SYNOPSIS

Name of Sponsor/Company: Astellas Pharma Europe Ltd.		
Name of Finished Product: FK 506 (Prograf®)		
Name of Active Ingredient: Tacrolimus		

Title of Study: An Open, Randomized, Multicentre, Exploratory Clinical Study to Compare the Safety and Efficacy of Tacrolimus in Combination with Monoclonal Anti-IL2R Antibodies or Steroids in HCV Positive Patients undergoing Liver Allograft Transplantation

Responsible Medical Officer: Dr. [redacted], Astellas Pharma Europe Ltd., [redacted], [redacted], UK. **Coordinating Investigator:** Dr. med. [redacted]

Investigator(s): 17 investigators in Europe participated in the study. These were: [redacted] Czech Republic; [redacted] Germany; [redacted] Spain; [redacted], France; [redacted] Italy; [redacted] Poland; [redacted], Sweden.

Study Center(s): The study was conducted in 17 European centers. These were located in: [redacted] Czech Republic; [redacted], Germany; [redacted], Spain; [redacted], France; [redacted], UK; [redacted], Italy; [redacted], Poland; [redacted], Sweden.

Publication (reference): None available to date.

Study Period: **Date of First Enrollment:** 13 June 2005 **Date of Last Evaluation:** 12 June 2008	**Phase of Development:** Phase II

Objectives: The objective of this exploratory study was to evaluate the impact of the elimination of steroids on viral hepatitis C (HCV) load at 12 months as measured by quantitative serum HCV-RNA determination in patients transplanted for HCV cirrhosis. A comparison was made between two regimens which both included tacrolimus, one with standard steroid administration (TAC/STR, reference treatment arm) and the other with daclizumab (TAC/DAC, experimental treatment arm) in which steroids were avoided for both prophylactic immunosuppression and anti-rejection treatment.

Study Design: This study was a multicentre, randomized, open, two-arm, parallel group, comparative, phase II explorative study. Patients about to undergo primary orthotopic liver or split liver allograft transplantation for HCV cirrhosis were randomized to receive tacrolimus and corticosteroids (for 3 months) or tacrolimus and daclizumab.

Diagnosis and Main Criteria for Inclusion: Study participants were adult patients (18-65 years old), HCV positive with HCV-cirrhosis, scheduled for a primary orthotopic liver or split liver allograft transplantation from a deceased donor.

Number of Subjects (planned and analyzed): There were no historical data on HCV viral load available for the calculation of sample size for this exploratory study. Therefore, study sample size was based on a compromise between the considerations of feasibility and reasonable statistical power to detect a signal for a positive effect of the avoidance of steroids on the HCV viral load. The HCV viral load at month 12 seems to be a surrogate marker for the activity of viral replication in the liver. It was planned to enroll 120 patients (60 per treatment arm) in approximately 8-12 centers. Actual total enrollment was 138 patients (68 patients in the TAC/DAC arm and 70 patients in the TAC/STR arm) at 17 medical centers. The full analysis set (FAS) was used for analysis of data and consisted of

67 patients in the TAC/DAC arm and 68 patients in the TAC/STR treatment arm.

Test Product, Dose And Mode of Administration:

Tacrolimus (Prograf®) 0.5 mg, 1.0 mg and 5.0 mg capsules. The initial daily dose was 0.10 to 0.15 mg/kg p.o. given orally in two doses (equals 0.05 to 0.075 mg/kg twice daily). The first dose was to be administered within 12 hours after skin closure. Subsequent tacrolimus doses were to be adjusted on the basis of clinical evidence of efficacy and occurrence of adverse events observing the following recommended blood trough level ranges: 10–15 ng/mL from Days 0–42, < 10 ng/mL from Days 43–365.

Daclizumab (TAC/DAC arm only). Two doses of daclizumab were administered. The first dose of 2.0 mg/kg was administered i.v. during the anhepatic phase. The second dose of 1.0 mg/kg was administered i.v. between postoperative days 7–10.

Lot Numbers: Tacrolimus 0.5 mg: [redacted]. Tacrolimus 1.0 mg: [redacted] Tacrolimus 5.0 mg: [redacted]. Daclizumab: [redacted].

Duration of Study and Treatment: Study duration per patient was 12 months. During this period a total of 9 regular assessment visits took place: Baseline, Day 1, Day 7, Day 14, Day 60 (month 2), Day 93 (month 3), Day 183 (month 6), Day 275 (month 9) and Day 365 (month 12).

Criteria for Evaluation: The primary study endpoint was the HCV viral load at 12 months post transplantation. The secondary efficacy and safety variables assessed were: incidence of and time to first biopsy-proven acute rejection and first biopsy-proven treatment-resistant acute rejection; overall frequency of biopsy-proven acute rejection episodes; severity of biopsy-proven acute rejection; incidence of and time to first acute rejection and first treatment-resistant acute rejection; overall frequency of acute rejection episodes; patient and graft survival at Month 12; incidence of and time to hepatitis C recurrence; histological fibrosis score/staging; histological HCV-grading; renal function; incidence of adverse events.

Statistical Methods: Analysis Sets: *Primary Analysis Set* (PAS) was defined as all randomized and transplanted patients with a HCV viral load above the limit of quantification at Baseline and was used for the analysis of the primary efficacy endpoint. *Full Analysis Set* (FAS) was defined as all randomized and transplanted patients and was used for the analysis of secondary study endpoints. Primary endpoint: HCV viral load at 12 months post transplant was analyzed after a transformation using the logarithm to base 10 which resulted in the unit log10 IU/mL. The one-sided Wilcoxon rank sum test was used to test for superiority of TAC/DAC over TAC/STR. All secondary efficacy and safety endpoints were summarized per treatment arm using appropriate descriptive statistics. Incidence and time to acute rejection and biopsy-proven acute rejection (BPAR) as well as patient and graft survival was analyzed using Kaplan-Meier methods. Wilcoxon-Gehan tests were used to compare survival functions in the two treatment arms. Adverse events were coded using MedDRA version 9.0 and compared using descriptive p-values from Fisher's exact test. All time to event related parameters were analyzed relative to Day 0 defined as the day of skin closure. Recurrence of hepatitis C was diagnosed by liver biopsy in patients with elevated liver enzymes or by protocol biopsy performed at months 6 and 12. Samples for the evaluation of qualitative and quantitative serum HCV-RNA were collected at Baseline and at subsequent visits. Additional samples were collected if clinically indicated or if a biopsy was performed.

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: Populations for Analysis and Disposition of Patients – Number (%) of Patients

	Tacrolimus/ Daclizumab	Tacrolimus/ Steroids (3 Mo)	Total
Randomized to treatment	68	70	138
Full analysis set	67 (100.0)	68 (100.0)	135 (100.0)
Primary Analysis Set	50 (74.6)	52 (76.5)	102 (75.6)
Completed	30 (44.8)	56 (82.4)	86 (63.7)
Total deaths†	11 (16.4)	3 (4.4)	14 (10.4)
During study	8 (11.9)	1 (1.5)	9 (6.7)
Withdrawn‡	29 (43.3)	11 (16.2)	40 (29.6)
Adverse event	16 (23.9)	5 (7.4)	21 (15.6)
Protocol violation	6 (9.0)	1 (1.5)	7 (5.2)
Retransplantation	1 (1.5)	3 (4.4)	4 (3.0)
Other	6 (9.0)	2 (2.9)	8 (5.9)

† 3 deaths in the TAC/DAC arm and 2 deaths in the TAC/STR arm occurred after study withdrawal which was due to an adverse event. ‡Withdrawn for reasons other than death.

Of the 138 patients randomized to receive treatment, 135 underwent transplantation and received at least one dose of study medication and were thus eligible for inclusion in the FAS. Further, a total of 33 patients (17 in the TAC/DAC arm and 16 in the TAC/STR arm) were excluded from the PAS because of a recorded HCV load below the level of quantification. Approximately 64% of the patients randomized in the FAS completed the study; the rate of study completion was lower in the TAC/DAC arm at 44.8% (30 patients) than in the TAC/STR arm at 82.4% (56 patients). More patients in the TAC/DAC arm were withdrawn from the study due to an adverse event (AE); there was no single AE which could be identified as the prominent cause of withdrawal. More patients in the TAC/DAC arm were withdrawn from the study due to a protocol violation: 5 of 6 violations were the administration of corticosteroids.

Demographics:

The mean (SD) age of patients in the TAC/DAC arm was 53.1 ± 9.6 and 55.3 ± 6.5 in the TAC/STR arm. Approximately 30% of all patients were ≥ 60 years. The majority of patients in each arm were male (73.1% of patients in the TAC/DAC arm and 66.2% in the TAC/STR arm). The 2 treatment arms were largely comparable in terms of patient demographics and viral status at baseline with one exception; patients in the TAC/DAC arm were significantly taller than those in treatment arm 2 (P = 0.011 for difference between arms, Student's t-test). All patients included in the FAS presented with a primary diagnosis of cirrhosis post hepatitis C infection. Of these, serum HCV-RNA was below the detection limit in 9 (15.3%) patients in the TAC/DAC and 3 (5.2%) patients in the TAC/STR arm.

Donor/recipient mismatch and characteristics of donor organs were similar between the treatment arms with the exception of a significantly higher number of organs from male donors in the TAC/DAC arm (P = 0.045, chi-square test). There is no clinical evidence that this difference would have had an effect on study results.

Study Drug Exposure:

Tacrolimus trough levels were used to measure drug exposure during the study. The per-protocol recommended tacrolimus trough level in both treatment arms was 10–15 ng/mL from Days 0–42 and < 10 ng/mL after Day 43. Mean tacrolimus exposure was higher during the first 3 months of the study in the TAC/STR arm then decreased to within pre-defined ranges at Day 183. In both treatment arms, mean tacrolimus trough levels were within pre-defined ranges at Day 365. At Day 365, tacrolimus trough levels were 7.12 ± 2.62 ng/mL in the TAC/DAC arm and 8.24 ± 3.83 ng/mL in the TAC/STR arm. The mean daily dose of tacrolimus at end of study was 0.060 ± 0.061 mg/kg in the TAC/DAC arm

and 0.046 ± 0.039 mg/kg in the TAC/STR arm.

All patients in the TAC/DAC arm received a first dose of daclizumab as defined in the protocol. However, only 52 (77.6%) of patients received a second dose and 1 patient received a third dose. One patient in the TAC/STR arm received 1 dose of daclizumab.

Corticosteroid administration. Six patients in the TAC/DAC arm erroneously received a bolus dose of corticosteroids between Days -1 and 1. During Week 1, 6 patients received maintenance doses of corticosteroids and small numbers of patients received corticosteroids as maintenance immunosuppression throughout the 12-month study. In total, 21 patients in TAC/DAC arm received at least 1 dose of corticosteroids administered as either maintenance, bolus or rejection treatment. The unusallyt high mean maintenance corticosteroid doses in the TAC/DAC arm were driven by 1 patient who received exceptionally high maintenance doses. Corticosteroids were not to be administered as maintenance immunosuppression after Month 3 in the TAC/STR arm. More than half of the patients (22/68 patients, 32.4%) in the TAC/STR arm received corticosteroids during Months 7 to 9 decreasing to one-quarter of patients (17/68, 25.0%) using maintenance corticosteroids during Months 10 to 12.

Efficacy Results:

Primary endpoint

No significant difference in median HCV viral load at 12 months was found between the treatment arms. A decision was made to perform an analysis to evaluate the maximum HCV load from Day 1 until the end of study. This decision was made by the study investigators and was defined in the Blind Data Review Meeting which took place prior to database closure. The analysis revealed a statistically significant difference in median maximum HCV load during the study in patients in the TAC/DAC arm compared to the TAC/STR arm. Results of the primary study endpoint are presented in the following table.

Table 2: HCV Viral Load and Maximum HCV Viral Load

	n	Tacrolimus/ Daclizumab	n	Tacrolimus/ Steroids (3 Mo)	*P* value†
HCV viral load - Month 12‡ (PAS):	19/50		35/52		
Mean (SD)		4.88 (1.54)		4.90 (2.06)	
Median (range)		5.46 (0.95-6.54)		5.91 (0.95-6.89)	0.294
HCV viral load - Maximum§ (PAS):	22/50		45/52		
Mean (SD)		6.03 (0.68)		6.42 (0.47)	
Median (range)		6.17 (4.78-6.89)		6.55 (5.04-6.89)	0.012
HCV viral load§ - Maximum (FAS):	30/67		56/68		
Mean (SD)		5.29 (1.77)		6.29 (0.88)	
Median (range)		5.83 (0.95-6.89)		6.53 (0.95-6.89)	<.001

PAS and FAS. Log_{10} of HCV viral load (IU/mL) was analyzed. Analyses was performed on all randomized and transplanted patients with baseline viral load above the limit of quantification who received at least one dose of either of the study medications (tacrolimus or daclizumab). PAS = Primary analysis set. FAS = Full analysis set.

†Wilcoxon rank sum test for superiority of TAC/DAC over TAC/STR with respect to HCV (central lab) viral load at 12 months or maximum HCV load during study. ‡Completers only using samples within Day 365 +/- 14 days but not after the day of last study visit or day of study withdrawal. In case of multiple samples, the closest one (and in case of ambiguity, the earlier one) was taken. §Completers only, maximum HCV load from Day 1 until end of study.

Acute rejection

The main analysis for BPAR was based on the Wilcoxon Gehan test and showed no statistical significant difference between the treatment arms. The overall rate of patients free from BPAR (Kaplan-Meier method) at Month 12 was higher with TAC/DAC (78.4%) than with TAC/STR (66.1%) (P = 0.118, chi-square test). The overall rate of patients free from acute rejection (Kaplan-Meier method) at Month 12 was higher with TAC/DAC (76.9%) than with TAC/STR (62.1%). The difference between arms was not significant (P = 0.084, chi-square test).

The overall frequency of BPAR and the frequency of acute rejection (diagnosed per clinical signs and symptoms) were significantly lower in the TAC/DAC arm than in the TAC/STR arm. Significantly fewer rejections in patients in the TAC/DAC arm resolved without a change of tacrolimus dose or the administration of MMF.

Table 3: Overall Frequency of Biopsy-proven and non Biopsy-proven Acute Rejections – Classification at End of Study or Study Withdrawal

	Number (%) of Patients				
	Tacrolimus/ Daclizumab N=67		**Tacrolimus/ Steroids (3 Mo) N=68**		***P* value†**
	N (%)	Episodes	N (%)	Episodes	
Biopsy proven acute rejection:	**11 (16.4)**	**11**	**21 (30.9)**	**25**	0.048
Resolved spontaneously	4 (6.0)	4	14 (20.6)	16	0.012
Sensitive to treatment	3 (4.5)	3	7 (10.3)	7	
Resistant to treatment	4 (6.0)	4	2 (2.9)	2	
Acute rejection:	**12 (17.9)**	**12**	**24 (35.3)**	**29**	0.022
Resolved spontaneously	4 (6.0)	4	15 (22.1)	17	0.007
Sensitive to treatment	3 (4.5)	3	9 (13.2)	9	
Resistant to treatment	5 (7.5)	5	3 (4.4)	3	

FAS. † Chi-square test comparing the numbers of patients.

Fewer BPARs occurred during Week 1 in the TAC/DAC arm than in the TAC/STR arm: 3 vs. 8, respectively. By Week 4, 9 BPARs had occurred in the TAC/DAC arm compared with 18 in the TAC/STR arm. The number of treatment resistant BPARs reported during Week 1 and by Week 4 was similar in the TAC/DAC and TAC/STR arm at 0 and 1 (during Week 1), and 3 and 2 (by Week 4), respectively.

Recurrence of hepatitis C

The incidence of acute and chronic HCV recurrence, diagnosed by central biopsy and counting the first recurrence for each patient, was numerically lower in the TAC/DAC arm (33 patients, 49.3%) than in the TAC/STR arm (52 patients, 76.5%). There was a significant difference between treatment arms in the overall estimated rate over 12 months to recurrence of HCV as confirmed by central biopsy. For the TAC/DAC and TAC/STR arms this was 19.1% and 13.8%, respectively (P = 0.020, chi-square test).

Histological fibrosis staging score and HCV grading

Using biopsy specimens, there were no differences between the treatment arms in the mean or median modified fibrosis staging score. Further, there were no clinical differences between the treatment arms in grading and staging scores of liver biopsies using the Modified HAI Grading and Staging of Liver Biopsy for Hepatitis C classification [Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995, 22 (6): 696-699].

Graft survival

The number of graft losses was higher in the TAC/DAC (13, 19.4%) than in the TAC/STR arm (9, 13.4%). In 11 patients in the TAC/DAC arm, graft loss was the result of patient death; of the patients who died, graft loss was related to complications of HCV recurrence in 2 patients. The cause of graft loss in the remaining 2 patients was primary non-function of the allograft. In 3 patients in the TAC/STR arm, graft loss was the result of patient death; no deaths were related to graft dysfunction. The causes of graft loss in the remaining 3 patients were retransplantation due to primary non-function of the allograft.

The overall estimated rate of graft survival (Kaplan-Meier method) in the TAC/DAC and TAC/STR arm was 80.1% and 91.1%, respectively (*P*=0.10, Wilcoxon-Gehan test).

Safety Results:

As expected for this population, 91.0% of patients in the TAC/DAC arm and 97.1% in the TAC/STR arm experienced at least one AE. The overall incidence of serious AEs was similar (67.2% and 66.2%) while the incidence of treatment-related AEs was lower in the TAC/DAC than in the TAC/STR arm (71.6% vs. 86.8%, respectively). Adverse events occurring with an incidence ≥ 10% per MedDRA Primary System Organ Class (SOC) and those occurring with a significant difference between treatment arms per MedDRA Preferred Term are presented in the following table.

Table 4: Overall Incidence of Adverse Events Regardless of Relationship to Study Medication

	Number (%) of Patients		
MedDRA Primary SOC MedDRA Preferred Term	**Tacrolimus/ Daclizumab N=67**	**Tacrolimus/ Steroids (3 Mo) N=68**	**P value†**
Overall	61 (91.0)	66 (97.1)	
Infections	40 (59.7)	49 (72.1)	
Hepatitis C	28 (41.8)	43 (63.2)	0.016
Metabolism/nutritional disorders	31 (46.3)	39 (57.4)	
Blood/lymphatic system disorders	34 (50.7)	35 (51.5)	
Thrombocytopenia	19 (28.4)	6 (8.8)	0.004
Renal/urinary disorders	34 (50.7)	30 (44.1)	
Gastrointestinal disorders	26 (38.8)	34 (50.0)	
Hepatobiliary disorders	30 (44.8)	24 (35.3)	
Vascular disorders	22 (32.8)	30 (44.1)	
Injury/procedural complications	24 (35.8)	25 (36.8)	
Respiratory/thoracic/mediastinal disorders	24 (35.8)	21 (30.9)	
Investigations	18 (26.9)	25 (36.8)	
Nervous system disorders	20 (29.9)	23 (33.8)	
General disorders/administration site conditions	16 (23.9)	19 (27.9)	
Psychiatric disorders	20 (29.9)	14 (20.6)	
Musculoskeletal/connective tissue disorders	11 (16.4)	17 (25.0)	
Cardiac disorders	16 (23.9)	11 (16.2)	
Skin/subcutaneous tissue disorders	8 (11.9)	16 (23.5)	

FAS. Adverse events occurring with an incidence ≥ 10% per MedDRA Primary System Organ Class (SOC) and those occurring with a significant difference between treatment arms per MedDRA Preferred Term. Coded using version 9.0 of MedDRA. †Fisher's exact test comparing the number of patients.

The overall incidence of AEs assessed by the investigator as being causally related to study medication was significantly higher in the TAC/STR than in the TAC/DAC arm. Causally related AEs occurring

with an incidence ≥ 10% per MedDRA SOC are presented in the following table. There was no significant difference in the incidence of any single causally related AE.

Table 5: Overall Incidence of Causally related Adverse Events

	Number (%) of Patients	
MedDRA Primary SOC	**Tacrolimus/ Daclizumab N=67**	**Tacrolimus/ Steroids (3 Mo) N=68**
Overall †	48 (71.6)	59 (86.8)
Renal/urinary disorders	27 (40.3)	27 (39.7)
Metabolism disorders	17 (25.4)	28 (41.2)
Infections	17 (25.4)	22 (32.4)
Nervous system disorders	17 (25.4)	15 (22.1)
Vascular disorders	14 (20.9)	14 (20.6)
Psychiatric disorders	16 (23.9)	11 (16.2)
Investigations	9 (13.4)	16 (23.5)
Blood/lymphatic system disorders	9 (13.4)	16 (23.5)

FAS. †P = 0.035, Fisher's exact test.

The incidence of causally related serious AEs was comparable between the treatment arms at 21 patients (31.1%) in the TAC/DAC arm and 20 patients (29.4%) in the TAC/STR arm.

The overall incidence of AE leading to premature study discontinuation was significantly higher in the TAC/DAC arm compared with the TAC/STR arm at 23 patients (34%) vs. 8 patients (11.8%), respectively (P = 0.002, Fisher's exact test). No single AE contributed to a majority of premature patient withdrawals in either treatment arm.

Patient deaths

There were 11 (6.4%) patient deaths in the TAC/DAC arm and 3 (4.4%) deaths in the TAC/STR arm. The deaths of 2 patients in the TAC/DAC arm were assessed by the attending investigator as being possibly related to the study medication. Patient #[redacted] had a history of hepatocellular carcinoma and tested CMV-positive at the time of transplant. On Day 290 he was diagnosed with disseminated aspergillus infection and treatment with antifungal agents was initiated. Immunosuppression could have contributed to the dissemination and severity of the aspergillus fungal infection. Patient #[redacted] had a history of hepatocellular carcinoma and tested CMV-positive at the time of transplant. This patient had a complicated post transplant course during which tacrolimus had to be interrupted several times because of renal problems and cardiac arrest. The patient died of a cerebrovascular accident which was assessed by the investigator as possibly related to study drug. The causes of death of the remaining 9 patients were: cerebral hemorrhage, cardiac disorder (3 patients), hemorrhagic shock, liver failure due to HCV recurrence (2 patients), portal vein thrombosis, and cholestatic hepatitis. The causes of death in the TAC/STR arm were: liver failure, multi-organ failure, and hemorrhage.

The overall estimated rate of patient survival (Kaplan-Meier method) was 83.1% in the TAC/DAC arm compared with 95.5% in the TAC/STR arm. The difference in 12 month survival between the treatment arms was significant (95% CI -0.227 to -0.019, P = 0.025).

Laboratory

At end of study, serum creatinine was slightly lower in the TAC/STR arm although mean values in both treatment arms were within reference limits. Mean calculated creatinine clearance values were comparable between the treatment arms. Blood counts were also comparable. Both mean and median values for tests of liver function (SGOT and SGPT) were higher in the TAC/DAC than in the TAC/STR arm whereas values for patients who completed the study showed similarity in measurements of liver function between the treatment arms. Serum lipid values were comparable

between the two treatment arms at Month 12.

CONCLUSIONS:

The analysis of the primary endpoint showed that there was no significant impact on median HCV viral load using an immunosuppressive regimen comprising tacrolimus and daclizumab induction compared with a standard regimen of tacrolimus and steroids which were administered for the first 3 months post transplant. There were numerically fewer recurrences of HCV with TAC/DAC and both BPAR and acute rejection incidences were lower with the TAC/DAC regimen.

Results of safety were largely comparable between the two treatment arms and the types of events reported reflected the known safety profiles of the study medications. More patients in the TAC/DAC arm prematurely discontinued the study, the majority because of an adverse event although no single event could be identified as the primary cause of discontinuation. Study withdrawal due to protocol violation was higher in the TAC/DAC arm, the administration of corticosteroids was the reason in 5 of the 6 patients withdrawn. The number of patient deaths was higher in the TAC/DAC arm. No patterns in patient demographic factors, type of events leading to or causing death, incidence of serious adverse events, or time of death could be identified to account for the higher number of patient deaths in this treatment arm. The overall estimated rate of patient survival (Kaplan-Meier method) was significantly higher in the TAC/STR than in the TAC/DAC arm. The number of grafts lost was higher in the TAC/DAC arm: in 11 of the 13 patients, graft loss was the result of patient death.

The conclusions derived from the results of this randomized clinical trial must remain guarded. The large numbers of premature patient withdrawals and the higher number of deaths in the TAC/DAC arm prevent any clear recommendations being made.

Date of Report: 8 June 2009