**SYNOPSIS**

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|---|---|---|
| **Name of Sponsor/Company:** Astellas Pharma Europe Ltd. | | |
| **Name of Finished Product:** FK506E (MR4) | | |
| **Name of Active Ingredient:** Tacrolimus | | |

**Title of Study:** A Multicenter, Open, Single Sequence Crossover Study to Assess the Safety and Efficacy of a Tacrolimus Modified Release, FK506E (MR4), Based Immunosuppressive Regimen in Stable Liver Transplant Patients Converted from a Prograf® Based Immunosuppressive Regimen

**Responsible Medical Officer:** Dr [redacted], Astellas Pharma Europe Ltd., [redacted]

**Coordinating Investigator:** Dr. [redacted] Germany.

**Investigator(s):** [redacted].

**Study Center(s):** 13 European centers participated in the study: Germany (2), France (3), Great Britain (1), Ireland (1), Poland (1), Spain (5).

**Publication (reference):** None available to date.

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|---|---|
| **Study Period:**<br>**Date of First Enrollment:** 17 October 2006<br>**Date of Last Evaluation:** 01 November 2007 | **Phase of Development:**<br>Phase IIIb |

**Objectives:** The primary objective was to assess the safety of a tacrolimus modified release, MR4 (Advagraf®), based immunosuppressive regimen in stable liver transplant subjects converted on a 1:1 (mg:mg) basis from a Prograf® based immunosuppressive regimen. Non-inferiority for creatinine clearance was assessed. The secondary objective was to assess the safety and the efficacy of this treatment.

**Study Design:** This was a multicenter, open, single sequence crossover study. On enrollment (Week -6), the twice daily Prograf® regimen was replaced by a twice daily Prograf regimen, as study medication. Subjects entered the 6-week Prograf-Treatment Phase to confirm compliance to regimen stability requirements and collect data under Prograf therapy.

On completion of the Prograf-Treatment Phase and qualifying for entry into the MR4-Treatment Phase, Prograf total daily dose (morning and evening dose) was converted to MR4 once daily (morning dose) on a 1:1 (mg:mg) basis on Day 1of the MR4-Treatment Phase. Subjects continued taking MR4 once daily treatment and were followed for 12 weeks with study assessments for safety and efficacy to evaluate the primary and secondary variables.

All concomitant immunosuppressive medications used in combination with Prograf® prior to enrollment were maintained at a constant dose throughout the duration of the study.

**Diagnosis and Main Criteria for Inclusion:** Male or female subjects with minimum 18 years of age; stable condition following a liver transplantation at least 12 months prior to study enrollment; unchanged dose of Prograf® over 12 weeks prior to enrollment; tacrolimus blood trough level between 5–15 ng/mL. Additional inclusion criteria to enter the MR4-Treatment Phase were: stable dose of Prograf; unchanged dose of concomitant immunosuppressive medications during the Prograf-Treatment Phase; mean tacrolimus blood trough levels within the recommended range of 5–15 ng/mL during the Prograf-Treatment Phase.

**Number of Subjects (planned and analyzed):** Assuming a decrease in creatinine clearance of 5% using MR4 treatment (corresponding to 4 mL/min) and a standard deviation for the pre-post difference

of 15% (12 mL/min) of the reference mean (Prograf), a total of 100 subjects would have a power of more than 90% to assess non-inferiority of MR4 with 10% pre-defined non-inferiorty margin. Assuming 20% as the rate of subjects to be excluded from the per protocol population, the analysis set for the primary analysis, approximately 125 subjects were to be enrolled to yield 100 evaluable subjects. The analyzed per protocol population consisted of 80 subjects.

**Test Product, Dose And Mode of Administration:**
Prograf 0.5 mg, 1.0 mg and 5.0 mg capsules.
Prograf administered orally:
Prograf capsules were administered twice daily (morning and evening).
Prograf dosing:
During the Prograf-Treatment Phase, the Prograf dose was to remain unchanged and the mean tacrolimus blood trough levels were to be between 5–15 ng/mL for the subject to enter into the MR4- Treatment Phase. Prograf was administered for the first 6 weeks of the study during the Prograf-Treatment Phase.
MR4: 0.5 mg, 1.0 mg and 5.0 mg capsules.
MR4 administered orally:
The total daily dose of Prograf at Day -1 was to be converted at 1:1 (mg:mg) on Day 1 to MR4 administered once daily in the morning.
MR4 dose modification:
Following conversion to MR4, tacrolimus blood trough levels were to be monitored and dose adjustments made to maintain a similar systemic exposure. Attempts were to be made to maintain a constant dose of MR4 for the duration of the study. Dose modifications could only be made if:

- indicated by clinical signs, and/or
- tacrolimus blood trough levels deviated by greater than 20% from the mean of the levels taken during the Prograf-Treatment Phase.

Recommended tacrolimus blood trough levels during the MR4-Treatment Phase were 5–15 ng/mL.

**Lot Numbers:** FK506: 0.5 mg, [redacted]; 1.0 mg, [redacted]; 5.0 mg, [redacted]
MR4: 0.5 mg, [redacted] 1.0 mg, [redacted]; 5.0 mg, [redacted].

**Duration of Study and Treatment:** Subjects were followed for 6 weeks during the Prograf-Treatment Phase of the study. Four assessment visits took place during this phase. Following conversion to MR4 subjects were followed for 12 weeks during which seven assessment visits took place.

**Criteria for Evaluation:** The primary study variable was the change in calculated creatinine clearance (Cockcroft and Gault formula) between Prograf and MR4 at steady state. The secondary variables assessed were: change in blood pressure, $HbA_{1c}$, total bilirubin, SGPT/ALT and/or SGOT/AST between Day -1 and Week 12; incidence of adverse events (AEs); frequency of biopsy-proven acute rejection episodes (BPAR); subject and graft survival.

**Statistical Methods:**

*Analysis sets*: Full analysis set (FAS): All subjects who received at least one dose of study drug in each treatment phase (Prograf and MR4) with sufficient data to derive the primary variable at least once during each treatment phase.

Per protocol set (PPS): All subjects in the FAS without major protocol violations and with sufficient data for the primary variable at least once during the steady state phase for each treatment (Week -6 to Day -1 for Prograf, and Week 6 to Week 12 for MR4).

Safety analysis set (SAF): All subjects who received at least one dose of study drug with any data reported thereafter.

Analysis of the primary variable as well as of relevant baseline characteristics was based on the FAS and PPS. The primary analysis was based on the PPS; an analysis based on the FAS was used to assess the robustness of the results of the primary analysis. Analysis of subject survival, graft survival and AEs was based on the SAF. Analysis of all other secondary variables was based on the FAS.

If non-inferiority is shown for any analysis set, then a test for superiority will be performed for the FAS and PPS sets [in accordance with CPMP/EWP/482/99: Points to Consider on Switching Between Superiority and Non-Inferiority, July 2000].

*Primary variable*: Creatinine clearance was calculated using the Cockcroft and Gault formula.

The comparison of creatinine clearance was based on the lower limit of the two-sided 95% confidence interval (CI) (corresponding to a one-sided significance level of alpha = 2.5%) for the relative difference of population means ($(\mu_{MR4} - \mu_{Prograf}) / \mu_{Prograf}$), which should lie above the acceptance limit of -10% of the Prograf mean for concluding non-inferiority. Analysis was performed using an appropriate ANOVA for repeated measurements of non-transformed data with Prograf as reference that included the data from scheduled visits Week -6 to Day -1 and Week 6 to Week 12.

*Secondary variables*: All safety parameters were summarized for the Prograf-Treatment Phase and the MR4-Treatment Phase using appropriate descriptive statistics. All time related analyses referred to the first day of the respective treatment during the study.

The change from Day -1 to Week 12 in blood pressure, $HbA_{1c}$, total bilirubin, SGPT/ALT, SGOT/AST was summarized for the PPS and FAS. The incidences of AEs were summarized for the SAF by MedDRA preferred term. A biopsy of the liver graft was to be performed if clinical or laboratory signs indicated the occurrence of a rejection episode. Subject and graft survival were summarized for the SAF by overall frequencies. Tacrolimus blood trough levels were summarized over time for the PPS and FAS populations.

**RESULTS:**

**Analysis Sets and Subject Disposition:**

The number of subjects included in each of the sets used for study analysis is presented in the following table:

**Table 1: Disposition of Subjects, Number (%)**

| | **Number of Subjects** |
|---|---|
| Enrolled | 112 (100.0) |
| Safety analysis set (SAF) | 112 (100.0) |
| Full analysis set (FAS) | 98 (87.5) |
| Per protocol analysis set (PPS) | 80 (81.6) |
| Completers | 96 (85.7) |
| Premature study withdrawal:<br>During Prograf -Treatment Phase<br>During MR4 -Treatment Phase | 16 (14.3)<br>14 (12.5)<br>2 (1.8) |

Fourteen subjects (12.5%) were not included in the FAS because they did not receive MR4. Further, 18 subjects (18.4%) in the FAS were excluded from the PPS. Reasons for exclusion from the PPS were: change of immunosuppressive medication for ≥7 consecutive days (3/3.1%), major violation of inclusion or exclusion criteria (4/4.1%), non-compliance to tacrolimus dose adjustment schedule (11/11.2%).

For purposes of this report and in adherence to the study protocol, results of the primary variable, study population demographics, and immunosuppressive therapy are reported for the PPS. Results of subject and graft survival and AEs are presented for the SAF. Results of all other secondary variables are presented for the FAS.

**Demographics:**

The mean (±SD) age of subjects was 52.5 years (±10.3), 72.5% of subjects were male, 97.5% were Caucasian, and 98.8% had received an organ from a deceased donor. CMV status of donors (D) and recipients (R) was D+/R- 64.6%/26.3%. The mean time since liver transplant was 41.2 months (±24.0) and the majority of subjects had a primary diagnosis of cirrhosis (65.0%) followed by carcinoma (18.8%) prior to transplantation.

**Study Drug Exposure:**

The mean daily dose of Prograf at Day -1 after 6 weeks of treatment was 0.05 mg/kg (±0.03). Similarly, the mean daily dose of MR4 at Week 12 after 12 weeks of treatment was 0.05 mg/kg (±0.03). During the MR4-Treatment Phase two subjects required a decrease in drug dose (each subject required one dose adjustment). Nine subjects required an increase in the dose of MR4: seven subjects required one dose adjustment and two subjects required two dose adjustments.

Mean tacrolimus trough levels were at the lower levels of the recommended range during both treatment phases of the study. Levels were lower during the MR4-Treatment Phase as depicted in Table 2. After one week of MR4 therapy, there was a decrease of approximately 1.25 ng/mL in the mean tacrolimus trough level; trough levels fluctuated less after Week 1.

**Table 2: Tacrolimus Trough Levels (ng/mL) during Study Time Periods (PPS)**

| Visit | N | Mean (SD) | Minimum | Median | Maximum |
|---|---|---|---|---|---|
| *Prograf-Treatment Phase* | | | | | |
| Week -6 | 70 | 7.63 (2.47) | 1.2 | 7.5 | 15.0 |
| Week -4 | 80 | 7.63 (2.80) | 3.4 | 7.3 | 16.9 |
| Week -2 | 80 | 7.74 (3.15) | 2.0 | 7.0 | 19.4 |
| Day -1 | 76 | 7.50 (2.82) | 2.6 | 7.1 | 15.8 |
| *MR4-Ttreatment Phase* | | | | | |
| Week 1 | 78 | 6.24 (2.15) | 2.3 | 5.8 | 11.4 |
| Week 2 | 75 | 6.45 (1.91) | 2.0 | 6.2 | 13.2 |
| Week 4 | 78 | 6.52 (2.10) | 2.5 | 6.2 | 13.0 |
| Week 6 | 80 | 6.52 (2.21) | 0.6 | 6.5 | 11.5 |
| Week 8 | 79 | 6.49 (2.30) | 2.2 | 6.2 | 13.4 |
| Week 10 | 79 | 6.63 (2.35) | 2.3 | 6.2 | 12.6 |
| Week 12 | 77 | 6.33 (1.91) | 1.8 | 6.2 | 10.8 |

**Primary Variable:** Non-inferiority of MR4 against Prograf was demonstrated with a relative difference in mean calculated creatinine clearance of -0.0% (±6.19), the 95% CI for relative difference [-1.4; 1.3] was well above the set non-inferior margin of -10% of the Prograf mean (PPS). Superiority of MR4 against Prograf was not shown as the CI crossed 0. Results of the primary variable are depicted in the following table:

**Table 3: Mean Creatinine Clearance during Steady State Phase: Results of the Test Procedure for Non-Inferiority (PPS)**

| | Steady State Phase | | Absolute Difference MR4 – Prograf (mL/min) | Relative Difference MR4 – Prograf (%) |
|---|---|---|---|---|
| | Prograf-Treatment Phase [a] (mL/min) | MR4-Treatment Phase [b] (mL/min) | | |
| N | 80 | 80 | 80 | 80 |
| Mean (SD) | 85.7 (24.2) | 85.5 (23.7) | -0.2 (5.2) | -0.0 (6.2) |
| Minimum | 45.3 | 44.5 | -11.5 | -16.8 |
| Median | 83.2 | 81.6 | -0.2 | -0.3 |
| Maximum | 198.9 | 187.4 | 14.0 | 19.7 |
| 95% CI[c] | [80.3; 91.0] | [80.2; 90.8] | [-1.3; 1.0] | [-1.4; 1.3] |

[a] Week -6 to Day -1; [b] Week 6 to Week 12; [c]arithmetic mean.

**Efficacy Results:**

There were no incidences of BPAR during either the Prograf-Treatment Phase or during the MR4-Treatment Phase. There were no incidences of clinical acute rejection (diagnosed per signs and symptoms) during either treatment phase.

**Safety Results:**

*Subject survival:* The estimated subject survival rate (Kaplan-Meier method) was 100% at the end of the study (based on the SAF population).

*Graft survival:* The estimated graft survival rate (Kaplan-Meier method) was 100% at the end of the study (based on the SAF population).

*Adverse events:* The incidence of serious AEs was low during each treatment phase as was the incidence of AEs leading to dose modification or to study withdrawal. There were two subjects who were withdrawn from the study during the Prograf-Treatment Phase due to an AE. No subjects withdrew prematurely during the MR4-Treatment Phase due to an AE.

**Table 4: Overall Summary of the Incidence of Adverse Events (SAF)**

| | **Prograf-Treatment Phase N=112** | | **MR4-Treatment Phase N=98** | |
|---|---|---|---|---|
| | Subjects | Events | Subjects | Events |
| | N (%) | Nr. | N (%) | Nr. |
| Adverse events | 41 (36.6) | 85 | 56 (57.1) | 105 |
| Causally-related adverse event | 18 (16.1) | 27 | 24 (24.5) | 38 |
| Serious adverse events | 3 (2.7) | 6 | 6 (6.1) | 6 |
| Causally-related serious adverse event | 1 (0.9) | 1 | 1 (1.0) | 1 |
| Adverse event leading to dose modification | 1 (0.9) | 1 | 3 (3.1) | 4 |
| Adverse event leading to premature withdrawal | 2 (1.8) | 2 | 0.0 | 0 |

The incidence of the most commonly reported AEs for the SAF set was generally higher during the MR4-Treatment Phase; however the duration of this treatment was 12 weeks compared with 6 weeks for the Prograf-Treatment Phase. Similar types of AEs were reported during both treatment phases. The most commonly reported AEs during both treatment phases were infection, metabolism/nutritional disorders, and gastrointestinal disorders. The AEs reported during the study are consistent with the established safety profile of tacrolimus.

**Table 5: Most Frequently Reported [a] Adverse Events Regardless of Relationship to Study Medication (SAF)**

| MedDRA Primary SOC<br>MedDRA High Level Term<br>*MedDRA Preferred Term* | **Prograf-Treatment Phase N=112** | | **MR4-Treatment Phase N=98** | |
|---|---|---|---|---|
| | Subjects | Events | Subjects | Events |
| | N (%) | Nr. | N (%) | Nr. |
| Infections/infestations | 10 (8.9) | 11 | 15 (15.3) | 18 |
| Upper respiratory tract, pathogen not specified | 2 (1.8) | 2 | 6 (6.1) | 6 |
| *Nasopharyngitis* | 2 (1.8) | 2 | 4 (4.1) | 4 |
| Bacterial infections NEC | 1 (0.9) | 1 | 3 (3.1) | 4 |
| Metabolism/nutrition disorders | 9 (8.0) | 13 | 13 (13.3) | 14 |
| Elevated triglycerides | 2 (1.8) | 2 | 4. (4.1) | 5 |
| *Hypertriglyceridaemia* | 2 (1.8) | 2 | 4 (4.1) | 5 |
| Diabetes mellitus (including subtypes) | 1 (0.9) | 1 | 3 (3.1) | 3 |
| Gastrointestinal disorders | 7 (6.3) | 9 | 11 (11.2) | 13 |
| Diarrhoea (excluding infective) | 4 (3.6) | 4 | 4 (4.1) | 4 |
| *Diarrhoea* | 4 (3.6) | 4 | 4 (4.1) | 4 |
| Nervous system disorders | 8 (7.1) | 10 | 7 (7.1) | 12 |
| Headache NEC | 2 (1.8) | 2 | 5 (5.1) | 10 |
| *Headache* | 2 (1.8) | 2 | 5 (5.1) | 10 |

(continued)

**Table 5 (continued)**

| MedDRA Primary SOC<br>MedDRA High Level Term<br>*MedDRA Preferred Term* | **Prograf-Treatment Phase N=112** | | **MR4-Treatment Phase N=98** | |
|---|---|---|---|---|
| | Subjects | Events | Subjects | Events |
| | N (%) | Nr. | N (%) | Nr. |
| Vascular disorders | 4 (3.6) | 4 | 8 (8.2) | 8 |
| Vascular hypertensive disorders NEC | 2 (1.8) | 2 | 7 (7.1) | 7 |
| *Hypertension* | 2 (1.8) | 2 | 7 (7.1) | 7 |
| Investigations | 7 (6.3) | 8 | 5 (5.1) | 5 |
| Musculoskeletal/connective tissue disorders | 7 (6.3) | 7 | 5 (5.1) | 6 |
| Musculoskeletal/connective tissue NEC | 2 (1.8) | 2 | 3 (3.1) | 4 |
| Skin/subcutaneous tissue disorders | 5 (4.5) | 5 | 4 (4.1) | 4 |
| General disorders/administrative site conditions | 1 (0.9) | 1 | 6 (6.1) | 6 |
| Hepatobiliary disorders | 1 (0.9) | 1 | 3 (3.1) | 3 |

[a] Reported in at least 3% of subjects during either treatment phase. Coded using MedDra version 6.1. SOC=system organ class. NEC=not elsewhere classified.

The incidence of treatment-related AEs was low during both treatment phases as shown in the following table:

**Table 6: Most Frequently Reported [a] Adverse Events Assessed by the Investigator as Causally-related to Study Medication (SAF)**

| MedDRA Primary SOC<br>MedDRA High Level Term<br>*MedDRA Preferred Term* | **Prograf-Treatment Phase N=112** | | **MR4-Treatment Phase N=98** | |
|---|---|---|---|---|
| | N (%) | Nr. events | N (%) | Nr. events |
| Metabolism/nutrition disorders | 4 (3.6) | 5 | 7 (7.1 ) | 8 |
| Infections/infestations | 4 (3.6) | 5 | 7 (7.1) | 7 |
| *Nasopharyngitis* | 1 (0.9) | 1 | 3 (3.1) | 3 |
| Nervous system disorders | 3 (2.7) | 4 | 4 (4.1) | 9 |
| *Headache* | 1 (0.9) | 1 | 4 (4.1) | 9 |
| Vascular disorders | 1 (0.9) | 1 | 4 (4.1) | 4 |
| *Hypertension* | 1 (0.9) | 1 | 4 (4.1) | 4 |

[a] Reported in at least 3% of subjects during either treatment phase. Coded using MedDra version 6.1. SOC=system organ class. NEC=not elsewhere classified.

There were no serious AEs of causal relationship to the study medication, as assessed by the investigator, which were reported in ≥3% of subjects during either treatment phase.

A statistically significant difference was found in mean blood pressure measurements (derived from 24-hour ambulatory blood pressure monitoring) between Week 12 and Day -1 (p=0.0084). The differences in other study secondary variables between those time points were minimal and stable over time.

**Table 7: Mean (SD) Differences in Blood Pressure, $HbAB_{1c}$, Total Bilirubin, SGPT/ALT, SGOT/AST from Week 12 to Day -1 (FAS)**

| | Arterial Blood Pressure (mmHg) [a] | $HbA_{1c}$ | Total Bilirubin (µmol/L) | SGPT/ALT (U/L) | SGOT/AST (U/L) |
|---|---|---|---|---|---|
| N | 84 | 51[b] | 98 | 98 | 98 |
| Day -1 | 101.9 (9.0) | 5.5 (0.6) | 12.9 (6.2) | 27.9 (18.7) | 24.4 (10.5) |
| Week 12 | 100.0 (8.7) | 5.5 (0.6) | 13.2 (6.5) | 29.4 (24.7) | 25.1 (11.4) |
| *Difference* | *-2.0 (6.7)* [c] | *0.1 (0.3)* | *0.3 (3.5)* | *1.5 (14.4)* | *0.7 (9.2)* |

[a] Derived from 24-hour ambulatory blood pressure monitoring. [b] Non-diabetic subjects. [c] p-value = 0.0084 [CI: -3.4; -0.5], one sample t-test.

**CONCLUSIONS:**
Non-inferiority of MR4 against Prograf was demonstrated using mean calculated creatinine clearance. Results of both safety and efficacy were excellent following treatment with Prograf for 6 weeks and MR4 for 12 weeks in this population of liver transplant recipients. The type of AEs reported during both treatment phases is consistent with the established safety profile of tacrolimus. The incidence of the most commonly reported AEs was generally higher during the MR4-Treatment Phase; however the duration of this treatment period was twice as long as the Prograf-Treatment Phase. Efficacy was good as evidenced by no incidences of BPAR during either treatment phase. Few subjects required either an increase or a decrease in dose of MR4 to maintain recommended trough levels. Mean tacrolimus levels were stable during both treatment phases. Mean trough levels decreased by approximately 1.25 ng/mL during the first week of MR4 treatment and remained lower during the MR4-Treatment Phase compared to the Prograf-Treatment Phase.

**Date of Report:** 31 October 2008