	10	9, 10, 11, 12, 13×2, 14×3, 16	13	—
	MMF days 0-4	6	20, >100×5	>100	0.0002
	MMF days 3-7	6	12, 13, 14, 16, 21, >100×1	15	0.10

* Pathology analysis showed bile duct proliferation and little evidence of rejection.

The results for syngeneic liver transplants and untreated TOL and REJ liver transplants have been published (8). P value compares treated with untreated groups.

MST, median survival time; MMF, mycophenolate mofetil; DA, Dark Agouti; PVG, Piebald Virol Glaxo; LEW, Lewis; TOL, tolerant (PVG→DA); REJ, rejecting (PVG→LEW).

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4.3.4. Effect of on intimal wall thickening:

The activity of ERL 080 and MMF on media (middle layer of the artery wall) and intimal wall thickening was evaluated in the rat aorta transplant model (Report # 98-00645). Transplantation procedures were done using DA rats as donors and Lewis rats as recipients. Under the appropriate conditions, a piece of abdominal aorta, 1 cm in length, was removed and transplanted into the abdominal aorta of the recipient animal. ERL 080 (10 and 20 mg/kg/day) or MMF (5, 10, and 20 mg/kg/day) were administered orally during the experimental period of 8 weeks. However, the exact time of drug administration was not mentioned in the protocol.

In placebo control animals, the mean media size of the aorta allograft was approximately 62 μm with signs of media necrosis in histology specimens. The average intimal size was reported to be approximately 58 μm . There were no significant differences in media thickening in animals treated with ERL 080 or MMF at a dose of 10 mg/kg/day. However, animals treated with ERL 080 at 5 and 20 mg/kg/day showed a slight increase in media thickening and a reduction in intimal thickening (Table 10). A significant reduction in intimal thickening was observed with ERL 080 at 20 mg/kg/day. All treatment groups showed an increase in body weight.

Table 10: Effect of ERL and MMF on aorta parameters:

	Own aorta Media (μm)	Grafted aorta				
		Media+ Intima (μm)	Media (μm)	Intima (μm)	Media (%)	Intima (%)
Placebo	104 \pm 12	120 \pm 42	62 \pm 9	58 \pm 37	56 \pm 17	44 \pm 17
ERL 5 mg/kg/d	106 \pm 11	114 \pm 37	71 \pm 14**	43 \pm 33	67 \pm 17*	33 \pm 17*
ERL 10 mg/kg/d	102 \pm 14	114 \pm 39	67 \pm 13	48 \pm 35	63 \pm 18	37 \pm 18
ERL 20 mg/kg/d	106 \pm 18	90 \pm 15**	77 \pm 12**	13 \pm 11**	87 \pm 11**	13 \pm 11**
MMF 10 mg/kg/d	103 \pm 11	104 \pm 47	64 \pm 12	39 \pm 43*	70 \pm 22*	30 \pm 22*
MMF 20 mg/kg/d	96 \pm 7*	100 \pm 30*	74 \pm 13**	26 \pm 23***	77 \pm 16***	23 \pm 16***

Data presented are mean values (each value is the average of 5 measurements in one longitudinal and one transversal section) \pm SD. Statistical significance of difference from placebo-treated control: * $p < 0.05$, ** $p < 0.01$. Statistical significance of difference between ERL and MMF at the same dose: + $p < 0.05$, ++ $p < 0.01$.

4.3.5. Effect on pancreatic islet graft:

The ability of MMF and $1\alpha, 25\text{-Dihydroxyvitamin D}_3$, (an antiproliferative agent known to induce transplantation tolerance), was investigated in rats (Gregori *et al.*, 2001). In this model, pancreatic islet from C56BL/6 mice was transplanted (350/mouse) under the kidney capsule of BALB/c recipient mice that were rendered diabetic by a single intravenous injection of streptozotocin (205 mg/kg). Graft survival was monitored by palpation. Islet graft rejection was defined by obtaining

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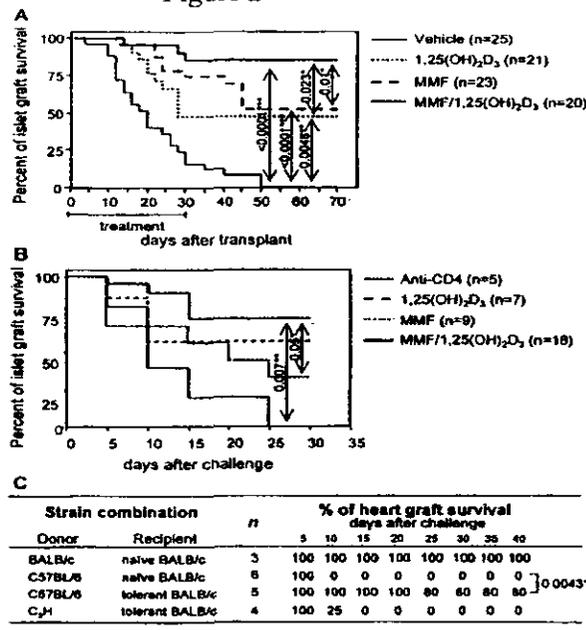
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two successive non-fasting blood glucose samples with levels of ≥ 200 mg/dl. Recipients mice were administered MMF (100 mg/kg) and or $1\alpha,25$ -Dihydroxyvitamin D_3 ($5 \mu\text{g/kg}$ three times per week) orally from the day before surgery (-1) up to day 30. The mean rejection time in vehicle-treated recipients was 23 ± 3 days. Animals treated with MMF prolonged islet graft survival in approximately 50% of the recipients. However, animals treated with both drugs demonstrated long-term islet graft acceptance of greater than 70 days (Figure 2A). ERL 080 and MPA were not tested in the study.

In another experiment, BALB/c recipients showing long-term (>70 days) allograft acceptance were challenged with intra-peritoneal (i.p.) injection of 10^6 donor type-BALB/c spleen cells (Figure 2B). Recipient mice treated with anti-CD4 (10 mg/kg/day) monoclonal antibodies (mAb) from day -1, 0, 1, and 2 demonstrated a mean survival time of 14 ± 2.4 days. Although the animals accommodated the islet transplant graft, they were not tolerant since they rejected the graft following challenge. Treatment with MMF and $1\alpha, 25$ -Dihydroxyvitamin D_3 resulted in resistance to rejection upon challenge in 73% of the allografts (Figure 2B). BALB/c mice demonstrating islet graft function for 4 weeks after challenge were transplanted, 100 days after the initial islet graft, with a vasuclarized heart from C57BL/6 or C_3H donors. The result demonstrated short term tolerance. BALB/c mice rejected C57BL/6 heart grafts in 10 days, and only one tolerant mouse of five rejected the graft in 25 days following transplant. Tolerant BALB/c mice rejected C_3H in 10-12 days (Figure 2C).

Figure 2

Tolerance induction by combined treatment with MMF and $1,25(\text{OH})_2\text{D}_3$. A. Long-term islet allograft survival induced by MMF and $1,25(\text{OH})_2\text{D}_3$ treatment. BALB/c mice were rendered diabetic by a single injection of streptozotocin (250 mg/kg i.v.) and transplanted with 350 B6 islets. Recipient mice were treated with MMF (100 mg/kg p.o. daily) and/or $1,25(\text{OH})_2\text{D}_3$ ($5 \mu\text{g/kg}$ p.o. three times per week) from day -1 to day 30. The function of islet allografts was monitored two times per week by blood glucose measurement. Values of p were determined by Fisher's exact test. B. Percent islet graft survival after B6 spleen cell challenge. Recipient mice were treated with MMF (100 mg/kg p.o. daily) and/or $1,25(\text{OH})_2\text{D}_3$ ($5 \mu\text{g/kg}$ p.o. three times per week) from day -1 to day 30. Alternatively, recipient mice were treated at days -1, 0, 1, and 2 with anti-CD4 mAb i.p. (10 mg/kg/day). Mice with functioning islet grafts 70 days after transplantation were injected i.p. with 10^6 B6 spleen cells. The function of islet allografts was monitored two times per week by blood glucose measurement. Values of p were determined by Fisher's exact test. C. Vasuclarized heart graft survival. Mice still normoglycemic 4 wk after spleen cell challenge were transplanted with B6 (donor-type) or C_3H (third party) vasuclarized heart grafts. As controls, naive BALB/c mice were transplanted with B6 (allograft) hearts. Heart function was monitored daily by palpation, and islet graft function was monitored two times per week by blood glucose measurement. The p value was calculated by Fisher's exact test.



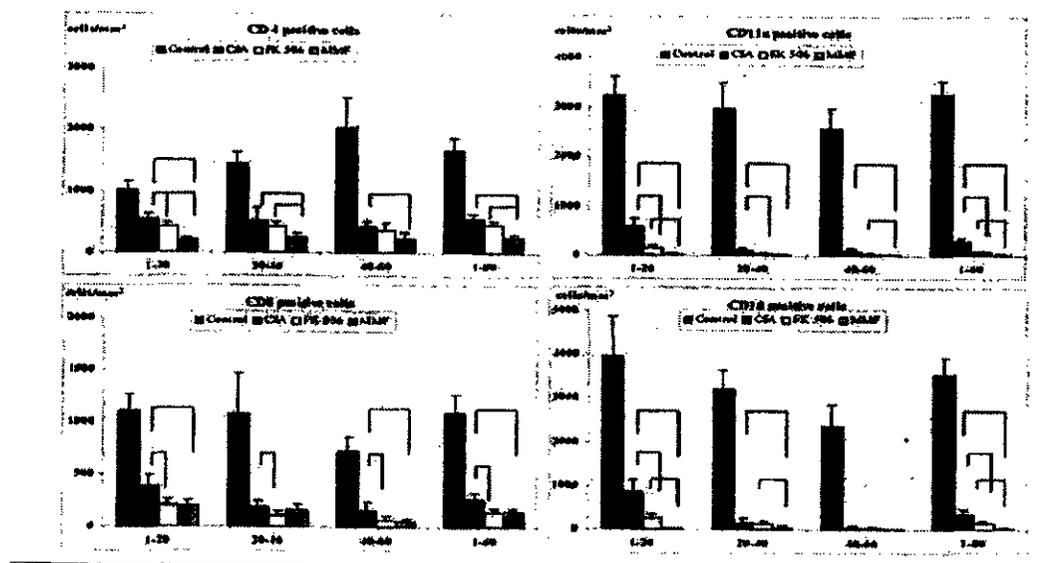
4.4. Effect on immune responses:

4.4.1. Effect on Leukocyte Graft infiltration:

The effect of MMF, CysA and FK506 on graft-infiltrating leukocytes after heterotopic heart transplantation was investigated in rats (Richter *et al.*, 2003). Hearts from Lewis rats were transplanted onto the abdominal cavity of Fisher rats. Graft viability was evaluated by daily palpation and function assessed on a scale of 0-4, with 4 being a normal heartbeat and 0 being the absence of a beat and rejection was concluded when the heart cease to function. Three hundred and forty animals were transplanted and following transplantation, animals were randomly placed in 4 study groups (control, CysA, FK506 and MMF). Three to four animals were sacrificed at intervals of 1-4 days up to day 60. All graft survived until the day of killing and immunohistochemistry was performed using monoclonal antibodies against different leukocytes (CD4, CD8, CD11a, and CD18).

The untreated controls had a continuous deterioration of palpable heart beat. An increase in leukocytes infiltrating in the perivascular space (PVS) of intra-myocardial arteries, throughout the 60 day study period (Figure 3), with an average cell accumulation of $1,674 \pm 192$ cells/mm² was also observed. All immunosuppressive drugs were shown to reduce CD4-positive cells infiltration when compared with controls. MMF treated animals demonstrated a reduction in the levels of cell infiltration when compared to both CysA and FK506 ($p < 0.05$). There was a decrease in the levels of CD-8, CD-11a and CD-18-positive cell accumulation with MMF being more superior to CysA and FK 506. ERL 080 and MPA were not included in the study.

Figure 3: Accumulation of leukocytes in the PVS of intramyocardial arteries within different time intervals and throughout the entire study period.



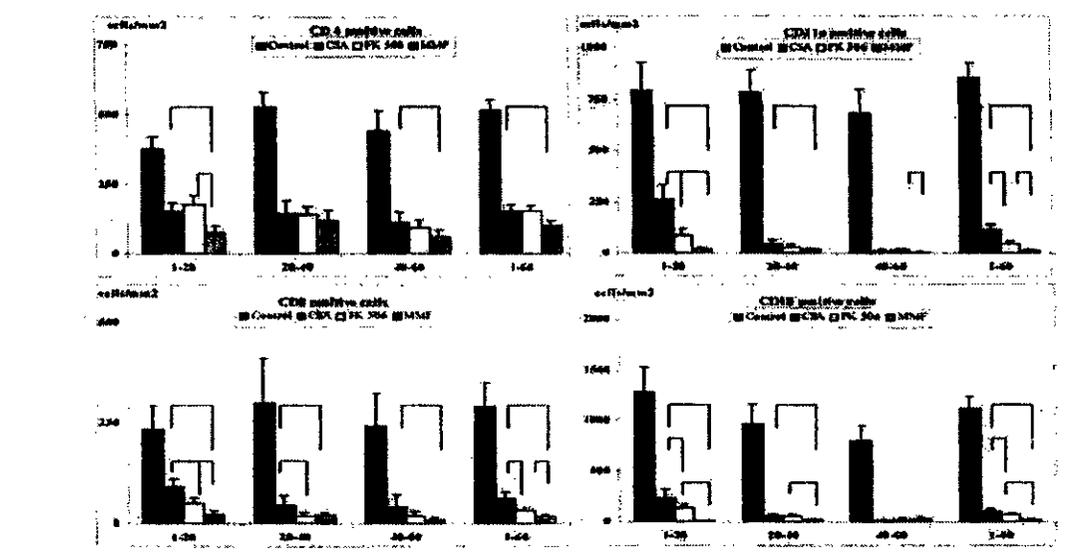
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An increase in the levels of CD4-positive cell accumulation was observed in the perivascular space of epicardial arteries. This increase was less than in intramyocardial perivascular space (Figure 4) with an average cell accumulation of 517 ± 36 cells/mm². A significant reduction in the levels of CD4-positive accumulation was observed with the administration of immunosuppressive agent (Figure 4). MMF was shown to prevent CD8, CD11a, and CD18-positive cell infiltration when compared to CysA and FK 506. ERL 080 and MPA were not included in the study.

Figure 4: Accumulation of leukocytes in the PVS of epicardial arteries within different time intervals and throughout the entire study period.



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4.4.2. Effect on antibody responses:

The ability of ERL 080 and MMF to inhibit B cell responses *in vivo* was investigated in mice (Report # 98-02542). Mice (4 weeks old OF₁ females) were intravenously immunized with DAGG-Ficoll (150 mg/kg) and TNP-LPS (150 mg/kg) followed by the oral administration of immunosuppressive (ERL 080 and MMF) therapy. Therapy began on the day of antigen injection and continued for 3 days, for a total of 4 applications. The compound 204-603 (Leflunomide) at 50 mg/kg body weight was included as a standard control. The *in vivo* immunosuppression was evaluated by ELISA for DNP/TNP specific IgM and IgG antibody. The sponsor states that antibodies directed against DNP and TNP are highly cross reactive; therefore the same readout can be used to determine antibodies induced by TNP-LPS or by DAGG-Ficoll. There are differences in the data obtained between four independent experiments (Table 11). With MMF the percent inhibition of IgM was -33% in experiment 37, -20% inhibition in experiment 34, and 62% inhibition in experiment 36. IgG was inhibited 82%, 90% and 33%, respectively (Table 11). In one study (experiment 34), MMF did not appear to inhibit IgM (Table 12).

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The addition of ERL 080 demonstrated no IgM inhibition in experiment 34. However, IgG was inhibited 78% (Table 11). The *in vivo* suppression of DAGG-Ficoll induced anti-DNP responses also varied between experiments for ERL 080 and MMF. However, inhibition values for IgG were more consistent between MMF and ERL 080 (Table 12).

Table 11: *In vivo* TNP-LPS induced antibody responses.

Substance	Dose (mg/kg)	EXP.#	% inhib.IgM ±SEM	% inhib.IgG ±SEM
PKF204-603	50	34	77	86
ERL 080	150	34	0	78
CellCept	150	34	-20	33
PKF204-603	50	36	75	85
ERL 080	150	36	79	91
CellCept	150	36	62	90
PKF204-603	50	37	33	96
ERL 080	150	37	72	94
CellCept	150	37	-33	82
PKF204-603	50	38	88	99
ERL 080	75	38	44	70
ERL 080	150	38	72	93

Suppression of the T1-1 induced anti-DNP response *in vivo*. Groups of 4 mice each were immunized with TNP-LPS i.v. and treated with the indicated drugs by daily oral application as described in Methods. IgM and the IgG anti-TNP antibodies in the serum were quantitated in an ELISA 6 days after immunization. Four mice per group.

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Table 12: *In vivo* DAGG-Ficoll induced antibody responses.

Substance	Dose (mg/kg)	EXP.#	% inhib.IgM	% inhib.IgG
PKF204-603	50	34	95	91
ERL 080	100	34	20	91
ERL 080	150	34	27	90
CellCept	100	34	-20	64
CellCept	150	34	40	91
PKF204-603	50	36	94	97
ERL 080	50	36	50	53
ERL 080	150	36	50	89
CellCept	50	36	67	47
CellCept	150	36	67	93
PKF204-603	50	37	94	98
ERL 080	50	37	50	44
ERL 080	150	37	80	95
CellCept	50	37	17	33
CellCept	150	37	50	85

Suppression of the T1-1 antigen (DAGG-Ficoll) induced anti-DNP response *in vivo*. Groups of five OF1 mice were immunized by intravenous injection of DAGG-Ficoll and treated with the listed drugs by gavage for four consecutive days as described in Methods. IgM and IgG anti-DNP antibodies were quantitated in an ELISA 6 days after immunization. Four mice per treatment group.

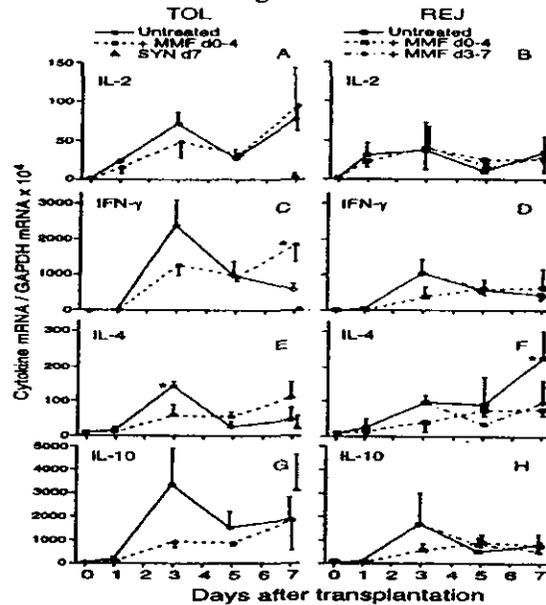
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In another study, the effect on antibody production and cytokine expression was also investigated in rats (Huang *et al.*, 2003). Briefly, immunohistochemistry analysis for the identification of rat B cells using anti-IgD, anti-IgG1, and anti-IgM antibodies was performed on the collected tissue samples. Rat α/β T cells were identified using the R73 marker, and the α -chain of the T cell receptors was identified by the OX39 marker. A summary of the experimental conditions is listed in section 4.4.3.

The result of the study shows that in the untreated TOL recipients, low levels of IgG1 were observed. IL-4 is important for antibody class switching to IgG and the low expression levels of IgG1 correlates with the inhibition of IL-4 gene expression (Figure 5).

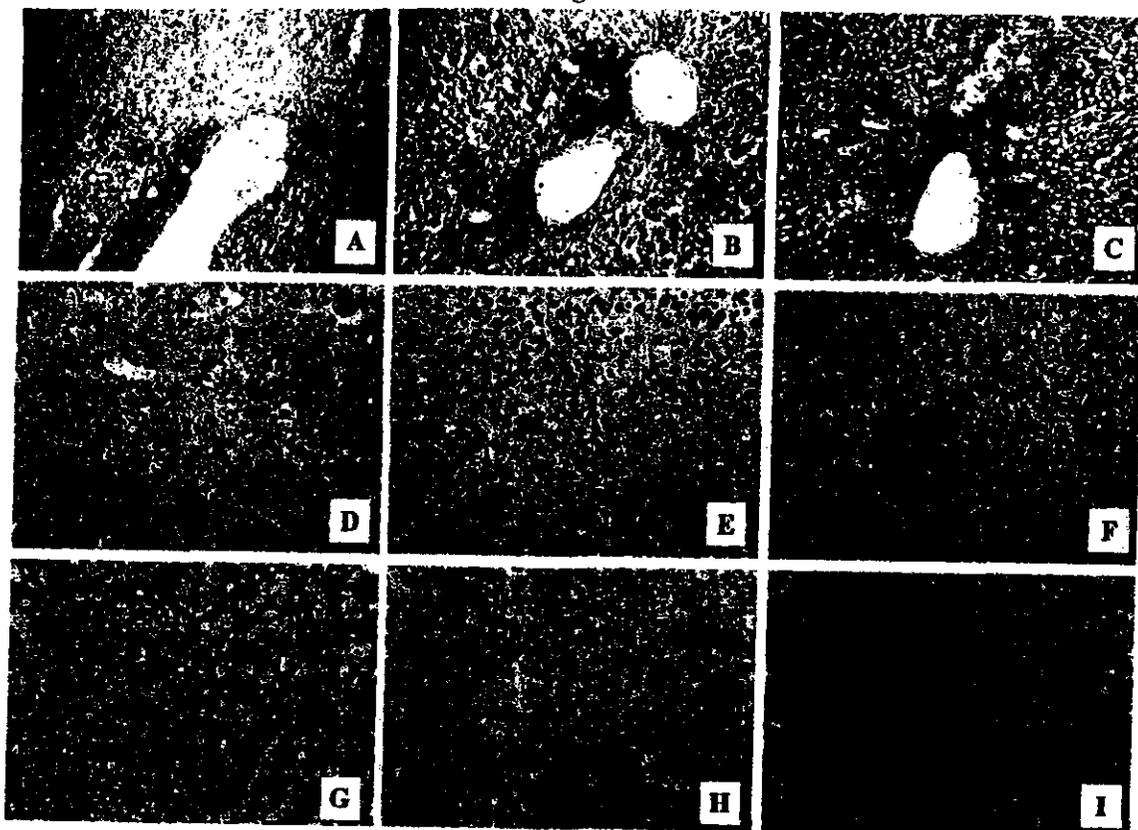
Figure 5



Effect of MMF treatment on mRNA expression of IL-2, IL-4, interferon (IFN)- γ , and IL-10 in TOL, REJ, and syngeneic liver transplants. The level of expression in normal PVG liver (day 0 figures). Cytokine expression is estimated from a cDNA standard curve and corrected for glyceraldehyde phosphate dehydrogenase expression according to the formula cytokine/glyceraldehyde phosphate dehydrogenase $\times 10^4$ as described in the *Methods* section. A significant difference in cytokine mRNA expression between untreated and MMF-treated grafts (*) is described in detail in the text.

This is in stark contrast with the high deposit observed in the lobular areas of untreated REJ liver allografts on day 7 (Figure 6). Early treatment of REJ animals with MMF significantly attenuated IgG expression levels as delayed treatment did not inhibit IgG deposition (Figure 6F). IgM were at similar levels in TOL and REJ animals and peaked at day 5 following transplantation (Figure 6G). Early MMF treatment in TOL and REJ recipients resulted in a reduction of IgM levels on day 7 (Figure 6H). Although a reduction in the IgM levels in the delayed MMF treatment arm was reported, it was not considered to be significant (Figure 6I). The activity of ERL 080 was not tested.

Figure 6



Characterization of the T-cell infiltrate and deposition of immunoglobulin (Ig)G1 and IgM in rats that reject (Piebald Virel Glaxo (PVG)→Lewis; REJ) livers after mycophenolate mofetil (MMF) treatment. Rat liver tissues obtained on day 7 after transplantation. REJ untreated (A, D, G); REJ plus MMF early treatment (B, E, H); REJ plus MMF delayed treatment (C, F, I). Infiltrates of α/β T cells (A-C), identified with R73, showed little difference between untreated REJ and early or delayed MMF-treated REJ recipients. IgG1 (D) and IgM (G) were both extensively deposited in REJ liver, and early treatment with MMF inhibited deposition of IgG1 (E). MMF inhibited IgM deposition but led to increased B-cell accumulation, identified by IgM membrane staining (H). Delayed MMF treatment of REJ liver was less effective in inhibiting deposition of IgG (F) and IgM (I).

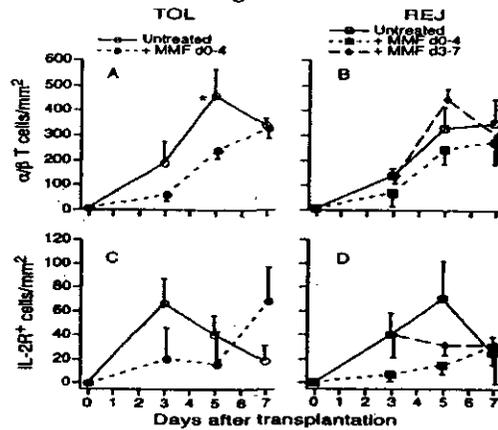
In PVG-Lewis (REJ) animals, immunohistochemistry staining with an α/β T cell marker showed heavy T cell infiltration in the portal tract areas (Figure 6A-C) and throughout the lobular areas of the liver from untreated animals that experienced rejection. MMF was effective at decreasing lobular T cells in TOL recipients. No significant differences were observed in the lobular T-cell infiltrate between MMF-treated and untreated REJ recipients (Figure 7). Immunohistochemistry staining with OX39, specific for CD 25 [α chain of the interleukin-2 (IL-2) receptor] also showed an increase in cellular infiltration in TOL grafts which appear to peak on day 3. Moreover, REJ graft showed a peak on day 5 (Figure 6). Although MMF treatment of both the TOL and REJ recipients reduced the extent of infiltration with IL-2 receptor positive cells, the reduction did not appear to be significant (Figure 7C and D). The activity of ERL 080 was not tested.

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As demonstrated in Figure 8, there was a significant difference between TOL and REJ animals in the extent of IgD infiltration. More B cell infiltration was observed in TOL livers on day 5 (Figure 8). Treatment with MMF also significantly increased liver B cell infiltrate in REJ animals over non treatment controls. Neither MPA nor ERL 080 was used in this study.

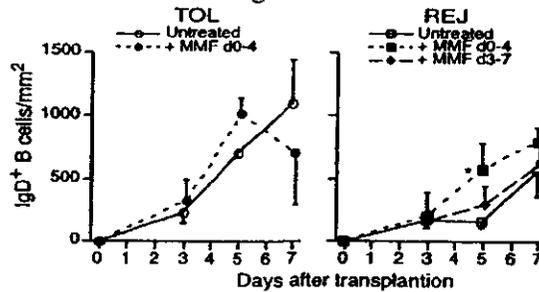
Figure 7



Effect of MMF on α/β T-cell (A, B) and interleukin (IL)-2R⁺ (CD25⁺) cell infiltrate (C, D) in liver allograft lobules. Positive cells in normal PVG livers (day 0 figures). (A, B) MMF treatment in all groups inhibited the lobular infiltrate of α/β T cells, although inhibition did not reach statistical significance apart from day 5 tolerated (PVG→Dark Agouti; TOL) livers ($P=0.03$). (C, D) The infiltrates of IL-2R⁺ cells, identified with OX39, were reduced during MMF treatment, although this was not statistically significant.

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Figure 8



Effect of MMF on B-cell (IgD⁺ cell) infiltrate in portal tracts of liver allografts. IgD⁺ cells in normal PVG livers (day 0 figures). There was greater IgD⁺ cell infiltrate in TOL compared with REJ recipients on day 5 ($P=0.0003$). Early MMF treatment of REJ animals led to significantly increased IgD⁺ cell infiltration on day 5 compared with untreated animals ($P=0.002$).

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During the first week following transplantation, MMF appears to have little effect on IL-2 and IL-10 gene expression in the transplanted liver. However, inhibition in the levels of IL-4 was observed in REJ animals treated with MMF (Figure 5). The result shows that there were no significant differences in the level of IL-10 expression in REJ and TOL animals at 3-5 days following transplant. However, although not significant, there was a slight increase in the level of IL-10 expression in TOL animals at day 7.

4.5. Drug combination:

4.5.1. Effect of ERL 080 or MMF plus CysA on renal graft:

The effect of combination of ERL 080 or MMF and CysA was evaluated in BN to Lewis rat orthotopic kidney transplant model (Report # -2001-00138). Graft survival was measured by the survival of the recipient animals. Based on the data obtained in the single dose study, doses were reduced by at least two fold the minimal effective dose. ERL 080 (5 mg/kg/day) in combination with CysA (2 mg/kg/day) resulted in acute cellular rejection in two animals. Long-term survival (98 days) without signs of histological rejection was achieved in one animal (Table 6). The data show that when MMF (10 mg/kg/day) was administered in combination with CysA (2 mg/kg/day) cellular rejection was observed in two animals. However, one animal survived for greater than 98 days without signs of rejection. The combination of MMF and CysA do not appear to offer any significant protection in the BN to Lewis model in two out of three animals tested (Table 6). Based on a small number of animals in each group, no synergistic or antagonistic interaction between the two drugs was observed (Table 6).

In another study, the effect of combination of ERL 080 and CysA was evaluated in DA to Lewis rat orthotopic kidney transplant model (Report # -2001-00138). Graft survival was measured by the survival of the recipient animals. The study shows that ERL 080 (5 mg/kg/day) and CysA at 2.5 mg/kg/day treatment were not effective at prolonging graft survival. ERL 080 (10 mg/kg/day) and CysA (1.25 mg/kg/day) were only effective at achieving long-term graft survival in two of three animals tested; the surviving animals lived for ≥ 98 days with no signs of rejection. In combination with CysA (2.5 mg/kg/day), long term survival (≥ 98 days) was achieved in 2 of 6 cases (Table 7). Animals that received an initial dose ERL 080 (20 mg/kg/day) and CysA (2.5 mg/kg/day) followed by a dose reduction of ERL 080 to 10 mg/kg/day on day seven also achieved long-term survival with no apparent signs of rejection (Table 7). The combination of ERL 080 and CysA appears to be more effective at prolonging kidney graft survival in the DA to Lewis model of kidney transplantation.

5. CONCLUSIONS:

The addition of MPA to PHA activated peripheral blood lymphocytes led to an inhibition of DNA synthesis. The number of cells at S phase increased with increasing concentration of MPA. PHA stimulation resulted in the slight increase of CD25 positive B- and T-lymphocyte, however, the expression of HLA-DR on T-cells were not influenced by PHA activation. The effects of PHA induction were reduced by the presence of MPA. MPA was shown to dose dependently inhibit IMP-DH in human lymphocytes, however, complete inhibition was not observed under the conditions tested.

The *in vitro* immunosuppressive effect of ERL 080 was compared with MPA, MMF and CysA, using mouse and human derived mononuclear cells. All were equally effective at inhibiting mouse and human mononuclear cells.

ERL 080 can suppress *in vitro* and *in vivo* antibody responses of spleen cells stimulated *in vitro* with T cell independent antigens TNP-LPS and DAGG-Ficoll. Although not significant, ERL 080 demonstrated a two-fold higher IC₅₀ value compared with MMF.

In the rat liver and heart transplant model, the administration of ERL 080 demonstrated a reduction of acute rejection episode and an increase in survival. ERL 080 was more effective than CysA and FK506 at reducing leukocyte graft infiltration in rat heterotopic heart transplant recipients. However, ERL 080 was less effective at prolonging the survival of renal graft and high doses were less tolerated and showed no efficacy at prolonging survival in these animals. ERL 080 in combination with CysA showed efficacy at prolonging the survival of renal graft in some rats.

6. LABEL**6.1. Sponsors proposed label:**

Mechanism of Action

└

└

6.2. Comments:

The sponsor states that [However, they did not provide enough data to support this claim. Therefore, this statement should be deleted.

The sponsor also states that [However, no data supporting this claim was provided.

In addition, the sponsor claims that ERL 080, [reference to this claim has not been described for other immunosuppressive drugs.

6.3. FDA's version of the label:

[MPA is an [uncompetitive, and reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH), and therefore inhibits the *de novo* pathway of guanosine nucleotide synthesis without incorporation to DNA. Because T- and B-lymphocytes are critically dependent for their proliferation on *de novo* synthesis of purines, whereas other cell types can utilize salvage pathways, MPA has potent cytostatic effect on lymphocytes. [

[Mycophenolate sodium has been shown to prevent the occurrence of acute rejection in rat models of kidney and heart allotransplantation. [

[Mycophenolate sodium also decreases antibody production in mice.]

7. REFERENCES:

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8. RECOMMENDATIONS:

This NDA is approvable with respect to the immunopharmacology, pending the accepted version of the label.

Microbiologist, HFD-590

CONCURRENCES:

HFD-590/Deputy Dir	_____	Signature	_____	Date
HFD-590/Micro TL	_____	Signature	_____	Date

CC:

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