First Human Trial of FTY720, a Novel Immunomodulator, in Stable Renal Transplant Patients

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Abstract. FTY720 is a novel immunomodulator to be developed for use in organ transplantation. The primary objective of this study was to measure safety, single-dose pharmacokinetics, and pharmacodynamics in stable renal transplant patients—the first human use of FTY720. This study used a randomized, double-blind, placebo-controlled design that explored single oral doses of FTY720 from 0.25 to 3.5 mg in 20 stable renal transplant patients on a cyclosporine-based regimen. Safety assessments and blood samples were taken predose and at multiple time points during a 96-h period postdose. Standard pharmacokinetic parameters were derived from the FTY720 whole blood concentrations, measured by HPLC/MS/MS. FTY720 was well tolerated, with no serious adverse events. Transient, asymptomatic bradycardia occurred after administration in 10 of 24 doses of FTY720. Pharmacokinetics are characterized by a prolonged absorption phase; the terminal elimination phase started 36 h after the administration, with elimination half-life (t1/2) ranging from 89 to 157 h independent of dose. Maximum plasma concentration and AUC were proportional to dose with low intersubject variability, the apparent volume of distribution (Vd/F) ranged from 1116 to 1737 L. FTY pharmacodynamics were characterized by a reversible transient lymphopenia within 6 h, the nadir being 42% of baseline. The lymphocyte count returned to baseline within 72 h in all dosing cohorts except the highest. Single oral doses of FTY720 ranging from 0.25 to 3.5 mg were well tolerated and caused a reversible selective lymphopenia. Transient, but asymptomatic bradycardia was the most common adverse event. The long t1/2 suggests less frequent dosing intervals. The size of Vd/F is in excess of blood volume, consistent with widespread tissue distribution.

The new immunosuppressant FTY720 (FTY) is a synthetic analogue of a natural compound derived from the fungus Isaria sinclairii (1,2). The mechanism of action of FTY appears to be specific and distinct from that of any other drug approved or being developed for use in solid organ transplantation. In experimental animals, FTY rapidly and transiently reduces circulating mature lymphocytes in peripheral blood (3), effecting T cells more than B cells; the CD3+, CD4+, and CD8+ T cell lines appear to decrease the most (6–8). It was initially thought that FTY reduced circulating lymphocytes via induction of lymphocyte apoptosis (4,5). However, recent data indicate that FTY induces the accelerated homing of lymphocytes to lymph nodes and Peyer’s patches (6–8). The lymphocyte sequestration might be mediated by an enhanced migratory response to homing chemokines (6–9).

Studies in allograft transplantation using different animal species and organs demonstrated the efficacy of FTY as a potent immunosuppressant at doses ranging from 0.05 to 10 mg/kg per day (9–15). In studies assessing its usefulness in combination therapy, synergy between FTY and cyclosporine (CsA) was demonstrated in multiple animal allograft models (9,10,12,14,16). Importantly, coadministration of FTY with CsA did not affect the blood concentration of either drug (15,17). To date, the pharmacokinetic data on FTY has been limited to animal studies. FTY bioavailability is 60 to 90% in rats and dogs, with a higher concentration in blood cells than plasma at steady state (data on file, Novartis Pharmaceuticals, East Hanover, NJ). The half-life of FTY in animals varies: 12 h in rats, 29 h in dogs, and 36 h in baboons (18). Maximal blood concentrations were reached after 7 to 8 h and 2 to 24 h in dogs and baboons, respectively. A near linear relationship between FTY dose and concentration was observed in these studies. FTY is metabolized to produce carboxylic acid derivatives that are devoid of immunosuppressive activity and ultimately excreted in urine and feces (19).
The objective of this study was to determine the safety, the lymphocyte response as a pharmacodynamic measure, and pharmacokinetics of whole blood FTY concentrations after single oral doses in stable human renal transplant patients. In preclinical studies, FTY was well tolerated in different species without evidence of renal, pancreatic, gastrointestinal, or bone marrow toxicity. In toxicology studies, increasing FTY exposure (>0.1 mg/kg for 1 mo) was associated first with reversible lung toxicity, second with heart, and then with central nervous system. Applying interspecies scaling, a no-toxic-effect dose in humans was estimated (20). The predicted FTY-AUC for the starting dose (0.004 mg/kg for 70-kg subject), using allometric modeling, falls 1.5 logs below the AUC associated with any organ toxicity in the most sensitive animal species. A consistent feature of all animal models treated at pharmacologic doses of FTY720 is a decrease in lymphocyte count, which returns to baseline with cessation of drug dosing. The pharmacodynamic effect of FTY720 is lymphopenia (13,14); therefore, a dose range was calculated that was one log-unit below the toxicity level. As a consequence, the pharmacodynamic response to FTY with the onset of significant lymphopenia was chosen as the stop point for further dose escalation.

Materials and Methods

Study Design

This was a randomized, double-blind, placebo-controlled, time-lagged, two-center, ascending single oral dose study. Entry criteria included subjects, aged 18 to 65 yr, who had received their first or second renal transplant ≥12 mo before randomization and who were treated with a CsA-based (Neoral; Novartis, Basel, Switzerland) immunosuppressive regimen. For inclusion, patients had to be medically stable for at least 3 mo with serum creatinine ≤3 mg/dl. All subjects weighed at least 50 kg and were between −15 and +40% of normal for their height and frame size. The study was approved by the local ethics committee, and subjects gave informed consent before study participation.

Subjects were excluded if graft rejection had occurred in the previous 6 mo, if they had received lympholytic therapy (OKT3, antithymocyte globulin) within the previous year, had consistent lymphocyte counts <1500/mm³, or had any significant medical condition, which might interfere with absorption, distribution, or metabolism. Concomitant immunosuppressive therapy with mycophenolate mofetil, azathioprine, or cyclophosphamide was prohibited.

Subjects were prohibited from strenuous physical exercise for 7 d before dosing and until after study completion. Subjects were domiciled from 24 h before dosing until 96 h after dosing. Subjects were required to fast overnight before study medication and to follow a standard weight maintenance diet. Unless performing a study assessment, subjects had to rest quietly in the upright position for the next 4 h after administration of the drug.

The doses selected for the study were 0.25, 0.5, 0.75, 1.0, 2.0, and 3.5 mg. For each dosing cohort, three patients were randomized to FTY and one to placebo. After subjects completed one dose, and if overall safety, tolerability, pulmonary function, and lymphocyte counts were acceptable, the next higher dose was administered. At the investigators discretion, the same dose could be used again in an additional cohort to expand the data for that particular dose. Dose escalation was terminated if significant toxicity occurred or if two or more subjects in a group developed a nadir lymphopenia ≤20% of the average baseline lymphocyte count. If only one of four subjects manifested lymphopenia, then testing at that dose was repeated in a different subject group. Subjects from a lower dose group could reenter into a higher dose group 1 mo later if all inclusion and exclusion criteria were still met and they reconsented.

Safety Assessments

Before oral dosing with FTY, all subjects had a physical examination with vital signs, a 12-lead electrocardiogram, pulmonary function testing, cardiopulmonary exercise testing, and a standard laboratory screen, including hematology and clinical chemistry. Vital signs, including supine and standing diastolic and systolic BP, radial pulse, and oral body temperature were recorded the day before dosing and immediately predose. Physical examinations, vital signs, an electrocardiogram, and standard laboratory tests were performed at regular intervals after dosing. BP and pulse were assessed after the subject has rested in the supine position for at least 3 min and after 3 min in the standing position. BP was measured on the same arm for each time of determination. Adverse events, serious adverse events, and co-medication were recorded as they occurred throughout the study according to good clinical practice guidelines.

Pulmonary function and cardiopulmonary exercise testing were repeated 48 and 96 h after dosing. Pulmonary function testing included measurements of the forced expiratory volume at 1 s (FEV₁), forced vital capacity (FVC), and diffusion capacity of the lung for carbon dioxide (DL CO). The tests were conducted in a manner consistent with standard pulmonary laboratory practice. Cardiopulmonary exercise testing was conducted to measure the degree of hypoxemia induced by a moderate level of exercise. The test was conducted by using a modified Bruce treadmill protocol to achieve and maintain a heart rate of 0.7 of maximal heart rate (=220-age) for 4 to 6 min. During this time, the oxygen saturation percentage was monitored by finger oximetry. If the subject was unlikely to achieve a moderate tachycardia, then the subject exercised to the point of moderate relative perceived exertion. A ≥20% decrease in pulmonary function tests and ≥3% decline from resting percentage oxygen saturation were defined as adverse events.

Methods of Measuring FTY

Samples of whole blood were drawn into ethylenediaminetetraacetic acid (EDTA)–containing tubes before FTY dosing and at multiple time points postdosing. Blood samples were frozen within 60 min of venipuncture and stored at −20°C pending analysis. Whole blood FTY concentrations were measured using HPLC/MS/MS. Within-study assay validation was performed by analysis of quality control samples together with the study samples. Limit of quantitation was 0.065 ng/ml. The method has been validated extensively with a mean accuracy and precision for different nominal concentrations of 104 to 109%, and 5 to 15%, respectively.

Pharmacodynamic Assessments

The lymphocyte count was the primary pharmacodynamic measure of this study. Whole blood was drawn by venipuncture from a peripheral forearm vein or indwelling venous cannula at screening (day −21 to day −2), baseline (day −1), 1 h and immediately predose, and at 0.5, 1, 2, 6, 12, 24, 48, 72, and 96 h postdosing into EDTA-containing tubes for differential blood counts, determining the absolute lymphocyte counts. Absolute counts were analyzed with a Micro-Diff II cell counter (Coulter-Immunotech Diagnostics, Hamburg, Germany).
Descriptive statistics were computed for lymphocyte variables. Results from placebo subjects were pooled across groups. The absolute lymphocyte count data collected over time was used to derive the following variables:

1. Average baseline lymphocyte count defined as the mean of the baseline lymphocyte count taken the day before dosing and the lymphocyte count at time −1 h and 0 h (predose).
2. Nadir lymphocyte count defined as the lowest lymphocyte count measured at any time after the dose through 96 h postdose.
3. Time to nadir lymphocyte count.
4. Lymphocyte and nadir lymphocyte count as a percentage of baseline defined as the lymphocyte or nadir lymphocyte count divided by the average baseline lymphocyte count multiplied by 100.

Pharmacokinetic and Statistical Analyses
Descriptive statistics were computed for all pharmacokinetic concentrations and for derived model-independent pharmacokinetic parameters separately by treatment, with and without log-transformation. The following parameters were determined for FTY by using noncompartmental methods: area under the concentration:time curve from time 0 to infinity (AUC_{0-\infty}), maximum plasma concentration (C_{max}), time to C_{max} (t_{max}), elimination half-life (t_{1/2}), apparent total oral clearance (CL/F), and apparent volume of distribution (V_{d}/F). Pharmacokinetic values are expressed as means together with the percentage coefficient of variation (CV%). The geometric means were reported for the log-transformed pharmacokinetic parameters, and the median was presented for t_{max}.

A regression analysis was conducted to investigate the dose proportionality of the AUC and C_{max}. The regression model was fitted to the raw and logarithmically transformed pharmacokinetic parameters with an intercept and a predictor variable in the model. For each model, the fit of the model was tested by using the ratio between the mean square of lack of fit and the mean square of pure error. The intercept of the regression line on the untransformed data were examined as to whether its 95% confidence interval included 0 (dose proportional). If the 95% confidence interval of the slope of the regression line for log-transformed data included 1, the pharmacokinetic parameter was considered dose proportional. Results from placebo subjects were pooled across groups. Descriptive statistics were computed for test results. Paired t tests were performed in normal distributed variables with a P < 0.05 considered to be significant.

Results
A total of 20 subjects were enrolled, with 12 re-enrolling in later dose groups, for a total of 32 subject profiles (24 on drug, 8 placebo). According to the protocol, four additional subjects were enrolled into the first two dose cohorts (0.25 mg and 0.5 mg) to more clearly define the safety and tolerability of FTY720 in the early phase of the trial.

The demographics of the subjects are summarized in Table 1. All patients received a Neoral-based immunosuppressive regimen, all but one in combination with low-dose steroids (prednisolone, n = 14; methylprednisolone, n = 5). Except for clinically insignificant abnormalities, the safety lab was unremarkable in pretreatment evaluations with normal leukocyte (7.19 ± 1.92 × 10^{9}/L) and lymphocyte (2.456 ± 0.796 × 10^{9}/L) counts and an acceptable kidney function as measured by calculated creatinine clearance of 70.3 ± 19.0 ml/min (range, 42 to 128 ml/min). At the inclusion, 19 subjects had hypertension; BP (134 ± 9 / 88 ± 7 mmHg) was well controlled in all patients. All 8 subjects who received placebo and 22 out of 24 subjects randomized to FTY treatment were receiving cardiovascular drug therapy. The most common drugs were β-blockers (n = 15), Ca-antagonists (n = 14), angiotensin-converting enzyme inhibitors/angiotensin II receptor blockers (n = 11), and diuretics (n = 9). Concomitant therapy remained unchanged throughout the dosing interval.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>19/1</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>43.2 (9.8)</td>
</tr>
<tr>
<td>White race</td>
<td>20</td>
</tr>
<tr>
<td>Time after Tx (yr)</td>
<td>8.7 (3.9)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>177 (6.7)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>84.7 (14.0)</td>
</tr>
<tr>
<td>Body mass index</td>
<td>26.8 (4.1)</td>
</tr>
</tbody>
</table>

Table 1. Demographics of subjects enrolled in study

Safety Assessments
Adverse Events. No serious adverse events were reported during or after the administration of FTY. One serious adverse event, transient diplopia lasting 1 h, was reported in a subject randomized to placebo. A neurologic evaluation of this subject was unremarkable. A total of 28 adverse events were recorded. These events, stratified by FTY treatment group, are presented in Table 2. Adverse events occurred in 91% of subjects randomized to FTY and in 75% if subjects randomized to placebo. The most common of the 28 reported adverse events were bradycardia (n = 10) and headache. Headache of mild to moderate severity occurred in two subjects receiving placebo and four receiving FTY. One patient on placebo was the only subject to receive concomitant medication as a result of an adverse event (headache), for which paracetamol was administered.

Heart Rate. Transient, asymptomatic bradycardia occurred in ten subjects randomized to FTY (42%) but none randomized to placebo (Figure 1A). Bradycardia was diagnosed in these subjects when heart rate decreased below baseline to an extent judged significant by the study physician. Higher doses of FTY were more frequently associated with bradycardia: 9 out of 12 subjects randomized to ≥0.75 mg of FTY developed bradycardia; however, only 1 of 12 subjects receiving 0.25 to 0.5 mg of FTY. Six out of ten subjects had baseline bradycardia (heart rate, ≤60). Only one of four patients with heart rate ≤60 receiving a lower dose of FTY (0.25 to 0.5 mg) developed bradycardia. However, in five of six patients with baseline bradycardia, the pulse rate further decreased after receiving higher doses of FTY (≥0.75 mg). In contrast, the pulse rate did not decrease in four patients with baseline bradycardia receiving placebo treatment. No apparent relationship was identified between subjects with bradycardia and their cardiovascular medical history or current cardiovascular drug therapy, including the use of β-blockers. Seven out
of 12 subjects in the lower FTY dose groups (0.25 to 0.5 mg) were on β-blockers; however, the subject who developed bradycardia was not on β-blocker therapy. In higher dose groups (≥0.75 mg) 8 of 12 subjects were on β-blocker treatment; all but one developed bradycardia. Again, two of four patients experienced bradycardia even though they were not on β-blocker treatment (baseline heart rate, 74 and 52 bpm). The observation of a relationship between bradycardia and FTY dose was strengthened by the 11 subjects who reenrolled in the study receiving a higher FTY dose (≥0.75 mg) during their second treatment period. Only 1 of 11 subjects had experienced bradycardia during the first treatment period (placebo treatment, n = 3; 0.25 to 0.5 mg FTY, n = 8), but 8 of 11 patients were reported to manifest bradycardia when rechallenged with a higher FTY dose (≥0.75 mg).

As shown in Figure 1B, heart rates reached nadir approximately 4 to 8 h after FTY dosing. Even the patients who were randomized to FTY but did not develop overt bradycardia (n = 14) had a small but significant (P < 0.05) decline in heart rate 4 to 12 h after the dosing (baseline, 67 ± 10 bpm; 8 h after dosing, 60 ± 10 bpm). Although placebo-treated patients had no significant changes in heart rate over time, FTY-treated subjects experienced a decline in heart rate as early as 2 h after dosing and lasting for up to 24 h. Again, low-dose–treated cohorts (n = 12) experienced only a minor decrease in heart rate (from baseline, 66 ± 11 bpm; to nadir after 8 h, 59 ± 12 bpm; P < 0.05). Patients treated with ≥0.75 mg of FTY had a more pronounced decline in heart rate (from 61 ± 10 bpm at baseline to 50 ± 8 bpm 8 h after dosing; P < 0.001).

It is important to point out that the patients did not experience any symptoms throughout the course of bradycardia. BP and standing BP were not affected, but standing heart rate declined as well (baseline, 71 ± 13 bpm; to nadir 58 ± 10 bpm; P < 0.001). All patients randomized to FTY had normal BP recordings at the time of nadir heart rate (supine BP, 128 ± 11/81 ± 8 mmHg; systolic range, 110 to 160 mmHg), and no patient developed an orthostatic reaction (standing BP, 130 ± 13/85 ± 8 mmHg; systolic range, 105 to 150 mmHg).

**Electrocardiogram.** One subject with a prolonged PR interval of 240 msec predose developed a transient prolongation of PR interval (320 msec; 24 h post-dose). Another subject had inverted T-waves in V5-V6 on electrocardiogram 48 h after dosing. Both patients were asymptomatic and had normal vital signs. All other electrocardiogram variables (including QRS time, QT time, and PR interval) remained within normal limits. The QT interval increased in concordance with the lower heart rates, but it remained within the normal range for all patients.

**Safety Laboratory Data.** Two subjects, randomized to 0.5 mg FTY, developed rising amylase and lipase after the FTY administration. In the first subject, the lipase and amylase returned to the normal range 1 wk after study completion. The second patient had elevated amylase and lipase at screening; therefore, later elevated values were judged as not clinically significant and unrelated to FTY exposure. In this patient, these levels were still elevated at screening for the next dosing period (2 mg): by 24 h post-dose, amylase and lipase were within the normal range. One patient (0.75 mg) experienced elevated lipase without raise in amylase. Patients did not manifest symptoms of pancreatitis. The safety laboratory studies (notably electrolytes and liver enzymes) of all other patients revealed only minor changes throughout the study, judged as clinically NS by the investigators. The kidney function, as measured by calculated creatinine clearance (predose, 68 ± 18 ml/min; 24 h after intake, 64 ± 16 ml/min) and serum creatinine (predose, 132 ± 34 μmol/L; 96 h, 125 ± 32 μmol/L) remained stable in FTY-treated subjects. Platelet and erythrocyte counts were not affected by FTY treatment. FTY had no effect on the white blood cells including differential blood count.

**Table 2. Summary of adverse events by FTY720 treatment group**

<table>
<thead>
<tr>
<th>Event</th>
<th>Placebo (n = 8)</th>
<th>0.25 mg (n = 6)</th>
<th>0.5 mg (n = 6)</th>
<th>0.75 mg (n = 3)</th>
<th>1.0 mg (n = 3)</th>
<th>2.0 mg (n = 3)</th>
<th>3.5 mg (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar pain</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Bradycardia</td>
<td></td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Prolonged PR interval</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweating</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decreased DlCO</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Increased lipase/amylase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold (upper respiratory tract infection)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>Ankle joint pain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transient diplopia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Dyspnea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tiredness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
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<tr>
<td>Abnormal T waves on ECG</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>1</td>
</tr>
<tr>
<td><strong>Total events</strong></td>
<td>6</td>
<td>1</td>
<td>6</td>
<td>5</td>
<td>2</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>
(predose, $6.62 \pm 1.59 \times 10^9$/L; 24 h, $6.59 \pm 1.47 \times 10^9$/L; 48 h, $6.82 \pm 1.49 \times 10^9$/L; 96 h, $6.72 \pm 1.62 \times 10^9$/L), with the exception of the highest dosing cohort. In this dose group white blood cells decreased 24 h after dosing in all 3 subjects ($7.4 \times 10^9$/L to $4.8 \times 10^9$/L). Differential blood count revealed 75% neutrophils ($3.7 \times 10^9$/L) but only 19% lymphocytes ($0.82 \times 10^9$/L) at this time point, a 54% decline (range, 42 to 63%) compared with baseline ($1.82 \times 10^9$/L lymphocytes). Low leukocyte numbers ($5.2 \times 10^9$/L) persisted throughout the study, with $3.84 \times 10^9$/L neutrophils and $0.96 \times 10^9$/L lymphocytes after 96 h in this cohort.

**Pulmonary Function Testing.** During the study, no subject manifested a worsening of FEV$_1$ or FVC of $\geq 10\%$ compared with baseline. A high degree of variability was seen with D$_L$CO. One subject in the 0.5-mg FTY group had a $20\%$ decrease in D$_L$CO compared with baseline ($20.7\%$ decrease) 96 h after dosing. This patient was asymptomatic and had normal FEV$_1$, FVC, and exercise oximetry at the same time point. In contrast, four patients (one placebo and three FTY treatment) had an increase of $\geq 20\%$ compared with baseline, changes interpreted as within the margin of error for the test. The most sensitive test for pulmonary dysfunction, cardiopulmonary testing with exercise oximetry, was normal for all subjects at 48 and 96 h postdosing.
**Pharmacodynamics**

The average baseline lymphocyte counts for all treatment groups (2.59 ± 0.76 × 10^9/L) were very near the normal average lymphocyte count of 2.50 ± 10^9/L. The mean coefficient of variation for the baseline values was 14% (range, 0.3 to 38%), similar across all dosing cohorts. Table 3 provides the mean and SD of baseline values for all dosing cohorts.

The mean lymphocyte count of the placebo group (n = 8) dropped from 2.73 ± 10^9/L to a nadir of 1.73 ± 10^9/L over the first day postdosing. This nadir lymphocyte count being 66% of the baseline count (Figure 2), and the range of this drop being from 49 to 13% of baseline with an average of 3.6 ± 2.6 h after administration of placebo (range, 0.5 to 6 h). As early as 1 h after intake of morning medication, lymphocytes started to decline (2.26 ± 0.41 × 10^9/L; 84% of baseline; P < 0.01). The maximal mean decrease of lymphocyte numbers was observed 6 h after intake of placebo (1.85 ± 0.30 × 10^9/L; P < 0.01; 71% of baseline; Figure 2), with a considerable variability between individuals (range, 4 to 49% decrease compared with baseline). No significant changes occurred in placebo-treated patients at any time thereafter. During the observational period, lymphocytes did not fall below 1.50 ± 10^9/L in any placebo-treated patient. The patient who did not receive any corticosteroid treatment exhibited only minor changes in lymphocyte counts (2.1 to 2.6 ± 10^9/L; maximal decrease, 13%) over 24 h, which was comparable with his baseline values (2.1 to 2.9 ± 10^9/L) and similar to his individual baseline coefficient of variation (11%).

All treated groups, 0.25 to 3.5 mg of FTY720, consistently manifested a more pronounced decrease in lymphocyte counts compared with the placebo group; the mean nadir range approximately 0.5 to 1.0 × 10^9/L lower than that measured in the placebo group (Table 3; Figure 2). The majority of FTY-treated subjects (83%) had a nadir below 1.5 × 10^9/L, with a

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**Table 3. Summary of lymphocyte count**

<table>
<thead>
<tr>
<th>FTY720 Dose (mg)</th>
<th>n</th>
<th>Baseline Lymphocyte Count (^b) (cells/μL)</th>
<th>Nadir Lymphocyte Count (^b) (cells/μL)</th>
<th>Time to Nadir (h)</th>
<th>Mean Decrease in Nadir (% baseline)</th>
<th>24-h Lymphocyte Count (cells/μL)</th>
<th>96-h Lymphocyte Count (cells/μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>8</td>
<td>2730 ± 585</td>
<td>1728 ± 215</td>
<td>3.6</td>
<td>66 ± 15</td>
<td>2771 ± 836</td>
<td>2861 ± 731</td>
</tr>
<tr>
<td>0.25</td>
<td>6</td>
<td>2284 ± 690</td>
<td>1057 ± 524</td>
<td>6.3</td>
<td>46 ± 17</td>
<td>1740 ± 353</td>
<td>2089 ± 759</td>
</tr>
<tr>
<td>0.5</td>
<td>6</td>
<td>2307 ± 585</td>
<td>1127 ± 298</td>
<td>8.0</td>
<td>48 ± 11</td>
<td>1927 ± 724</td>
<td>2165 ± 524</td>
</tr>
<tr>
<td>0.75</td>
<td>3</td>
<td>2457 ± 435</td>
<td>1220 ± 303</td>
<td>4.7</td>
<td>50 ± 9</td>
<td>2057 ± 340</td>
<td>2357 ± 424</td>
</tr>
<tr>
<td>1.0</td>
<td>3</td>
<td>2760 ± 393</td>
<td>1203 ± 267</td>
<td>8.0</td>
<td>44 ± 13</td>
<td>2353 ± 278</td>
<td>2643 ± 678</td>
</tr>
<tr>
<td>2.0</td>
<td>3</td>
<td>2844 ± 616</td>
<td>853 ± 239</td>
<td>6.0</td>
<td>31 ± 8</td>
<td>2163 ± 425</td>
<td>2430 ± 557</td>
</tr>
<tr>
<td>3.5</td>
<td>3</td>
<td>3002 ± 1491</td>
<td>730 ± 212</td>
<td>8.0</td>
<td>27 ± 11</td>
<td>1007 ± 133</td>
<td>1180 ± 327</td>
</tr>
</tbody>
</table>

\(^a\) Values are mean ± SD, except Time to Nadir (mean).

\(^b\) Average baseline: baseline, −1 h, and predose.

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**Figure 2.** Lymphocytes as percent of baseline (mean for different dosing cohorts) versus hours postdose.
mean nadir of 42 ± 14% compared with baseline (range, 17 to 77%). All FTY-randomized groups manifested a temporal pattern of relative lymphopenia, detected at the latest by 6 h postdose. Similar to placebo-treated patients, most patients (71%) throughout the different dosing cohorts had the lymphocyte nadir after 6 h (mean, 6.9 ± 2.9; range, 2 to 12 h). Twenty-four hours after dosing, the lymphocyte numbers had returned to the normal range, with values of approximately 80% of baseline in most patients, a consistent finding in all groups except the highest. By 72 and 96 h postdose, the lymphocyte numbers of all dose groups 2.0 mg FTY had returned to baseline values (96 h, 2.28 ± 0.59; 93 ± 14% of baseline).

At FTY doses ranging from 0.5 mg to 3.5 mg, no clear dose response relationship was detected, but the two highest dose groups exhibited a more pronounced decline in lymphocyte numbers. FTY doses of ≥2.0 mg were associated with a more rapid onset of lymphopenia (31 to 43% decrease after 2 h). The three subjects treated with 3.5 mg FTY manifested the most prolonged and intensive lymphopenia. The mean lymphocyte count decreased from 3.00 × 10^9/L at baseline to a nadir of 0.73 × 10^9/L. Two subjects in this group manifested nadir percentage of baseline values of 17% and 24%, respectively, at which time dose escalation was terminated. This degree of lymphocyte drop met the study termination criterion according the protocol. This dosing cohort exhibited a much more sustained response with persistent lymphopenia after 96 h (mean, 1.18 ± 0.33; range, 0.93 to 1.55 × 10^9/L; 42% of baseline). One subject randomized to 3.5 mg FTY had the lowest absolute lymphocyte count nadir, 0.49 × 10^9/L at 6 h postdose. Lymphopenia persisted through discharge at 96 h in this subject, but the lymphocyte count at 240 h postdose was normal again (1.60 × 10^9/L).

The observed difference between placebo and FTY was further confirmed in four patients who received placebo and

### Table 4. Number of subjects and pharmacokinetics of single oral doses of FTY at each dose level

<table>
<thead>
<tr>
<th>Dose mg</th>
<th>n</th>
<th>t_{max} h</th>
<th>C_{max} ng/ml (CV%)</th>
<th>AUC ng · h/ml (CV%)</th>
<th>t_{1/2} h (CV%)</th>
<th>V_d/F L (CV%)</th>
<th>Cl/F ml/min (CV%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>4</td>
<td>18</td>
<td>0.158 (12)</td>
<td>28 (26)</td>
<td>119 (43)</td>
<td>1489 (30)</td>
<td>155 (23)</td>
</tr>
<tr>
<td>0.5</td>
<td>6</td>
<td>18</td>
<td>0.282 (10)</td>
<td>42 (12)</td>
<td>89 (18)</td>
<td>1526 (13)</td>
<td>200 (12)</td>
</tr>
<tr>
<td>0.75</td>
<td>3</td>
<td>36</td>
<td>0.520 (21)</td>
<td>91 (17)</td>
<td>93 (21)</td>
<td>1116 (17)</td>
<td>140 (18)</td>
</tr>
<tr>
<td>1.0</td>
<td>3</td>
<td>36</td>
<td>0.713 (9)</td>
<td>120 (16)</td>
<td>104 (17)</td>
<td>1264 (7)</td>
<td>142 (18)</td>
</tr>
<tr>
<td>2.0</td>
<td>3</td>
<td>36</td>
<td>1.195 (22)</td>
<td>275 (33)</td>
<td>157 (12)</td>
<td>1737 (24)</td>
<td>130 (31)</td>
</tr>
<tr>
<td>3.5</td>
<td>3</td>
<td>12</td>
<td>2.823 (6)</td>
<td>434 (20)</td>
<td>100 (24)</td>
<td>1162 (7)</td>
<td>139 (22)</td>
</tr>
<tr>
<td>Mean</td>
<td>22</td>
<td>24</td>
<td></td>
<td></td>
<td>108 (30)</td>
<td>1407 (23)</td>
<td>158 (24)</td>
</tr>
</tbody>
</table>

a t_{max}, time to maximum plasma concentration; C_{max}, maximum plasma concentration; t_{1/2}, elimination half-life; V_d/F, apparent volume of distribution; Cl/F, apparent total oral clearance.

![Figure 3](image1.png)

**Figure 3.** Mean (± SD) blood FTY720 concentration versus time profiles at different doses.

![Figure 4](image2.png)

**Figure 4.** FTY720 maximum concentration (A) and area under the concentration time curve (B) compared with dose.
different doses of FTY (0.25 to 2.0 mg). All four patients had a lower nadir after receiving FTY (1.21 ± 0.35 × 10^9/L; 48% of baseline) compared with placebo (1.81 ± 0.29 × 10^9/L; 71% of baseline). A trend of increased lymphopenia with increasing doses of FTY was observed in 8 patients who reenrolled during the course of the study and receiving a higher dose during their second treatment period. Five patients received during their first course a low dose of FTY (0.25 mg of FTY, n = 4; 0.5 mg of FTY, n = 1) before an intermediate dose of FTY (0.75 to 2.0 mg) during their second treatment period. Again, mean lymphocyte decrease was more pronounced in the intermediate dose cohorts (1.16 ± 0.568 versus 0.89 ± 0.17 × 10^9/L; 47 versus 35% of baseline). However, two out of five had slightly lower lymphocyte counts after receiving the low dose of FTY (0.25 mg). All three subjects randomized to the highest dose group had previously received 0.5 mg of FTY. As stated above, the highest dose group exhibited a much stronger response to FTY in all three subjects.

Pharmacokinetics

During the study, 22 evaluable pharmacokinetic profiles of FTY were obtained. The FTY blood concentrations of two subjects after 0.25 mg dose were below the limit of quantitation at several time points. The pharmacokinetic parameters for these two subjects could not be estimated. The descriptive statistics of the pharmacokinetic parameters for this group is based on four subjects. Table 4 and Figure 3 summarize the number of subjects and pharmacokinetics of single oral doses of FTY at each dose level. The blood concentrations showed a steady increase over the first 12 h after oral administration. After initial absorption, FTY blood concentrations remained high during the next 24 h; in some dose groups, C_max was reached as late as 36 h after intake. As a consequence, t_max was highly variable and was reached between 8 and 36 h after administration. Approximately 36 h after intake, the FTY blood concentrations started to decline monoexponentially, with a t_1/2 ranging from 89 to 157 h. The t_1/2 was independent of dose with a mean value of 108 h across different dosing cohorts. Except for the lowest dosing cohort, the t_1/2 demonstrated low intersubject variability with a coefficient of variation ranging from 12 to 24%. The maximal concentration (C_max) and the systemic exposure of FTY720 (AUC_0,∞) increased with the dose and ranged from 0.16 to 2.8 mg/ml and from 28 to 434 ng/h/ml, respectively. As depicted in Figure 4, both C_max (R^2 = 0.966; P < 0.001) and AUC (R^2 = 0.916; P < 0.001) were dose proportional over the dose range of 0.25 to 3.5 mg, indicating that at these doses the pharmacokinetics of FTY are linear. FTY’s pharmacokinetics exhibited low inter-subject variability with respect to C_max (6 to 22%) and AUC (12 to 33%). The mean oral clearance (Cl_oral/F) of FTY was 158 ml/min and varied little between dose groups and individuals (CV, 24%). The mean apparent volume of distribution (V_d/F) was 1407 L, or approximately 20 L/kg, with a variability similar to the oral clearance (CV, 23%).

Discussion

Immunosuppressive drugs with distinct mechanisms of action but without overlapping toxicity offer the best options for synergistic antirejection therapy. FTY has a novel mechanism of action (3), is highly effective in animal models of transplantation without added toxicities, and is synergistic in combination with other immunosuppressants (9–17). Thus, FTY has significant potential as a new immunosuppressive treatment. This is a report of the first use of FTY in humans conducted in a randomized, double-blind phase I trial. Several unique features of FTY have been discovered in this study regarding this drug’s safety, its effect on peripheral lymphocyte numbers, and the pharmacokinetic variables in humans.

Single oral doses of FTY were well tolerated. No serious adverse events were seen in any subjects treated with FTY. Bradycardia was the most common adverse event, but this was transient and asymptomatic. An increased frequency of this unexpected adverse event was observed in the higher dosing cohorts, mainly in patients with borderline bradycardia at baseline. Interestingly, although bradycardia was reversible and required no clinical intervention, the effect was not related to FTY blood concentrations, because the systemic exposure to FTY coinciding with the loss of effect remained high, suggesting that the effect on the heart rate is not solely dependent on the blood concentration. Most importantly, all patients were apparently without any symptoms during the transient period of bradycardia, BP remained within normal limits, and no orthostatic reaction was observed in any patient. The extensive safety tests revealed no additional organ toxicity in this single-dose study. In animal studies, multiple high doses of FTY produced dose-dependent increases in lung weights (data on file, Novartis Pharmaceuticals). No clinical signs or symptoms of pulmonary dysfunction were identified in any subject. In summary, the data from this phase I trial suggest that higher doses of FTY may cause bradycardia. As a consequence, a close monitoring of heart rate is advised in future studies with this compound, especially in patients with borderline bradycardia receiving a higher dose of FTY. Further studies are planned to more fully explore the safety profile of this new immunomodulatory drug.

The second objective of this study was to determine the pharmacokinetics of this novel compound. Several important features of FTY have been discovered in this study regarding the pharmacokinetic characteristics of FTY in humans. First, the absorption phase of FTY is prolonged, characterized by a t_max of >12 h. This finding is consistent with the capacity of FTY to be absorbed over a long length of the gastrointestinal tract, an observation consistent with the results of experiments in the animal models. The absolute bioavailability of FTY in different animal species is high (60 to 90%), with maximal blood concentrations attained in 2 to 24 h (18,19). FTY manifests chemical structure motifs similar to those of sphingolipids. The data on the absorption and metabolism of sphingolipids in vivo are scarce (21). Schmelz et al. (22) described a slow systemic uptake of sphingolipids over the whole intestine. Some components are initially associated with intestinal cells;
others are transported through the mucosa and appear in the lymph after several hours. It is unknown whether FTY is mainly absorbed via the portal vein or via the intestinal lymph or is initially associated with intestinal cells. Mechanistically, such factors may contribute to the slow systemic appearance after oral intake. Further studies are needed to better characterize the absorption and the absolute bioavailability of this molecule.

Second, after the initial absorption, the FTY blood concentrations remain high over a sustained period of time. Several factors may be involved in the concentration plateau observed in all dose groups: the process of ongoing slow intestinal absorption, distribution, and redistribution of FTY in different body compartments and a rather slow elimination of the drug. However, given the limited scope of pharmacokinetic evaluations in this single dose study, it is not possible to draw any further conclusions from the current study. In general, it is not uncommon to observe qualitatively similar drug concentration versus time profiles, particularly in the area of sustained or controlled release formulations providing stable concentrations at steady state and requiring less frequent dosing intervals, the characteristics pertinent to FTY.

Third, FTY has a very long half-life in humans, approximately 4 to 5 d. The half-life of any drug is a function of two independent variables, clearance and volume of distribution. Although the magnitude of FTY’s mean oral clearance of 158 ml/min is a typical value for a number of drugs used in clinical practice, it is the very large apparent volume of distribution of FTY, that in large part contributes to the long half-life of this compound. Given the chemical structure of FTY, this large apparent volume of distribution is not unexpected. With FTY’s long half-life comes the potential for an extended pharmacologic effect with significant accumulation. The clinical advantage is that FTY can be given once-a-day with little fluctuation over the dosing interval at steady state. Typical of long half-life drugs, the use of a loading dose of FTY would be useful if the clinical setting requires rapid attainment of therapeutic drug levels, for example in the setting of de novo renal transplantation.

Finally, the interdose and interpatient variability of FTY is low. Over the 1.5 log dose range studied in this trial; a close, linear relationship exists between the dose and both the systemic exposure and the maximal concentration of FTY. In clinical use, adjusting the dose of FTY will result in proportionate changes in FTY exposure and whole blood concentration. The low intersubject variability indicates that there is consistent absorption and disposition of the drug among subjects and should allow for the future development of simple standardized dosing guidelines without the need for frequent blood level monitoring or individualized dose titration.

Both the absorption and disposition properties of FTY likely relate to the sphingolipid-like features of this compound. These features include a polar head group and a hydrophilic, aliphatic tail; giving FTY the capacity to be incorporated into the cell membrane. Sphingolipids may bind to membrane proteins, lipoproteins, and other lipid-rich structures (21). They are critical for the maintenance of membrane structure and may modulate the behavior of receptors. The molecular mechanism of action of FTY is not known; however, given our current understanding of this compound, the modulation of certain membrane-bound receptors may in part explain FTY’s pharmacologic activity (23). FTY has been shown to be highly effective in prolonging graft survival in animal allograft transplant models (9–17,24). The doses of FTY, which were clearly associated with synergistic improvement in graft survival when used with low doses of either cyclosporine or everolimus as described above, typically resulted in FTY systemic exposures of approximately 10 to 300 ng·h·ml. Thus, the range of FTY exposures measured in this first-in-humans trial, 28 to 434 ng·h·ml, are well within the range of AUC associated with effective synergy with either cyclosporine or mTOR inhibitors in preclinical models (9–17,24).

FTY induces a dose-dependent lymphopenia in animals (6–9,18,23–25); therefore, the anticipated pharmacodynamic response in this phase I study was a reduction in lymphocyte counts. In fact, all FTY-treated groups manifested lymphopenia that was more profound and followed a different time course compared with placebo. Among placebo-treated subjects, the nadir lymphocyte count dropped to 66% of the baseline count. It is well known, that lymphocytes and lymphocyte subsets exhibit a diurnal rhythm (26) and fluctuate with strenuous physical activity or rest (27). A modulation of lymphocyte subsets during corticosteroid therapy has been described as well (28,29). Four to six hours after intake, the maximal drop in total lymphocyte counts, T cells and T-helper cells was recorded. Because our patients were resting for at least 4 h after intake and strenuous physical exercise was prohibited, it is suggestive that the transient lymphopenia was the result of the concomitant corticosteroid treatment in seven out of eight placebo-treated subjects. The patient without corticosteroid treatment exhibited only minor changes in lymphocyte counts, which were within the normal diurnal fluctuation.

Similar to its pharmacodynamic effect in animals (6–9,18,23–25), single doses of FTY resulted in a transient decrease in lymphocyte count in humans. The degree and kinetics of lymphopenia clearly differed from placebo. This observation was strengthened by the four patients who were exposed to both FTY and placebo. This lymphopenic effect is first measured at approximately 2 to 6 h after the dose and lasted up to 72 h with intermediate doses of FTY and >96 h with the highest FTY dose tested. The time course, with a rapid onset and slow recovery of peripheral lymphodepletion has been observed in all animal models tested (6–9,18,23–25); therefore, peripheral lymphocyte numbers reflect the pharmacodynamic effect of the drug. Ultimately, peripheral lymphocyte counts may prove to be a useful parameter for monitoring the immunosuppressive effect of FTY in treated patients (18,23–25).

As been described for nonhuman primates, the lymphopenic effect of FTY has no linear dose-response relationship (18). In baboons, the response onset was faster and duration was longer at higher dose, but the maximal peripheral lymphodepletion was similar within the large dose range (0.03 to 0.3 mg/kg) administered. The results of the present study support this
observation; higher doses caused a more rapid and more sustained lymphopenia; however, the degree of lymphopenia showed only minor differences. Quesniaux et al. (18) observed decreasing interindividual variability of the pharmacodynamic response with increasing dosage. Especially during the recovery phase, interindividual variability was noted. Similar observations were made in the present study. Due, however, to small numbers, this cannot be statistically proven. The interaction of single-dose FTY pharmacokinetics and pharmacodynamics is complex. A poor correlation between blood levels and the extent of peripheral lymphodepletion has been observed in nonhuman primates (18) similar to the present study. The rapid onset of lymphopenia with measurable effects on lymphocyte subsets occurred at time points when drug levels started to rise. However, at maximal drug levels (after 12 to 36 h), the lymphocyte counts already returned slowly to baseline values. Although FTY exhibits very predictable pharmacokinetics with a linear dose response relationship, the pharmacodynamic effect was not linear over the dose range tested in this study.

In summary, single oral doses of FTY were well tolerated with transient asymptomatic bradycardia as the most common adverse event. FTY appears to have a slow absorption and a long half-life of up to 5 d, and the single-dose pharmacokinetics are linear with low intersubject variability, characteristics conducive to standardized dosing recommendations. Single oral doses of FTY in doses ranging from 0.5 mg to 3.5 mg caused a dose-dependent, reversible lymphopenia. Together, these data support further clinical trial-based investigations of the capacity of FTY to provide safe, potent, and synergistic immunomodulatory activity in organ transplantation.

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References


