of at least 2 hours at 37°C. The rates of drug efflux from erythrocytes to plasma increased with increasing temperature from 4 C to 37C

14C rapamycin: single intragastric dose placental transfer in pregnant rats (protocol 95557)
GTR-33266
29 June 1998

14C-Rapamycin was administered as a single oral (intragastric) suspension (0.5 mg/kg) to timed-pregnant Sprague-Dawley rats on day 15 of gestation. Following 14 C-rapamycin administration, groups of rats (3 rats per group) were sacrificed and maternal blood samples were taken to determine the concentration of rapamycin in blood and the concentration of radioactivity in blood and plasma. Amniotic fluid, placentas and fetuses were collected and pooled by tissue and by litter for total radioactivity determination. Peak concentrations (Cmax) of 14C-rapamycin-related radioactivity in the maternal plasma, maternal blood, and amniotic fluid were 11.2, 14.6, 1.70 ng equiv./mL, respectively, and their corresponding time to reach peak concentrations (tmax) were 0.5, 0.5, and 6 hr post-dosing. The Cmax values of 14C-rapamycin-related radioactivity in the fetal and placental tissues were 6.84 and 21.3 ng equiv./g, respectively, and their tmax values were both 6 hr post-dosing. The Cmax value of rapamycin in the maternal whole blood was 1.70 ng/mL which occurred 0.5 hr post-administration. The AUC0-∞values of the 14 C-rapamycin-related radioactivity in the maternal plasma, maternal blood, and amniotic fluid were 81.6, 111, 59.4 ng equiv.•hr/mL, respectively, and those in the placental and fetal tissues were 691 and 161 ng equiv.•hr/g, respectively. The AUC0-∞ of rapamycin in the maternal whole blood was 6.15 ng•hr/mL. The AUC 0-∞ values of the rapamycin related-radioactivity in maternal plasma and maternal blood were 13- and 18-fold higher, respectively, than that of rapamycin in maternal blood. Radioactivity AUC0-∞ values in placental and fetal tissues were 6.2- and 1.5-fold higher than in maternal blood, respectively, while the AUC0-∞ in amniotic fluid was 0.5-fold that of maternal blood. Rapamycin-related radioactivity crosses the placental barrier providing measurable exposures in placental and fetal tissues. In general, the pharmacokinetics of rapamycin in gravid rats in this study were similar to the pharmacokinetics of rapamycin reported in previous studies.

Rapamycin (rapamune)TM, AY-22989, (sirolimus) 14C-rapamycin: single oral (PO) dose breast milk transfer study in lactating rats
GTR-33214 non glp
8 July 1998

Twenty-five lactating rats (post-partum day 10) received a single oral dose of 0.5 mg/kg of 14C-rapamycin. Milk and blood samples were collected from 4 rats per timepoint at 0.5, 1, 4 and 8 hours after dosing. Transfer of 14C-rapamycin to nursing rat pups was studied in 9 lactating rats and their litters by collecting blood samples from 3 lactating rats/timepoint and their nursing pups (from 2-4 pups/timepoint) at 0.5, 1, 2, 4, 6, 8, 12 and 24 hr after dosing. Milk samples were analyzed by [redacted] for [redacted]. Whole blood was analyzed by [redacted] and by an [redacted] 14C-Rapamycin-derived radioactivity was readily excreted into the milk of lactating rats. However, with one exception, concentrations of total radioactivity were not detected in the blood of nursing pups.

Sirolimus (rapamycin): effect on the liver microsomal drug metabolizing capacity of rats following repeated administration of IG or IV vehicles used with sirolimus
GTR-27830 non glp
6 July 1998

Twenty rats were randomly assigned to the following four treatment groups: iv rapamycin vehicle, i.g rapamycin vehicle, saline and phenobarbital. The iv rapamycin vehicle contained a mixture of propylene glycol (16.6%, v/v), PEG 400 (21.7%, v/v) and polysorbate 80 (0.9%) in sterile water. The i.g rapamycin vehicle consisted of Phosal 50 PG (9.9%, v/v) and polysorbate 80 (0.1%, v/v) in sterile water. Physiological saline was administered to the control animals, and phenobarbital in

saline (20 mg/ml) was administered intraperitoneally (80 mg/kg). Each treatment group received single daily doses for 7 days except the phenobarbital group, which received daily injections for only 3 days. The liver microsomal cytochrome P-450 content for individual animals within the various treatment groups was similar for the two vehicle groups (iv and i.g) and the saline control group, with mean values between 0.65 and 0.91 nmol/mg protein. The vehicle groups were not significantly different from the saline group. The cytochrome P-450 concentration for the phenobarbital positive induction group was statistically different from the saline control group with a mean value of 1.61 nmol/mg. Liver microsomal aminopyrine N-demethylase activity for the various treatment groups was also examined. The vehicle groups and saline control group had similar mean values ranging between 4.29 and 4.61 nmol/min/mg protein. Phenobarbital pre-treatment produced an increase in the aminopyrine N-demethylase activity relative to the saline control group with a mean of 10.42 nmol/min/mg. The in vitro metabolism of rapamycin was examined in liver microsomes that were obtained from various treatment groups. The amount of rapamycin remaining after a one hour incubation period was used to assess the extent of metabolism. Based on the amount of rapamycin remaining after the incubations, all treatment groups, including the phenobarbital group, showed similar extents of metabolism.

***In vitro* rat hepatic microsomal metabolism of rapamycin**
GTR-21275 non glp
19 May 1992

The *in vitro* transformation of rapamycin was examined using liver microsomes isolated from control and induced rats. Phenobarbitone or 3-methylcholanthrene did not induce the in vitro metabolism of rapamycin. Rapamycin was transformed to at least 8 products (peaks A-H). Two of these peaks (A and E) appeared to be due to degradation. With negative controls (heat inactivated protein, no added NADPH), the remaining peaks appeared to be formed by enzymatic processes, possibly catalyzed by the cytochrome P-450 dependent mixed function oxygenates. The major route of in vitro transformation of the drug was degradation to [redacted]. The extensive first-pass clearance of rapamycin may be due to a combination of both oxidative and degradative processes.

***In vitro* rat, dog, monkey and human microsomal metabolism of rapamycin**
GTR-21607 non glp
13 July 1992

The *in vitro* transformation of rapamycin was examined using liver microsomes isolated from beagle dogs, cynomolgus monkeys and from human transplant donors. Negative controls (heat inactivated protein, no added NADPH) indicated that in all of the investigated species, rapamycin is transformed by both NADPH dependent processes, most probably involving the cytochrome P-450 dependent mixed function oxygenates, and by simple non-enzymatic decomposition. [redacted] of the rat *in vitro* microsomal products with those formed by dog, monkey and human microsomes indicated that the degradation products A and E and metabolites C, D, G, and H were common to all species, but the metabolic profile differed between species. Dog microsomes produced at least two unique metabolites of rapamycin. Both monkey and four of the nine human livers appeared to form a large number of more polar rapamycin metabolites. Qualitative differences were seen in the metabolic profiles of rapamycin with microsomes from the human livers, although the rate of rapamycin turnover varied only 2.2 fold. One human liver was unique in its ability to form large amounts of a metabolite which co-eluted with [redacted] formed in the rat. This [redacted] was absent or very small with microsomes from all of the other human livers. [redacted] of the products of rat microsomal rapamycin incubations with the product of rapamycin excreted in rat bile indicated that the *in vitro* microsomal and biliary metabolites and degradation products of rapamycin are identical. Based on characterization of the rat biliary rapamycin metabolites, the cytochrome P-450 dependent microsomal metabolites are formed by hydroxylation, demethylation or a combination of these processes. The major *in vitro* degradation product of rapamycin [redacted] co-elutes with a major biliary peak which is an isomer of rapamycin. The combination of both cytochrome P-450 dependent metabolism and nonenzymatic decomposition contribute to the extensive first pass clearance and low bioavailability of rapamycin.

Rapamycin: isolation and characterization of two major *in vitro* degradation products
GTR-21857
14 December 1992

Two major degradation products, compounds A and B were isolated after incubation of rapamycin in rat bile. Both A and B were previously observed in the bile of a rat receiving rapamycin intravenously. B had less than 4% of the potency of rapamycin in the thymocyte proliferation assay, while A was inactive. Both compounds are non-enzymatically formed ring-opened metabolizes of rapamycin that occur in significant amounts in rat bile but are biologically inactive. Their formation occurs by hydrolysis of the ester linkage at the C25 ester bond. This instability may account for the low bioavailability and high first pass catabolism of rapamycin in rats and other species .

Biliary rapamycin metabolites in rats receiving rapamycin intravenously (II)
GTR-21776 non glp
31 August 1992

Twelve rats were dosed intravenously with rapamycin at 1.3 mg/kg. The ethyl acetate extracts of bile collected from three rats at 0-4, 4-8, 8-12 and 12-24 hr after dosing were analyzed by [redacted] The biliary metabolize profiles were similar in the three animals. The relative concentration of the metabolizes varied with time. Trace amounts of rapamycin were detected in the 0-4 hr bile, and virtually no rapamycin could be found in the 4-8, 8-12 and 12-24 hr bile samples. At least sixteen rapamycin metabolites or isomers were recognized by [redacted] Five of the major metabolites were further investigated by [redacted] analysis. Structures were proposed for these five metabolites. The major types of metabolic transformations were monohydroxylation, dihydroxylation, and demethylation plus monohydroxylation. Rapamycin appears to be extensively metabolized in the rat.

Rapamycin (sirulimus): isolation of drug-derived products from dexamethasone-induced rat liver microsomal incubation of rapamycin-structural elucidation by [redacted] and the assay of *in vitro* immunosuppressive activity
GTR-30346 non glp
8 December 1997

In this study, six rapamycin metabolite fractions were isolated from an incubation of rapamycin in dexamethasone-induced rat liver microsomes, which were shown to generate a metabolite profile similar to that in the systemic circulation of renal transplant patients or normal volunteers after rapamycin administration. These fractions appeared as the major [redacted] in the incubation, and were characterized by [redacted] as didemethyl rapamycin/hydroxy rapamycin [redacted] 7-O-demethyl rapamycin/hydroxy rapamycin (peak 3), 'southern'-hydroxy rapamycin [redacted] 'southern'-hydroxy rapamycin [redacted] seco-rapamycin (peak 8), 41-O-demethyl [redacted] and unchanged drug. The structures of [redacted] were confirmed by [redacted] as 11-hydroxy rapamycin and 41-O-demethyl rapamycin, respectively. Control samples were isolated similarly from incubations in the absence of rapamycin. The *in vitro* immunosuppressive activity of isolated rapamycin fractions was determined in a thymocyte proliferation assay [redacted] (mean IC50 of 2.1 nM) and [redacted] (mean IC50 of 3.6 nM) were the most potent rapamycin metabolites tested, representing approximately 30% and 20%, respectively, of the activity of rapamycin (mean IC50 of 0.67 nM) isolated under identical conditions. The activity of other drug-derived [redacted] (mean IC50 of 17 nM to 45 nM) was approximately 25-70 fold less than that of rapamycin isolated similarly. These results support the sponsor's claim of the relative inactivity of rapamycin metabolites. It would be of interest to determine if these metabolites interfere with the activity of the parent compound.

Rapamycin (sirolimus): metabolite characterizations in rat and human liver microsomes, and in trough whole blood of renal transplant patients following multiple (26 weeks) oral administration of rapamycin (1-5 mg/m^2/day) and cyclosporine (3-6 mg/kg/day)
GTR-27030
15 March 1996

In vitro findings were used to facilitate the determination of rapamycin metabolite profiles in trough (24 hour) whole blood of renal transplant patients, who received (26 weeks) oral doses of rapamycin (1-5 mg/m 2 /day) and cyclosporine (3-6 mg/kg/day). In rat and human liver microsomal incubations with rapamycin, the oxidative rapamycin metabolites were a characterized as three demethylated and at least eight monohydroxylated species. At least seven dihydroxy and two didemethylated metabolites were also detected, though at low levels. In contrast, virtually all monohydroxylations occurred on the 'southern' fragment of rapamycin. Demethyl rapamycin were characterized as 7-O-demethyl rapamycin (WAY-138769 and WAY-138772) and 32-O-demethyl rapamycin (WAY-125286), respectively, due to their with the respective reference standards. The ring-open rapamycin isomer 'seco-rapamycin' (WAY-126792) was produced in a non-P-450-dependent manner, which is consistent with previous data. Preliminary evidence showed that presumably 41- 2,3,4 O-demethyl rapamycin) and authentic WAY-125286 (32-O-demethyl rapamycin) were converted to their corresponding seco-isomers in human liver microsomal incubations. The retention time of the seco-isomer of H (presumably 41-O-demethyl rapamycin) was also similar to that of demethyl rapamycin suggesting that consisted of both 7-O-demethyl rapamycin and seco-41-O-demethyl rapamycin.

Sirolimus (rapamycin): biotransformation in male rats after a single oral (gavage) dose of the 14C-radiolabeled drug (6 mg/kg)
GTR-33024 non glp
20 April 1998

In this study, the biotransformation of rapamycin was studied in male rats after a single oral (gavage) 6 mg/kg dose of the 14C-radiolabeled drug. Blood samples (n = 4 per time point) were collected prior to drug administration and at 1, 4 and 8 hr post-dose concentrations of total radioactivity in blood (143.6 ± 34.2 ng parent equivalent/mL, mean ± SD) and in plasma (123.5 ± 34.8 ng parent equivalent/mL, mean ± SD) occurred at 4 hours after drug administration. The blood to plasma (B/P) concentration ratios of radioactivity remained relatively constant between 1 hour (B/P ratio of 1.4 ± 0.1, mean ± SD) and 8 hours (B/P ratio of 1.4 ± 0.2, mean ± SD) post-dose. The observed B/P ratios of radioactivity suggested that drug-derived products also partitioned into formed blood elements, though apparently to a lesser extent than the parent drug itself (B/P ratios reported to range from 3 to 9). The metabolite profiles of rapamycin in blood and plasma were determined by with radioactivity detection and by In blood, rapamycin was the most dominant drug-related product present, while other drug-derived products were characterized as 7-O-demethyl rapamycin (C), 32-O-demethyl rapamycin (I), 41-O-demethyl rapamycin (H), hydroxy rapamycin (A, B and C', D, E', F and G) and the macrocyclic ring-opened isomer seco-rapamycin. In plasma, rapamycin and seco-rapamycin appeared to be the major drug-related products present, while metabolites such as demethyl metabolites C, H and I, and hydroxy metabolites D, E', F and G, were also detected. Little didemethyl rapamycin, hydroxy-demethyl rapamycin or dihydroxy rapamycin were detected in blood or plasma. Based on integration of major drug-derived in the rapamycin was estimated to represent approximately 40% to 60% of total blood radioactivity between 1 hour and 8 hours post-dose, while the contribution of other drug-derived products individually ranged from approximately 4% to 11%. Due to the relatively low levels of radioactivity in plasma extracts, the contribution of rapamycin and drug-derived products was not determined. Rapamycin underwent primarily demethylation and hydroxylation in rats after a single oral dose of the drug. Rapamycin remained the major drug-related product present in blood.

Sirolimus (rapamycin): biotransformation in cynomologus monkeys after a single oral (gavage) dose of the 14C-radiolabeled drug (5 mg/kg)
GTR-32952 non glp
31 March 1998

The biotransformation of rapamycin was studied in three male cynomolgus monkeys after a single oral (gavage) 5 mg/kg dose of the 14C-radiolabeled drug (approximately 100 mCi). Blood samples were collected prior to drug administration and at 2, 4, 12, 24 and 48 hours post-dosing. The absorption of drug-related material was rapid; concentrations (Cmax) of total radioactivity in blood (126 ±19 ng equiv./mL, mean ± SD) occurred at 2 hours (the earliest time point) after drug administration. Peak plasma radioactivity concentrations (77±17 ng equiv./mL) occurred between 2 hours (the earliest time point) and 12 hours, and may in part be related to inter-animal variability in blood to plasma partitioning of drug-related products and in plasma drug clearance. The blood to plasma (B/P) concentration ratios of radioactivity declined steadily between 2 hours (B/P ratio of 1.7 ± 0.3) and 48 hours (B/P ratio of 0.4 ± 0.2) after drug administration. The higher concentrations of total radioactivity in blood than in plasma suggested that drug-derived products also partitioned into formed blood elements, though apparently to a much lesser extent than the parent drug itself (B/P ratios reported to be >20). The observed decline of radioactivity B/P ratio with time may also reflect a change in the relative concentrations of rapamycin and its metabolites over time, together with differences in their partitioning into formed blood elements. In blood, rapamycin metabolites were characterized as didemethyl rapamycin (B'), 7-O-demethyl rapamycin (C), 41-O-demethyl rapamycin (H), hydroxy rapamycin (A, B and C'), hydroxy-demethyl rapamycin (A') and several dihydroxy rapamycin metabolites. Rapamycin was approximately 26% and 42% of total blood radioactivity at 2 hours and 12 hours, respectively. Due to the co-elution of some metabolites, the individual contribution of these metabolites could not be determined. The metabolite in blood decreased between 2 hours and 12 hours after drug administration, consisting of up to 20% of radioactivity in blood. Rapamycin underwent primarily demethylation and hydroxylation in cynomolgus monkeys after a single oral dose of the drug, while rapamycin remained the major drug-related product present in blood. The metabolite profile of rapamycin in blood of monkeys is very similar to that of healthy human subjects or renal transplant patients after drug administration.

Rapamycin (sirolimus): biotransformation in healthy male subjects after a single oral dose of the 14C-radiolabeled drug (nominal 40 mg)
GTR-29528 non glp
15 September 1997

The metabolic disposition of rapamycin was studied in six healthy male subjects after a single nominal 40 mg oral dose of the 14C-radiolabeled drug (100 mCi). Blood samples were collected prior to drug administration (1 x 10 mL) and at 0.5, 1, 2, 4, 12, 24, 48, 72, 96 and 144 hours (4 x 10 mL each) post-dosing. Plasma was prepared from aliquots of blood samples. Urine was collected at 0-12, 12-24 and each 24 hour interval after drug administration until day 15. Feces were collected until day 15. Rapamycin metabolite profiles in blood of individual subjects at 1 and 4 hours post-dosing were determined by whereas those of pooled samples at 2, 12 and 24 hours were in addition analyzed by The absorption of drug-related material was rapid, with peak concentrations (Cmax) of total radioactivity in blood (267±35 ng equiv./mL, mean ± SD) and plasma (68 ± 17 ng equiv./mL) at 1.3 ±.5 hours and 2.0 ± 1.1 hours, respectively. Mean exposure of radioactivity in blood based on Cmax was approximately 4-fold higher than in plasma, while exposure based on AUC0-144h was approximately 2.7-fold higher than in plasma (5371 vs. 2004 ng equiv. h/mL). The higher concentrations of total radioactivity in blood than in plasma suggested that drug-derived products also partitioned into formed blood elements, though apparently to a much lesser extent than the parent drug itself. Rapamycin concentrations in blood were measurable over the entire 144 hour time interval, while plasma concentrations were measurable only over the initial 0.5 to 24-hour period. Rapamycin showed rapid absorption and a long elimination half-life (t1/2), as reflected by estimated tmax of 1.3 ± 0.5 hours (mean ±SD) and t1/2 of 59.8 ± 9.7 hours in blood. Rapamycin plasma exposure (Cmax 2.13 ±1.1 ng/mL, AUC0-24h 5.98 ± 3.67 ng.h/mL) was much lower than blood exposure (Cmax 144 ± 32 ng/mL, AUC 0-24h 909 ± 240 ng.h/mL). The rapamycin B/P ratio of 142 ±39 is consistent with the extensive partitioning of the

drug into formed blood elements. Rapamycin represented an average 68% of total blood radioactivity at 0.5 hours after drug administration, and decreased to an average 33% at 24 hours. Based on AUC0-144h values, rapamycin represented approximately an average 35% of total radioactivity in blood. Major metabolites were characterized as 41-O-demethyl rapamycin, 7-O-demethyl rapamycin, and several hydroxy, hydroxy-demethyl and didemethyl rapamycin derivatives. Rapamycin metabolites were estimated to individually represent less than an average 15% of total drug-derived products monitored between 2 and 24 hours following drug administration. In plasma, rapamycin represented only <5% of total radioactivity at 0.5 hours and <1% at 24 hours following drug administration. In plasma samples pooled from all subjects at 1 and 2 hours after drug administration, low levels of unchanged drug, 41-O-demethyl rapamycin and several monohydroxylated metabolites were present. Most (average >90%) of the orally administered dose of 14C-rapamycin was excreted into feces, with most of the radioactivity excreted within the first several days. Rapamycin metabolite profiles were determined in representative fecal samples (1-2 or 2-3 days of five subjects) [redacted] indicated that virtually all the radioactivity eluted at the polar region of the [redacted] In the fecal samples, low levels of unchanged drug, seco-rapamycin and oxidative metabolites were found. In urine, analyses of representative samples (0-0.5, 0.5-1 or 2-3 days of five subjects) also indicated a preponderance of polar drug-derived product(s). Glucuronide or sulfate conjugates of rapamycin metabolites did not appear in any of the samples analyzed. Rapamycin was extensively metabolized in healthy human male subjects after a single oral dose.

Sirolimus (rapamycin): biotransformation of its primary metabolites 41-O-demethyl sirolimus, 32-O-demethyl sirolimus and 7-O-demethyl sirolimus, and the macrocyclic ring-opened isomer seco-sirolimus, in human liver microsomes
GTR-29756 no glp
20 May 1998

The biotransformation of sirolimus has been extensively studied *in vivo* in mice, rats, monkeys and man , and *in vitro* in liver microsomes from rats, dogs, monkeys and humans. Sirolimus is metabolized primarily by cytochrome P450 3A (CYP3A) to a large number of monodemethylated, monohydroxylated, didemethylated, dihydroxylated, and demethylated-hydroxylated metabolites. In human liver microsomes, 41-O-demethyl sirolimus, but apparently not 32-O-demethyl sirolimus, 7-O-demethyl sirolimus or seco-sirolimus, underwent NADPH-dependent oxidations to form various didemethyl and demethyl-hydroxy metabolites. The major metabolites were didemethylated (B') and demethyl-hydroxy (A') metabolites. In addition, 41-O-demethyl sirolimus and 32-O-demethyl sirolimus, and to a much lesser extent 7-O-demethyl sirolimus, were converted to their respective macrocyclic ring-opened isomers in human liver microsomes in a non-NADPH dependent manner. The more dominant ring-opened hydroxy metabolite (peak C', formerly referred as C) was detected in the blood of cynomolgus monkeys and human subjects, and to a lesser extent in the blood of rats and mice after sirolimus administration. The primary metabolic pathways are cytochrome P450 (primarily CYP3A4)-catalyzed demethylations at the 41-O-, 32-O- and 7-O-methyl positions to form the corresponding O-demethylated metabolites, and hydroxylations on the southern fragment of sirolimus to form various hydroxy metabolites. 41-O-Demethyl sirolimus is further oxidized to various didemethyl or demethyl-hydroxy metabolites. Sirolimus and its demethyl and hydroxy metabolites also underwent non-CYP-catalyzed process to form their corresponding macrocyclic ring-opened isomers. With the possible exception of macrocyclic ring-opening to form seco-sirolimus, the in vitro metabolite profile of sirolimus determined in this study is similar to that reported in the blood of humans after drug administration.

Sirolimus (rapamycin); excretion of radioactivity by cynomologus monkeys following a single intravenous dose (0.1 mg/kg) of the [14C] labelled drug
GTR-25740 non glp
16 July 1998

Four male cynomolgus monkeys were administered a single intravenous (0.1 mg/kg) dose of [14C] rapamycin. Urine, cage wash and feces were collected daily for 17 days. Urine and cage wash samples were analyzed directly by [redacted] Feces were homogenized, digested with Soluene-350,

decolorized and then analyzed by [redacted] The recovery of radioactivity was virtually complete, with a mean ±SD value of 97.9 ± 0.4% by 17 days. Greater than 90% of the radioactive dose was recovered by 7 days post-dose, while only 39% was recovered during the first 48 hours. Individual animals showed very low variability in the overall recovery (97.3 to 98.2%). Most of the recovered radioactivity (90.3± 2.0%) was found in the feces. Only a small amount of the administered dose (7.6 ±1.6%, mean ±SD) appeared in the urine by day 17 after dosing. It appears that after intravenous administration of [14C]-sirolimus, fecal excretion was the predominant route of elimination and renal excretion was a minor pathway. Excretion of most radioactivity occurred within 7 days after dosing.

Rapamycin (*sirolimus*): mass balance and excretion of radioactivity following a single oral dose of 14C-rapamycin (42 mg) in healthy male subjects
GTR-26642
4 December 1997

Six normal male volunteers each received a 42 mg (116.5 µCi) oral dose of 14 C-Rapamycin. Blood was drawn at intervals through 144 hours. Blood and the corresponding plasma were analyzed for concentrations of radioactivity. Urine and feces were collected over 15 days following dosing and analyzed for radioactive content. The results of blood and plasma analysis are to be reported under separate cover. The mean total recovery from urine and feces was 93.2% of the administered radioactivity, with the majority of the recovered radioactivity, 91.1%, recovered from the feces. The urine was a minor route of elimination, with 2.2% excreted by this route. Excretion was protracted, with 6.6% recovered in the first 24 hours and 89.7% in the first 5 days. The results of this study are similar to mass balance data in the rat.

Microbiological assay for rapamycin in cynomolgus monkey serum
GTR-18889 non glp

A microbiological assay for the determination of rapamycin in cynomolgus monkey serum was developed using *Candida albicans* ATCC 38247. The calculated detection limit was 0.0028 pg/ml. The experimental detection limit was =0.006 pg/ml.

Microbiological assay for rapamycin in human serum and plasma
GTR-19511 non glp

A microbiological assay for the determination of rapamycin in human serum and plasma was developed using *Candida albicans* ATCC 38247 The standard curve points are 100, 50, 25, and 12.5 ng/ml. Based on statistical analysis of data from 20 separately weighed rapamycin standard curves including control serum, a good model fit was observed using a nonlinear model [linear regression on log (concentration + C); correlation coefficient (R2) > 0.9851. The calculated detection limit was 2.48 ng/ml. The experimental detection limit was 6 ng/ml. Employing the same experimental design and statistical analysis, data from the rapamycin plasma standard curves also showed a good model fit. The calculated detection limit was 3.76 ng/ml. The experimental detection limit was 6 ng/ml. The presence of therapeutic levels of azathioprine, dexamethasone, prednisone, acyclovir, and sulfamethoxazole/trimethoprim did not interfere with the assays.

A [redacted] method for the determination of AY-22989 (rapamycin) in mouse plasma
GTR-26291 non glp
14 September 1995

Rapamycin and an internal standard (AY-24668) were extracted from mouse plasma on a [redacted]
Quantitation was based on [redacted] Using a 0.2 ml sample, the assay was linear from 50 to 3000 ng/ml. Intra-day precision and accuracy ranged from 4.7 to 10.1% and 6.9 to 18.0%, respectively. Inter-day precision and accuracy values ranged from 9.1 to 17.4% and -7.2 to 3.5%, respectively. Blinded

samples produced results within 20% of expected values. In mouse plasma the minimum quantifiable concentration was 50 ng/ml.

AY-22989/rapamycin/siolimus/rapamune [TM]: rapamycin: a [redacted] method for the determination of rapamycin in mouse whole blood
GTR-29789
29 April 1997

This method uses a [redacted] for detecting rapamycin in whole mouse blood. Using a 0.2 mL whole blood sample, the assay is linear over the range of 50 to 6000 ng/mL for rapamycin and the minimum quantifiable concentration for rapamycin is 50 ng/mL in mouse whole blood with this method.

Measurement of rapamycin in whole blood using reverse phase [redacted]
GTR-20653 non glp manuscript
20 May 1992

[redacted] analysis was performed [redacted] Using 2.0 ml of human whole blood for extraction, a sensitivity of 1 ng/ml could be achieved. Using a blood volume of 4.0 ml, sensitivity could be increased with the limit of detection of 0.5 ng/mL. The assay was found to exhibit linearity up to a concentration of 250 ng/l.

Method validation for the determination of rapamycin (sirolimus) in human whole blood by [redacted]
GTR-33556 non glp manuscript
11 June 1998

This report describes an [redacted] method used [redacted] to determine rapamycin concentrations in human whole blood. A concentration range of 5 to 50 ng/ml was validated.

Rapamune [TM]: an [redacted] method for the quantitation of rapamune in rat whole blood [range 0.05 to 2 ng/ml] (protocol 96991)
GTR-32861 non glp
1 April 1998

This [redacted] method utilized 0.5 mL of rat whole blood spiked with internal standard and extracted with 1-chlorobutane. The assay was linear over the range of 0.5 to 100 ng/mL. The intra-day coefficient of variation (precision) of the assay ranged from 4.6 to 8.8%. The accuracy of the method, expressed as % bias, ranged from -12.5 to 10.7%.

Rapamune [TM]: an [redacted] method for the quantitation of rapamune in rat whole blood [range 0.05 to 2 ng/ml] (protocol 97744)
GTR-33157 non glp

An [redacted] method utilized 0.5 mL of rat whole blood diluted with an equal volume of physiological saline, spiked with internal standard, and extracted with 1-chlorobutane. The assay was linear over the range of 0.05 to 2 ng/mL. The intra-day coefficient of variation (precision) of the assay ranged from 2.1 to 9.9%. The accuracy of the method, expressed as % bias, ranged from -7.3 to 16.4%. Inter-day variation was done with independently prepared quality control samples. The overall coefficients of variation ranged from 5.9 to 9.9% and the overall accuracy for the low, mid, and high QC samples, expressed as the % bias, ranged from -0.7 to 8.4%.

Rapamune [TM]: an [redacted] method for the quantitation of rapamune in rabbit whole blood [range 0.05 to 2 ng/ml] (protocol 97788)
GTR-33090 non glp
20 April 1996

This [redacted] method utilized 1.0 mL of rabbit whole blood spiked with internal standard and extracted with [redacted] using 1-chlorobutane. The assay was linear over the range of 0.1 to 50 ng/mL. The intra-day coefficient of variation (precision) of the assay ranged from 0.8 to 4.2%. The accuracy of the method, expressed as % bias, ranged from -12.3 to -4.0%. Inter-day variation was done with independently prepared quality control samples. The overall coefficients of variation ranged from 7.0 to 8.7% and the overall accuracy for the low, mid, and high QC samples, expressed as the % bias, ranged from -2.7 to 7.8%.

Method validation for the quantitation of rapamycin in pig whole blood by [redacted] conducted at [redacted]
GTR-30230 non glp
1 August 1997

This report presents the validation data for an [redacted] method for the determination of rapamycin in pig whole blood. The method was cross validated from the previously validated method for human whole blood. This method is linear between 0.400 and 400 ng/mL.

[redacted] methods for the determination of rapamycin (AY-22989) in human whole blood and plasma. [redacted] assay
GTR-22130 non glp
28 October 1992

This [redacted] method for the quantitation of rapamycin in human plasma and whole blood employs a [redacted] and incorporates both a [redacted] The whole blood assay monitors the sodium adducts of rapamycin and the internal standard (29- O-desmethoxy rapamycin, AY-24,668) by selected [redacted] The different adduct formation is achieved by replacing the sodium acetate in the sheath flow buffer in the whole blood assay with ammonium acetate for the plasma assay. For both assays, there was no interference by endogenous components with the detection of either rapamycin or internal standard. Based on a one ml sample size, the limitation of quantitation for both assays was 250pg/ml. The method was linear from 250 to10,000 pg/ml in plasma and from 250 to 50,000pg/ml in whole blood.

Validation report: the quantitation of rapamycin in human whole blood by [redacted]
GTR-29630 non glp
7 May 1997

This report presents the validation data for a [redacted] method for the determination of rapamycin in whole blood by [redacted] This method, is linear from 0.100 to 100 ng/mL for use on samples collected during clinical studies.

Assay validation report for the quantitation of sirolimus in human whole blood using an [redacted] method
GTR-25539
10 November 1995

This report describes an [redacted] method used by the Huddinge University Hospital to determine rapamycin concentrations in human whole blood. A concentration range of 0.253 to 50.6 ng/ml was validated.

Validation of [redacted] method for analysis of rapamycin in human whole blood
GTR-32096 non glp
6 February 1998

This report presents the validation data for a [redacted] method for the determination of rapamycin in whole blood by [redacted] This method, is linear from 0.100 to 100 ng/mL for use on samples collected during clinical studies.

Method validation for the [redacted] analysis of sirolimus in human whole blood
GTR-33558 non glp
11 June 1998

This report describes an [redacted] method used by the [redacted] Australia, to determine rapamycin concentrations in human whole blood. A concentration range of 0.2 to 100 ng/ml was validated.

AY-22989/siroliimus/rapamune: an [redacted] assay for the determination of rapamune in whole blood (protocol no. 96935)
GTR-31395 non glp
1 December 1997

This report describes an [redacted] assay for the detection of rapamycin in human whole blood based on [redacted] technology. Based on a 0.15 mL whole blood sample, the calibration range was 3 to 30 ng/mL.

Validation of the [redacted] to measure sirolimus in human whole blood samples
GTR-33032 non glp
15 January 1998

This report describes the validation of the [redacted] from [redacted] in human whole blood samples over a concentration range of 1.5 to 30 ng/mL.

The validation of an [redacted] assay to measure AY-22989 (rapamycin) in blood
GTR-34316 non glp
21 September 1998

This report describes an [redacted] method used by [redacted] to determine rapamycin concentrations in human whole blood. A concentration range of 6.5 to 356.4 ng/ml was validated.

Validation of sirolimus [redacted] conducted at the [redacted]
GTR-34138
21 August 1998

This report describes the validation of the [redacted] from Centre for Clinical and Experimental Therapeutics, Department of Medicine, University of Queensland at Princess Alexandra Hospital, Australia in human whole blood samples over a concentration range of 1.5 to 30 ng/ml.

Sirolimus (AY-22989), rapamycin, rapamune™: stability and additional validation data for the quantification of sirolimus in human whole blood and plasma using [redacted] (protocols DM931093, 95774, DM941018, 95775, 96766, 96964)
GTR-33371
13 August 1998

Long-term storage stability studies were conducted to evaluate the stability of rapamycin in human whole blood and plasma stored at -20°C and -80°C. Rapamycin was stable in whole blood for 231 days at -20°C and for at least 1366 days at -80°C. At concentrations ranging from 0.3 ng/mL to 5 ng/mL rapamycin in plasma was stable at -20°C for 568 days. At a concentration of 40 ng/mL plasma samples were stable for at least 6 days when stored at -20°C. At all concentrations tested, rapamycin in plasma stored at -80°C was stable for 1017 days. Rapamycin concentrations were determined by [redacted] method. Rapamycin spiked in whole blood at concentrations from 0.3 ng/mL to 40 ng/mL and whole blood samples prepared from pools of dosed patients were stored at 25°C and 37°C. The samples were stable for up to 28 days when stored at 25°C. Samples stored at 37°C were not stable. The rapamycin stock solution stored at -80°C was stable for 762 days when assayed in whole blood at concentrations ranging from 0.3 ng/mL to 40 ng/mL. Diluted standard solutions stored at -80°C and assayed in whole blood were stable for at least 11 days. Extracts from whole blood and plasma were stable when stored at -80°C for 12 days. Quality control sample extract from whole blood were not stable when stored at room temperature for 12 days. Samples were stable when extracted before or after freezing. The rapamycin assay was not affected by the presence of cyclosporine in the whole blood samples.

Sirolimus (rapamycin): inhibition of CYP3A catalyzed demethylation and hydroxylation by cyclosporine in human liver microsomes-estimate of in vivo drug interaction
GTR-32184
2 April 1998

The kinetics of the demethylation and hydroxylation of rapamycin, and the inhibition of its biotransformation by cyclosporine, were examined in human hepatic microsomes prepared from three individual donor livers. Liver microsomal CYP3A4 activities were assessed by measuring the rates of testosterone 6b-hydroxylation. Apparent Km's (mean ± SD of three livers) were 8.7 ± 3.9 mM for Metabolite C (7-O-demethylation/hydroxylation), 6.7 ± 2.8 mM for Metabolite D (hydroxylation), 2.1 ± 0.13 mM for Metabolite F (hydroxylation), and 7.7 ± 2.8 mM for Metabolite H (41-O-demethylation). Corresponding Vmax's (mean ± SD of three livers) were 13.8 ± 13.0 pmol/mg/min, 34.6 ± 30.0 pmol/mg/min, 11.9 ± 1.9 pmol/mg/min, and 36.6 ± 22.6 pmol/mg/min. Cyclosporin competitively inhibited the formation of Metabolites C, D and H but not F. Ki's for the inhibition of C, D and H formation by cyclosporin were estimated by plotting the slopes of the Lineweaver-Burk plots versus inhibitor concentrations. Ki values (mean ± SD of three livers) obtained were 18.9 ± 10.5 mM for C, 10.1 ± 3.1 mM for D and 8.3 ± 1.6 mM for H. Ketoconazole markedly inhibited the formation of C, D and H but not F, which suggested that the formation of F was catalyzed by CYP isozyme(s) other than CYP3A. Using an overall Ki of approximately 10 mM determined in vitro for cyclosporin, and reported peak cyclosporin blood concentrations of approximately 2.5 mM in renal transplant patients, cyclosporin was predicted to inhibit the metabolism of rapamycin by approximately 20%. Since cyclosporin and rapamycin are metabolized by small intestinal mucosa, the contribution of the small intestine to total drug clearance and drug interactions in vivo should be considered. Both cyclosporin and rapamycin are also substrates of P-glycoprotein. In addition, cyclosporin is an inhibitor of the transporter protein. The inhibition of P-glycoprotein by cyclosporin in the small intestine and liver could lead to an increased absorption and decreased biliary elimination of rapamycin, respectively, in renal transplant patients given the two drugs.

Rapamune™: 13 week oral (gavage) phospholipidosis study in male rats with a 4 week recovery: bioanalytical report (protocol 96248)
GTR-33812 non glp
20 July 1998

Male Charles River CD rats (40 /group) received either control vehicle or 6 mg/kg/day of rapamycin by oral gavage for at least 12 weeks after which time 20 animals in each group were sacrificed and alveolar macrophages were harvested. After a recovery period of at least 4 weeks, the remaining animals were sacrificed and the alveolar macrophages harvested. One rat in the treated group died during recovery; reason not provided. Measurable concentrations of rapamycin were only present in samples from the group sacrificed after at least 12 weeks of dosing without a recovery period. These concentrations ranged from 0.161 to 3.85 ng/million cell lysate equivalent. Samples from animals in the rapamycin treated group following at least 4 week recovery period did not show measurable concentrations of rapamycin.

TOXICOLOGY SUMMARY

Nonclinical toxicology studies performed with rapamycin included acute PO and IV studies in mice and rats; 1-month repeated-dose IV studies in rats and monkeys; and repeated-dose PO studies in mice for up to 3 months, in rats for up to 1 year (with 1- and 3-month recovery periods in the 3- and 6-month studies, respectively), and in monkeys for up to 6 months (with a 3-month recovery period in the 6-month study). Repeated-dose PO, IV, and intravaginal studies for up to 1 month were conducted in dogs. Additional studies were conducted to investigate the nature of findings seen in rat toxicity studies, such as the occurrence of lameness/bone effects, pulmonary lesions, testicular atrophy and myocardial degeneration. Studies in dogs as a non rodent species were not utilized due to findings in studies of up to 1 month duration in which the species sensitivity of dogs was demonstrated. Myocarditis and gastrointestinal tract ulceration leading to study deaths and sacrifices in extremis, as well as necrotising fibrinoid vasciculitis of arterioles, precluded further studies. A synopsis of the individual organ system toxicities follows, with the majority of toxicity information from rat studies.

Lymphoid atrophy (spleen, lymph nodes, thymus) was seen throughout studies conducted in mice, rats, dogs and monkeys as the pharmacologic effect of rapamune. Studies in cynomologus monkeys typically had findings including increased fibrinogen, diarrhea/loose stool, increased red blood cell counts, hemoglobin and hematocrit (up to 3 months). Diarrhea was attributed to drug-induced colitis. Colitis (with diarrhea and loose stool) was also seen in 6 month studies as well as increased fibrinogen (doses ≥ 0.25 mg/kg). Red blood cell counts, hematocrit and hemoglobin were decreased, however (doses ≥ 0.25 mg/kg). Cardiovascular toxicity was manifested as myocardial degeneration in rats, in a 1 month study at 1 mg/kg, 3 and 6 month studies at 0.1 mg/kg and 1 year at 0.65 mg/kg. The exacerbation of endogenous orphan parvovirus by immunosuppression was attributed to this toxicity, which had increased severity in combination with cyclosporine. Nephrotoxicity, a concern with other immunosuppressive agents, was not observed with rapamune. In combination with cyclosporine, nephrotoxicity inherent to cyclosporine may be enhanced. Cataracts were seen throughout the rat studies, at exposures ranging from 5 mg/kg for 1 month, 2 mg/kg for 3 months and 0.2 mg/kg for 1 year. This may be related to dose-related increased glucose levels, as pancreatic islet cell vacuolation and atrophy was observed at 0.25 mg/kg for 1 month, 2 mg/kg for 3 months and 0.65 mg/kg for 1 year. Effects on lipid metabolism, including elevated triglycerides and cholesterol, seen at 1 mg/kg after 1 month, 0.1 mg/kg at 6 months and 0.2 mg/kg at 1 year. Phospholipidosis and alveolar macrophage accumulation occurred at 1mg/kg at 1 month, 0.5 mg/kg at 3 months and 0.2 mg/kg at 1 year. Lamellar inclusion bodies were typical of the affected macrophages. Fibrinogen was increased after 6 months at 0.5 mg/kg but reversed after recovery. Effects on red blood cells were seen at 1 and 3 months with increases in RBC counts and hemaglobin. At one year these were not seen but hematopoiesis in the liver and spleen and hemosiderosis of the kidney, lung, lymph node and spleen were seen at 0.2 mg/kg. Bone toxicity was seen in male rats following observation of lameness (0.2 mg/kg, 1 year study). Bone parameters affected by rapamune included bone mineral density, cortical thickness, bone strength and bone mineral area. Coincidentally to these findings, testosterone levels were decreased, possibly associated with testicular effects (see below). One study demonstrated the bone affects to occur in young male rats rather than in older male rats. Replacement testosterone partially alleviated the

toxicity, as did a 6 month recovery (2, 6 mg/kg, 13 weeks). Deficiency in testosterone has been associated with suppression of testicular mitochondrial side-chain cleavage enzyme, the rate limiting enzyme in steroidogenesis, by rapamune. Combination studies with cyclosporine did not produce new or increased toxicities in a 13 week study at 0.5 mg/kg rapamune/2mg/kg cyclosporine. Recent publications (e.g. Andoh et al., Transplantation 62:311, 1996) have raised the possibility of synergistic nephrotoxicity between cyclosporin and rapamycin.

It should be noted when relating animal toxicities and corresponding exposure to rapamycin to clinical exposure that exposure to rapamune as determined by whole blood AUC in rats is less than human exposure at a 5 mg dose; exposure in monkeys was similar to that seen in humans-see table below

REPRODUCTIVE TOXICOLOGY SUMMARY

Treatment of male rats with rapamune had compelling effects on reproductive organs. Decreases in testicular, epididymes and prostate size accompanied by testicular tubular degeneration and oligospermia at doses of 2 and 6 mg/kg for 13 weeks, as well as reduced sperm counts, were demonstrated Recovery of up to 6 months allowed partial recovery. In fertility studies these effects were accompanied by slight decreases in fertility rates and no effects on offspring from untreated females. Female rats had decreased weight gain on gestation days 14 to 20 and decreased gravid uterine weights (0.5 mg/kg). Embryo/fetal toxicity occurred at 0.5 mg/kg with increased resorptions, live fetuses and total implants. At delivery, live pup number was decreased as was litter size. F1 pups weights were lower on day 4 postpartum and remained lower than controls. In developmental studies, these effects were repeated at 0.5 mg/kg. At 1.0 mg/kg, fetal vertebral ossification was reduced and variations increased. In radioactive label distribution studies, rapamune entered the placenta and fetus of gravid rats, resulting in exposures up to 6 times greater than maternal blood exposure. Developmental effects did not occur. Rapamune in combination with cyclosporine (0.5/2 mg/kg) increased fetal mortality in addition to the effects of rapamune alone. Radiolabeled rapamune was detected in milk of lactating rats but not in pups receiving the milk. Pregnant rabbits treated with rapamune had maternal toxicity at doses of 0.05 mg/kg including decreased body weight and food consumption, blood in pan, no feces, decreased activity, early delivery and abortion.

GENETIC TOXICOLOGY SUMMARY

A full battery of genetic toxicology studies was performed with rapamune. These included (1) bacterial reverse mutation assay, with and without metabolic activation, (2) forward mutation (mouse lymphoma assay), with and without metabolic activation, (3) chromosomal aberration assay, with and without metabolic activation and (4) mouse micronucleus assay. All assays had negative findings for genetic toxicity.

Carcinogenicity

Studies in mice were curtailed due to skin lesions on the ears and back at week 29 in males and after dose reduction in females, at week 86. These lesions may be due to immunosuppression by rapamune, as bacterial infections were found associated with the lesion in a dose-dependent manner. Due to the shortening of the studies, interpretation was not possible and these are being reattempted. In rats, males had increased incidence of testicular interstitial cell adenomas at 0.1 and 0.2 mg/kg. Interstitial cell hyperplasia was increased at these doses and 0.05 mg/kg as well. Carcinogenicity studies are in progress in mice. Refer to phase IV commitments.

PHARMACOKINETIC SUMMARY

The pharmacokinetics of rapamycin were studied in mice, rats, rabbits, monkeys, and human tissues. [14C]-Labeled rapamycin was used to characterize the pharmacokinetics, metabolism, tissue distribution, and excretion of rapamycin in selected studies. The ability of rapamycin to induce the cytochrome P450 (CYP) system was assessed in rats. Single-dose studies and repeated-dose studies for up to 1 year were conducted

to characterize the toxicokinetics of rapamycin in mice, rats, and monkeys. Pharmacokinetic interaction studies with cyclosporin were conducted in rats. The effect of rapamycin on steroidogenic enzymes in rats was also studied. Plasma protein binding (whole blood/plasma ratio) over a concentration range of 10 to 100 ng/ml provided a relationship of human (9.3 to 13.6) > monkeys (8.0 to 13.3) > pigs (5.7 to 6.8) > rabbits (5.5 to 8.2) > rats (1.7 to 6.6) > mice (0.9 to 1.8). In humans and monkeys, this makes for rapid disappearance of rapamycin from plasma into red blood cells, necessitating development of analytical methods for whole blood to quantify rapamycin and its metabolites. The nonclinical metabolism of rapamycin is most similar to human metabolism in the monkey. Oral absorption was slow, with radiolabeled rapamycin remaining in the gastrointestinal tract for 6 hr. The major sites for radioactivity deposition were the esophagus, lungs, liver, kidneys, spleen, bladder, and salivary glands. Radioactivity was transferred to the placental and fetal compartments in gravid rats. In all species tested, unchanged rapamycin was the major constituent found via biliary excretion in radiolabel studies. In blood, the major metabolites characterized are products of O-demethylation, and/or hydroxylation, while unchanged drug was the major product present. In contrast, very little unchanged drug or recognizable drug-derived products were detected in feces or urine, such that rapamycin likely underwent further biotransformation and/or degradation prior to or after its excretion. Seco rapamycin is a ring cleavage product of metabolism, and a degradation product of drug product. A 28 day study in rats with up to 12 % seco-rapamycin did not show any additional toxicity but this does not qualify this impurity as clinical exposure will be chronic. See phase IV commitments (below) for attempts to resolve this issue. Pharmacokinetic interaction between rapamycin and cyclosporin was found and has implications for possible immunosuppressive synergy. Such synergy may be a function of inhibited metabolism by each drug for the other, as was seen in rat studies in which each the Cmax and AUC of each drug was increased by coadministration of the other. In human liver microsomes cyclosporin inhibited both demethylation and hydroxylation of rapamycin. In studies relating to the testicular effects of rapamycin, both rapamycin and cyclosporin inhibited testicular mitochondrial side-chain cleavage activity. Rapamycin and cyclosporin had similar effects on rate-limiting enzymes of steroidogenesis in rats, and this may be the mechanistic basis for lower testosterone concentrations reported in rats receiving rapamycin or cyclosporin.

Major rapamycin-related findings in multiple dose oral toxicity studies for rats and monkeys

	Findings	Study duration	Doses used Mg/kg/day	LOEL Mg/kg/day	NOAEL Mg/kg/day
RATS	Bone loss	3 month male female 1 year	 0.05 to 5 0.05 to 5 0.2 to 6	 5 not seen 0.2	 2 >5 <0.2
	Hematopoisis (liver, spleen) hemosiderosis (kidney, lung, lymph node, spleen)	1 year	0.2 to 6	0.2	<0.2
	Lymphoid/thymic atrophy	1 month 1 year	0.05 to 5 0.2 to 6	0.25 0.65	0.1 0.2
	Myocardial degeneration	1 month 3 and 6 month 1 year	0.05 to 5 0.05 to 5 0.2 to 6	1 0.1 0.65	0.25 0.05 0.2
	Ovarian atrophy	1 year	0.2 to 6	0.2	<0.2
	Pancreatic islet cell vacuolation	1 month 3 month 1 year	0.05 to 5 0.5 to 2 0.2 to 6	0.25 2 0.65	0.1 0.5 0.2
	Pulmonary alveolar macrophages	1 month 3 month 1 year	0.05 0.5 to 5 0.2 to 6	1 0.5 0.2	0.25 <0.5 <0.2
	Testicular tubular atrophy/degeneration	3 month 1 year	0.5 to 5 0.2 to 6	2 0.65	0.5 0.2
MONKEYS	Colitis	3 and 6 month	0.05 to 10	0.25	0.05
	Lymphoid/splenic/thymic atrophy	1 month 3 and 6 month	0.05 to 15 0.05 to 10	0.25 0.25	0.1 0.1

LOEL =lowest observable effect level
NOEAL=no observable effect level

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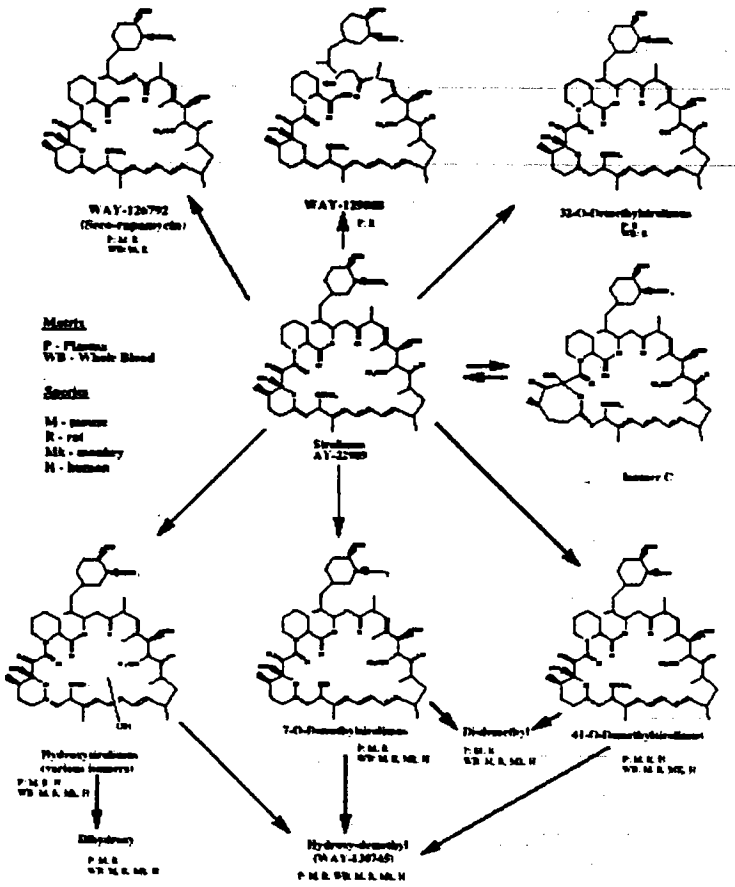
Systemic exposure (AUC) and exposure ratio (ER) for rapamycin at dosages used in animal toxicity studies

			AUC (ng.hr/ml)					
species	Study	Oral dose Mg/kg	Measured whole blood (ER) M	F	Estimated plasma (ER) M	F	Estimated plasma unbound (ER) M	F
mouse	micronucleus	5000[a]	49800 (126)		93962 (8542)		1128 (1240)	
	Carcinogenicity Wk 52	6[b]	6162 (16)	10174 (26)	11626 (1057)	19196 (1057)	140 (154)	230 (253)
	Wk 53	25[b]		9264 (23)		17479 (1589)		210 (230)
rat	Toxicity 1 yr Wk 50	0.2[c]	10.7 (<1)	7.1 (<1)	1.78 (<1)	1.18 (<1)	0.12 (<1)	0.08 (<1)
	Wk 41	6[c]	277 (<1)	281 (<1)	46.2 (4.2)	46.8 (4.3)	3.0 (3.3)	3.0 (3.3)
	Carcinogenicity Wk 50	0.2[d]	10.7 (<1)	7.1 (<1)	1.78 (<1)	1.18 (<1)	0.12 (<1)	0.08 (<1)
	Reproductive/ developmental	0.1[e]		3.42 (<1)		0.57 (<1)		0.04 (<1)
		0.5[e]		16.5 (<1)		2.75 (<1)		0.18 (<1)
rabbit	Developmental toxicity	0.025[f]		45.8 (<1)		5.59 (<1)		NA
monkey	6-month toxicity	0.5[h]	218 (<1)		16.4 (1.5)		1.38 (1.5)	
human	Phase III	2 mg (0.04mg/kg	158		4.39		0.36	
		5 mg (0.1 mg/kg)	396		11		0.96	

a: Highest dosage; exposure data obtained from a single-dose toxicokinetic study.
b: The initial highest dosage of 50 mg/kg/day was lowered to 6 mg/kg/day in females at week 31; exposure data for the 6 mg/kg/day dosage group obtained from the toxicokinetic results of the second carcinogenicity study (in progress). The initial intermediate dosage of 25 mg/kg/day was maintained throughout the study; exposure data for the 25 mg/kg/day dosage group obtained from the toxicokinetic results of the first carcinogenicity study.
c: The dosages of 0.2 and 6 mg/kg/day represent the lowest and highest dosages, respectively; although an NOAEL was not established in this study, there was generally no clinically relevant toxicity.
d: The dosage of 0.2 mg/kg/day represents the highest dosage; exposure data obtained from the 1-year toxicity study . For 0.05 mg/kg/day, exposure data after repeated-dosing was not available.
e: The dosage of 0.1 mg/kg/day represents the NOAEL dosage for maternal toxicity in the reproductive performance study and for maternal and embryo/fetal toxicity in the developmental toxicity study; in the perinatal/postnatal study, the NOAEL for maternal and postnatal toxicity was 0.09 mg/kg/day. The dosage of 0.5 mg/kg/day represents the highest maternal dosage in the reproductive performance study and the perinatal/postnatal study. Exposure data from a 10-day toxicokinetic study in gravid rats dosed from gestation days 6 through 15.
f: The dosage of 0.025 mg/kg/day represents the NOAEL dosage for maternal toxicity; there were no fetal effects up to the highest dosage of 0.05 mg/kg/day. Exposure data from a 13-day toxicokinetic study in gravid rabbits dosed from gestation days 6 through 18.
g: The plasma protein binding of sirolimus was not evaluated in rabbits.
h: Highest dosage; pharmacological effects at all dosages. Exposure data from a 2-week toxicokinetic study.
AUC = Area under the concentration-versus-time curve; ER = Exposure ratio (AUC value in animals/AUC value in humans at 5 mg/day); F = Females; M = Males; NA = Not available; NOAEL = No-adverse -effect level

Metabolic pathways for rapamycin



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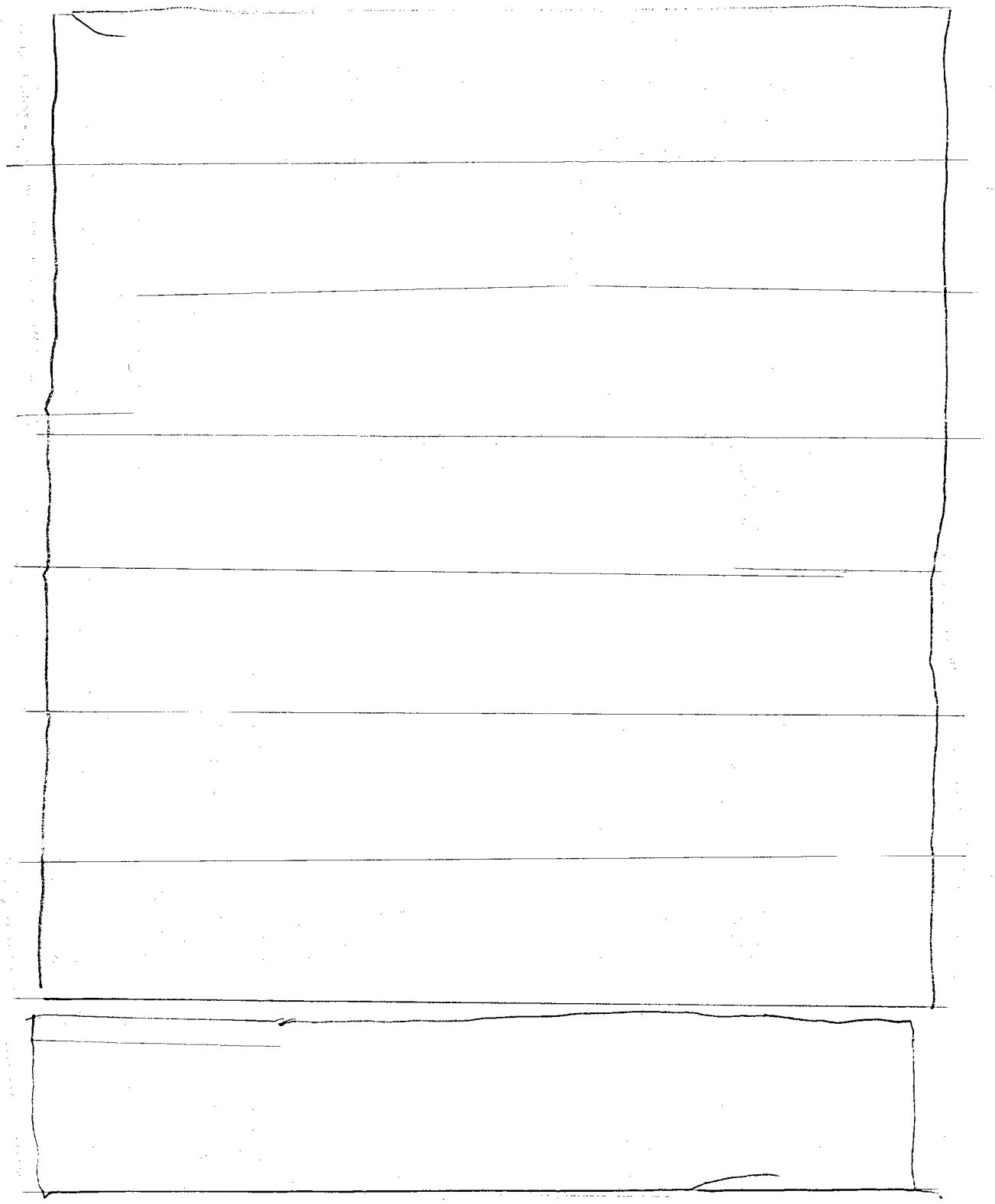
REQUESTS

Label issues were resolved in teleconference with the sponsor.



CONCLUSIONS

This submission is acceptable with regards to pharmacology with the inclusion of labeling changes and Phase IV commitments.

/S/

S.C. Kunder, Ph.D.
Reviewing Pharmacologist

concurrences:
HFD-590/ADir/RAlbrecht
HFD-590/SPharm/KHastings /S/ 8/25/99
Steven C. Kunder/Pharm/

disk:
HFD-590/KHastings
cc:
HFD-590 (original)
HFD-590 Division file
HFD-340
HFD-590/MBacho
HFD-590/RTiernan
HFD-590/MSeggel
HFD-590/SBala
HFD-345

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PHARMACOLOGY / TOXICOLOGY REVIEW AND EVALUATION

NDA #: 21-083
Type: Pharmacology / Toxicology Review
Date of Submission: 12/15/98

Review Division: Special Pathogen and Immunologic Drug Products
HFD-590
Reviewer: Stephen Hundley, Ph.D., Pharmacologist / Toxicologist

Review Completion Date: 8/24/99

Sponsor: Wyeth-Ayerst Research
P.O. Box 8299
Philadelphia, PA 19101 – 8299

Phone: (601) 902-3710

Drug Information:

Trivial Name:	Rapamycin
Product Name:	Rapamune®
Chemical Name:	Refer to NDA review by primary pharmacologist
CAS Number:	53123-88-9
Molecular Weight:	914.19
Structure:	Refer to NDA review by primary Pharmacologist

Drug Category: Immunosuppressant

Related Submission: IND [redacted]

Indication: Prevention of renal transplant rejection.

BACKGROUND

A review was requested by Dr. Stephen Kunder, the primary Pharmacologist / Toxicologist Reviewer of NDA 21-083, of rat and mouse carcinogenicity studies contained within the submission. The mouse carcinogenic study was inadequate because all male mice were sacrificed and the study terminated by Week 29 due to excessive toxicity. Female mice were terminated at Week 86 with the high dose level being lowered from 50 mg/kg/day to 6 mg/gk/day at Week 31. The sponsor, in an amendment to IND [redacted] indicated that a second carcinogenicity study with mice was initiated in Feb. 1997. The terminal sacrifice was conducted in Feb. 1999, and the final report is scheduled to be issued by March, 2000. The rat carcinogenicity study was carried to completion for all male and female dose groups. A significant number of vehicle-control and rapamycin-dosed animals were found dead or sacrificed *in extremis* prior to the

scheduled sacrifice date. Sufficient numbers lived to the terminal sacrifice to produce a valid study for statistical analysis. Both the rat and mouse studies are evaluated in this review.

Nonclinical Toxicology Studies (Carcinogenicity)

1. Two Year Oral (Gavage) Carcinogenicity Study in Rats, GTR 32266.

2. Rapamune: A Twenty-Nine Week Chronic (Male) and Eighty-Six Week Carcinogenicity Study in Mice, GTR 32267.

Nonclinical Studies Reviews

Two Year Oral (Gavage) Carcinogenicity Study in Rats, GTR 32266.

The carcinogenic activity of rapamycin was examined in male and female Charles River CD rats in a 2-year oral-gavage carcinogenicity study. Each dosing group consisted of 65 male and 65 female rats, all animals were scheduled to be on study for 104 to 106 weeks. Each rat received single daily oral gavage doses of a vehicle control or different dose levels of rapamycin, seven days a week for 104 weeks. The dosing groups were: vehicle-control group 1, vehicle-control group 2, and 0.05, 0.1, and 0.2 mg rapamycin/kg body weight per day dose groups. Animals were selected for the dose groups by computer randomization procedures. The lot (or batch) number for the rapamycin used in this study was OC5634. The study was conducted in accordance with GLP Guidelines and was audited by a Quality Assurance group.

In-life monitoring consisted of the following: twice daily monitoring for mortality; daily general clinical observations; detailed physical examination once weekly through Week 13, then once every 4 weeks until study completion; body weights and food consumption were determined weekly through Week 13, then once every 4 weeks until completion; digital palpations once every 4 weeks from Week 29 through 53, then once every two weeks until completion. Blood samples were drawn during Week 52 from 5 animals of each dose group approximately 2 hours after dosing; these samples were assayed for rapamycin concentrations.

Complete necropsies were conducted on all animals on study and included animals found dead, sacrificed *in extremis*, and terminal (scheduled) sacrifices. Bone smears were taken at necropsy and stored for possible hematological analysis. The following organs and tissues were removed from all animals and preserved for histopathology.

Organs and Tissues Histopathology Table

Adrenal Glands	Jejunum	Spinal Cord
Aorta	Kidneys	Spleen
Bone (femur)	Liver	Stomach

Femoral-Tibial Joint	Lungs	Testes
Bone Marrow (sternum)	Cervical Lymph Nodes	Thymus
Brain	Mesenteric Lymph Nodes	Thyroid Gland
Cecum	Mammary Glands	Tongue
Cervix	Ovaries	Trachea
Colon	Pancreas	Urinary Bladder
Epididymides	Parathyroid Gland	Uterus
Esophagus	Sciatic Nerve	Vagina
Eyes	Pituitary Gland	
Harderian Gland	Prostate Gland	
Heart	Salivary Gland	
Ileum	Seminal Vesicles	

Histopathological evaluations for neoplastic and non-neoplastic lesions were conducted on these organs and tissues from all animals on study including those animals found dead and sacrificed *in extremis* (unscheduled sacrifices).

The sponsor detailed various statistical analytical procedures used in the study. Survival was assessed by a non-parametric time-adjusted log-rank method that developed Kaplan-Meier survival curves. Body weight was analyzed by a one-way analysis of variance and a one-sided non-parametric trend test. Benign and malignant neoplasms were analyzed by a time-adjusted Peto method (Peto analysis) incorporating a one-sided dose-related trend test. Subsequent neoplasm analyses included an exact trend test and pairwise tests to establish significance for tumor incidence rates between two dose groups. The incidence rates for non-neoplastic lesions were statistically analyzed by a Mantel-Haenszel type test.

Clinical observations associated or related to rapamycin included opacity of the eyes in male rats at the intermediate and high dose levels (40 and 43% incidence rate), thin appearance in male rats at the intermediate and high dose levels (28 and 31% incidence rate), lameness in males possibly due to loss of bone density (approximately 15% incidence rate at each rapamycin dose level), and decubital ulcers in female rats. Statistical analysis of mortality rates did not indicate an elevated mortality rate due to rapamycin although rapamycin was associated with the cause of death in a number of male rats. Urinary and reproductive tract inflammation resulted in early death for the following groups; zero-level controls – 2/76, 0.05 mg/kg dose – 3/36, 0.1 mg/kg dose – 4/27, and 0.2 mg/kg – 9/35. Histiocytic sarcomas were the cause of death in a total of four male rats (1 male rat at the 0.1 mg/kg dose and 3 males at the 0.2 mg/kg dose) and considered related to rapamycin dosing. Several causes of early death in female rats such as pituitary adenomas, fibroadenomas, and mammary gland adenocarcinomas were not associated with rapamycin dosing. The following animal survival table numerically demonstrates similar survival rates between the two zero-level controls and the rapamycin-dosed male and female rats. In addition, the number of surviving males and females at Week 91 and Week 104 are adequate for statistical analyses in a rodent carcinogenicity study.

ANIMAL SURVIVAL TABLE

	Number of Surviving Animals							
	Males				Females			
	C1/C2	0.05	0.1	0.2	C1/C2	0.05	0.1	0.2
Week 26	65 / 63	65	65	64	65 / 65	65	65	65
Week 52	65 / 63	65	62	60	65 / 62	65	61	61
Week 78	57 / 55	56	54	52	52 / 50	53	51	54
Week 91	35 / 46	43	52	46	37 / 36	44	38	35
Week 104	22 / 35	30	38	33	21 / 24	27	23	23

Control Group 1 and Control Group 2 are represented by C1/C2.

Body weight gain differences between the zero-level control groups and the rapamycin dosed animals were reduced by more than 10% in the 0.1 and 0.2 mg/kg male dose groups and by approximately 10% for the 0.2 mg/kg female dose group. The data for differences in body weight gain are presented in the following table.

DIFFERENCE IN BODY WEIGHT GAIN
COMPARED TO CONTROLS

	Percent Change From Controls					
	Males			Females		
	0.05	0.1	0.2	0.05	0.1	0.2
Week 25	0%	+1.3%	-1.0%	-8.2%	-2.9%	-6.2%
Week 53	-5.1%	-8.2%	-13%	-9.8%	-2.9%	-8.5%
Week 104	+6.1%	-19.8%	-29%	-3.2%	+6.9%	-10.2%

The deviation from control values for body weight gain was approximately -13% for the 0.2 mg/kg male group by Week 53. The differences in mean absolute body weights were also calculated and exceeded 10% reduction for both the 0.1 and 0.2 mg/kg male groups, however the difference was less than 10% for the 0.2 mg/kg female group. The following table displays the percent differences in group mean body weight from the zero-level control groups.

PERCENT DIFFERENCES IN GROUP MEAN BODY WEIGHT FROM CONTROLS

	Males			Females		
	0.05	0.1	0.2	0.05	0.1	0.2
Week 29	- 0.7%	- 0.2%	- 2%	- 5%	-2%	- 3%
Week 53	- 4 %	- 6%	- 10%	- 7%	-2%	- 5%
Week 77	- 9%	- 15%	-23%	- 3%	-1%	- 6%
Week 104	+ 4%	- 15%	- 22%	- 2%	+5%	- 7%

Gross pathologic lesions for all males (scheduled plus unscheduled sacrifices) included opacity of eyes (15, 37, and 43 percent incidence rates for the 0.05, 0.1, and 0.2 mg/kg dose levels, respectively), hydronephrosis (21 percent incidence rate for zero-level controls vs. 28 and 26 percent for the 0.1 and 0.2 mg/kg dose levels), enlarged liver at the 0.2 mg/kg dose level, discolored lungs at the 0.1 and 0.2 mg/kg dose levels, small prostate and seminal vesicles at the 0.1 and 0.2 mg/kg dose levels, and small thymus at the 0.1 and 0.2 mg/kg dose levels. Female rats exhibited marginally elevated incidences of discolored and enlarged adrenal cortex at the 0.2 mg/kg dose level and higher incidences of abrasions of the feet at the 0.1 and 0.2 mg/kg dose levels (14 and 18 percent incidence rates). Opacity of the eyes was observed at a marginally higher rate (5 percent) in females from the 0.1 and 0.2 mg/kg dose levels compared to control values, however the incidence rates were much less than observed in male rats.

The two zero-level control groups of both males and females were pooled for analysis of non-neoplastic and neoplastic lesions. The following table contains the percent incidence rates for selected non-neoplastic lesions in male rats that were associated with rapamycin.

Percent Incidence Rates for Non-neoplastic Lesions in Male Rats				
	Controls	0.05 mg/kg	0.1 mg/kg	0.2 mg/kg
Lungs				
Alveolar Macrophage	43	62	77	71
Cervical Lymph Node				
Plasma Cell Hyperplasia	39	32	58	51
Lymphoid Atrophy	14	25	45	34
Spleen				
Hemosiderosis	60	80	80	74
Lymphoid Atrophy	8	23	8	26
Mesenteric Lymph Nodes				
Ceroid-laden Macrophages	50	59	55	63
Lymphoid Atrophy	10	11	32	28
Thymus				
Atrophy	76	82	81	90
Eyes				
Cataracts	3	17	31	42
Retinal Degeneration	2	12	30	32
Pancreas				
Islet Cell Loss/Atrophy	1	9	25	30
Testes				
Tubular Cell Degeneration	24	35	45	35
Arterial Mineralization	9	37	45	46
Interstitial Cell Hyperplasia	2	5	14	9
Epididymides				
Hypospermia	12	15	26	25

	Controls	0.05 mg/kg	0.1 mg/kg	0.2 mg/kg
Prostate				
Atrophy	1	3	12	9
Seminal Vesicle				
Atrophy	6	8	19	17
Vesiculitis	4	7	9	11
Kidney				
Pyelitis	8	11	12	18
Pelvic Cell Hyperplasia	2	5	2	11
Urinary Bladder				
Cystitis	5	9	14	25
Epithelial Hyperplasia	1	3	16	16
Feet				
Dermal Fibrosis	14	17	19	38
Hyperostosis	2	13	5	19
Periosteal Bone Formation	40	52	52	62

Selected non-neoplastic lesion incidence rates in female rats associated with rapamycin are listed in the following table.

Percent Incidence Rates for Non-neoplastic Lesions in Female Rats				
	Controls	0.05 mg/kg	0.1 mg/kg	0.2 mg/kg
Lungs				
Alveolar Macrophage	43	54	48	65
Cervical Lymph Node				
Lymphoid Atrophy	21	27	32	45
Mesenteric Lymph Node				
Ceroid-laden Macrophages	60	54	63	81
Plasma Cell Hyperplasia	11	9	11	19
Adrenal Cortex				
Cystic Degeneration	81	89	85	92
Mammary Gland				
Galactocele	16	22	14	26
Feet				
Ulcer	54	57	75	54
Spleen				
Lymphoid Atrophy	13	23	23	28

Alveolar macrophage infiltration was observed in lungs as clusters or degenerate sheets from both male and female rats at each rapamycin dose level. This observation was consistent with pulmonary alveolar macrophage infiltration detected in a previous one-year toxicity study with rapamycin. The immunosuppressive activity of rapamycin resulted in lymphoid atrophy in spleen and the mesenteric and cervical lymph nodes from both sexes at all rapamycin dose levels. Male rats were more sensitive to the ocular effects of rapamycin as indicated by the high incidence rate of cataracts and retinal degeneration at each rapamycin dose level. The sponsor attributed the ocular effects to

the diabetic condition caused by rapamycin as supported by the elevated level of islet cell loss or atrophy in the pancreas at each rampamycin dose level.

Rapamycin-induced effects to the urogenital tract were seen in male rats. The histopathology included tubular cell degeneration, arterial mineralization, and interstitial cell hyperplasia in the testes; atrophy in the prostate and seminal vesicle; and hyperspermia in the epididymides. Cystitis and epithelial hyperplasia were observed in the urinary bladder while elevated incidence rates for pyelitis and pelvic cell hyperplasia were seen in the kidneys. Histopathology specific to female rats included galactocele in the mammary glands and ulcers of the feet.

[redacted]. The sponsor combined the two zero-level control groups for each sex then recorded and statistically evaluated the background tumor incidences for the combined control animals. The following table lists neoplastic tumors that were statistically significant by trend analysis.

Incidence of Neoplastic Tumors				
	Controls	0.05 mg/kg	0.1 mg/kg	0.2 mg/kg
Males				
Testes				
Interstitial Cell Adenomas	7/130	0/65	8/65	10/65
Multisystemic				
Histiocytic Sarcomas[a]	1/76	0/36	1/27	3/36
Lungs				
Bronchioalveolar Adenomas	0/130	1/65	0/65	2/65
Females				
Cervix				
Fibroma	0/130	1/65	4/65	1/65

[a] Observed only in animals found dead or sacrificed *in extremis*, therefore animals at the final sacrifice were not included.

The incidence rates for multisystemic histiocytic sarcomas and bronchioalveolar adenomas in male rats were statistically significant based upon trend analysis. However, the incidence rates were not statistically significant by pairwise analysis against the zero-level controls. Both of these tumor types occur spontaneously in older rats and the tumor rates in the current study fall within the range of historical control rates. Testicular interstitial cell adenomas were statistically significant by trend analysis and the incidence rate for the 0.2 mg/kg group was statistically different from the zero-level control based upon pairwise analysis. The incidence rates for fibromas of the cervix were statistically significant by trend analysis and by pairwise analysis of the 0.1 mg/kg dose group, however, there was no dose response with increased rapamycin dose levels (0.1 to 0.2 mg/kg). None of the other observed neoplastic tumors were statistically significant by trend analysis or exact tests.

Comments

The study design and test animal survivability were adequate to assess the carcinogenic activity of rapamycin in laboratory rats. The study was conducted in accordance with the protocol and provided sufficient histopathological data from the designated organs and tissues to evaluate both the non-neoplastic and neoplastic effects of rapamycin at all dose levels including the zero-level controls. Rapamycin did not cause increased mortality in male and female rats compared to the concurrent zero-level control groups. Body weight depression was more pronounced in males compared to females as a result of rapamycin. The group mean body weights for male rats were depressed 15 and 22% for the 0.1 and 0.2 mg/kg groups, respectively. Group mean body weight depression for females was approximately 7% for the 0.2 mg/kg group.

The sponsor indicated that the two control groups for each sex were pooled before statistical analyses were conducted for neoplastic tumor incidence. The Executive Carcinogenicity Assessment Committee indicated that the control groups should not have been pooled but rather assessed individually by the different statistical procedures

The sponsor presented the argument that testicular interstitial cell adenomas resulted from chronically reduced testosterone levels and resultant elevation in luteinizing hormone levels due to rapamycin dosing. In the 52-week rapamycin toxicity study with male rats, testicular toxicity resulted in decreased testosterone levels leading to elevated production of luteinizing hormone by interstitial cells. Interstitial cell hyperplasia was observed at terminal sacrifice of the 52-week study. Interstitial cell hyperplasia was considered a pre-neoplastic lesion by the sponsor that resulted in the observed interstitial cell adenomas in the 2-year bioassay. The mechanism proposed by the sponsor of reduced testosterone production leading sequentially to increased luteinizing hormone production followed by interstitial cell hyerplasia progressing to adenomas is plausible. However, the sponsor would need to supply additional and definitive mechansism of action data specifically for rapamycin to support this theory.

Rapamune: A Twenty-Nine Week Chronic (Male) and Eighty-Six Week Carcinogenicity Study in Mice, GTR 32267.

The carcinogenicity study in CD mice was designed to include two zero-level controls for each sex and the following rapamycin dose levels; 12.5, 25, and 50 mg/kg/day (orally by gavage). Each control group consisted of 60 or 75 animals; each rapamycin dose group consisted of 75 animals. The Lot (or Batch) of rapamycin was OC5644 and OC5634. The study was conducted in accordance with GLP guidelines and audited by a Quality Assurance Group.

Males suffered from severe skin lesions in the rapamycin dose groups. The severity of these lesions progressed resulting in early deaths and sacrifices *in extremis*. All males were sacrificed during Week 29 due to the poor health of the animals in the rapamycin groups. Female mice were also affected to a lesser extent. The declining health of the

50 mg/kg dose group at Week 31 forced the sponsor to reduce the dose level to 6 mg/kg. The entire study was terminated at Week 86 due to the severity of skin lesions in female mice at all rapamycin dose levels and the reduced survivability rates.

The following organs and tissues were removed from each female mouse on study (from both scheduled and unscheduled sacrifices) and preserved for histopathology.

Adrenal Glands	Jejunum	Skin
Aorta	Kidneys	Spinal Cord (Cervical)
Bone (femoral-tibial joint)	Liver	Spinal Cord (Lumbar)
Bone Marrow (sternum)	Lung	Spleen
Brain	Lymph Nodes (Cervical)	Stomach
Cecum	Lymph Nodes (Mesenteric)	Thymus
Cervix	Mammary Glands	Thyroid Glands
Colon	Ovaries	Tongue
Duodenum	Pancreas	Trachea
Esophagus	Parathyroid Glands	Urinary Bladder
Eyes	Sciatic Nerve	Uterus
Gall bladder	Pituitary Gland	Vagina
Heart	Salivary Gland	
Ileum	Skeletal Muscle	

The mean group body weight for female mice was reduced 10 to 15 percent in the rapamycin dose groups by Week 53, and 14 to 21 percent by Week 85. By Week 86, the survivability percentages for female mice were: zero-level controls – 81 percent; 12.5 mg/kg group – 54 percent; 25 mg/kg group – 42 percent; and the 50 / 6 mg/kg group – 37 percent. The actual number of surviving animals for the rapamaycin groups were; 30, 23, and 23 for the 12.5, 25, and 50 / 6 mg/kg dose levels, respectively. The number of surviving female mice and the duration of study were adequate to evaluate the carcinogenic activity of rapamycin in female mice.

Unscheduled sacrifices or animals found dead were due primarily to lymphomas or skin ulcers in the rapamycin dosed animals. Hematology revealed a general elevation of white blood cells possibly due to enhanced inflammation [neutrophils, monocytes, and eosinophils were increased]. An interim sacrifice was scheduled at Week 54 for histopathological evaluations. The following histopathology was observed in the rapamycin-dosed female mice; alveolar infiltration into lungs, lymphoid atrophy of the cervical and mesenteric lymph nodes and thymus, atrophy of the uterus, skin ulcers, dermatitis and ulcers of the ear, and myeloid hyperplasia in bone marrow. Blood pharmacokinetic values from these animals were; Cmax of 0.69 and 0.82 μg/ml for the 12.5 and 25 mg/kg dose levels, respectively, and $AUC_{0-24\,hr}$ values of approximately 9.2 μg · hr/ml for each dose level; essentially no differences in the AUC between the two dose levels were observed.

The following table presents the percent incidence rate of non-neoplastic lesions observed at the terminal sacrifice of female mice (Week 86).

Percent Incidence of Non-neoplastic Lesions in Female Mice				
	Controls	12.5 mg/kg	25 mg/kg	50/6 mg/kg
Lungs				
Alveolar Macrophage	33	50	63	37
Cervical Lymph Node				
Lymphoid Atrophy	45	72	85	68
Spleen				
Hematopoiesis	11	19	32	23
Thymus				
Lymphoid Atrophy	28	53	51	52
Mesenteric Lymph Node				
Lymphoid Atrophy	27	64	70	54
Uterus				
Atrophy	1	45	60	43
Skin				
Ulcer	1	11	25	15
Bone Marrow				
Myeloid Hyperplasia	9	20	41	32
Eyes				
Uveitis	1	4	13	0
Keratitis	4	7	12	5
Ear				
Ulcer	25	53	56	27

The only statistically significant neoplastic lesion (tumor) in female mice was multisystemic lymphoma. The incidence rates were ; 5 percent for zero-level controls, 14 percent for the 12.5 mg/kg group, 12 percent for the 25 mg/kg group, and 19 percent for the 50/6 mg/kg group. These values were statistically significant by both trend and pairwise analysis.

The non-neoplastic lymphoid atrophy in lymph nodes and thymus is an expected response to immunosuppressive agents such as rapamycin. Several effects such as pulmonary macrophage infiltration, hematopoiesis in the spleen, and myeloid hyperplasia are responses to inflammation which is secondary to the immunosuppressive activity of rapamycin. Ocular inflammation also results from infection due to the immunosuppressive activity of rapamycin. Uterine atrophy, however, is not as readily explained by the immunosuppressive activity of rapamycin. Possible mechanisms, such as altered FSH/LH secretion or peripheral activity affecting estrogen secretion, are briefly offered by the sponsor in absence of any direct cause-effect mechanistic data with rapamycin. A no-effect level for uterine atrophy was not demonstrated in this study.