SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion for the approval of Rapamune. This scientific discussion has been updated until 1 October 2004. For information on changes after this date, please refer to module 8B.

1. Chemical, pharmaceutical and biological aspects

Composition

Oral Solution

The product is formulated as a clear viscous solution of 1 mg/ml sirolimus as active ingredient. It contains polysorbate 80 as dispersant and Phosal 50 PG as a solubiliser consisting of phosphatidylcholine, soya-fatty acids, ethanol, mono/diglycerides, propylene glycol, and ascorbyl palmitate. Polysorbate is derived from tallow. Furthermore, several materials of animal origin are used in the production of the master and/or working cell banks. The measures to prevent transmission of TSE are sufficient. It is packed in either single-dose aluminium laminate sachets or multidose amber glass bottles with dosing syringe. The packaging materials comply with the PhEur specification.

Coated Tablets

The product is approved in tablet form consisting of 2 strengths, 1mg and 2mg. Rapamune 2 mg coated tablets differs from Rapamune 1 mg coated tablets in that the tablets contain yellow and brown iron oxides (E172) therefore resulting in a different colour to the 1 mg tablets namely yellow to beige compared to white.

The inert tablet cores for both tablet strengths are composed of lactose monohydrate (diluent, PhEur), macrogol 8000 (binder, PhEur), magnesium stearate (lubricant, PhEur), and talc (glidant, PhEur).

The tablet coating for both strengths is composed of several layers of coatings consisting of the Nanodispersion (sirolimus and stabiliser), macrogol 20000 (plasticizer, in-house specification), glyceryl monooleate (plasticizer, in-house specification), pharmaceutical glaze (shellac, coating agent), calcium sulphate anhydrous (coating agent), microcrystalline cellulose (reinforcing agent, PhEur), sucrose (filler, PhEur), titanium dioxide (colorant, PhEur), povidone (binder, PhEur), carnauba wax (polishing agent, PhEur) with a red printing ink. The composition of this proprietary ink is presented. In addition, Rapamune tablets 2mg contain iron oxides as colouring materials to distinguish them from the 1mg (white) tablets.

The tablets are presented in both PVC/PE/Aclar/foil blisters within cartons and two pack sizes are available, 30 & 100.

Active substance

Sirolimus is chiral (15 stereogenic centres) but the method of manufacture described in the dossier uniformly produces a single defined stereoisomer in the solid state. However, in solution this interconverts between three isomers: A, B and C. In solution sirolimus exists primarily as isomer B, which contains an intramolecular hemiketal, forming a six-membered ring. Isomer C also contains an intramolecular hemiketal, but with a neighbouring keto group, and thus forming a seven-membered ring (oxepane). Isomer A is believed to be the transient intermediate, lacking hemiketal.

Sirolimus is produced by *Streptomyces hygroscopicus*. For the commercial fermentation a two-tiered cell bank system is employed. A master cell bank (MCB) of strain B-1251 consisting of a spore suspension in glycerol has been prepared and this cell bank is used to generate working cell banks (WCBs). The MCB is tested for lack of microbial contamination, culture homogeneity and the ability

to successfully produce a WCB able to give rise to a specified sirolimus production. The master cell bank is stored at –60°C. In process controls consist of tests with regard to identity, assay, purity, residual solvents, and water content.

Raw material used during preparation of the sirolimus crude oil has been specified. The specifications are acceptable.

Five raw materials of animal-derived origin are used in the culture media for either the master cell bank, the working cell bank or in the production of the sirolimus crude oil. Gelatine is porcine-derived. Casein and the casein hydrolysate, NZ-amine Type A, are Category IV materials with no detectable infectivity. Beef extract is derived from bovine skeletal muscle, which is considered a Category IV material with no detectable infectivity. (Animal material used in the preparation of this product is sourced from Australia). Glycerol (derived from tallow from animal from USA, Japan, Canada & China) is used in both the master and working cell banks but is not used in the spore inoculation process. All bovine-derived raw materials used in the fermentation process are sourced in compliance with Directive 1999/82/EC amending the Note for Guidance for Minimizing the Risk of Transmitting Agents causing Spongiform Encephalopathy via Medicinal Products.

All components and/or the media are sterilised in an autoclave. All of the sterilising conditions were validated.

The end-point of the fermentation process is sirolimus crude oil.

Further purification techniques result in a crystalline solid, which is the active substance.

The conditions employed in the recovery and purification steps, e.g., solvent extraction, evaporation at high temperature; crystallisation from organic solvents will result in denaturation and degradation of DNA from the production strain. It is highly unlikely that intact DNA would be carried through these purification steps. In addition, the *S. hygroscopicus* production strain is not known to be pathogenic to humans, animals or plants or to contain plasmids or other potential gene transfer vectors such as viruses that confer resistance to known therapeutic antibiotics.

Active Substance Specification

The sirolimus particle size was not a concern for the initial product, that is, the oral solution. However, all batches were monitored and routinely milled.

Process impurities are individually controlled in the active ingredient specifications. In addition, there is a limit for “Total impurities” and a limit for “Any single unknown impurity”.

The risk for carrying over of living matter from the fermentation is considered to be negligible.

Since this is a fermentation product, the ICH guideline on impurities in drug substances is formally not applicable. Nevertheless the proposed specification limits have been justified with reference to all batches used in the toxicological studies and in clinical trials.

Results of batch analyses for three batches are reported and the results confirm a consistent quality and in general justify the proposed limits.

Some changes to the specification and controls of the active substance were required for the solid dosage form. At the same time, changes were made to generally update the active substance sections of the dossier. A separate limit was added to the specification for Group II impurities (impurities/degradants) as these are observed to increase with storage time of the coated tablets. The method used is that previously approved for the purity determination. A test and limits for particle size was included, the limit for the largest single impurity was tightened, and new limits were included for both sirolimus isomer A and total unknown impurities. Three new or revised methods were

presented and these were satisfactorily validated. Additional batch analyses data were also provided, from more recently produced batches.

Stability

Formal stability studies on three batches of sirolimus, which have been stored at 5°C and 25°C/60%RH for eighteen months in a package simulating the bulk storage package, were provided. The studies at 5°C will continue. Studies at higher temperatures (40°C, 40°C/75%RH, 51°C) and in light have also been performed. The strength remained essentially unchanged at 5°C and 25°C/60%RH, but losses were found when exposed to light in flint glass bottles. These changes are not considered significant for sirolimus stability.

The stability data presented for the active substance was extended (during the application for the coated tablets) from that initially presented (for the oral solution formulation) by more recently produced data, and the claimed retest period was fully justified.

Other ingredients

Oral Solution

Nitrogen and polysorbate 80 comply with the current PhEur. Phosal 50 PG is not described in a pharmacopoeia. It is prepared from propylene glycol, phosphatidylcholine derived from soy lecithin and some additional compounds needed for processing, preservation and stability purposes.

Coated Tablets

With the exception of the macrogol 20 000, glyceryl monooleate, calcium sulphate anhydrous, pharmaceutical glaze and the proprietary printing ink, all the excipients comply with the appropriate specifications of the current PhEur. The calcium sulphate anhydrous complies with the current PhEur specification for calcium sulphate dihydrate except for the limits for water; hence the proposed specification is satisfactory.

Satisfactory control specifications are provided for the macrogol 20 000 (for which the PhEur specification is not suitable as it is only for a linear chain polymer, which is not suitable for use with this product), glyceryl monooleate and the proprietary printing ink.

A PhEur Certificate of Suitability (TSE) is provided for the magnesium stearate from the stated manufacturer. An assurance is provided that the lactose is TSE-free and that all other substances in the coated tablet formulation are not of animal origin.

Solvents such as methanol which are used during the manufacture of the product, for example, for the preparation of the tablet polish suspension, for the preparation of the shellac seal coating and for preparation of the printing ink solution, have all been demonstrated to be removed during processing, resulting in no detectable levels in the finished product. Suitable specifications have also been provided.

The containers selected for the coated tablets are (PVC/PE/Aclar) film/aluminium foil blisters and adequate details have been provided of their specifications.

Product development and finished products

Oral Solution

The development pharmaceutics have been addressed satisfactorily. The use of a non-aqueous vehicle eliminates major concerns with regard to the solid-state properties of the drug substance, such as potential for polymorphic forms, particle size, surface area and intrinsic dissolution rate. Phosal 50

PG, a propylene glycol-based vehicle containing phosphatidylcholine, was chosen as the major vehicle. Polysorbate 80 was included to enhance the solubility upon dilution.

No antimicrobial preservative was required since it was determined that the active drug solution was self-preserving and passed the PhEur test for efficacy of antimicrobial preservative.

The clinical trials for sirolimus oral solution were conducted with 1 mg/ml (proposed commercial formulation), 2.5 mg/ml, 5 mg/ml and 10 mg/ml oral solutions, 6 mg/ml and 50 mg/ml injection concentrates, and several solid dosage forms. However, the intended sirolimus oral solution market formulation (1 mg/ml) is quantitatively identical to the formulation used in Phase III studies.

The manufacturing process basically consists of dissolving the ingredients, whilst stirring under nitrogen.

Validation data generated from three production batches provided show that all batches met the acceptance criteria, demonstrating that each manufacturing stage can be reproducibly carried out.

Coated Tablets

In order to facilitate dosing and patient compliance, different approaches to finding a stable solid dosage form of sirolimus were investigated. In order to improve the stability and bioavailability of the coated tablet, the active substance, sirolimus, has been incorporated in a NanoCrystal Colloidal Dispersion (Nanodispersion) in which the drug particle size is reduced to nanometer dimensions in the presence of a stabiliser (poloxamer 188). The Nanodispersion containing sirolimus and the stabiliser is added to a sugar coating suspension, used to coat inert tablet cores previously overcoated with shellac and inert filler coats. The last coat is the colour coat. The specification proposed for the Nanodispersion is considered adequate to control the relevant physico-chemical characteristics. Batch analyses data are also provided.

Initially three different tablet strengths were developed (1 mg, 2 mg and 5 mg). The 2 mg tablets were not registered as the same time as the 1 mg tablets, but they were later proposed for marketing in order to reduce the pill burden and aid patient compliance and convenience.
(Proprietary formulation information – should not be included)

The inert tablet cores were prepared by a direct compression manufacturing process typical for this type of product. Then two different coating layers were applied, a shellac seal coating and a filling suspension coating. The manufacturing process of the 2 mg strength is almost identical to the process for the 1 mg strength apart from the thin sugar seal introduced between the colour and the active coat.

For the 1 mg strength, the change from the initially oval shaped tablet to the final triangular shape was supported by extensive dissolution data obtained from physiologically relevant, discriminatory dissolution conditions from pre-change and post-change batches. Equivalence between the oval and triangular shapes, to a high level of statistical confidence, was shown. Additionally an *in vivo/in vitro* correlation (IVIVC) study provides some support for equivalence between the batches used in clinical trials (oval-shaped) and the batches proposed for marketing (triangular-shaped).

Satisfactory process validation data have been provided.

Finished product specifications

Oral Solution

The finished product specifications include tests for identity (TLC), assay (HPLC) (sum of isomers B and C), seco-rapamycin (HPLC), purity (total other degradation impurities, largest single other degradation impurity), peroxide value, deliverable volume for sachets, deliverable volume for bottles, moisture (KF), total aerobic count including yeast and mould, presence of *E. coli*, presence of *Salmonella* species, and presence of *P. aeruginosa*.

The purity specification for the active substance limits the amount of related impurities in the finished product. All the methods applied to this product are reasonable, sensitive, accurate, and validated.

On-going stability studies of both clinical and registration lots show seco-rapamycin (seco) is the major degradation product. The specification limit for this impurity is supported for safety studies. Regarding the level of unknown impurities in toxicological/clinical studies data from repeated-dose toxicity studies, which support the proposed limit for largest single unknown impurity, have been included.

Satisfactory certificates of analysis have been provided for three batches of the finished product.

Coated Tablets

The finished product specifications are comprehensive and include justified tests and limits for identity (active substance and colorant), content uniformity, microbial contamination, assay, impurities (specified, unidentified single, and total impurities) and moisture content. The dissolution specification includes limits at 30 minutes and 120 minutes, and these have been justified. The lack of a test for residual solvents has been justified.

All the methods have been adequately validated.

Batch analyses data have been provided for seven batches both tablet strengths and these demonstrate consistency of manufacture and compliance with the proposed specifications.

Stability of the products

Oral Solution

Stability studies were conducted on three commercial sized batches for up to 18 months at 5°C, 15°C/60%RH and 25°C/60% RH. The packaging systems used for these batches were 2-oz (60 ml) and 5-oz (150 ml) amber glass bottles with child-resistant caps and 1, 2 and 5 ml polyester/low density polyethylene aluminium foil laminate sachets (pouches), all with nitrogen headspaces.

Testing was performed periodically for up to eighteen months at 5°C, twelve months at 15°C/60%RH and six months at 25°C/60%RH.

Batches were tested for physical appearance and description, strength, degradation products, identity, deliverable volume, peroxide value, ethanol content, moisture, microbial limit and antimicrobial effectiveness. Stability on exposure to light, freeze/thaw cycling and under the simulated conditions of use were also determined.

At 5°C total sirolimus content decreased, but all samples were within specifications. At 15°C/60%RH, the intermediate accelerated storage condition, samples were still within specifications, but the loss of sirolimus content was greater. At 25°C/60%RH (primary accelerated conditions), the decrease in active substance content was more pronounced, with one sample below the specification limit at six months. All results for the simulated use, photostability and freeze/thaw tests, in the proposed packaging for marketing, were satisfactory. These results support a shelf life of 18 months at 5°C.

Coated Tablets

1 mg coated tablets

The major stability studies were performed on four batches of the triangular shaped tablets, packed in both 40 ml HDPE bottles with child-resistant caps (used only for development studies – the bottles will not be marketed at this time) and PVC/PE/Aclar/foil blisters. 18 months data are provided for

tablets stored at 25°C/60%RH although the studies will continue up to 36 months. The tablets have also been stored for 12 months at 30°C/60%RH and for 6 months at 40°C/75%RH, respectively. Photostability studies under ICH option 2 light conditions and room light have also been performed on batches packed as above.

Generally, all results for the triangular tablets comply with the specifications, independent of the storage conditions and time. Exposure of tablets to ICH option 2 light conditions or room light resulted in little or no change in the Group II degradant levels. However, with increasing temperature an increase in Group II degradant levels was observed. The product information therefore carries a recommendation to "Keep the blister in the outer carton".

Supportive stability data are provided for three batches (of the same composition) but where the tablets were oval shaped. Since the difference in size and exposed area between the oval and triangular tablets is negligible, these results strongly support the previous results. From representative batches, 24 months data are available at 25°C/60%RH and these studies will continue for 36 months. 12 months data at 30°C/60%RH and 6 months data at 40°C/75%RH are also available. Also the results for oval shaped tablets comply with the specifications, independent of storage conditions and time.

The limited number of stability studies on commercial scale batches does not yet provide sufficient data on which to tighten the shelf-life specification for sirolimus content. However, when further commercial scale batch data become available the data will be re-examined and the shelf-life specification tightened or justified.

In conclusion, the stability studies submitted supported a shelf life of 18 months for the triangular tablets, independent of storage conditions. The absence of a temperature-specific storage recommendation is therefore justified.

2 mg coated tablets

23 months stability data were provided for one production scale batch and two pilot scale batches under long-term study (25C/60% H.R). Accelerated stability studies were performed at 40C/75% H.R over 6 months duration. Additional long-term stability studies were conducted at the intermediate condition of 30C/70% H.R. over a 18 months duration. A photostability study was also performed. The methods used for drug product stability have been validated, and shown to be stability indicating. The results presented support the proposed shelf life and storage conditions defined in the SPC.

2. Toxico-pharmacological aspects

Pharmacodynamics

Pharmacodynamic effects related to the proposed indication

Sirolimus was initially identified as an antifungal agent. Further pharmacological evaluation revealed its immunosuppressive activity in a rat model of T cell-mediated autoimmune disease. Additional *in vitro* and *in vivo* pharmacology studies demonstrated that sirolimus is an immunosuppressive agent that inhibits lymphocyte proliferation and prevents allograft rejection in animal models of organ transplantation.

The macrocyclic immunosuppressive agents, including sirolimus, tacrolimus, and CsA bind to specific cytosolic proteins called immunophilins to gain their immunosuppressive activity. The complex of tacrolimus or CsA with their respective immunophilins inhibits calcineurin, a phosphatase required for the production of cytokines and early activation of T cells (G_0 to G_1). Although sirolimus binds to the same immunophilin as tacrolimus, the sirolimus:immunophilin complex has no effect on calcineurin activity. Rather, this complex binds to a specific cell cycle regulatory protein called the *m*ammalian *t*arget *o*f *r*apamycin (mTOR) and inhibits its activation. It is likely that phosphorylation mediated activation of one or more phosphatases or kinases by mTOR is the first step in the cascade of events

that are affected by sirolimus. The inhibition of mTOR supresses cytokine-induced cell proliferation, inhibiting the progression from G_1 to the S phase of the cell cycle.

In vitro studies

Sirolimus inhibits cell proliferation induced by specific stimuli including cytokines and growth factors and non-specific stimuli such as lectins and phorbol ester. This effect is observed in T cells, B cells, fibroblasts, smooth muscle cells, keratinocytes, and tumor cells.

In vivo studies

Sirolimus prolongs graft survival in different models of transplantation (mouse, rat, dog, rabbit, pig, primates), and for heterotopic and orthotopic organ grafting given orally and/or intravenously. Additional studies have demonstrated the efficacy of sirolimus in preventing the rejection of skin grafts, islet cells, and bone marrow in the mouse.

The efficacy of two different tablet formulations (spray-dried powder and NanoSystems dispersions) were compared with that of the oral liquid formulation (all given at 1.5 mg/Kg/day for 14 days) in a heterotopic heart transplant model in male rats. Controls were either untreated or given phosal-based liquid formulation. There was no difference among sirolimus-treated groups as regards mean survival time of allografts, which was 30.5±3.7 days, 30.5±2.4 days, and 28.7±2.6 in animals treated with spray-dried powder, NanoSystems and oral liquid formulation, respectively, as compared with 6.3±0.5 days in controls.

Pharmacodynamic drug interactions

The majority of *in vitro* and *in vivo* studies indicate that, sirolimus and CsA act synergistically to inhibit T cell responses to different stimuli.

General and safety pharmacology programme

Studies in rats, mice and guinea pigs did not indicate any major effects of sirolimus on the CNS, the cardiovascular or respiratory system.

In contrast to the structurally related tacrolimus and CsA, renal effects seem to be significantly less marked with sirolimus. These observations are in line with the fact that nephrotoxic effects of tacrolimus and CsA have been suggested to involve inhibition of calcineurin, and sirolimus does not seen to interact with calcineurin. However, combination toxicity studies of sirolimus in conjunction with CsA resulted in a potentiation of CsA-induced nephrotoxic effects in rats. It is not clear whether this is a consequence of the increased blood levels of CsA due to the combination.

The nephrotoxic effects of CsA have been suggested to include effects in vasodilatory and vasoconstrictor substances. The effect of sirolimus and tacrolimus on prostacyclin and endothelin release was determined in cultured rabbit mesangial and endothelial cells. Sirolimus significantly increased release of prostacyclin (PGI2) and endothelin in TGF-β treated endothelial cells while tacrolimus caused a decrease in PGI2, but an increase in endothelin. Both sirolimus and tacrolimus have the capacity to affect release of vasoactive substances *in vitro.*

Haematological effects

Human platelet aggregation induced by Ca^{2+}-dependent and Ca^{2+}-independent pathways were not affected by sirolimus or tacrolimus at concentrations up to 100 nM. Mitochondrial respiration and induced swelling was not affected, while 10 μM CsA partially inhibited respiration.

Other effects

Sirolimus has no antibacterial effects. Neither sirolimus nor CsA reactivated latent murine cytomegalovirus infection; the ability of the host to control active, replicating virus was, though, impaired.

In a receptor binding study sirolimus showed no activity in most assays, but histamine I binding was inhibited with IC_{50} values of 100-500 nM.

Sirolimus inhibited cell proliferation in several models *in vivo* and *in vitro* and was active in various models of acute and chronic inflammation (e.g. arthritis, uveoretinitis, encephalomyelitis, myocarditis, carrageenan oedema) in the rat.

Pharmacokinetics

Studies performed

Pharmacokinetic studies of sirolimus have been done primarily in mice, rats and cynomolgus monkeys, these being the species used in the toxicity studies. The concentrations of sirolimus were determined by validated high-performance liquid chromatographic or liquid chromatographic/tandem spectrometric methods. In some studies radiolabelled sirolimus was employed.

Linearity

After repeated oral dosing of sirolimus, exposure to the drug was generally comparable to that after a single dose. In rats receiving repeated sirolimus, for a seven-day dosing period there was only a modest increase (34%) in sirolimus exposure compared to a single dose.

Pharmacokinetic characteristics

The main pharmacokinetic characteristics are given in Table 1.

Table 1: Animal pharmacokinetic data

Absorption	Low oral bioavailability.
Presystemic metabolism	Studies in rats have indicated that limited absorption from the gut and first-pass hepatic and extra-hepatic oxidation and degradation play a role in limiting the bioavailability of oral sirolimus.
Distribution	Uptake into blood. High protein binding. High volume of distribution. Tissues that accumulated radioactivity after IV [^{14}C]-sirolimus were the gastrointestinal tract (due to biliary excretion of metabolites), liver, lungs, pancreas, brain, lymph nodes, testes and urinary bladder.
Half-life	The $t_{1/2}$ of the unchanged drug in the rat is about 31 h and in the monkey about 14 h.
Metabolism	Extensive metabolism in all species. Mainly O-demethylation and hydroxylation. CYP3A the major isozyme involved. In addition to CYP3A dependent metabolism in liver, intestinal biotransformation may occur.
Excretion	The majority excreted in faeces in form of metabolites.
Pharmacokinetic drug interactions	In rats concomitantly given sirolimus and cyclosporin the bioavailability of sirolimus in terms of blood AUC was increased by 82% and that of cyclosporin by 76%, compared with the two drugs given alone.
Isomeric composition	A study in rats given a single oral or intravenous dose of isomer C showed extensive isomerisation to isomer B.

Transferability of compound across the placenta and distribution into milk

Following administration of radiolabelled sirolimus, radioactivity crosses the placental barrier and distributes into the amniotic fluid and foetal tissues with relatively slow elimination of radioactivity from these tissues, suggested that accumulation might occur with repeated dosing. Radioactivity is also excreted in the milk of lactating rats.

Rapamune should not be used during pregnancy. Breast-feeding should be discontinued during therapy.

Drug interactions

Ketoconazole, nicardipine and CsA, inhibitors of CYP3A4, inhibited sirolimus metabolism in human liver microsomes.

The bioavailability of sirolimus is increased by a factor 2-11 and the bioavailability of CsA by a factor 2-3 when both compounds are administered together. Combination toxicity studies in rats with sirolimus and CsA also indicated potentiated toxicity that is in line with exposure increasing in combination.

Systemic exposure in animals

Exposure to sirolimus in patients is generally higher than in animals.

Bioequivalence

The bioavailability of various oral formulations of Rapamune was evaluated in a non-randomised crossover study in six male cynomolgus monkeys at doses from 0.25 to 1 mg/kg. The formulations tested were the liquid formulation, the spray-dried tablet formulations and the Nanosystems® (an intermediate developed for processing into tablet formulations).

The absolute bioavailability of the drug from all oral formulations was determined by comparing their respective (dose-normalised) area under the blood concentration-time curve (AUC) to the AUC from an intravenous dose of 0.025 mg/kg. The relative rates of absorption from the oral formulations were determined by comparing maximum concentrations (C_{max}) and the time of its occurrence (t_{max}). Statistical analysis included descriptive statistics by formulations on AUC, C_{max} and t_{max} and on the extent of absorption (absolute bioavailability). Analysis of variance was used to assess the significance of differences in absolute bioavailability between the oral formulations. Blood samples were analysed by LC/MS at TexMS, Houston.

The absolute bioavailability of the clinical oral liquid formulation (2.1%) was consistent with that seen in previous studies in monkeys (2.4%) (GTR-24235). However, it gave lower bioavailability than the solid oral formulations and the Nanosystem dispersions. Two of the solid oral formulations yielded bioavailability around 6% and the remainder, including the three strengths of the Nanosystems tablets (1, 2 and 5 mg), gave values in the range of 10-15% as evidenced by the late t_{max} values, which ranged from 6 to 11 h.

The rate and extent of Rapamune Nanodispersion tablets manufactured from two different particle size dispersions were evaluated in a further study in six male cynomolgus monkeys. Each monkey received a single oral dose of 3 x 1 mg tablets according to a randomised two-period, two-treatment crossover design. The relative bioavailability of the drug for the two tablets with different particle size dispersions was assessed by comparing the blood AUC_{0-24}, C_{max} and t_{max}. Differences in pharmacokinetic parameters for the two lots were less than 20%, indicating that both particle size dispersions were bioequivalent in the monkey (GTR-37178).

The study in monkey indicated that the tablet formulation could enhance bioavailability compared with the liquid formulation. This observation does not seem relevant to the human situation as such a difference was not noted in renal transplant patients.

Toxicology

The toxicity of sirolimus has been characterised in studies up to one year, in mouse, rat, dog and monkey. In general, mice tolerated higher doses than other species. Overall exposure to the different intermediates seemed to be covered in the species used in toxicology studies. There were no obvious specifics indicating a particular relevance of one species.

A developmental toxicity study in rats indicated an increased risk to foetal development (mortality, curved/kinked tail) of the combination sirolimus and cyclosporine, and embryotoxicity seemed thus enhanced in comparison with sirolimus alone.

A 28-day repeated dose toxicity study in rats with Group II impurities at levels from 0.4 to 11%, was conducted. Group II impurities consisted of multiple oxidation/hydrolysis degradation products found at relative retention times from 0.0 to 0.6 and which could not be resolved because of >100 different impurities. There were no relevant differences in the toxicological profile between 0.4 and 11% impurities. Further, Group II and other relevant impurities have been present in batches used in earlier studies, including studies on genotoxicity.

Single dose toxicity

Sirolimus had low acute toxicity in mouse and rat. In rats, single oral doses did not significantly influence CNS, cardiovascular, respiratory or gastrointestinal function, but effects were only monitored for a short time period in some studies.

Repeated dose toxicity

Introduction

Toxicity studies with sirolimus were conducted in mice, rats (up to 1 year), dogs and monkeys (1-6 month studies). A wide range of toxic responses, in a few cases partially reversible, was recorded. Effects such as increases in red cell blood parameters and decreases in lymphocytes and platelets seemed primarily related to haemoconcentration secondarily to a diabetogenic state and the immunosuppressant activity. Prominent changes in clinical chemistry included increases in glucose (males), triglycerides (females) and fibrinogen levels. The toxicity of sirolimus according to organs affected is outlined below. Generally no relevant margins of exposure relative to the expected clinical exposure could be identified. However, the toxicity to become first manifest was usually directed at reproductive organs (atrophy), heart (myocardial degeneration), pancreas (islet cell vacuolation), spleen, thymus, lymph nodes (atrophy), gastrointestinal tract (diarrhoea, colitis, typhlitis), lungs (phospholipidosis) and eyes (cataracts). At higher doses involvement of skin (ulcers), bone (fractures) and liver (haematopoiesis) became apparent.

Pancreas

Islet cell vacuolation was seen in rats and associated posterior polar cataracts; increased food intake and decreased body weights were noted. Effects were also accompanied by changes in clinical chemistry parameters consistent with an uncontrolled diabetic state and males seemed particularly sensitive. There are reports that tacrolimus and CsA also affect pancreatic function although apparently cataracts were not evident in those studies. Effects of sirolimus on the pancreas were considered as primary effects and morphological changes were reported in rat studies, only. Glucose levels were increased in female monkeys given intravenous doses; however, there were no pancreatic lesions.

Heart

Myocardial degeneration was observed in rat studies. In rats, the finding was ascribed to an exacerbation of a spontaneous lesion related to endogenous orphan parvovirus. This was supported by results from special toxicity studies. Overall, males seemed more sensitive although this was not coupled to differences in systemic exposures. CsA and tacrolimus have also been reported to induce myocardial degeneration. Cardiotoxicity with sirolimus was noted in dogs in 1-month intravaginal studies. Effects included ECG changes. In the dog, cardiac toxicity was considered secondary to acute necrotising fibrinoid vasculitis. Inconsistent findings of cardiotoxicity have also been reported in mice.

Reproductive organs

In male rats testicular tubular atrophy and giant cells in tubules were reported in intravenous oral studies. Similar changes were found in mouse studies at oral doses of 5 mg/kg and also, less consistently, in the monkey. Some reports also suggest that tacrolimus has adverse effects on testes function and morphology. Priapism in rat studies with sirolimus was considered a non-specific effect. The effects on male reproductive organs *per se* are unlikely to constitute a clinical safety concern during treatment, but in as much a decrease in testosterone levels may be involved, depressed hormone levels may result in an increased risk for secondary adverse effects.

Thymus, spleen, lymph nodes

Lymphoid atrophy and decreases of lymphocyte numbers in mouse, rat, dog and monkey were observed as expected for the mechanism of action.

Gastrointestinal tract

Acute gastritis was reported in a 3-month rat study and diarrhoea was occasionally evident in rat as well as mouse studies. In dogs, colonic cecal lymphoid necrosis, gastric ulcers and oral mucosal ulceration occurred. Colitis and typhlitis with diarrhoea were observed in monkey and considered secondary to immunosuppression or the antifungal effect. Tacrolimus may cause severe diarrhoea in monkey.

Kidney

Effects on renal function seemed minor in repeated dose studies with sirolimus given orally. In a 3-month study in rats non-specific renal effects, haemosiderosis in kidney and renal mineralisation were reported. In one dog study, kidney tubular degeneration was recorded and kidney tubular vacuolation in one mouse study was considered artifactious. Findings were not consistent and overall sirolimus seems to have a lower potential for renal toxicity than tacrolimus or CsA. In combination toxicity studies, CsA induced renal toxicity was potentiated by sirolimus in one study, possibly in part due to a pharmacokinetic interaction.

Lung

Upon treatment with sirolimus pulmonary alveolar macrophages gained characteristics common to phospholipidosis.The clinical relevance of this change is not clear. Further responses specific for sirolimus were haemosiderosis and increased haematopoiesis in liver and spleen.

Bone

Six studies were performed to elucidate the reduction of bone strength. The mechanism of this effect is not fully known, the effects of sirolimus on body weight, cytokines, testosterone and also its diabetogenic effect may contribute to the detrimental effects on bone structure. The results indicated partial reversibility and a particular sensitivity of young rats. Testosterone replacement only partially counteracted bone effects.

Combination studies

Combination toxicity studies showed that CsA had additive effects on the severity of pancreas islet cell vacuolisation, thymic atrophy and testicular atrophy while sirolimus induced hyperglycaemia was potentiated. Further, sirolimus potentiated CsA induced tubular basophilia. A pharmacokinetic interaction between CsA and sirolimus may partly explain the toxic interactions. Although CsA is also known to affect bone integrity, limited studies in rats with low doses of sirolimus and CsA did not indicate any interactions on bone effects.

Conclusion

Sirolimus induced a variety of toxic effects in laboratory animals. Most of the toxic effects have been reported with other immunosuppressants or represented expected exaggerated pharmacological effects. Reactions that appeared "unique" for sirolimus were pulmonary alveolar macrophages, increased haemosiderosis and haematopoiesis, indicative of an increase in red blood cell turnover, and possibly skin toxicity (ulcers mainly in mouse).

Reproduction toxicity

Effects of sirolimus on the different stages of the reproductive process have been adequately studied. In most studies maximum tolerated doses were reached, but exposure levels were lower than expected clinical levels. Male rats treated had decreased fertility and atrophy of testes; giant cells in testes and hypospermia in testes and epididymides were evident. These effects on male reproductive organs are not unexpected with an agent with antiproliferative properties. Female fertility was not affected, but early resorptions, decreased uterine weights, foetal body weights and foetal toxicity were noted. In a rat developmental toxicity study no teratogenic effects of sirolimus were observed. Reduced vertebral ossification and vertebral variations were increased. In rabbits, treatment with sirolimus seemed related to an increase in abortions.

Interactions of sirolimus with oral contraceptives are expected due to the involvement of CYP3A. Special studies in lactating rats showed that the compound-derived radioactivity passes into milk to a large extent.

Genotoxicity

Sirolimus did not induce any significant mutagenic or clastogenic effects in the standard battery of *in vitro* and *in vivo* tests for genotoxicity with or without metabolic activation.

Carcinogenicity

One carcinogenicity study in mice was terminated at week 29 (males) and week 86 (females) due to severe skin ulcerative lesions. The low dose resulted in C_{max} levels approx. x14 the expected clinical levels. An increased incidence of lymphomas, involving multiple organs, was recorded in the female groups at all doses, but particularly in the high dose group, where lymphomas were a major cause of death. In males the causes of death or moribundity were mainly attributed to skin lesions, GI distension, meningitis/meningoencephalitis and gavage errors.

In the second mouse carcinogenicity study the maximum dose reduced survival to approximately 20% due to the occurrence and progression of skin lesions which occurred early in the study and progressed throughout. A variety of haematological and histological changes were reported including lymphoid atrophy, testicular tubular degeneration and myeloid hyperplasia in bone marrow. Neoplastic changes consisted of hepatocellular tumours and lymphomas in males, lymphomas and granulocytic leukaemia in females. These were considered related to the immunosuppression. Liver tumours, including carcinomas, although statistically significantly increased were within historical control ranges. Liver tumours were seen only in male mice and it was concluded that these were due to non-genotoxic promotion of spontaneous neoplasms. A no-effect level for this change could not be identified as an increase was noted at the lowest dose that corresponded to approx. 2x expected clinical exposure.

The high dose in the rat carcinogenicity study was based on the maximum tolerated dose in a 52-week study. In terms of systemic exposure, margins to expected clinical levels were less than 1. An increase in testicular interstitial cell adenomas was recorded. Although not directly measured in the study, it is conceivable that this increase may be related to altered LH levels secondary to decreased serum testosterone levels. As luteinising hormone receptors are different in humans and rats and the age of onset of the tumours is also different, the increased incidence in rats may therefore be considered not predictive for humans.

Overall it can be concluded that carcinogenicity studies with sirolimus indicate that the intrinsic potential for carcinogenicity was secondary to that induced by the pharmacological action.

Skin cancers

Immunosuppressants may enhance UV induced skin tumours. Clinical observations are in line with that of combinations of CsA and immunosuppressants such as azathioprine and prednisolone, which hasten development of skin cancers. Clinical data with sirolimus are available for 2 years and to date do not indicate an increase over control therapy.

The potential phototoxicity of sirolimus has been assessed in a study in rabbits given oral doses of 8-methoxypsoralen (8-MOP), vehicle or sirolimus. The results were overall inconclusive and the study will be repeated in a different model. The available clinical experience to date would indicate that acute photoirritant reactions are not a major concern.

Local tolerance

The irritation potential of sirolimus and vehicle was similar (mild to moderate) in rabbits given the compound intravenously for one week. Rabbits treated with a single dermal application of sirolimus in petrolatum did not show any dermal irritation or other adverse effects.

Impurities

In a 28-day study a specific impurity (WAY-124854) was tested. The toxicological profile of rapamycin did not change in the presence of this impurity. In studies on the genotoxic potential high concentrations and high doses of sirolimus were used and impurities were present at considerable amounts. Furthermore, carcinogenicity studies with sirolimus showed that doses or systemic exposures lower than expected therapeutic levels resulted in positive findings and therefore the potential effects of the impurities may be considered less relevant.

A 28-day repeated dose toxicity study in rats with Group II impurities at levels from 0.4 to 11% was conducted. Group II impurities consisted of multiple oxidation/hydrolysis degradation products found at relative retention times from 0.0 to 0.6 and which could not be resolved because of >100 different impurities. There were no relevant differences in the toxicological profile between 0.4 and 11% impurities. Further, Group II and other relevant impurities have been present in batches used in earlier studies, including studies on genotoxicity**.**

4. Clinical aspects

Clinical pharmacology

Pharmacodynamics

Mechanism of action

Sirolimus is an immunosuppressive agent that exerts anti-rejection activity *in vitro* and *in vivo* through inhibition of the proliferation of T- and B-lymphocytes. Sirolimus also inhibits the proliferation of a variety of transformed cell lines and inhibits growth factor-mediated proliferation of smooth muscle cells *in vivo*.

Pharmacodynamic analyses indicate that the sirolimus formulation (tablet or solution) is not a predictor of efficacy or safety events. Tablet and solution can be used interchangeably and the same target trough concentrations can be used for therapeutic drug monitoring. In the open-label study 309, the treatment formulation was not a significant predictor of acute allograft rejection or laboratory results. HLA mismatches and average concentrations of sirolimus (tablet or solution) were the only meaningful predictors of acute rejection and significant associations were found between sirolimus exposure (measured by concurrent trough concentrations) and the laboratory parameters LDH, aspartate aminotransferase (AST), and blood urea nitrogen (BUN).

Pharmacokinetics

Oral solution

Introduction
Pharmacokinetic information was gathered in 44 clinical studies (n=690), of which 16 were performed in healthy volunteers (n=335). Twenty clinical studies were carried out in patients with stable allograft function (n=117). Other studied groups were renal allograft patients with renal complications (n=32), renal allograft patients at high risk of chronic rejection (n=12), psoriasis patients (n=77), stable liver allograft recipients (n=8), hepatically impaired patients (n=19), post renal transplant recipients (0-12 months after transplantation, n=70) and paediatric dialysis patients (n=20). The majority of studies displayed full pharmacokinetic profiles, whereas a few studies measured sirolimus trough levels only.

Three different analytical techniques were used to analyse whole blood and plasma samples from clinical trials. The methods were HPLC with mass spectrometric detection, HPLC with UV detection and an immunoassay. Cross-validations have been performed. Based on results from the European central laboratory, both chromatographic techniques yield results 0.8 times the immunoassay. The immunoassay is not currently available. In order to assist centres in establishing an HPLC-UV or LC/MS assay, the Applicant has developed a program to assure that Therapeutic Drug Level Monitoring (TDM) is available to transplant centres throughout Europe. The program consists of providing analytical standards, assay methods, technical assistance and TDM documentation.

Absorption
Following oral administration, sirolimus is rapidly absorbed, with a time to peak concentration of 1 hour in healthy subjects receiving single doses and 2 hours in patients with stable renal allografts receiving multiple doses. Upon repeated administration, the average blood concentration of sirolimus is increased approximately 3-fold. The absolute/oral availability of sirolimus in combination with simultaneously administered CsA is approximately 14%. The bioavailability was estimated in the presence of CsA administered simultaneously to sirolimus. In the clinical situation, CsA will be administered 4 h apart during the initial 2-3 months, and without CsA thereafter. It can therefore be expected that the bioavailability in the clinical situation will be lower.

Distribution
Sirolimus is extensively partitioned into formed blood elements; the blood to plasma ratio is 36. The *in vitro* protein binding studies demonstrated that sirolimus is approximately 92% bound, mainly to serum albumin (97%). No significant relationship has been found between hematocrit values and clearance and any changes in RBC count over time are therefore unlikely to have an effect. In the healthy population, the apparent distribution volume (V_{ss}/F), based on whole blood concentrations, was 19 l/kg, whereas in the target population (renal allografts) V_{ss}/F was significantly lower, 11.6 l/kg.

Metabolism
Sirolimus is extensively metabolised. The primary enzyme involved in the metabolism of sirolimus was CYP3A4, catalysing demethylation at the 41-O-, 32-O- and 7-O-methyl positions to form the corresponding O-demethylated metabolites and the hydroxylation of sirolimus. The immunosuppressive activity of the metabolites is less than 30% of the sirolimus activity.

Excretion
After a single dose of [^{14}C] sirolimus in healthy volunteers, the majority (91.1%) of radioactivity was recovered from the faeces, and only a minor amount (2.2%) was excreted in urine. Approximately 90% of the radioactivity was excreted after 5 days. After multiple oral doses, the terminal half-life in stable renal transplant patients receiving CsA and steroids concomitantly was 62±16h. The effective half-life, however, is shorter and mean steady-state concentrations were achieved after 5 to 7 days.

Special populations

Impaired renal function
Renal excretion of sirolimus is very small (in a study in healthy volunteers only 2.2% was excreted in the urine, see above). The pharmacokinetics of sirolimus are similar in various populations with renal function ranging from normal to absent (dialysis patients).

Impaired hepatic function
In mild and moderate hepatically impaired patients (Child-Pugh classification of A or B), mean values for sirolimus AUC and $t_{1/2}$ were increased 61% and 43% respectively and CL/F was decreased 33% compared to normal healthy subjects. Sirolimus pharmacokinetics were not evaluated in patients with severe hepatic impairment. It is recommended that sirolimus whole blood trough levels be especially closely monitored in patients with impaired hepatic function.

Race and gender effects

Black recipients
There is limited information indicating that black renal transplant recipients (predominantly African-American) require higher doses and trough levels of sirolimus to achieve the same efficacy as observed in non-black patients. Currently, the efficacy and safety data are too limited to allow recommendations for use of sirolimus in black recipients.

Women
A gender effect was detected for CL/F and $t_{½}$, in the two-stage analysis of Phase I, II and III-data. CL/F was increased in women (260 ml/h/kg) compared with men (233 ml/h/kg) and, consequently, $t_{½}$ was shortened. The difference was not considered clinically important.

Elderly
Clinical studies of sirolimus did not include a sufficient number of patients > 65 years of age to determine whether they will respond differently than younger patients. Sirolimus trough concentration data in 35 renal transplant patients > 65 years of age were similar to those in the younger population (n=822) from 18 to 65 years of age.

Children and adolescents
In paediatric patients on dialysis (30% to 50% reduction in glomerular filtration rate) within age ranges of 5 to 11 years (n=9) and 12 to 18 years (n=9), the mean weight-normalised CL/F was larger for younger paediatric patients (580ml/h/kg) than for older paediatric patients (450ml/h/kg) or adults (287ml/h/kg). There was a large variability for individuals within the age groups. There is insufficient experience to recommend the use of sirolimus in children and adolescents.

Interaction studies

Interactions with other medicinal products
Ten interaction studies were performed in healthy volunteers mainly regarding substrates of CYP3A4 and/or P-glycoprotein (PgP). The majority of studies included healthy males and females, 18-45 years of age, and sirolimus concentrations were followed for 144h. It should be noted that CsA, being a substrate and also an inhibitor of both CYP 3A4 and PgP, was not administered together with sirolimus, which is likely to be done in the clinical situation. Hence, any recommendations based on these studies must be interpreted having this in mind. In order to address this issue, Therapeutic Drug concentration Monitoring (TDM) in all patients is advised and co-administration of sirolimus with

strong inducers (e.g. rifampin, rifabutin) or inhibitors (e.g. ketoconazole) of CYP3A4 is not recommended unless the benefit outweighs the risk (see below*)*.

No clinically significant interactions were detected for acyclovir, atorvastatin, glibenclamide, nifedipine, digoxin and trimethoprim/sulphamethoxazole. Sirolimus slightly increased the glibenclamide exposure by 14 % and C_{max} by 20%.

Cyclosporin A (CsA) (CYP3A4/P-gp substrate and inhibitor): The rate and extent of sirolimus absorption was significantly affected by CsA. Microemulsion CsA, administered 4 hours prior to sirolimus, increased the sirolimus AUC, C_{max}, and t_{max} 1.8-fold, 1.4-fold, and 1.6-fold, respectively. Single-dose sirolimus did not affect the pharmacokinetics of CsA (microemulsion) in healthy volunteers when administered simultaneously or 4 hours apart. Based on the design of large Phase III clinical trials, it is recommended that sirolimus be administered 4 hours after CsA (microemulsion). Implementation of TDM in all patients and the recommendation to give CsA and sirolimus at a consistent time interval are adequate measures.

Rifampicin (strong CYP3A4/P-gp inducer): Administration of multiple doses of rifampicin decreased sirolimus whole blood concentrations following a single 10 mg dose of sirolimus oral solution. Rifampicin increased the clearance of sirolimus by approximately 5.5-fold and decreased AUC and C_{max} by approximately 82% and 71%, respectively. Co-administration of sirolimus with rifampicin is not recommended unless the benefit outweighs the risk due to this interaction. If rifampicin is administered with sirolimus, the dose of sirolimus should be initially increased to 8-times the maintenance dose, followed by trough sampling within 5 to 7 days for purposes of therapeutic drug monitoring. Upon termination of rifampicin therapy, the dose of sirolimus should be gradually reduced to the original maintenance dose.

Ketoconazole (strong CYP3A4/p-gp inhibitor): Multiple-dose ketoconazole administration significantly affected the rate and extent of absorption and sirolimus exposure as reflected by increases in sirolimus C_{max}, t_{max}, and AUC of 4.3-fold, 1.4-fold, and 10.9-fold, respectively. Co-administration of sirolimus with ketoconazole is not recommended unless the benefit outweighs the risk due to this interaction. If ketoconazole is administered with sirolimus, the dose of sirolimus should be initially reduced to 1/6th the maintenance dose, followed by trough sampling within 5 to 7 days for purposes of therapeutic drug monitoring. Upon termination of ketoconazole therapy, the dose of sirolimus should be increased to the original maintenance dose.

Diltiazem (CYP3A4/P-gp substrate and inhibitor): The simultaneous oral administration of 10 mg of sirolimus oral solution and 120 mg of diltiazem significantly affected the bioavailability of sirolimus. Sirolimus C_{max}, t_{max}, and AUC were increased 1.4-fold, 1.3-fold, and 1.6-fold, respectively. Sirolimus did not affect the pharmacokinetics of either diltiazem or its metabolites desacetyldiltiazem and desmethyldiltiazem. If diltiazem is administered, sirolimus blood levels should be monitored and a dose adjustment may be necessary.

Other possible interactions in connection with cytochrome P_{450} : Inhibitors of CYP3A4 and P-gp may increase the absorption of sirolimus and increase sirolimus blood levels (e.g. calcium channel blockers: nicardipine, verapamil; antifungal agents: clotrimazole, fluconazole, itraconazole; macrolide antibiotics: clarithromycin, erythromycin, troleandomycin; gastrointestinal prokinetic agents: cisapride, metoclopramide; other substances: bromocriptine, cimetidine, danazol, protease inhibitors). Inducers of CYP3A4 and P-gp may decrease the absorption of sirolimus and decrease sirolimus blood levels (e.g. St. John's wort (*Hypericum perforatum),* anticonvulsants: carbamazepine, phenobarbital, phenytoin; antibiotics: rifabutin). Although sirolimus inhibits human liver microsomal cytochrome P_{450} CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 *in vitro*, the drug is not expected to inhibit the activity of these isozymes *in vivo* since the sirolimus concentrations necessary to produce inhibition are much higher than those observed in patients receiving therapeutic doses of sirolimus.

Norgestrel/ethinyl estradiol: No clinically significant pharmacokinetic interaction was observed between sirolimus and 0.3 mg norgestrel/ 0.03 mg ethinyl estradiol. The results of a single dose drug interaction study with an oral contraceptive cannot exclude the possibility of changes in the

pharmacokinetics that might affect the efficacy of the oral contraceptive during long-term treatment with sirolimus. This is of particular relevance in view of the fact that effective contraception must be used during sirolimus therapy and for 12 weeks after sirolimus has been stopped.

Interactions with food and drink

The influence of orange juice, Tang and water on the blood profile of sirolimus was investigated in 19 healthy volunteers. Compared to water, orange juice did not affect either the rate or extent of absorption of sirolimus. Tang increased the sirolimus t_{max} and AUC by 20% and 17%, respectively. Grapefruit juice is known to significantly affect the activities of CYP3A4 and P-pg in the intestine and must therefore be avoided.

The pharmacokinetics was significantly changed after a high fat meal as compared with fasting conditions. In healthy volunteers not receiving concomitant CsA, there was a 34% decrease in the peak concentration (C_{max}), a 3.5-fold increase in the time to peak concentration (t_{max}), and a 35% increase in total exposure (AUC). The half-life did not change as a result of the different feeding conditions. It is recommended that sirolimus be taken consistently either with or without food. In phase III trials, sirolimus was given approximately 4 hours after the morning dose of CsA.

Coated Tablets

The pharmacokinetic characteristics of sirolimus tablet formulations were evaluated in seven studies, two of which were conducted in renal transplant patients. The oval 1mg tablet and the solution were not bioequivalent after single dose (1mg) to healthy volunteers. AUC and t_{max} were increased by 27% and 82%, respectively, whereas C_{max} was decreased by 35% for the tablet compared with the solution. The bioavailability of the tablet was approximately 17%, which should be compared with the 14% estimated for the solution. The half-life, approximately 70h, was comparable between the two formulations, which suggests that the elimination of sirolimus was not affected by formulation. After a high-fat meal, the increase in bioavailability is in the same range as that seen for the oral solution. It is recommended that the tablet should be taken consistently with or without food to minimise intra-individual variability. Triangular 1 and 2mg tablets were bioequivalent in C_{max}, and AUC after single doses to healthy volunteers, but t_{max} for 2mg tablets was increased 18%. The exposure was essentially linear in the studied dose range of 5 – 40mg (oval tablets) and 2 – 5 mg (triangular tablets). However, the dose-linearity study with oval tablets was performed using a 5mg tablet.

In patients, an oval tablet formulation was comparable with the oral solution comparable with respect to C_{max} and AUC, but t_{max} was prolonged for the tablet. In Study 310, the sirolimus dose without CsA was approximately four times the dose given in combination with CsA. The dose increase after CsA withdrawal reflects the increase in trough levels needed when sirolimus is administered without CsA and the vanished interaction effect of CsA. The distribution of trough levels was comparable between formulations and the same therapeutic interval as for the solution may be used also for the tablet. As described for the solution, the intra- and inter-individual variability in trough levels is high (40-50%). When switching formulations, blood concentration monitoring is recommended after 1-2 weeks.
CsA given 4h prior to the tablet had a significant effect on absorption and bioavailability of sirolimus in healthy subjects. The effect was, however, less pronounced than for the solution. Thus, the formulation affects the interaction potential of sirolimus.

In the clinical trial, an oval tablet was used, whereas a triangular form is proposed for marketing. The triangular shaped tablet tends to give slightly higher drug levels compared with the oval form (inter-study comparison). No formal bioequivalence study was conducted. Given the inter-patient variability, the difference between the two formulations is not considered of clinical importance. Indeed, any minor differences in bioavailability between the oval- and triangular-shaped tablets would be extremely small compared to the large inter-individual differences in bioavailability, the consequences of which are minimised by therapeutic drug monitoring (TDM). The applicant re-iterated that Study 310, in which a tablet (oval) was the only sirolimus formulation given to patients, was the largest concentration-controlled trial that tested the feasibility of cyclosporine withdrawal. Therefore, the

sirolimus trough concentrations observed are the basis for the recommendations in the current SmPC for the oral solution.

Sirolimus whole blood trough concentrations should be monitored in all patients. In the SmPC, separate target ranges are recommended for the initial period when sirolimus is given with cyclosporine and for the maintenance period after cyclosporine is withdrawn. Recommended loading and starting doses of sirolimus are 6 mg and 2 mg, respectively. This regimen has been tested in a large number of patients who received either the oral solution or tablet formulations.

In the assessment, the following is considered relevant:

- Sirolimus pharmacokinetics is characterised by high variability (modest bioavailability, food effect, CsA interaction) resulting in intersubject CV ≥40%. The clinical relevance of differences between formulations must be viewed in the light of this high variability.
- TDM has been accepted as an adequate tool for dosing of sirolimus, based on the good correlation between C_{min} and AUC. TDM should be used in all patients and the applicant has previously documented that methods and equipment for this are adequately available to transplantation centres.
- The currently approved dosing regimen for sirolimus oral solution is based mainly on experience from trial 310, which used the oval Rapamune tablet as the only sirolimus formulation. In view of the reasoning above, CPMP considered adequate an extrapolation of these data to the commercially available oral solution.
- The new triangular tablet has shown *in vitro* sirolimus release rates not significantly different from those obtained with the clinically tested oval tablet.
- Compared with the oval tablet (inter-study), the triangular tablet showed 21% higher AUC and 45% higher C_{max}. The triangular tablet showed lower dose normalised C_{max} (57%) and moderately higher dose normalised AUC (14%), in an inter-study comparison with the oral solution. Variability was, as expected, high. Compared with overall variability, these mean differences are of questionable relevance.
- Other relevant pharmacokinetic characteristics were shown to be equivalent between triangular tablet and oral solution.

In summary, the proposed triangular tablet has been shown to provide an adequate source of sirolimus. Any small difference between tablet and oral solution could be of no matter whatsoever during TDM-based maintenance therapy, recommended for all patients. The single dose comparative trial between tablet and oral solution, simulating the loading dose situation, indicated a lower C_{max} with the tablet, which should be reassuring from the toxicity viewpoint and adequate exposure to sirolimus (AUC), which should be the first priority in this situation.

To confirm the relative bioavailability and pharmacokinetic linearity of the 1-mg and 2-mg tablets, a study was conducted in healthy volunteers. It was a single-dose, open-label, randomised, 3-period crossover study. Study 0468H1-186-UK assessed the dose proportionality of sirolimus triangular tablets over a dose range of 2 to 5 mg, following single-dose administration of 2 mg (2 x 1-mg tablets), 4 mg (2 x 2-mg tablets), and 5 mg (5 x 1-mg tablets) of sirolimus in healthy subjects. The pharmacokinetics for the 1- and 2-mg tablets was evaluated, although not at equivalent doses. Following dose corrections, AUC and C_{max} were considered bioequivalent. For the 2mg-tablet, t_{max} was slightly longer, but this is not considered to be of clinical relevance.

Clinical efficacy

Oral solution

Introduction

Efficacy of sirolimus combined with CsA and steroids was primarily studied in two large, double blind trials (301 and 302). Altogether 1,295 patients were included, of whom 1,004 were randomised to sirolimus at fixed doses of 2 or 5 mg/d as add-on to CsA and corticosteroids (CS). Six-month and one-year data from these trials were provided in the initial submission.

Supplementary submissions provided data on sirolimus as base immunosuppressant after discontinuation of CsA and with dosing based on concentration monitoring (studies 212 and 310). These trials included a total of 770 patients, all treated with sirolimus. Six-month data was submitted for study 212 and 12-month data was available for study 310. Subsequent updates on study 310 have provided follow up through 36 months.
The clinical trials were performed according to GCP standards and agreed international ethical principles.

Dose-response studies and main clinical studies

Dose response study of the sirolimus-CsA combination (203)

The daily sirolimus doses of 2 and 5 mg were chosen for the phase III studies, based on the phase II study 203-GL. In this study, there was little difference among the actual doses of sirolimus administered within a given dose group, suggesting that dose administration by body surface area was unnecessary. Therefore, two fixed dose levels of 2 mg/day and 5 mg/day, which represented central values for the two surface area dose levels of 1mg/m^2/day and 3 mg/m^2/day, were chosen for phase III. The need for a loading dose was suggested by the performed Phase I studies.

Pilot studies comparing sirolimus to CsA (207 and 210)

Two pilot studies in renal transplantation compared sirolimus to CsA in combination with either azathioprine (AZA) or mycophenolate mofetil (MMF). All patients received corticosteroids. Sirolimus therapy was concentration-controlled by adjusting doses to obtain sirolimus blood troughs of 30 ng/mL for 8 weeks, and 15 ng/mL thereafter. The outcomes in sirolimus and CsA, treatment arms regarding 12-month patient and graft survival was comparable whereas the renal function was better with sirolimus at most time-points (see table below).

Table 2 Mean ± SEM calculated GFR (mL/min, Nankivell method): Studies 207 and 210 combined

Week	Sirolimus	CsA	ANOVA
4	54.54 ± 2.53[a] (66)[b]	50.55 ± 2.22 (61)	0.241
12	64.56 ± 2.21 (55)	55.38 ± 2.05 (57)	0.003
24	66.28 ± 2.30 (48)	58.17 ± 2.05 (52)	0.009
36	66.65 ± 2.60 (47)	60.07 ± 2.35 (51)	0.063
52	68.60 ± 2.52 (43)	59.70 ± 2.27 (53)	0.010
104	69.00 ± 3.1 (31)	56.80 ± 2.76 (40)	0.005
a: Standard error of the mean. b: Number of patients is shown in parentheses.			

Phase III studies of the sirolimus-CsA combination (301 and 302)

There were two trials (301-US and 302-GL) of similar design. The main characteristics of these studies are given in Table 3. In both trials, sirolimus 2 mg/d and 5 mg/d, started as a loading dose of 6 mg and 15 mg, respectively, 24-48 h post-transplant were evaluated as add-on therapy to conventionally dosed CsA and corticosteroids. Antibody induction therapy was prohibited, as was the addition of other immunosuppressive agents. The comparators, azathioprine (AZA, trial 301) and placebo (trial 302) are not fully representative of current medical practice, but have been accepted in recent applications in this therapeutic field.

Table 3: Characteristics of studies 301 and 302

Study number	Design, blinding, randomisation	Randomisation and stratification criteria	Number of patients, doses, and control used	Duration of the treatment	Inclusion and exclusion criteria	Efficacy end-points
301-US	Randomised, double-blind, parallel group	The randomisation took place within 24-48 hours post-transplant and used a computerised randomisation/enrolment system to assign patients in a 2:2:1 ratio to the two dose levels of sirolimus and to AZA, respectively. Stratification by investigator and race	719 patients sirolimus (2 and 5 mg/day): 558; AZA (2-3 mg/kg/day): 161	Two year	The major inclusion and exclusion criteria were identical. The trials enrolled primary recipients ≥13 years of age of cadaveric or (HLA-mismatched) living-donor grafts. Patients with a history of malignancy or with recent severe atherosclerotic heart disease (unstable angina, recent acute MI) were excluded. Concomitant, protocol-defined, anti-infectious therapy was similar. All patients were required to use *pneumocystis carinii* (PCP) prophylaxis (preferentially TMP-SMX) during the first year post-transplant, and continued prophylaxis after the first year was recommended. All CMV –ve patients receiving grafts from CMV +ve donors were required to use CMV prophylaxis for the first three months post-transplant, and such prophylaxis was recommended in all patients. The use of antilymphocyte antibody induction therapy was prohibited.	<u>Primary end-point:</u> Efficacy failure within the first 6 months after transplantation. The 6-month window was defined from 154 to 194 days after transplantation. Efficacy failure was defined as the first occurrence of 1) biopsy-confirmed acute rejection, 2) graft loss (functional [>56 days of continuous dialysis] or physical), or 3) death. Patients defined as lost to follow-up were scored as efficacy failures, regardless of treatment assignment. The <u>secondary end-points</u> included time to first acute rejection episode
302-GL	Randomised, double-blind, parallel group	This trial used a pre-translantation randomisation procedure (2:2:1). Stratification by investigator and donor origin	576 patients sirolimus (2 and 5 mg/day): 446 Placebo, 130	Three year		

Study populations/accountability of patients

Demographic and baseline characteristics for the 301 and 302 trials are given in Tables 4 and 5. Regarding patient distribution with respect to ethnic and graft origin, a higher proportion of black patients were enrolled in study 301 than in study 302 (23% and 11%, respectively), and a lower proportion of patients received cadaveric grafts in study 301.

The patient populations were, generally, not at high immunological risk; mean percentage of PRA was <4% in all treatment groups. The average number of HLA mismatches was approximately 3.5 in all treatment groups. Only primary transplants were enrolled.

During the period of primary evaluation (months 0-6), the percentage of patients withdrawing without reaching an endpoint was 12-14% in sirolimus 2 mg groups, 18-26% in sirolimus 5 mg groups, and 14-19% in control therapy groups. The higher figures for sirolimus, 5 mg were accounted for by a higher incidence of adverse events in this group. These withdrawal rates were high but not exceptional for this type of trial.

Table 4: Demographic and baseline characteristics of patients enrolled in protocols 0468E1-301-US and 0468E1-302-GL

Characteristic	Sirolimus 2 mg/day Study 301 [a]	Sirolimus 2 mg/day Study 302[b]	Sirolimus 5 mg/day Study 301 [a]	Sirolimus 5 mg/day Study 302 [b]	AZA Study 301	Placebo Study 302
Total enrolled	284	227	274	219	161	130
Sex, n (%)[b]						
Female	76 (27)	79 (35)	104 (38)	70 (30)	70 (43) [b]	39 (30)
Male	208 (73)	148 (65)	170 (62)	149 (68)	91 (57)	91 (70)
Ethnic origin, n (%)						
White	160 (56)	172 (76)	154 (56)	175 (80)	92 (57)	103 (79)
Black	63 (22)	26 (11)	62 (23)	27 (12)	41 (25)	13 (10)
Hispanic	48 (17)	6 (3)	42 (15)	7 (3)	15 (9)	3 92)
Asian	7 (2)	10 (4)	10 (4)	2 (<1)	10 (6)	4 (3)
Australian aborigine		3 (1)		1 (<1)		
Other	6 (2)	10 (4)	6 (2)	7 (3)	3 (2)	7 (5)
Age (y)						
Mean	44.9	45.6	46.8	45.1	45.6	46.0
SD	13.6	12.3	13.0	12.2	13.0	13.1
Minimum	16	15	13	17	12	16
Maximum	79	71	76	68	69	71

a: There were no statistically significant differences among treatment groups in any baseline characteristic in study 0468E1-301-US, with the exception of sex ($p < 0.001$).
b: There were no statistically significant differences among treatment groups in any baseline characteristic in study 0468E1-302-GL.

Table 5: Donor source and primary aetiology of renal failure of patients enrolled in protocols 0468E1-301-US and 0468E1-302-GL[a]

Characteristic	Sirolimus 2 mg/day Study 301	Sirolimus 2 mg/day Study 302	Sirolimus 5 mg/day Study 301	Sirolimus 5 mg/day Study 302	AZA Study 301	Placebo Study 302
Total enrolled	284	227	274	219	161	130
Donor source, n (%)						
Cadaver	180 (63)	173 (76)	167 (61)	174 (79)	119 (74)	99 (76)
Living unrelated donor	18 (6)	15 (7)	24 (9)	16 (7)	9 (6)	4 (3)
Living related donor	86 (30)	39 (17)	83 (30)	29 (13)	33 (20)	27 (21)
Primary aetiology of renal failure, n (%)						
Autoimmune disease	13 (5)	7 (3)	7 (3)	8 (4)	13 (8)	5 (4)
Diabetes mellitus	59 (21)	28 (12)	53 (19)	34 (16)	32 (20)	17 (13)
Glomerulonephritis	64 (23)	65 (29)	50 (18)	51 (23)	18 (11)	32 (25)
Hypertension	72 (25)	35 (15)	77 (28)	27 (12)	47 (29)	2 (17)
IgA nephropathy (Berger's)	12 (4)	19 (8)	12 (4)	18 (8)	7 (4)	12 (9)
Interstitial nephritis/pyelonephritis	7 (2)	13 (6)	6 (2)	6 (3)	3 (2)	8 (6)
Obstructive uropathy/reflux	15 (5)	14 (6)	16 (6)	17 (8)	9 (6)	6 (5)
Other/unknown	19 (7)	23 (10)	21 (8)	25 (11)	13 (8)	10 (8)
Polycystic disease-kidney	23 (8)	23 (10)	32 (12)	33 (15)	19 (12)	18 (14)

a: There were no statistically significant differences among treatment groups in any baseline characteristic in either study.

Efficacy analysis

The primary efficacy endpoint was efficacy failure (defined as biopsy-proven rejection, death, or functional or physical graft loss at six months). A biopsy-proven acute rejection was any episode grade ≥1, according to the Banff 1993 criteria, irrespective of whether anti-rejection treatment was instituted. Local biopsy readings were used to define acute rejection. Based on historical data and the results of a phase II study (study 0468E1-203-GL), the efficacy failure rates were estimated to be 18% for sirolimus, 40% for the placebo group and 36% for the AZA group. Each comparison of a sirolimus treatment group to the control was made by the Cochran-Mantel-Haenszel (CMH) test stratified by investigator and by either race (301) or donor source (302). A Bonferroni correction for comparisons between each dose and controls was used at the α-level of 0.025. These studies were not powered to show a difference in the primary end-point between 2 and 5 mg/day. All randomised patients were included in the primary efficacy evaluation on an Intent-To-Treat (ITT) basis.

With this, sirolimus therapy (2 and 5-mg/day) was found statistically significantly superior to placebo at 6 and 12 months for both the primary endpoint of efficacy failure and the secondary end-point of acute rejection. In comparison with AZA (trial 301), superiority regarding efficacy failure was not maintained at 12 months. The incidences of efficacy failure and of acute rejection are given in the tables below.

Table 6: Incidence of efficacy failure (6 and 12 months; studies 301 and 302)

	Study 301			**Study 302**		
	AZA (n = 161)	**SRL 2 mg (n = 284)**	**SRL 5 mg (n = 274)**	**Placebo (n = 130)**	**SRL 2 mg (n = 227)**	**SRL 5 mg (n = 219)**
6 months	32.3 (52)	18.7 (53)	16.8 (46)	47.7 (62)	30.0 (68)	25.6 (56)
p-value	-	0.002	<0.001	-	0.002	<0.001
12 months	33.5 (54)	26.4 (75)	19.7 (54)	50.0 (65)	33.0 (75)	31.1 (68)
p-value	-	0.175	<0.003	-	0.004	<0.001

Table 7: Incidence of acute rejection (3, 6 and 12 months; studies 301 and 302)

	Study 301			**Study 302**		
	AZA (n = 161)	**SRL 2 mg (n = 284)**	**SRL 5 mg (n = 274)**	**Placebo (n = 130)**	**SRL 2 mg (n = 227)**	**SRL 5 mg (n = 219)**
3 months	29.8 (48)	15.1 (43)	8.4 (23)	40.8 (53)	22.0 (50)	18.7 (41)
p-value		0.0004	< 0.001		0.0002	< 0.001
6 months	29.8 (48)	16.9 (48)	12.0 (33)	41.5 (54)	24.7 (56)	19.2 (42)
p-value	-	0.002	<0.001	-	0.003	<0.001
12 months	31.1 (50)	21.8 (62)	14.6 (40)	43.1 (56)	26.9 (61)	23.3 (51)
p-value	-	0.046	<0.001	-	0.007	<0.001

The lack of statistical significance in the rate of efficacy failure for the 2-mg/day cohort in study 301 at month 12 (versus AZA) was mainly due to a disproportionate number of events in the 2-mg/day group between months 6 and 12 (intent-to-treat analysis).

In both studies, the rate of acute rejection remained significantly lower for both doses at month 12. This held true for the intent-to-treat as well as the on-therapy analyses. The data from two CsA withdrawal studies (212-GL and 310-GL, see below) provided additional data on the efficacy of sirolimus as add-on to CsA in preventing acute rejection in the early post transplant period.

Graft function

Group means calculated GFR up to 12 months are given in Table 8. The findings provide documentation that the sirolimus – CsA combination is associated with inferior average graft function, compared with CsA alone or CsA plus AZA. This effect is more pronounced at the 5 mg/day dose. At the 2 mg/day dose, there was no difference with placebo at 3 months.

Table 8: Mean values (± sem) for Nankivell calculated GFR (ml/min): studies 301 and 302 combined

	Sirolimus		AZA	Placebo	ANOVA
Time	**2 mg/day**	**5 mg/day**			
Month 1	43.09 ± 0.98[a] (496)[b]	42.63 ± 1.03 (471)	42.87 ± 1.90 (157)	43.66 ± 1.91 (124)	0.970
Month 3	60.85 ± 0.84[c,e] (379)	56.84 ± 0.93[c,d] (349)	66.04 ± 2.11 (91)	61.12 ± 1.39 (75)	< 0.001
Month 6	61.25 ± 0.92[c,e] (323)	57.98 ± 1.09[c] (283)	68.78 ± 2.13[f] (80)	62.58 ± 1.77 (66)	< 0.001
Month 12	60.33 ± 0.95[c,d,e] (275)	55.19 ± 1.16[c,d] (245)	67.51 ± 1.83 (78)	66.29 ± 1.86 (65)	< 0.001

a: Mean value is the mean on-therapy value. SEM is the standard error of the mean.
b: Number of patients.
c: Pairwise significant p-value comparison for a sirolimus treatment group versus the azathioprine group.
d: Pairwise significant p-value comparison for a sirolimus treatment group versus placebo.
e: Pairwise significant p-value comparison for 2 mg/day sirolimus versus 5 mg/day sirolimus treatment group.
f: Pairwise significant p-value comparison for placebo group versus azathioprine treatment group.

An attempt was made to correlate graft function to CsA and sirolimus levels. It could be noted that, in both trials 301 and 302, mean CsA trough levels at different time points were at the upper end of the intended concentration window. There was, however, no difference in CsA levels between sirolimus and AZA/placebo groups at any time point post-transplantation. A mixed linear regression analysis was performed, which did indicate a moderate impact of CsA and sirolimus trough levels on GFR.

Special populations

Race and gender effects

In study 301, black patients treated with sirolimus 5-mg/day, but not those treated with sirolimus 2-mg/day, had efficacy failure and acute rejection rates lower than those treated with azathioprine. Patients in this study were prospectively stratified by ethnic origin, in anticipation of safety and efficacy differences in black versus non-black patients.

Table 9: Incidence of efficacy failure (6 and 12 months) stratified by ethnic origin; study 301

	Black Patients			Non-black Patients		
	AZA	**SRL 2 mg**	**SRL 5 mg**	**AZA**	**SRL 2 mg**	**SRL 5 mg**
6 months	14/42 (33.3)	22/63 (34.9)	11/61 (18.0)	38/119 (31.9)	31/221 (14.0)	35/213 (16.4)
p-value	-	1.00	0.102	-	<0.001	0.001
12 months	16/42 (38.1)	27/63 (42.9)	13/61 (21.3)	38/119 (31.9)	48/221 (21.7)	41/213 (19.2)
p-value	-	0.688	0.077	-	0.049	0.011

Table 10: Patient and graft survival rates in black patients (study 301) – 12 months

	AZA	SRL 2 mg	SRL 5 mg
Patient survival	41/42 (97.6%)	61/63 (96.8%)	60/61 (98.4%)
Graft survival	38/42 (90.5%)	57/63 (90.5%)	57/61 (93.4%)

The information above indicates that black renal transplant recipients (predominantly African-American) require higher doses and trough levels of sirolimus to achieve the same efficacy as observed in non-black patients, although there are no indications that the dose/exposure relationship is different in black, compared with non-black individuals. Compared with AZA, a moderately effective immunosuppressive in this setting, there is no apparent benefit of the 2-mg dose of sirolimus in the black population. Currently, the efficacy and safety data are too limited to allow specific recommendations for use of sirolimus in black patients.

Elderly patients, children and adolescents

As expected in this type of trials, the enrolment of elderly patients was limited. Very few patients below 18 years old were studied and the documentation is considered insufficient for a paediatric indication for sirolimus.

Sirolimus as base immunosuppressant after discontinuation of CsA (212 and 310)

Trials 212 and 310 provide data for the dosage regimen finally proposed by the applicant.

Trial 212-GL
This was a 12-month Phase II trial. Six-month data were reported in the submission. The trial enrolled recipients of a primary cadaveric renal allograft and randomised only those who had established graft function within seven days post transplantation. Two sirolimus-containing regimens were compared:

- Group A: A fixed dose of sirolimus, 2 mg/d (following an initial loading dose of 6 mg) as add-on to conventional doses of CsA and corticosteroids. The targeted CsA through level was slightly lower than that used in trials 301and 302 (200-400 ng/ml during the first month, 150-250 ng/ml after month four).
- Group B: In this group, sirolimus, 20 mg/d was given for three days, followed by 10 mg/d during days 4-9. After this, dosing was based on Therapeutic Drug Monitoring, targeting trough levels of 10-20 ng/ml. CsA was given with target trough levels of 100-175 ng/ml during the first two months. During month three, CsA was tapered to discontinuation in patients who had stable graft function, stable and adequate sirolimus levels, and no history of recent rejection.

A "rescue" group C was available for patients who had not established graft function by seven days, or who could not be managed according to the intended regimens.

The primary analysis was for graft function at six months in the evaluable efficacy population, defined as those patients who were on assigned therapy and had not experienced an acute rejection since transplantation. Results are given in Table 11.

Table 11: Renal function parameters at Month 6 (on therapy patients without rejection); Trial 212-GL

Parameter	Group A Standard-dose CsA + 2 mg/day Sirolimus	Group B CsA Elimination + Concentration-controlled Sirolimus	p-value
Serum creatinine (µmol/l)	150.4 ± 7.3 (70)	126.6 ± 4.7 (64)	0.007

Calculated GFR (ml/min)	59.0 ± 1.9 (70)	69.4 ± 1.9 (64)	< 0.001
Measured GFR (mL/min/1.73m^2)	54.7 ± 5.6 (40)	72.4 ± 6.5 (45)	0.044

Biopsy-confirmed acute rejection at six months was evaluated as a secondary parameter (ITT population). Results are given in Table 12.

Table 12: Biopsy-confirmed acute rejection (ITT-population), Trial 212-GL

	Group A (n=95)	**Group B (n=99)**	**Group C (n=46)**
Acute rejection, n (%)			
Month 2	12 (12.4)	8 (8.0)	7 (14.3)
Month 6	15 (15.5)	18 (18.0)	9 (18.4)

Graft and patient survival was high and similar in groups A and B.

Trial 310-GL

This is a trial, extended to 5 years follow up, with the primary objective of comparing functional graft survival for two sirolimus-containing regimens. The submission now contains 36-month data for all patients. The trial enrolled recipients of primary or secondary renal allografts from cadaveric or living donors (see demography table below). The study population was strongly dominated by Caucasians (93-95%). Initial therapy for all patients during the first 3 months was CsA (target trough concentration during month 1: 200-400 ng/ml, thereafter 150-300 ng/ml) plus sirolimus ([SRL] nominal daily dose 2 mg, adjusted to target trough concentration >5 ng/ml, immunoassay) plus corticosteroids (CS).

Randomisation occurred at 3 months ± 2 weeks in patients who

- had not had Banff grade 3 rejection or vascular rejection during the last four weeks
- had serum creatinine <400 µmol/l
- were not on dialysis
- had sufficient renal function to support CsA withdrawal according to investigator's discretion.

Randomisation was to one of two groups:

- Group A: CsA reduced to target trough concentration 75-200 ng/ml through month 24 and then 50 – 150 ng/ml thereafter, plus sirolimus 2mg/d, adjusted to targeted trough concentration >5 ng/ml (immunoassay), plus CS
- Group B: CsA tapered to discontinuation plus sirolimus increased to targeted trough concentration 20-30 ng/ml (immunoassay) until month 12 then 15 to 25 ng/mL, thereafter, plus CS.

Discontinuation of CsA (group B) was to take place over 4-6 weeks, but took longer in 48%. Discontinuation was eventually successful in 92% of patients randomised to this treatment arm. By day 116, all patients in group B had discontinued CsA or left the study (23%). Patients that had failed initial treatment (n=79) or were not eligible for the second randomisation (presence of Banff grade 3 rejection or vascular rejection during the last 4 weeks, serum creatinine > 400 µmol/l or insufficient renal function to support CsA withdrawal according to investigator's discretion; n=16) discontinued protocol treatment (n=95). These patients are being followed through 5 years on an intent-to-treat (ITT) basis for graft survival, patients survival, acute rejection, renal function and malignancy. The demographic characteristics of patients enrolled into study 310 were similar to those in recent registration trials for other immunosuppressive agents. There were no notable differences between patients randomised to groups A and B. Moreover, the demographics were representative of the European patient population. Thus, an important selection bias due to patient withdrawal prior to randomisation was excluded.

Table 13. Demographic Characteristics – Study 310

Characteristic	Non-randomized (SRL-CsA) (n=95)	SRL-CsA (n=215)	SRL (n=215)	Total (n=525)
Recipient sex (% male)	63.2	66.5	61.9	64.0
Recipient ethnic origin (%)				
White	94.7	93.5	95.3	94.5
Asian	3.2	1.9	1.4	1.9
Black	1.1	2.3	0.9	1.5
Other	1.1	2.3	2.3	2.1
Recipient mean age (years)	48.8	45.8	44.6	45.9
Donor mean age (years)	48.0	44.0	41.7	43.8
Secondary transplants (%)	12.6	8.4	9.8	9.7
Source of donor organ (%)				
Cadaver	93.7	87.9	88.4	89.1
Living related donor	4.2	8.8	8.4	7.8
Living unrelated donor	2.1	3.3	3.3	3.0
Ischemia time, mean (hours)	17.8	17.8	16.4	17.2
HLA mismatches, mean	2.9	2.9	3.0	2.9
Delayed graft function (%)	48.4	21.9*	19.1*	25.5
Cause of end-stage renal disease (%)				
Glomerulonephritis	31.6	22.8	20.5	23.4
Polycystic disease-kidney	12.6	14.4	9.5	12.0
IgA nephropathy (Berger's)	9.5	11.6	13.5	12.0
Diabetes mellitus	11.6	6.5	7.9	8.0
Interstitial nephritis/pyelonephritis	7.4	7.4	8.8	8.0
Hypertension	6.3	7.0	5.1	6.1
Other/unknown	21.1	30.2	34.9	30.5
Fisher's exact test for pairwise comparison with the non-randomized group: * $p < 0.001$.				

Patient disposition

There was no difference in the rates of discontinuation at month 24 (Figure 1); however, there were significantly more discontinuations in the SRL+CsA group by month 36 (Table 14). The low-risk nature of the randomised population is evident in comparison with the non-randomised subset.

FIGURE 1. PATIENTS REMAINING ON THERAPY AT 24 MONTHS (775 DAYS)- STUDY 310

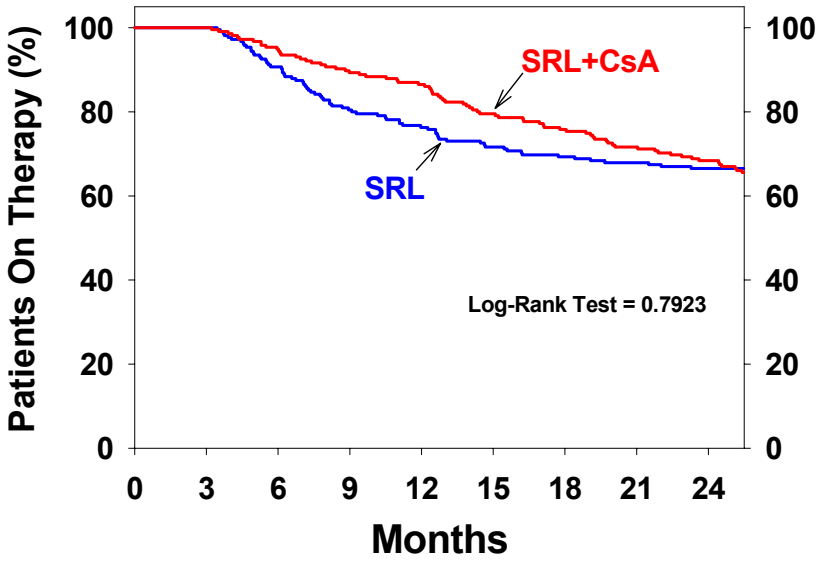


Table 14. Number (%) of patients who discontinued during by 36 months by primary reason and treatment group – study 310

Reason for Discontinuation	Nonrandomized SRL + CsA (n = 95)	Group A SRL + CsA (n = 215)	Group B SRL (n = 215)	A-B % (95% CI)	A vs B p-Value[a]
Total	95 (100)	103 (48)	81 (38)	10.2 (0.9, 19.5)	0.041*
Adverse reaction	70 (74)	68 (32)	54 (25)		
Other	1 (1)	3 (1)[b]	1 (< 1)		
Other nonmedical event	2 (2)	8 (4)	1 (<1)		
Patient request	4 (4)	5 (2)	9 (4))		
Protocol stipulation	2 (2)	0	0		
Protocol violation	4 (4)	4 (2)	5 (2)		
Unsatisfactory response – efficacy	12 (13)	15 (7)	11 (5)		

a: Fisher's exact test; *p < 0.05.

b: Includes patient 310-3703 whose reason for discontinuation was incorrectly reported as "other" instead of "adverse event: cardiac arrest."

Graft survival – study 310

The primary endpoint was graft survival at 12 months, and the noninferiority of group B compared to group B was established. Graft survival at 36 months was a secondary endpoint. According to the protocol, non-inferiority of group B to group A would be established at 36 months if the 95% confidence interval of the difference (rate in group A minus rate in group B) of graft survival crossed

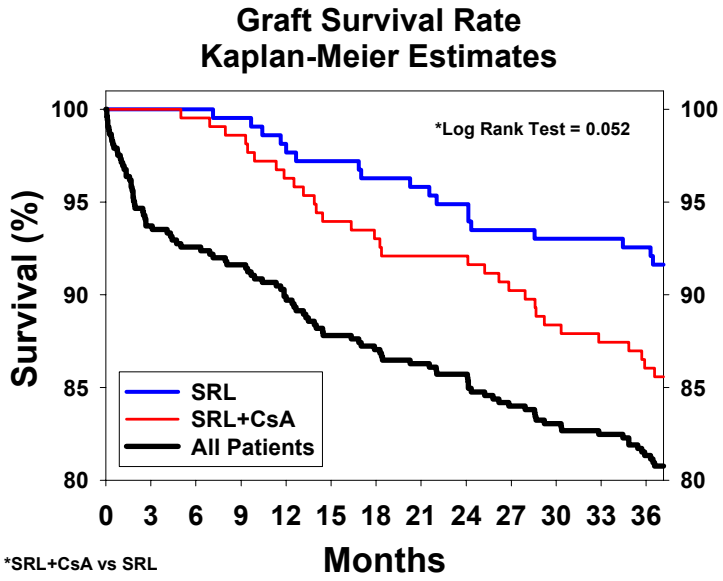
zero and if the upper limit of the confidence interval was no more than 10%. Graft survival at 36 months is given in Table 15 and the time to graft loss is plotted in Figure 2. These data confirmed the non-inferiority of group B compared to group A and growing difference in graft survival in favour of group B (CsA withdrawal).

Table 15. Summary of graft survival at 36 months – study 310

Status	Nonrandomized SRL + CsA (n = 95)	Group A SRL + CsA (n = 215)	Group B SRL (n = 215)	Total (n = 525)
Overall rate of graft survival	43 (45.3)	183 (85.1)[a]	196 (91.2)[a]	422 (80.4)
Pure graft loss	34 (35.8)	15 (7.0)	7 (3.3)[b]	56 (10.7)
Death with functioning graft	14 (14.7)	10 (4.7)	8 (3.7)	32 (6.1)
Lost to follow-up	4 (4.2)	7 (3.3)	4 (1.9)	15 (2.9)

a: Difference between groups (A-B) = -6.0, (-12.1, 0.0)
b: Difference between groups (A-B) = 3. (-0.4;7.9)
Abbreviations: CsA = cyclosporine, SRL = sirolimus, CI = confidence interval.

FIGURE 2. TIME TO GRAFT LOSS (INCLUDING LOST TO FOLLOW-UP) – STUDY 310



Graft and patient survival rates were similar between groups and compare well with overall UNOS data. There were no differences between groups A and B regarding cause of graft loss

Patient survival – study 310
At 36 months, there were more deaths in group A, but the difference in patient survival was not significant (Table 16).

Table 16 patient survival at 36 months – Study 310

Status	Nonrandomized SRL + CsA (n = 95)	Group A SRL + CsA (n = 215)	Group B SRL (n = 215)	Total (n = 525)
Overall patient survival n (%)	67 (70.5)	190 (88.4)[a]	201 (93.5)[a]	458 (87.2)
Deaths	20 (21.0)	12 (5.6)	8 (3.7)	40 (7.6)
Lost to follow-up	8 (8.4)	13 (6.0)	6 (2.8)	27 (5.1)

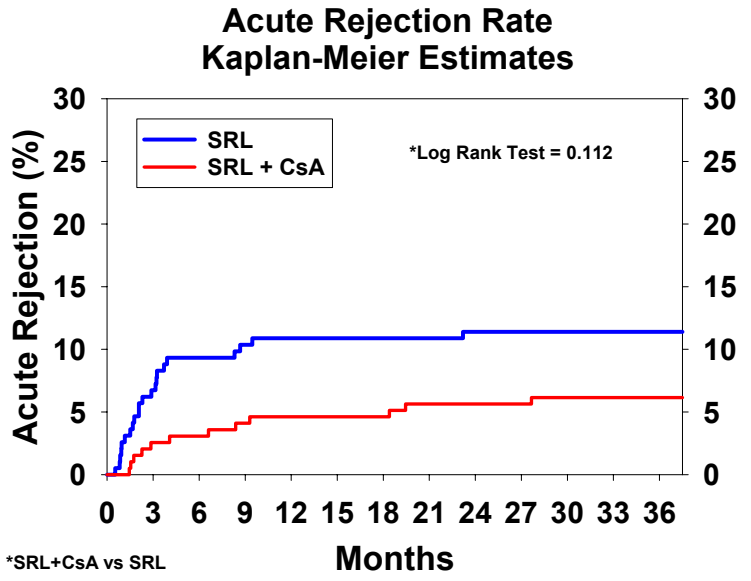
a: Difference between groups (A-B) = -5.1, (-10.5, 0.3)
b: Difference between groups (A-B) = -1.9, (-5.8;2.1)

Biopsy proven acute rejection – study 310

Period	Nonrandomized SRL + CsA	Group A SRL + CsA	Group B SRL	A-B % (95% CI)	A vs B p-Value [a]
Prerandomization	-	20/215 (9.3)	22/215 (10.2)	-0.9 (-6.5, 4.7)	0.871
Postrandomization [b]	-	12/215 (5.6)	22/215 (10.2)	-4.7 (-9.7, 0.4)	0.107
Total	36/95 (37.9)	32/215 (14.9)	44/215 (20.5)	-5.6 (-12.8, 1.6)	0.164

a: Fisher's exact test.
b: Includes both on-therapy period after randomization and follow-up after discontinuation period.

FIGURE 3. TIME TO FIRST ACUTE ALLOGRAFT REJECTION FOLLOWING RANDOMIZATION- STUDY 310



Renal function – study 310

Serum creatinine levels at 36 months were significantly better after CsA withdrawal, either when measured in patients remaining on therapy (163 vs 127 µmol/L, $P < 0.001$) or when including values from discontinued patients in the ITT analysis (168 vs 145 µmol/L, $P = 0.002$). The same was true of calculated GFR; the on therapy values are plotted in Figure 4 and the ITT analysis are presented in Table 18). Additional analyses of renal function over time (slope analyses) indicated that the slopes were significantly negative for SRL+CsA therapy, indicating a loss of renal function, whereas the slopes were positive for SRL therapy. The differences in slopes were significant ($p<0.001$) in favour of CsA withdrawal.

FIGURE 4. OBSERVED MEAN VALUES FOR CALCULATED NANKIVELL GFR (ML/MIN) - STUDY 310

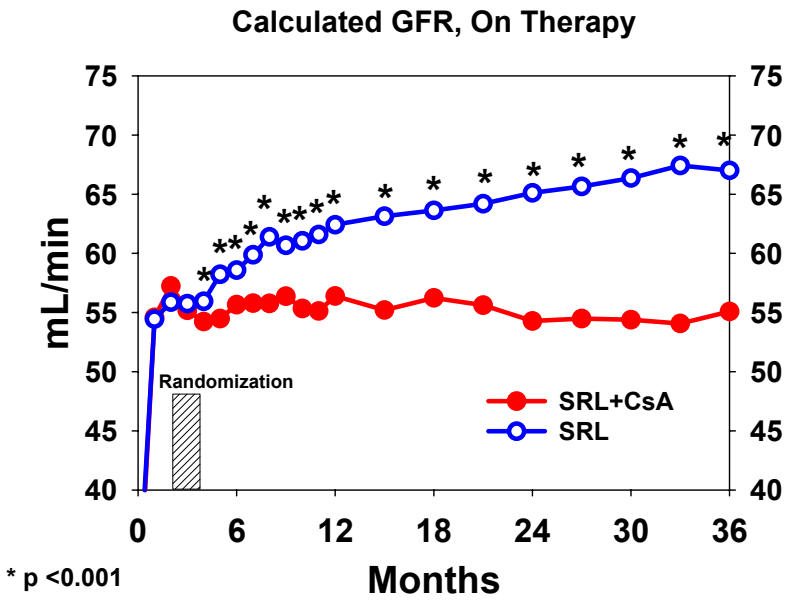


Table 18. Observed mean values (± SEM) for calculated nankivell GFR (mL/min): ITT Analysis (36 months)

Time (Posttransplant)	Nonrandomized SRL + CsA (n = 95)	Group A SRL + CsA (n = 215)	Group B SRL (n = 215)	A vs B ANCOVA p-Value
Month 6	1.98 ± 1.44 (39)[a]	55.35 ± 1.35 (189)	58.11 ± 1.31 (191)	< 0.001
Month 12	23.98 ± 3.00 (82)	53.17 ± 1.46 (208)	59.25 ± 1.46 (203)	< 0.001
Month 24	23.77 ± 3.22 (84)	48.38 ± 1.67 (203)	58.35 ± 1.60 (201)	< 0.001
Month 36	18.74 ± 3.00 (78)	47.26 ± 1.83 (194)	59.38 ± 1.82 (194)	< 0.001

a: Number of observations used to calculate the mean.

Additional follow up after 36 months – study 310In addition to the 36-month, the CPMP also received cumulative data (up to 54 months) for graft survival, acute rejection and patient survival. These data were reviewed during September 2003 meeting. Of note, the cumulative graft survival rate was significantly better in group B (81.4% vs 89.8%; difference [CIs], -8.4% [-15.0, -1.8%]). The 36-month and cumulative data provide further indications that SRL plus standard or near-standard dose CsA, as used in group A, is an unsuitable combination for long-term use, especially as regards evolution of graft function and some other indices of CsA toxicity.

Based on the growing differences in renal function and graft survival as well as lower blood pressure in the favour of the CsA-withdrawal group (group B), the MAH took the decision to discontinue treatment arm A. These patients have been transferred to treatment according to group B or to other regimen according to investigator's discretion. All enrolled patients will be followed for five years on an ITT basis.

Coated Tablet

Introduction

The clinical experience with the tablet formulation of Rapamune derives from trials 309 and 310. The data from trial 310 were extensively reviewed during the initial dossier evaluation and appeal process for Rapamune.

Main clinical studies

Trial 309

Description of the study

This was a phase III, randomised, open-label, dose-controlled, comparative study conducted at multiple centres in the United States, Canada and Australia, it compared the efficacy and safety of tablet (1 mg strength) and solution (1 mg/mL) formulations of sirolimus for the prevention of acute rejection in renal allograft recipients. The primary objective was to compare the efficacy and determine the equivalence of sirolimus oral solution (liquid and sirolimus tablets administered concomitantly with cyclosporine (CsA) and corticosteroids in *de novo* renal allograft recipients. The primary efficacy end-point was the rate of the composite efficacy failure (first biopsy-confirmed acute rejection, graft loss or death) within the first three months after transplantation.

Before surgery, recipients of a primary or non-primary renal allograft were randomly assigned in a 1:1 ratio to receive either sirolimus solution (2 mg/day) or sirolimus tablets (2 mg/day). An initial loading dose of 6 mg sirolimus 24 to 48 hours after transplantation was followed by a 2-mg dose once a day. The 1-mg tablets were given together, to equal the intended daily dose of 6 mg initially followed by 2 mg once a day. Both groups received a standard immunosuppressive regimen consisting of CsA microemulsion (Neoral) and protocol-defined corticosteroids. In the event of acute tubular necrosis (ATN) or delayed graft function in the early post-operative period, patients were allowed anti-T lymphocyte antibody therapy with sirolimus until renal function improved and CsA could be started. Sirolimus could also be given concomitantly with anti-T lymphocyte antibody therapy for the treatment of acute rejection. Pre-planned induction therapy with anti-T lymphocyte antibody preparations was, however, a criterion for exclusion.

Four hundred and seventy-seven (477) patients were enrolled, including those who received primary (92%), secondary (7%), or tertiary (<1%) renal allografts from cadaveric (69%) or living /31%) donors. The principal ethnic origins were white (58%) and black (24%). There were no significant differences in demographic variables between the two treatments. Of these 238 were randomly assigned to sirolimus solution and 239 to the tablets, and were evaluated for efficacy. Twenty (9 randomly assigned to solution and 11 randomly assigned to tablet) actually never received the study drug; nevertheless, they were included in the intent-to-treat analysis for the primary efficacy endpoint.

Primary endpoints

The next table summarises the incidence of the primary composite endpoint (efficacy failure) in each treatment group after transplantation, at time-point 12 months.

At time-point 3 months, the overall rate of failure in the tablet group (24.7%) was the same as with the solution (23.5%). The criteria for equivalence were met: the 95% confidence interval (CI) for the difference in rates (-6.5 to 8.8) included zero and the upper limit was less than 20%. The rate of failure was also equivalent between treatment groups when stratified by HLA mismatches, donor origin, age, ethnic origin, sex, and primary or non-primary transplant. Similarly, at 6 months, there was an equivalent overall rate of failure in the tablet (27.2%) and in the solution groups (26.1%) and, again, the criteria for equivalence were met.

The incidence of the primary endpoint (efficacy failure) in each treatment group (tablet: 31.4%; solution: 30.3%) was also equivalent at 12 months of follow-up.

Looking at the more specific analysis of efficacy sub-populations, while the incidence of biopsy-proven acute rejection or death was comparable in the two groups at the three time points of analysis, the incidence of graft loss in patients given sirolimus tablets was numerically higher than with the solution at 3 months (6.3% versus 3.4%), 6 months (6.3% versus 3.4%) and 12 months (6.7% versus 3.4%). These differences, however, did not reach statistical significance.

It was noted that efficacy failure occurred more often in patients with living donor transplants when treated with the tablet formulation (difference 9-11% to solution). These differences were observed at

6 months (rates at 6 months in patients with living donor were 18.8% for the oral solution and 27.5% for the tablet with a difference of 8.5%) and at 12 months (rates at 12 months were 22.5% for the oral solution and 33.3% for the tablet with a difference of 10.8%). Among the 18 sirolimus oral solution-treated patients (22.5%) who had efficacy failure, 17 (21.3%) were due to acute rejection and 1 (1.3%) due to graft loss. Among the 23 sirolimus tablet-treated patients (33.3%) who had efficacy failure, 19 (27.5%) were due to acute rejection, 3 (4.3%) due to graft loss, and 1 (1.5%) due to death. Neither the overall rates of efficacy failure nor the distributions of the individual components contributing to the efficacy failure were significantly different between the treatment arms.

As a matter of comparison, in study 310 the efficacy failure rate at 12 months in 57 tablet-treated patients with transplants from living donors was 21.1%. This rate was close to the efficacy failure rate of 22.5% in the oral solution-treated patients with transplants from living donors in study 309. Therefore, the efficacy failure rate of 33.3% observed for the tablet-treated patients with living donors in study 309 was not confirmed in the subsequent clinical study 310.

The secondary objectives of study 309 included patient and graft survival over the 12-month follow-up. At three months, patient survival rates in the tablet (98.7%) and solution groups (97.5%) were not significantly different (p=0.339). There was also no difference between formulations in patient survival at six months (tablet 97.9% versus solution 97.1%) and 12 months (tablet 96.2 versus solution 95.8%).

Similarly, at six months, the graft survival rates in the tablet and solution groups were not significantly different (90.8% and 94.1%) and (p=0.225). The results were confirmed at 12 months (tablet 88.7%; solution 92.0%). Overall, the most common causes of graft loss were death with a functioning graft and acute tubular necrosis (ATN). Both groups had comparable rates of graft loss due to death with a functioning graft. However, there was a numerically higher rate of graft loss due to ATN in the tablet group (2.8% vs 0.8%). This was not confirmed in study 310; only 1.0% of 525 patients receiving the tablet formulation experienced graft loss due to ATN.

Trial 310

Description of the study

This was one of the key studies in the original submission. This was an open-label study conducted in Europe, Australia, and Canada. A total of 525 primary (90%) or secondary (10%) renal allograft recipients from cadaveric (89%) or living (11%) donors received sirolimus (SRL) 2 mg (trough level >5 ng/mL, immunoassay), CsA and corticosteroids when enrolled. At 3 months $\pm$ 2 weeks, eligible patients were randomised (1:1) to remain on sirolimus-CsA-corticosteroids (SRL-CsA-CS), or to have CsA withdrawn and continue maintenance therapy with SRL (trough level: 20 to 30 ng/mL) and corticosteroids (SRL-CS).

The study was designed to allow for the randomisation of 408 patients: 204 in each treatment group. Enrolment was monitored during the course of the study to achieve this goal as closely as possible. However, given the time lag between enrolment and randomisation, a total of 430 patients were randomly assigned to either group A or B after three months.

The efficacy results have been discussed earlier in this document. (Tables 13-18 and Figures 1-4). All patients have completed 36 months;post-transplantation follow-up is still ongoing through 5 years. Treatment arm A has been discontinued.

Discussion on clinical efficacy

Nearly all data submitted for assessment referred to kidney transplant recipients at low to moderate immunological risk.

Sirolimus as add-on to CsA

Trials 301 and 302 tested fixed doses of sirolimus, 2 or 5 mg/d as add-on to conventionally dosed CsA and CS. Both doses were efficacious.

The immunosuppressive efficacy of sirolimus added to CsA and CS is considered acceptably documented as regards prevention of acute rejection. Beyond three months, average graft function was lower in the arms combining sirolimus with CsA than in the control groups. This is probably due to potentiation by sirolimus of CsA nephrotoxicity. Long-term use of the combination of sirolimus plus CsA is, thus, not recommended. There is limited information indicating that black renal transplant recipients (predominantly African-American) require higher doses and trough levels of sirolimus to achieve the same efficacy as observed in non-black patients. At present, the efficacy and safety data are too limited to allow recommendations for use of sirolimus in black recipients.

In trial 309, sirolimus was given as add-on to dual therapy of CsA and CS, at a nominal dose of 2 mg/day. The regimen was, thus, similar to that used in trials 301 and 302 as well as the first 3 months of study 310. The data from study 309 documented the similar efficacy profiles of oral solution and tablet formulations.

Sirolimus as base immunosuppressant after early discontinuation of CsA

This is the regimen proposed by the Applicant for use in patients of low to moderate immunological risk. Trials 212 and 310 provided evidence that sirolimus added to CsA and corticosteroids for 2 to 3 months, followed by progressive elimination of CsA, is associated with:

- low rates of acute rejection up to 3 months in a general Caucasian population of recipients of cadaveric and living-donor allografts.
- a small and transient rise in mild to moderate acute rejection in association with CsA tapering, but high graft and patient survival in patients suited for CsA withdrawal, which represented approximately 80% of the enrolled patients.
- improvements in graft function and blood pressure following CsA withdrawal.

In these trials, sirolimus was used initially at 2 mg/d (212) or at a nominal 2 mg/d with target trough levels >5 ng/ml (310). In study 310, this regimen resulted in an initial average daily dose of 2.1 mg and a mean trough sirolimus level of 9.8 ng/ml. The CsA regimen used resulted in exposures 30-40% lower than those in the previous 301 and 302 trials. Observed CsA troughs (10^{th} - 90^{th} percentiles) in study 310 were 155-424 ng/ml and 139-330 ng/ml during month 1 and months 2-3, respectively. When CsA was withdrawn, sirolimus trough levels were adjusted to 10-20 ng/mL (212) or 20-30 ng/mL (310). The average sirolimus doses following CsA withdrawal through month 12 were 6.1 and 8.2 mg/d in studies 212 and 310, respectively. The proposed sirolimus trough range for maintenance therapy after eliminating CsA is 15-25 ng/mL by immunoassay, which corresponds to 12-20 ng/mL by HPLC-UV or LC-MS/MS.

With regard to the perceived need for new therapies, sirolimus acts selectively on activated T-cells instead of on all T-cells. There is a need for agents with the same efficacy as calcineurin inhibitors and a different safety profile. Furthermore, an alternative is needed for those patients who fail on calcineurin inhibitors and MMF, as well as agents that may be safer for marginal kidneys and patients with delayed graft function.

Trial 310 (performed entirely with the tablet formulation) was considered the pivotal evidence supporting the positive CPMP opinion for Rapamune oral solution. In this sense, the clinical usefulness of a tablet formulation of Rapamune, when used according to the approved indication and in a TDM-based regimen, had already been acknowledged at time of initial approval.

Clinical safety

Oral solution

Patient exposure

In the overall clinical programme, more than 3,500 patients and subjects were enrolled in 50 studies (2,247 of whom received at least one dose of sirolimus). The primary source of data for safety is the combined twelve-month data from the two phase III blinded trials, in which 977 (976 actually received drug) patients received at least one dose of sirolimus (500 initially received 2 mg/day and 477 initially received 5 mg/day). The control groups were 159 patients who received at least one dose of AZA and 124 patients who received at least one dose of placebo. A total of 771 patients received sirolimus in the CsA withdrawal trials (212 and 310). Additional safety data are available for 811 patients who received one or more doses of sirolimus in the completed phase I, completed phase II, and ongoing phase II and phase III studies. Three hundred sixty-five (365) healthy subjects were given sirolimus in clinical pharmacology studies and >200 patients have been treated with sirolimus on an emergency or investigator-sponsored basis. The principal populations contributing safety information are listed in Table 19.

Table 19: Principal patient populations for the safety summary

Studies Included	Description	Study Drugs	Total Patients (Total drug)
0468E1-301-US (12-month data) 0468E1-302-GL (12-month data)	Phase III trials of continuous sirolimus + CsA treatment in renal allograft recipients	Sirolimus, AZA (0468E1-301-US), placebo (0468E1-302-US), CsA, prednisone	1260[a] (sirolimus = 977)[b] (AZA = 159)[c] (placebo = 124)
0468E1-207-EU,-210-EU (12-month data)	Comparative studies to CsA in renal allograft recipients	Sirolimus or CsA with AZA (207) or MMF (210) and steroids	161 (sirolimus = 81) (CsA = 80)
0468E1-212-GL (6-month data) 0468H1-310-GL (12-month data)	Studies in renal transplantation randomizing patients to remain on sirolimus+CsA or have CsA withdrawn from 2 to 3 months	Sirolimus, CsA and steroids	771

a: 1295 patients enrolled; 1260 patients received treatment.
b: 977 patients are tabulated in the database as having received sirolimus but only 976 actually received drug.
c: 159 patients are tabulated in the database as having received drug; actually 160 received drug.

Adverse events and serious adverse events/deaths

Adverse events in Phase III studies of the sirolimus-CsA combination (301 and 302)

For treatment Emergent Adverse Effects (TEAEs), occurring in at least 20% of patients, hypercholesterolemia, hyperlipemia, and peripheral oedema were significantly increased in patients treated with both 2 mg/day or 5 mg/day sirolimus, when compared with either the AZA or placebo groups. Anaemia, arthralgia, diarrhoea, and thrombocytopenia were significantly more common with 5 mg/day sirolimus than with 2 mg/day sirolimus, AZA (except anaemia), or placebo (except diarrhoea). Hyperkalemia was significantly less common in both sirolimus groups when compared with either AZA or placebo. Nausea was significantly more common in the AZA group than either sirolimus group. Two other common events that differed significantly by treatment were dyspepsia (more common in the placebo group than all other groups), and fever (less frequent in the sirolimus 2 mg/day group than the sirolimus 5 mg/day group or AZA group).

For TEAEs occurring at a frequency of < 20%, lymphocele was significantly more common in both sirolimus groups compared with both control groups. Epistaxis and increased LDH were significantly more common in the 5 mg/day sirolimus group compared with all other groups. Chills, face oedema, hypotension, and rash were more common in the 5 mg/day sirolimus group compared with the 2 mg/day sirolimus and AZA groups. Insomnia was significantly more frequent in the 5 mg/day sirolimus group compared with the 2 mg/day sirolimus or placebo groups. Leukopenia was significantly more common in the 5 mg/day sirolimus group compared with the 2 mg/day group; leukopenia was also significantly more common in the AZA group than in the 2 mg/day sirolimus and placebo groups. Hypokalemia was more common in the 5 mg/day sirolimus group than in the AZA or placebo groups. Tachycardia was significantly more common in both the sirolimus groups compared with the AZA group and significantly more common in the 5 mg/day sirolimus group compared with the placebo group.

Adverse events in pilot studies comparing sirolimus to CsA (207 and 210)

There was a significantly higher incidence of the following common TEAEs in the sirolimus group than in the CsA group: pain, diarrhoea, leukopenia, thrombocytopenia, hyperlipemia, hypercholesteremia, hypokalemia, and increased LDH. For less frequently occurring TEAE (between 5% and 20% of patients), the sirolimus group had a significantly higher incidence of sepsis, hypophosphataemia, increased AST, arthralgia, epistaxis, kidney tubular disorder; while the CsA group had a significantly higher incidence of asthenia, chest pain, gum hyperplasia, hyperuricemia, and tremor. There was no difference between the sirolimus and CsA groups in the incidence of diabetes. The sirolimus group had a lower incidence of increased creatinine level and hypertension, but these differences were not significant.

Adverse events with sirolimus as base immunosuppressant after discontinuation of CsA (310)

The table below presents the treatment-emergent adverse events that were significantly different between randomized groups in study 310. It includes 36- month data for all patients.

Table 20. Number (%) of patients with significantly different treatment-emergent adverse events at 36 months – study 310

Adverse Event	Group A SRL+CsA (n = 215)	Group B SRL-ST (n = 215)	p-Value[a]
Higher in group A			
Creatinine increased	71 (33.0)	41(19.1)	0.001
Hypertension	52 (24.2)	22 (10.2)	<0.001
Hyperuricemia	36 (16.7)	16 (7.4)	0.005
Abnormal kidney function	38 (17.7)	16 (7.4)	0.002
CsA nephrotoxicity	22 (10.2)	6 (2.8)	0.003
Edema	22 (10.2)	9 (4.2)	0.024
Toxic nephropathy	13 (6.0)	2 (0.9)	0.006
Gingival hyperplasia	15 (7.0)	5 (2.3)	0.037
Hyperkalemia	6 (2.8)	0	0.030
Higher in group B			
Thrombocytopenia	12 (5.6)	26 (12.1)	0.026
ALT increased	11 (5.1)	35 (16.3)	<0.001
AST increased	8 (3.7)	27 (12.6)	0.001
Hypokalemia	7 (3.3)	22 (10.2)	0.006
Healing abnormal	3 (1.4)	12 (5.6)	0.032
Rectal disorder	2 (0.9)	10 (4.7)	0.036
Ileus	0	6 (2.8)	0.030

[a] Fisher exact test, group A vs Group B in randomly assigned patients.

Graft loss in Phase III studies of the sirolimus-CsA combination (301 and 302)

Graft survival was very similar in the different treatment arms. Causes of graft loss are given in Table 21.

Table 21: Aetiology of graft loss following transplantation: number (%) of patients (studies 301 & 302): 12 months

Aetiology of graft loss	Sirolimus 2 mg/day (n = 511)	Sirolimus 5 mg/day (n = 493)	AZA (n = 161)	Placebo (n = 130)	Total (n = 1295)
Death with functioning graft	15 (2.9)	17 (3.4)[a]	2 (1.2)	6 (4.6)[b]	40 (3.1)
Acute rejection	5 (1.0)	8 (1.6)[b]	3 (1.9)	4 (3.0)	20 (1.5)
Acute tubular necrosis	3 (0.6)[b]	4 (0.8)	3 (1.9)	2 (1.5)	12 (0.9)
Renal vein or artery thrombosis	8 (1.6)[c]	3 (0.6)	1 (0.6)	2 (1.5)[b]	14 (1.0)
Infected graft	0 (0.0)	2 (0.4)[d]	0 (0.0)	0 (0.0)	2 (0.2)
Other	7 (1.4)	6 (1.2)	1 (0.6)	2 (1.5)[b]	16 (1.2)

a: 2 patients did not receive randomised treatment
b: 1 patient did not receive randomised treatment
c: 3 patients did not receive randomised treatment
d: Same donor for both patients at a single centre; donor kidneys were determined to be infected with vancomycin-resistant *Enterococcus* spp.

Graft loss with sirolimus as base immunosuppressant after discontinuation of CsA (310)

The reasons for graft loss in study 310 at 12 months (see table below) were similar to other large studies in renal transplantation.

Table 22. Aetiology of graft loss: number (%) of patients (study 310): 12 months

	Non-randomized (SRL-CsA) (n=95)	SRL-CsA (n=215)	SRL (n=215)	Total (n=525)
Death	13 (13.7)	4 (1.9)	4 (1.9)	21 (4.0)
Renal thrombosis	10 (10.5)	0	0	10 (1.9)
Chronic dysfunction	6 (6.3)	3 (1.4)	1(0.5)	10 (1.9)
Primary nonfunction	5 (5.3)	0	0	5 (1.0)
Acute rejection	1 (1.1)	1 (0.5)	0	2 (0.4)
Lost to follow-up	1 (1.1)	0	0	1 (0.2)
Other	6 (6.3)	1 (0.5)	0	7 (1.3)

Malignancies in Phase III studies of the sirolimus-CsA combination (301 and 302)

The rates for post-transplantation lymphoproliferative disease in the cumulative safety database are given in Table 23. The rates observed with sirolimus do not appear markedly different from those published for other agents in this therapeutic area.

Table 23: Rates of post-transplantation lymphoproliferative disease and lymphoma-studies 301 and 302

Incidence Rate	Sirolimus 2 mg/day (n = 511)	Sirolimus 5 mg/day (n = 493)	AZA (n = 161)	Placebo (n = 130)
0 to 12 Months				
Incidence rate, n (%)	2 (0.39)	7 (1.42)	1 (0.62)	0
95% CI	0.05-1.41	0.57-2.90	0.02-3.41	0-2.80
Cumulative				
Rate, n (%)	5 (0.98)	10 (2.03)	1 (0.6)	1 (0.8)
95% CI	0.32 -2.27	0.98 -3.70	0.02 -3.41	0.02 -4.21

Malignancies with sirolimus as base immunosuppressant after discontinuation of CsA (310)

The overall rate of malignancy was 11.2% in group A and 5.6% in group B (see Table 24).

Table 24. Number (%) of patients with malignancy including lymphoproliferative disease at 36 months – study 310

	Nonrandomized (SRL+CsA) (n=95)	Group A SRL+CsA (n = 215)	Group B SRL+ST (n = 215)	p-Value[a]
Total	6 (6.3)	21 (11.2)	12 (5.6)	0.054
Skin cancer	4 (4.2)	14 (6.5)	8 (3.7)	0.274
PTLD/lymphoma	1 (1.1)	3 (1.4)	1 (0.5)	0.623
Other	1 (1.1)	7 (3.3)	3 (1.4)	0.338

[a] Fisher exact test for pairwise comparison, group A vs group B.
PTLD=posttransplant lymphoproliferative disease

Interstitial lung disease/pneumonitis

The review of the global safety database for sirolimus disclosed 30 cases of interstitial lung disease/pneumonitis. Nine cases emanated from one centre (compassionate use programme), but other cases were found in the majority of clinical trials (enrolling altogether more than 3,000 patients). It is pointed out that similar cases have been reported with AZA, CsA and mycophenolate mofetil (MMF) in transplant populations. Of the 30 cases, 11 had few or no obvious confounding factors and evidence of a positive dechallenge.

Opportunistic and other severe infections in Phase III studies of the sirolimus-CsA combination (301 and 302)

Twelve-month data for opportunistic infections for studies 301 and 302 are given in Table 25. Low rates of CMV disease are reflective of prophylaxis given, recommended to be continued three months post transplantation. *Pneumocystis carinii* pneumonia (PCP) was seen only in sirolimus-treated patients, which were not receiving anti-PCP prophylaxis. Observed rates during the first 12 months were low, supporting efficacy of prophylaxis, recommended as mandatory for that period.

Of 10 cases of mycobacterial infections, nine were seen with sirolimus. Overall, the incidence is not different from that reported for CsA in some published trials. According to the review of published data provided, the incidences of Herpes simplex and zoster infections seen with sirolimus are in the same range as that observed with mycophenolate mofetil (MMF) and tacrolimus in this population.

Overall, the risk for opportunistic infections during the first year with sirolimus appears comparable with that observed with MMF and tacrolimus, but higher than with the IL-2 receptor antibodies.

Table 25: Incidence of opportunistic infections: 12- month data, studies 301 and 302

Opportunistic Infection	sirolimus 2 mg/day (n = 511)	sirolimus 5 mg/day (n = 493)	Placebo (n = 130)	AZA(n = 161)
CMV[a] (generalised)	3.9	5.1	5.4	5.7
CMV (tissue-invasive)	0.8	1.0	0.8	1.2
Herpes zoster	3.3	4.5	3.8	5.0
Herpes simplex[b]	6.5	14.2	6.9	4.4
Epstein-Barr virus	0.6	0.6	0.8	0
PCP[c]	0.4	0.2	0	0
Mycobacterial	1.2	0.6	0	0.6

a: Cytomegalovirus
b: $p < 0.05$ for sirolimus 5 mg/day versus sirolimus 2 mg/day, azathioprine, and placebo
c: *Pneumocystis carinii* pneumonia

An analysis was provided of the overall risk of infection in relation to sirolimus trough levels recorded. As expected a trend towards higher rates of infections was noted with increasing sirolimus levels for the first six months post transplantation

Opportunistic and other severe infections with sirolimus as base immunosuppressant after discontinuation of CsA (310)

At 36 months, the only significant difference between groups was a higher incidence of Herpes Zoster (6.5% vs 0.9%) in patients continuing on triple-therapy, group A.

Deaths

In studies 301 and 302, the overall patient survival at 12 months for 1004 patients receiving sirolimus was 96.2%, comparable with that for patients receiving either comparator (96.6%). Overall patient survival at 12 months in the 525 patients enrolled in the larger of the CsA-withdrawal studies (310) was 94.7%, and there was no difference in rates between patients randomised to remain on triple-therapy (97.2%) and those in whom CsA was withdrawn (98.1%).

Laboratory findings

Cholesterol and triglycerides
Mean serum cholesterol and triglycerides for studies 301 and 302 are provided in the tables below. Serum lipid concentrations, which peaked at 2 months after transplantation in most sirolimus-treated

and control patients, decreased progressively in both groups thereafter. The magnitude of the differences in blood lipid concentrations between the groups also tended to decrease progressively.

Table 26. Adjusted mean values (± SEM) for fasting cholesterol (mmol/L) - (combined 301 and 302)

Time	Sirolimus 2 mg/day	Sirolimus 5 mg/day	Azathioprine	Placebo	ANCOVA p-Value
Month 1	5.025 ± 0.044[a,c,d,e] (415)[b]	5.168 ± 0.045[c,d] (401)	4.805 ± 0.079 (131)	4.744 ± 0.088 (107)	< 0.001
Month 3	7.303 ± 0.112[c,d,e] (254)	7.971 ± 0.113[c,d] (248)	6.486 ± 0.230 (60)	6.327 ± 0.225 (63)	< 0.001
Month 6	6.742 ± 0.103[c,d,e] (223)	7.353 ± 0.107[c,d] (206)	5.852 ± 0.229 (45)	6.042 ± 0.211 (53)	< 0.001
Month 12	6.405 ± 0.108[c,e] (199)	6.727 ± 0.114[c,d] (179)	5.903 ± 0.221 (47)	6.010 ± 0.212 (51)	0.001

a: Adjusted mean value is the mean on-therapy value for a patient with an average baseline. SEM is the standard error of the adjusted mean.
b: Number of pairs used for adjusted mean.
c: Pairwise significant p-value comparison for a sirolimus treatment group versus azathioprine.
d: Pairwise significant p-value comparison for a sirolimus treatment group versus placebo.
e: Pairwise significant p-value comparison for 2 mg/day sirolimus versus 5 mg/day sirolimus treatment group.

Table 27. Adjusted mean values (± SEM) for fasting triglycerides (mmol/L) - (combined 301 and 302)

Time	Sirolimus 2 mg/day	Sirolimus 5 mg/day	Azathioprine	Placebo	ANCOVA p-Value
Month 1	1.901 ± 0.046[a,e] (414)[b]	2.124 ± 0.047[c,d] (402)	1.907 ± 0.083 (129)	1.912 ± 0.091 (107)	0.004
Month 3	3.467 ± 0.168[c,d,e] (238)	4.228 ± 0.168[c,d] (237)	2.561 ± 0.352 (54)	2.316 ± 0.329 (62)	< 0.001
Month 6	3.100 ± 0.158[c,d,e] (221)	3.911 ± 0.168[c,d] (195)	2.192 ± 0.354 (44)	2.265 ± 0.326 (52)	< 0.001
Month 12	3.153 ± 0.163[d,e] (198)	3.648 ± 0.174[c,d] (174)	2.669 ± 0.351 (43)	2.325 ± 0.324 (50)	0.001

a: Adjusted mean value is the mean on-therapy value for a patient with an average baseline. SEM is the standard error of the adjusted mean.
b: Number of pairs used for adjusted mean.
c: Pairwise significant p-value comparison for a sirolimus treatment group versus azathioprine.
d: Pairwise significant p-value comparison for a sirolimus treatment group versus placebo.
e: Pairwise significant p-value comparison for 2 mg/day sirolimus versus 5 mg/day sirolimus treatment group.

Fasting cholesterol and triglycerides were lower in the 310 CsA-withdrawal study (see below), probably due to better recommendations for treatment with lipid-lowering agents. HDL-cholesterol levels were also measured in this study; they were normal or high in most patients.

Table 28. Observed mean values (± SEM) for fasting cholesterol (mmol/L) – study 310

Time (Posttransplant)	Nonrandomized SRL + CsA (n = 95)	Group A SRL + CsA (n = 215)	Group B SRL (n = 215)	ANCOVA A vs B p-Value[a]
Month 1	6.6 ± 0.3 (42)[b]	7.0 ± 0.1 (173)	6.9 ± 0.1 (179)	-
Month 2	7.8 ± 0.3 (31)	7.4 ± 0.2 (173)	7.2 ± 0.1 (162)	-
Month 3	6.1 ± 0.5 (12)	6.9 ± 0.2 (162)	7.0 ± 0.1 (161)	0.071
Month 6	-	6.4 ± 0.1 (154)	6.4 ± 0.1 (147)	0.464
Month 9	-	6.1 ± 0.1 (148)	6.4 ± 0.1 (139)	0.011*
Month 12	-	6.0 ± 0.1 (156)	6.3 ± 0.1 (142)	0.281
Month 18	-	5.9 ± 0.1 (127)	6.3 ± 0.1 (126)	0.054
Month 24	-	5.9 ± 0.1 (128)	6.2 ± 0.1 (129)	0.060
Month 30	-	6.0 ± 0.2 (104)	6.4 ± 0.1 (109)	0.005**
Month 36	-	5.9 ± 0.2 (103)	6.3 ± 0.1 (116)	0.059

a: *p <0.05, **p < 0.01.
b: Number of observations used to calculate the mean.

Table 29. Observed mean values (± SEM) for fasting triglycerides (mmol/L) – study 310

Time (Posttransplant)	Nonrandomized SRL + CsA (n = 95)	Group A SRL + CsA (n = 215)	Group B SRL (n = 215)	ANCOVA A vs B p-Value
Month 1	3.0 ± 0.3 (41)[a]	2.7 ± 0.2 (171)	2. 5 ± 0.1 (176)	-
Month 2	3.7 ± 0.4 (29)	3.1 ± 0.2 (173)	2.7 ± 0.1 (161)	-
Month 3	3.6 ± 0. 5 (11)	2.7 ± 0.1 (162)	2.8 ± 0.1 (161)	0.136
Month 6	-	2.7 ± 0.1 (153)	2.7 ± 0.1 (147)	0.534
Month 9	-	2.5 ± 0.1 (145)	2.6 ± 0.1 (138)	0.394
Month 12	-	2.2 ± 0.1 (156)	2.5 ± 0.1 (141)	0.066
Month 18	-	2.2 ± 0.1 (127)	2.4 ± 0.1 (125)	0.146
Month 24	-	2.2 ± 0.1 (129)	2.3 ± 0.1 (129)	0.942
Month 30	-	2.2 ± 0.1 (104)	2.2 ± 0.1 (107)	0.610
Month 36	-	2.3 ± 0.1 (102)	2.4 ± 0.1 (115)	0.403

a: Number of observations used to calculate the mean.

Table 30. Observed mean values (± SEM) for fasting HDL cholesterol (mmol/L) – study 310

Time (Posttransplant)	Nonrandomized SRL + CsA (n = 95)	Group A SRL + CsA (n = 215)	Group B SRL (n = 215)	ANCOVA A vs B p-Value
Month 1	1.4 ± 0.1 (33)[a]	1.7 ± 0.06 (146)	1.7 ± 0.05 (152)	-
Month 2	1.7 ± 0. 1 (22)	1.7 ± 0.05 (154)	1.8 ± 0.05 (144)	-
Month 3	1.3 ± 0.2 (8)	1.6 ± 0.04 (147)	1.7 ± 0.04 (150)	0.133
Month 6	-	1.5 ± 0.04 (141)	1.6 ± 0.04 (137)	0.215
Month 9	-	1.5 ± 0.05 (136)	1.6 ± 0.04 (127)	0.776
Month 12	-	1.5 ± 0.04 (144)	1.6 ± 0.05 (136)	0.766
Month 18	-	1.5 ± 0.04 (119)	1.7 ± 0.05 (117)	0.113
Month 24	-	1.5 ± 0.04 (119)	1.7 ± 0.05 (121)	0.026*
Month 30	-	1.5 ± 0.05 (96)	1.7 ± 0.05 (99)	0.045*
Month 36	-	1.5 ± 0.05 (95)	1.6 ± 0.06 (104)	0.952

a: Number of observations used to calculate the mean.

Efficacy and safety of concomitant lipid-lowering therapy

In trials 301 and 302, statins were used in 47 and 27% of patients receiving sirolimus and control therapy, respectively. Fibrates were given to 11% of sirolimus-treated patients and 4% of control patients. In study 310 at 36 months, cumulative use of statins (75% vs 78%) and fibrates (25% vs 26%) to control serum lipids was comparable between group A vs group B, respectively.

Lipid-lowering therapy is indicated in the majority of transplant recipients treated with sirolimus. The experience so far is that statins and/or fibrates are efficacious and can be given without remarkable increases in incidence of adverse events. In study 310, the most frequently used statin and fibrate were atorvastatin and gemfibrozil, respectively.

There were no observations supporting an increased risk of pancreatitis, disturbed liver function or thrombosis with sirolimus, compared with controls receiving lipid-lower therapy or related to serum lipid elevations.

Other laboratory findings

Serum levels of LDH, AST, ALT, and CK were followed. In studies 301 and 302, mean levels of all four enzymes were generally higher in the sirolimus arms. AST and ALT levels were also higher in group B patients of study 310; these patients received higher doses of sirolimus. Generally, the observed group mean differences were of moderate magnitude and doubtful clinical relevance.

Fasting glucose was not affected by sirolimus in any of the studies. Overall, there are no indications of a specific diabetogenic effect of sirolimus.

Sirolimus results in lower serum potassium levels and increases the frequency of hypokalemia. This is not considered to be of major clinical importance.

A dose-dependent, reversible reduction in platelet counts was noted with sirolimus. Over a period of 12 months in studies 301 and 302, the incidence of treatment discontinuation due to thrombocytopenia was 0.6% and 1.7% in the sirolimus 2 mg/day and 5 mg/day groups, and 1.9% and 0.8% in the azathioprine and placebo groups, respectively. As a TEAE, thrombocytopenia was reported in 13% and 24% of patients in the sirolimus 2 mg/day and 5 mg/day groups, respectively, and epistaxis, in 5% and 9% of patients, respectively. Only 1 patient in the 5-mg/day sirolimus group was reported to have experienced thrombocytopenia and mild epistaxis concurrently. Two cases of severe thrombocytopenia (<20 x 10^9/L) were observed. Discontinuations due to thrombocytopenia were mainly seen in patients subsequently diagnosed with HUS/TTP. Leukopenia was less frequent with sirolimus compared with AZA in trial 301(discontinuation for leukopenia <1% with sirolimus, 4% with AZA).

In study 310 at 36 months, mean hemoglobin values (126.4 vs 132.4 g/L, $P < 0.001$) were significantly higher in group B patient, perhaps secondary to better renal function. One (1) patient in the group A (posttransplantation day 378) and 2 in the group B (days 113 and 217) discontinued because of anemia. White blood cell counts (7.81 vs 7.45 x 10^9/L, group A vs group B) and platelet counts (228 vs 227 x 10^9/L) were not significantly different between treatment groups at month 36. One (1) SRL+CsA patient discontinued because of leukopenia (posttransplantation day 395) and 1 discontinued because of thrombocytopenia (posttransplantation day 792); no patients in the SRL group discontinued for either leukopenia or thrombocytopenia.

Blood pressure

In studies 301 and 302, blood pressure in the sirolimus-treated patients was not significantly different from controls. In study 310, both diastolic and systolic blood pressures were significantly lower shortly following CsA withdrawal. At 36 months, the differences in systolic (140.1 vs 131.3 mm Hg, $P = 0.002$) and diastolic blood pressures (81.2 vs 76.3 mm Hg, $P = 0.006$) remained statistically and clinically significant (group A vs group B, respectively). These differences were observed despite significantly ($P = 0.001$) lower use of antihypertensive medication in-group B.

Coated Tablets

The safety profile of sirolimus tablets was assessed in the same two trials as efficacy endpoints. In both instances the safety cannot be compared formally with non-sirolimus-containing immunosuppressive regimens.

Of the 477 patients enrolled in study 309, 457 received at least one dose of sirolimus; 229 received the solution formulation and 228 received the tablet.

The overall safety profile of sirolimus was similar to that in the previous blinded trials (301 and 302). There were no differences between the tablet and solution groups in the incidence of clinically relevant adverse events including tachycardia, diabetes mellitus, leukopenia, thrombocytopenia, TTP, hyper- and hypokalemia, arthralgia, epistaxis, and rash. The incidence of selected, clinically important infections (sepsis, CMV infection, herpes simplex, herpes zoster, and EBV) was also similar:

The overall number of deaths is 19 in the first 12 months after transplantation. Twelve patients died during the first six months after transplantation and an additional seven died within the next 6 months. There was no significant difference in the incidence of death in the tablet (3.8%) and solution groups (4.2%). Malignancies were few: nineteen patients had histologically-confirmed malignancies during the first 12 months after transplantation. Twelve were from the tablet group and seven from the solution group. However, the incidence of skin carcinoma was higher in Australian patients treated with sirolimus compared with patients from outside Australia, and overall more patients in the sirolimus tablet-group developed a skin carcinoma compared with patients in the solution-group (irrespective of the country)**.** No reason to suspect the tablet formulation is evident, which is corroborated by findings from trial 310. In study 310, the rate of skin carcinoma at 12 months was 1.9% among the 525 enrolled patients; very similar to the rate of 1.7% observed for the oral solution arm in study 309. Furthermore, no skin carcinoma cases were reported in study 310 from the time of

randomisation (month 3 ± 2 weeks) through month 12, in those patients who had an increase in sirolimus tablet dose and withdrawal of CsA, as specified in the SmPC. With respect to this issue, the scientific Committee observed that skin tumour numbers are too small for any conclusions. However, this issue should be further monitored.

At 3 months in study 309, there was a numerically higher incidence of renal graft loss in the tablet-treated group than in the oral solution-treated group (8.4% vs 5.5%, tablet vs oral solution, p = 0.270). This difference was due principally to the higher incidence of graft loss secondary to acute tubular necrosis (ATN) in the tablet-treated group (incidence, 2.9%) than in the oral solution-treated group (incidence, 0.8%). In study 310, in which more patients received the tablet formulation than in study 309 (525 in study 310 and 239 in study 309), only 1.0% of patients had a graft loss that was related to ATN. There is no evidence to suggest that the rate of ATN-related graft loss is increased by the tablet formulation.

In study 309 at 3 months, the incidence of myocardial infarction for the tablet formulation was 1.3% (3 events; n = 228) and 0% for the oral solution formulation (n = 229). At the study's conclusion (12 months), the incidence of myocardial infarction was 2.2% (5 events) and 0.4% (1 event) for the tablet and oral solution formulations, respectively. In both cases, the differences were not significant.
Again, interstudy comparison with trial 310 does not suggest any reason to suspect the sirolimus formulation as a causative factor.

For laboratory adverse events, no difference was found between the tablet and the solution in serum creatinine, fasting serum cholesterol and triglyceride levels. Statistically significantly higher mean liver enzymes (AST and ALT) were noted with the tablet compared with the solution formulation at 12 months post-transplant. However, the difference with the tablet formulation was not considered to achieve clinical significance. The most likely explanation is the slightly higher average exposure to sirolimus obtained with the tablet formulation in this dose-controlled study. Findings from trial 310 and, indeed, the cumulative experience with sirolimus provide clear evidence that higher sirolimus doses/trough concentrations expose patients to a greater risk of liver toxicity.

Post-authorisation study 316 in high-risk and low-risk patients

Trial 316-EU is an open label, multicentre trial over 208 weeks to evaluate a randomised switch from calcineurin inhibitor-based [CNI: Ciclosporin A (CsA) or tacrolimus] triple therapy to sirolimus-based (SRL, target trough 12-20 ng/ml) immunosuppression in stable renal transplant recipients 6-120 months post transplantation. The study aims to enrol 750 patients and was originally designed to stratify to two groups: low-risk (GFR at baseline >40 ml/min) and high-risk (GFR at baseline 20-40 ml/min). The primary endpoint is change from baseline in GFR at 52 weeks, and the aim was to show superiority for SRL conversion in the low risk stratum and non-inferiority in the high- risk group.

At routine Data and Safety Monitoring Board (DSMB) evaluation after enrolment of 400 patients, poor outcomes were noted in high-risk patients switched to SRL and inclusion into this stratum was stopped in August 2003 when 90 patients had been recruited. These patients will be allowed to continue in the trial and the remaining 660 patients will come from the low-risk group. The protocol has been amended accordingly and SRL target trough has been adjusted to 8-20 ng/ml to better comply with current practice among investigators. According to the MAH, subsequent DSMB evaluation (data cut-off 11/02/2004 based on 551 patients treated for at least three months) revealed no unexpected findings and the recommendation was to continue the trial.

Discussion on clinical safety

In the overall clinical programme, more than 3,500 patients and subjects were enrolled in 50 studies (2,247 of whom received at least one dose of sirolimus).

The most common side effects are lymphocele, abdominal pain, diarrhoea, anaemia, thrombocytopenia, hypercholesterolemia, hypertriglyceridemia (hyperlipidemia), hypokalaemia;

increased lactic dehydrogenase (LDH), increased transaminases, arthralgia, acne and urinary tract infection.

As expected, sirolimus is associated with dose/concentration related risks for over immunosuppression with infections, including opportunistic infections. These have been reported at rates which appear comparable with those seen with MMF and tacrolimus, but higher than what has been reported with IL-2 receptor antibodies in this indication. CMV and PCP appear manageable with the recommended prophylactic regimens.

Haematological toxicity with sirolimus does not appear a major concern with the proposed dosing regimen.

The period of observation available is too limited for definite evaluation regarding the risk for malignancy, including PTLD, but the submitted data do not provide any signals.

The majority of patients treated with sirolimus would be considered as likely candidates for concomitant use of a statin or fibrate. Lipid elevations decrease with time and lipid-lowering therapy.

For the evaluation of the tablet formulation, the overall safety profile of sirolimus was similar to that found in the previous blinded trials. There were no real differences between the tablet and solution groups in the incidence of clinically relevant adverse events including tachycardia, diabetes mellitus, leukopenia, thrombocytopenia, TTP, hyper- and hypokalemia, arthralgia, epistaxis, and rash. The incidence of selected clinically important infections such as sepsis, CMV infection, herpes simplex, herpes zoster, and EBV infection was also similar. Incidence of deaths at 12 months post-transplant was comparable in both groups. Malignancies were few, with lymphoproliferative disease/lymphoma reported in two patients in the tablet group and three in the solution group. With respect to skin carcinoma, the CPMP observed that numbers are too small for any conclusions. However, this issue should be continuously monitored in PSURs.

There were no significant differences in the rates of discontinuation during treatment. Although no significant difference between the groups was reported for most of the adverse events, it should be noted that during the first three months post-transplant the incidence of myocardial infarction was numerically higher in the tablet (1.3%) than in the solution group (0).

For laboratory adverse events, no difference was found between the tablet and the solution in serum creatinine, fasting serum cholesterol and triglyceride levels. Of some concern are the statistically significantly higher liver enzymes (AST and ALT) with the tablet than the solution formulation particularly at 12 months post-transplant. However, the difference with the tablet formulation was not considered to achieve clinical significance.

The findings from trial 310 indicate that high dose sirolimus, while preventing graft rejection in a regimen of CsA withdrawal, may expose patients to a greater risk of liver toxicity. Cumulative data obtained from trial 310 indicates that the main safety concerns of hepatotoxicity, pulmonary toxicity, hyperlipidaemia, thrombocytopenia and overimmunosuppression do not show trends to increase over time. The trial is ongoing to provide long-term safety and patient outcome data

Overall, studies 309 and 310 provide evidence of equivalence between sirolimus tablets and solution in terms of safety. However, the findings also confirm a potential risk of toxicity, particularity hepatotoxicity, when sirolimus is employed at high doses. The need for close drug monitoring has to be considered.

In a study evaluating the safety and efficacy of conversion from calcineurin inhibitors to sirolimus (target levels of 12 - 20 ng/ml) in maintenance renal transplant patients, enrollment was stopped in the subset of patients with a calculated baseline glomerular filtration rate (Nankivell method) of < 40 ml/min due to a higher rate of serious adverse events.

Safety issues observed during trials for other transplant types (no authorised indication)

The use of sirolimus in combination with tacrolimus was associated with excess mortality and graft loss in a study in *de novo* liver transplant patients. Many of these patients had evidence of infection at or near the time of death. In this and another study in *de novo* liver transplant recipients, the use of sirolimus in combination with cyclosporine or tacrolimus was associated with an increase in hepatic artery thrombosis (HAT); most cases of HAT occurred within 30 days post-transplantation and most led to graft loss or death. The safety and efficacy of sirolimus as immunosuppressive therapy have not been established in liver transplant patients, and therefore such use is not recommended.

Cases of bronchial anastomotic dehiscence, mostly fatal, have been reported in *de novo* lung transplant patients when sirolimus has been used as part of an immunosuppressive regimen.

5. Overall conclusion and benefit risk assessment

Quality

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

Preclinical pharmacology and toxicology

Overall, the primary pharmacodynamic studies provided adequate evidence that sirolimus is an immunosuppressive and antiproliferative agent that prevents allograft rejection in animal models of organ transplantation.

The kidney is a target organ for toxicity of the related macrocyclic, tacrolimus, but pancreatic toxicity has also been reported. In the case of tacrolimus, renal and pancreatic toxicity were considered of greatest concern. The general pharmacology studies indicated that renal toxicity with sirolimus is a minor effect. Although the potential for nephrotoxic effects appears lower with sirolimus than for CsA or tacrolimus, the toxicity was enhanced in combination with CsA.

Toxic effects of sirolimus were evident at systemic exposure levels providing (little or) no margins of exposure to expected clinical levels. Species differences in tolerance were evident. The effect on bone is a concern. Young animals were more susceptible to this effect.

The toxicology programme revealed that reactions that appear “unique” for sirolimus were pulmonary alveolar macrophages, increased haemosiderosis and haematopoiesis, indicative of an increase in red blood cell turnover, and possibly skins toxicity (ulcers mainly in mouse).

Sirolimus did not induce any significant mutagenic or clastogenic effects, with or without metabolic activation, in the standard battery of *in vitro* and *in vivo* tests for genotoxicity.

Carcinogenicity studies with sirolimus indicate that the intrinsic potential for carcinogenicity was secondary to that induced by the pharmacological action.

Sirolimus has a predictable toxicity profile with both similarities and differences to other immunosuppressants. Most of the toxic effects have been reported with other immunosuppressants or represented expected exaggerated pharmacological effects.

Clinical Efficacy

All data submitted for assessment referred to kidney transplant recipients at low to moderate immunological risk.

Sirolimus as add-on to CsA
Trials 301 and 302 tested fixed doses of sirolimus, 2 or 5 mg/d as add-on to conventionally dosed CsA and CS. Both doses were efficacious. In the risk/benefit evaluation, no benefit of the 5 mg/d dose was demonstrated, and this was subsequently withdrawn by the Applicant. Renal function was reduced compared to control therapy when the CsA-sirolimus combination was used beyond 3 months.

Studies 212 and 310 provided supportive evidence that sirolimus as add-on to CsA and CS is associated with low rates of acute rejection and high graft and patient survival. These trials, moreover, used lower CsA exposures than studies 301 and 302. Immunosuppressive efficacy of sirolimus as add-on to CsA and CS is considered acceptably documented as regards prevention of acute rejection in patients of low to moderate immunological risk. Due to the unfavourable effect of the CsA-sirolimus combination on graft function, maintenance therapy with sirolimus is not recommended in patients where CsA cannot be discontinued.

Sirolimus as base immunosuppressant after discontinuation of CsA

The observed, adverse effects on graft function of the sirolimus plus CsA combination prompted renewed exploration of sirolimus as monotherapy in combination with CS after tapering and discontinuation of CsA in trials 212 and 310. Twelve-month data from trial 310 were available during the marketing authorisation procedure. These data indicated that, in the population studied, this regimen is compatible with good graft and patient survival, despite a transient increase in the rate of mild to moderate acute rejections. Longer-term 24 support this conclusion and 36-month data supplied post-marketing. Graft function and blood pressure improved when CsA was withdrawn. The observed adverse interaction of sirolimus and CsA on graft function would mandate that CsA be progressively withdrawn after 2-3 months, if patients remain on sirolimus maintenance therapy.

Coated tablet

The clinical experience with a tablet formulation of Rapamune derives from trials 309 and 310. Both studies used an oval tablet presentation and the currently proposed triangular tablet has not been tested in clinical trials.

Trial 310 (performed entirely with the tablet formulation) was considered the pivotal evidence supporting the positive CPMP opinion for Rapamune oral solution.

The other trial (309) was a one-year, multicentre, parallel group study that enrolled 477, largely primary, recipients of a renal allograft. This trial presented compared sirolimus tablets (oval) and solution in a combination regimen (fixed-dose sirolimus plus continuous full-dose CsA) that has been clearly identified as unsuitable. In this study, no statistically significant differences in primary efficacy outcome could be seen between tablets and oral solution. Considering the specific indication and TDM-based regimen approved by CPMP for sirolimus oral solution and which are also applicable to the proposed tablet formulation, these findings are not considered important for determination of suitability of a tablet formulation *per se* and, thus, not critical to the approval of the tablet formulation.

Safety
In the overall clinical programme, more than 3,500 patients and subjects were enrolled in 50 studies (2,247 of whom received at least one dose of sirolimus)
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Sirolimus is associated with dose/concentration related risks for over immunosuppression with infections, including opportunistic infections. These have been reported at rates which appear comparable with those seen with MMF and tacrolimus, but higher than what has been reported with IL-2 receptor antibodies in this indication. CMV and PCP appear manageable with the recommended prophylactic regimens.

Haematological toxicity with sirolimus occurs in a dose-related manner, but does not appear a major concern with the proposed dose.

The period of observation available is too limited for definite evaluation regarding the risk for malignancy, including PTLD, but the submitted data do not provide any signals.

A majority of sirolimus-treated patients develop hyperlipidemia. By 12 months, lipid-lowering therapy was effective in lowering serum lipids to values similar to those in the 301/302 control groups, without notable increases in adverse events. When used with the proposed regimen of CsA withdrawal at 3 months, sirolimus maintenance therapy was associated with lower blood pressure and normal to high HDL-cholesterol levels. The long-term impact of sirolimus on cardiovascular risk has not been established.

Coated tablets

Overall, studies 309 and 310 provide evidence of equivalence between sirolimus tablets and solution in terms of safety. However, the findings also confirm a potential risk of toxicity, particularity hepatotoxicity, when sirolimus is employed at high doses. The need for close drug monitoring has to be considered.

Benefit/risk assessment

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus *that* the benefit/risk profile of Rapamune was favourable. Rapamune is indicated for the prophylaxis of organ rejection in adult patients at low to moderate immunological risk receiving a renal transplant. It is recommended that Rapamune be used initially in combination with cyclosporine microemulsion and corticosteroids for 2 to 3 months. Rapamune may be continued as maintenance therapy with corticosteroids only if cyclosporine can be progressively discontinued..