# Multiple-Dose FTY720: Tolerability, Pharmacokinetics, and Lymphocyte Responses in Healthy Subjects

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*FTY720 is a sphingosine-1-phosphate receptor agonist being developed as an immunomodulator for acute rejection prophylaxis after organ transplantation. This study was performed to characterize the pharmacokinetics of and lymphocyte response to multiple-dose FTY720. In this randomized, double-blind study, three groups of 20 healthy subjects each received either placebo, 1.25 mg/day FTY720, or 5 mg/day FTY720 for 7 consecutive days. FTY720 blood concentrations and lymphocyte counts were assessed over the weeklong treatment phase and over a month-long washout phase. The relationship between FTY720 blood concentrations and lymphocyte counts was explored by an inhibitory $E_{max}$ model. First-dose exposure was consistent with dose proportionality between the low- and high-dose groups. Blood levels accumulated fivefold over the treatment period. Exposure on day 7 was dose proportional for $C_{max}$ (5.0 ± 1.0 vs. 18.2 ± 4.1 ng/mL) and for AUC (109 ± 24 vs. 399 ± 85 ng•h/mL). Washout pharmacokinetics after the last dose indicated an elimination half-life averaging 8 days. Lymphocyte counts decreased by 80% in subjects receiving the lower dose to a nadir of $0.4 \pm 0.1 \times 10^9$/L and by 88% in subjects receiving the upper dose to a nadir of $0.2 \pm 0.1 \times 10^9$/L. Descriptive exposure-response modeling estimated that the lymphocyte response at 5 mg/day is near the maximal response achievable. By the end-of-study evaluation on day 35, lymphocyte counts had recovered to within 75% and 50% of baseline in the low- and high-dose groups, respectively. In summary, systemic exposure to FTY720 was consistent with dose-proportionality after both single- and multiple-dose administration. Total lymphocyte counts decreased from baseline by 80% and 88% at regimens of 1.25 and 5 mg/day, respectively. Exposure-response modeling provided evidence that 5 mg/day FTY720 resulted in a near-maximal dynamic effect of this drug on lymphocytes.*

**Keywords:** *FTY720; sphingosine-1-phosphate receptor agonists; pharmacokinetics; lymphocyte response; immunosuppressants; tolerability*

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Classical immunosuppressants used in organ transplant medicine inhibit either the activation or proliferation of T lymphocytes. A new paradigm in transplantation drug development focuses on agents that alter lymphocyte trafficking to impede T lymphocytes from reaching the grafted organ, thereby preventing acute rejection episodes.[1,2] FTY720 is the first in a new class of immunomodulators called sphingosine-1-phosphate receptor agonists. This immunomodulator has a unique mode of action that protects transplanted grafts by reducing the recirculation of lymphocytes to blood and peripheral tissues without impairing activation, expansion, or memory at clinically relevant drug concentrations. This mode of action is in marked contrast to classical immunosuppressants.[3,4] Early development trials in renal transplantation used FTY720 in multidrug regimens with corticosteroids and either cyclosporine or everolimus. These trials provided initial evidence of the effectiveness of FTY720 in preventing acute rejection episodes when dosed in the range 2.5 to 5 mg once daily.[5,6]

Characterizing the pharmacokinetics and pharmacodynamics of a new drug in the target patient population is an essential component of rational drug development. Nonetheless, it can be particularly challenging in

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organ transplant patients to discern drug-specific tolerability concerns and pharmacological effects against a background of multiple comorbidities and comedications.[2] Moreover, in the case of FTY720, coadministration of corticosteroids and other immunosuppressants that affect lymphocytes could obscure the FTY720-specific effect on these target cells. Accordingly, we conducted a multiple-dose, placebo-controlled study of FTY720 in healthy subjects to assess short-term tolerability and safety and to characterize the lymphocyte response under conditions unencumbered by comedications. We chose a subtherapeutic and a therapeutic dose of FTY720 to span a broad range of exposures and responses.

## METHODS

### Study Design

This was a randomized, double-blind, placebo-controlled study in healthy subjects. The protocol was approved by a local medical ethics committee, and the study was performed at PPD Clinical Development (Austin, TX). Subjects were judged eligible for the study based on their past medical history, a physical examination, vital signs, standard clinical laboratory parameters, and an electrocardiogram performed at a screening visit. Subjects gave written informed consent to participate in the trial.

The study was divided into three phases: (1) a baseline phase, during which clinical evaluations were performed; (2) a treatment phase in which subjects received once-daily dosing of 1.25 mg FTY720 ($n = 20$), 5 mg FTY720 ($n = 20$), or placebo ($n = 20$) capsules for 7 days, with an end-of-study evaluation on day 8; and (3) a month-long washout phase in which subjects returned to the clinic for follow-up pharmacokinetic blood sampling and lymphocyte counts. Subjects fasted for 10 hours before drug administration until 4 hours after the dose on day 1. On other study days, breakfast was served 1 hour after the drug was administered. Subjects were domiciled at the study center throughout the baseline and treatment phases and were discharged on day 8.

### Clinical Assessments

Safety assessments included physical examinations, vital signs, clinical laboratory parameters (biochemistry, hematology, urinalysis), and electrocardiograms at baseline, at multiple time points during the treatment phase, and at the end-of-study evaluation. Total lymphocyte counts were obtained each morning during the treatment phase and at each visit during the washout period. Lymphocyte counts were part of the routine complete blood count determined at the clinical laboratory of the study center. Extensive cardiac function testing was performed during the baseline and treatment phases; these will be reported separately.

### Pharmacokinetic Assessments and Bioanalytics

Venous blood samples were obtained before FTY720 administration and then at 1, 2, 4, 6, 8, 12, 16, 20, and 24 hours after the first dose on day 1 and at these same time points after the last dose on day 7. On days 2 to 6, blood samples were obtained before (trough) and 6 to 8 hours after the daily dose (peak). During the washout phase, subjects returned to the clinic on days 10, 14, 21, 28, and 35 for pharmacokinetic blood sampling. Each blood sample was 2.7 mL in volume collected in an EDTA-containing vacuum tube. Sample tubes were inverted several times to mix the tube contents and frozen at –20°C. FTY720 whole-blood concentrations were determined by a validated liquid chromatography method with mass spectrometry, as described previously.[7] Assay performance was judged on the basis of three quality control concentrations: 0.02, 1.5, and 40 ng/mL. Assay accuracy ranged from 97.5% to 108.7% and precision coefficients of variation from –2.5% to 8.7%. The lower limit of quantification was 0.08 ng/mL.

### Data Evaluation and Statistics

Standard noncompartmental pharmacokinetic parameters were derived from the concentration-time data. These included the predose concentration, $C_{(0)}$; the peak concentration, $C_{max}$; the time of its occurrence, $t_{max}$; the area under the concentration-time curve over the 24-hour dose interval, $AUC_\tau$; the average concentration over the dose interval, $C_{avg}$ ($C_{avg} = AUC_\tau/24$); the percent peak-trough fluctuation, PTF (PTF = $[C_{max} - C_{(0)}] / C_{avg}$); and the elimination half-life, $t_{1/2}$. Dose-normalized $C_{max}$ and $AUC_\tau$ were log-transformed and tested in an ANOVA for dose proportionality.

Total lymphocyte counts were graphed with respect to time and inspected for temporal trends. Response parameters derived from the lymphocyte-time curves included the lymphocyte nadir count and the time of its occurrence. Exposure-response data were explored using an inhibitory $E_{max}$ model, including a baseline parameterized as $E = E_0 - [(E_{max} \bullet C)/(EC_{50} + C)]$, where $E_0$ is the baseline response, $E_{max}$ is the maximal response, $EC_{50}$ is the exposure at which half-maximal response occurs, and C is the FTY720 exposure met-

ric as described below. Both pharmacokinetic and exposure-response evaluations were performed in WinNonlin, version 4.0 (Pharsight Corporation, Mountain View, CA).

## RESULTS

### Study Population, Tolerability, and Safety

A total of 66 subjects were enrolled in the study. Six of these subjects withdrew from participation during the baseline phase before receiving FTY720 because of abnormal cardiac rhythms ($n = 4$), viral infection ($n = 1$), and family emergency ($n = 1$). The remaining 60 subjects completed the study. The full study population consisted of 32 men and 34 women. They were 28.7 ± 7.0 years old (range: 18-44) and weighed 74.2 ± 12.5 kg (range: 50-111). The study population was 52% white, 4% black, 2% Asian, and 42% other ethnicities.

Multiple-dose FTY720 was generally well tolerated. There were a total of 114 adverse events reported by 36 subjects. The temporal distribution was 14% during the baseline phase, 65% during the treatment phase, and 21% during the washout phase. Roughly half the events consisted of headache (31%), dizziness (9%), and nausea (7%). Adverse events did not show a particular treatment distribution with 36% in the placebo group, 16% in the 1.25-mg FTY720 group, and 48% in the 5-mg FTY720. There were no clinically relevant changes in biochemistry or urinalysis parameters over the course of the study. Among hematology parameters, there was a mean decrease in platelets and a mean increase in monocytes, neutrophils, and basophils on day 7; however, most values remained in the normal range. The effects of FTY720 on total lymphocyte counts are described below. Effects of FTY720 on cardiac function will be described in a separate report.

### First-Dose Pharmacokinetics

Table I and Figure 1 summarize the pharmacokinetic data. FTY720 concentration profiles exhibited a biphasic rise in blood concentrations, with the first occurring between administration and 4 hours and the second between 4 and 12 hours. During the second, broad rise in concentrations, the peak concentration occurred at a median of 12 hours at both dose levels, with an individual range from 8 to 16 hours. $C_{max}$ was consistent with dose proportionality based on the geometric mean ratio (95% confidence interval [CI]) of the dose-normalized values of 1.04 (0.92-1.17). After reaching the maximum, concentrations declined very slightly over the remainder of the 24-hour dosing interval before the next dose administration. $AUC_\tau$ was also consistent with dose proportionality after the first dose, with a ratio of 1.00 (0.90-1.12). Both $C_{max}$ and $AUC_\tau$ exhibited moderate interindividual variability, with coefficients of variation between 16% and 20%.

**Table I** Pharmacokinetic Parameters

| Parameter | 1.25 mg/day | 5 mg/day |
|---|---|---|
| Day 1 | | |
| $t_{max}$ (h) | 12 (12-16) | 12 (8-16) |
| $C_{max}$ (ng/mL) | 1.1 ± 0.2 | 4.2 ± 0.8 |
| $C_{(24)}$ (ng/mL) | 0.9 ± 0.2 | 3.3 ± 0.5 |
| $AUC_\tau$ (ng•h/mL) | 20 ± 4 | 79 ± 13 |
| Day 7 | | |
| $C_{(0)}$ (ng/mL) | 3.7 ± 0.8 | 14.0 ± 2.7 |
| $t_{max}$ (h) | 12 (6-16) | 12 (6-16) |
| $C_{max}$ (ng/mL) | 5.0 ± 1.0 | 18.2 ± 4.1 |
| $AUC_\tau$ (ng•h/mL) | 109 ± 24 | 399 ± 85 |
| $C_{avg}$ (ng/mL) | 4.5 ± 1.0 | 16.6 ± 3.5 |
| $C_{(24)}$ (ng/mL) | 4.3 ± 1.1 | 15.7 ± 3.5 |
| PTF (%) | 27 ± 8 | 25 ± 6 |
| $t_{1/2}$ (days) | 7.9 ± 2.2 | 8.1 ± 2.0 |

Data are mean ± standard deviation, except for $t_{max}$, which is median (range). $C_{(x)}$, blood concentration × hours postdose; $t_{max}$, time to reach peak; $C_{max}$, peak blood concentration; $AUC_\tau$, area under the concentration-time curve over the dosing interval; $C_{avg}$, average concentration; PTF, peak-trough fluctuation; $t_{1/2}$, half-life.

### Multiple-Dose Pharmacokinetics

Figure 1 shows that predose trough concentrations $C_{(0)}$ and approximate peak concentrations at 6 to 8 hours postdose $C_{(8)}$ increased daily over the multiple-dose treatment phase. From day to day, the ratio of $C_{(0)}$s between dose levels remained relatively constant and averaged 3.8, reflecting the fourfold difference in doses. A similar pattern occurred for $C_{(8)}$. Drug accumulation in blood over the full study course from days 1 to 7 was quantified by the day 7 to day 1 ratio of $AUC_\tau$, which averaged 5.5 ± 0.8 at 1.25 mg/day and 5.0 ± 0.7 at 5 mg/day. If $C_{(24)}$ was used to assess accumulation, a similar result was obtained: 4.9 ± 0.7 and 4.7 ± 0.6 at the two dose levels, respectively. Hence, the overall accumulation in blood over 7 days of daily dosing was fivefold regardless of dose level.

Comparison of the mean concentration profiles on day 7 with those from day 1 emphasizes the flatness of drug exposure over the dose interval during multiple dosing. Nonetheless, the biphasic nature of drug input was still evident, with $t_{max}$ reached at a median 12 hours postdose, similar to the pattern seen after the first dose. On day 7, the difference in exposure between dose lev-

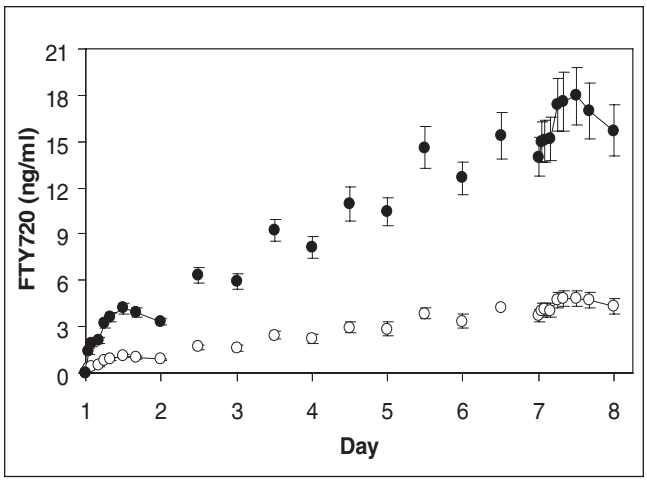


*Figure 1. Mean FTY720 concentration profiles over the dosing interval from days 1 to 7 at 1.25 mg/day (○) and 5 mg/day (●). A full AUC profile was obtained after the first and last doses and trough and peak concentrations on the intermediate days. Bars represent 95% confidence intervals.*

els was consistent, with the fourfold difference of doses based on the dose-normalized parameter ratio (95% CI) for $C_{(0)}$ of 1.06 (0.94-1.21), $C_{max}$ of 1.09 (0.96-1.25), and $AUC_{\tau}$ of 1.09 (0.95-1.25). The flatness of the profiles was underscored by the remarkably narrow peak-trough fluctuation of about 25%. The fact that $C_{(24)}$ was higher than the predose $C_{(0)}$ indicated that steady state was not reached in 7 days, as anticipated.

The decline in concentrations after the last dose was gradual and parallel at the two dose levels. Accordingly, the half-lives were similar between doses and averaged about 8 days. Inasmuch as these values were derived from six concentrations obtained over the month-long washout phase, they are likely robust estimates.

## Lymphocyte Responses

Table II summarizes the lymphocyte response data, and Figure 2 shows the lymphocyte trajectories. The baseline lymphocyte count was similar in all groups. In subjects receiving placebo, mean lymphocyte counts exhibited small fluctuations around the baseline during the 7-day treatment period. The daily mean counts on treatment days ranged from 1.7 to $1.9 \times 10^9$/L. In the washout period, mean counts rose slightly to $2.2 \times 10^9$/L by the end of the study on day 35.

In subjects receiving FTY720, mean lymphocyte counts were decreased at the first postdose lymphocyte sampling time point on day 2 to 0.8 and $0.4 \times 10^9$/L in the low- and high-dose groups, respectively. These represented 58% and 76% reductions from baseline. The absolute lymphocyte nadir subsequently occurred between days 3 to 7 across all FTY720-treated subjects. In subjects receiving 1.25 mg/day, the nadir averaged $0.4 \pm 0.1 \times 10^9$/L, corresponding to an 80% decrease from baseline. In subjects receiving 5 mg/day, the mean nadir count was half that measured in the low-dose group, $0.2 \pm 0.1 \times 10^9$/L, or an 88% decrease from baseline.

Since the lymphocyte nadir occurred across subjects on different days over the course of the weeklong treatment, we chose the cumulative area under the curve of FTY720 trough concentrations from days 1 to 8, or $AUC_{(1-8)}$, as an overall uniform exposure metric. This was used to explore for an exposure-response relationship to lymphocyte nadir. Figure 3 shows that an inhibitory $E_{max}$ model including a baseline parameter could reasonably describe the data. The model estimated a baseline $E_0$ of $1.53 \times 10^9$/L (coefficient of variation [CV] = 4.7%), with a cumulative exposure yielding half-maximal inhibition of 3.0 ng•days/mL (CV = 74.6%). The maximal lymphocyte response ($E_{max}$) was a decrease by $1.37 \times 10^9$/L (CV = 70.0%) to an estimated minimal nadir count of $0.16 \times 10^9$/L.

A gradual lymphocyte recovery was evident during the washout phase between days 9 to 35, as seen in Figure 2. The recovery was more rapid at the lower compared with the higher dose group. At the end-of-study visit, mean absolute lymphocyte counts were $1.4 \pm 0.4 \times$

**Table II** Lymphocyte Response Parameters

| Parameter | Placebo | 1.25 mg FTY720 | 5 mg FTY720 |
|---|---|---|---|
| Baseline lymphocytes ($10^9$/L) | 1.9 ± 0.5 | 1.9 ± 0.5 | 1.7 ± 0.4 |
| Day 2 lymphocytes ($10^9$/L) | 1.8 ± 0.4 | 0.8 ± 0.2 | 0.4 ± 0.2 |
| Nadir lymphocytes ($10^9$/L) | 1.5 ± 0.5 | 0.4 ± 0.1 | 0.2 ± 0.1 |
| Nadir lymphocytes (% of baseline) | 81 ± 20 | 20 ± 6 | 12 ± 4 |
| Time of nadir (day) | 4 (2-8) | 7 (4-7) | 4 (3-7) |
| Day 8 lymphocytes ($10^9$/L) | 1.8 ± 0.6 | 0.5 ± 0.2 | 0.3 ± 0.2 |
| Day 35 lymphocytes ($10^9$/L) | 2.2 ± 0.7 | 1.4 ± 0.4 | 0.8 ± 0.4 |

Values are mean ± standard deviation, except for time of nadir, which is median (range).

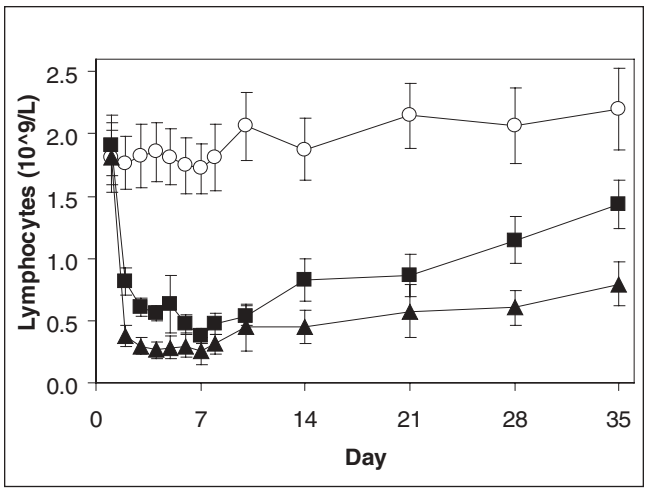


*Figure 2. Mean lymphocyte trajectories over the treatment phase (days 1-8) and during the washout phase (days 9-35) in subjects receiving placebo (○), 1.25 mg/day FTY720 (■), and 5 mg/day FTY720 (▲). Bars represent 95% confidence intervals.*

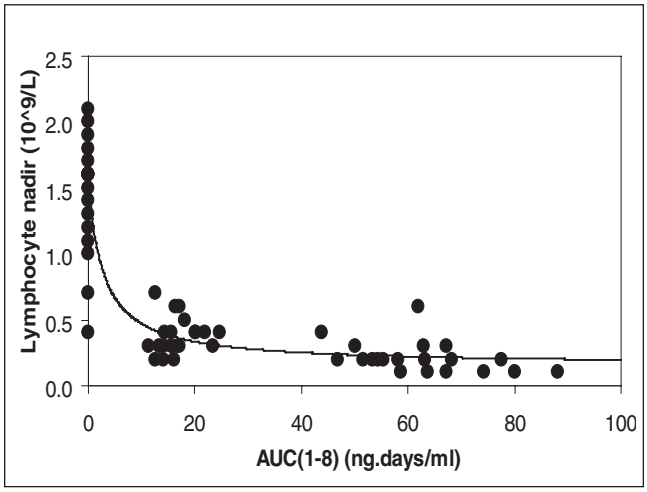


*Figure 3. Relationship between FTY720 cumulative AUC over the weeklong treatment phase (days 1-8) versus the nadir lymphocyte count. Shown is the line from the fit of an inhibitory $E_{max}$ model to the data.*

$10^9$/L, or 74% of baseline in the low-dose group, and $0.8 \pm 0.4 \times 10^9$/L, or 47% of baseline in the high-dose group.

## DISCUSSION

Daily administration of FTY720 over 1 week was well tolerated up to the maximal dose used in this study of 5 mg/day. There were generally mild, transient adverse events for which none of the subjects required treatment. These results are encouraging inasmuch as daily doses up to 5 mg are currently used in clinical trials in renal transplantation. In addition, the disposition of FTY720 observed in these healthy subjects is similar to that in transplant recipients.[8] For example, after a 2-mg single dose (a dose intermediate between those we used in this study), the $C_{max}$ in patients was $1.2 \pm 0.3$ ng/mL, $AUC_{(0\text{-}24)}$ was $23 \pm 5$ ng•h/mL, and half-life was $6.5 \pm 0.8$ days.[8] Hence, healthy subject studies are an appropriate vehicle for learning about the clinical pharmacology of FTY720 under controlled conditions and applying these lessons to the clinical setting with appropriate confirmatory data in the target patient population.

FTY720 peak and total exposure on day 1 at the two dose levels were consistent with dose-proportional disposition. $C_{max}$ and $AUC_{\tau}$ exhibited moderate interindividual variability of 20% or less. The delayed $t_{max}$ that ranged from 8 to 16 hours postdose appears consistent with the slow absorption of dietary sphingolipids inasmuch as FTY720 is a structural analog of this lipid class. Over the treatment phase, blood concentrations accumulated fivefold. The concentration profile during multiple dosing was remarkable for its flatness, as manifested by the narrow peak-trough fluctuation of around 25%. This is consistent with FTY720's prolonged half-life relative to the 24-hour dosing interval. The moderate interindividual variation in exposure observed after the first dose was also the case on day 7, with $C_{max}$ and $AUC_{\tau}$ variation around 22%.

It was anticipated that steady state would not be attained in a weeklong treatment phase, and this was confirmed by the pharmacokinetic data. Rather, this dosing period was chosen to gain short-term experience with multiple dosing in healthy subjects and to provide normative data on the lymphocyte response to FTY720, which reaches its maximal effect in individual patients within a few days after starting treatment and is maintained with continued dosing. Hence, a weeklong treatment phase was deemed adequate to explore this exposure-response relationship. Moreover, the broad range of doses from 1.25 to 5 mg/day and AUCs from 15 to 111 ng•h/mL constituted a robust database for exploring the associated lymphocyte response to FTY720.

Daily morning lymphocyte counts remained relatively stable over the treatment phase in placebo-treated subjects and rose slightly thereafter. In FTY720-treated subjects, lymphocyte counts decreased from baseline by about 60% in the low-dose group and by about 75% in the high-dose group by the morning after

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