SYNOPSIS

Name of Sponsor/Company: Astellas Pharma Europe B.V. (Successor in interest to Fujisawa GmbH)		
Name of Finished Product: FK506		
Name of Active Ingredient: Tacrolimus		

Title of Study: AN OPEN, MULTICENTRE, RANDOMISED PARALLEL GROUP CLINICAL STUDY TO COMPARE SAFETY AND EFFICACY OF TACROLIMUS (FK506) WITH MONOCLONAL ANTI-IL2R ANTIBODIES (DACLIZUMAB) VS TACROLIMUS (FK506) WITH STEROIDS AND EVALUATE PHARMACOKINETICS IN LIVER ALLOGRAFT RECIPIENTS RECEIVING SUBOPTIMAL LIVERS

Study Center(s): Italy: [redacted]; Italy: [redacted]; Italy: [redacted], Italy: [redacted]; Italy: [redacted]; Italy: [redacted]; Italy: [redacted]; Italy: [redacted]

Publication (reference):

Study Period: 4Q 2004 – 4Q 2007 **Date of First Enrollment: 12 May 2005** **Date of Last Evaluation: 25 July 2008**	**Phase of Development:** Phase III

Objectives: To explore the safety, efficacy of two different tacrolimus based immunosuppressive regimens in recipients of suboptimal livers, by measuring the occurrence of acute rejection episodes, liver function, patient and graft survival, infections and adverse events. Moreover, FK506 pharmacokinetics and bile composition were to be investigated in a subgroup of 30 patients (15 patients per arm), but foreseen number was not achieved (total patients were 25 only, 14 in arm 1 and 11 in arm 2) and data collected were heavily incomplete in many patients, hence they have not jet analysed, as no significant results are foreseen.

Study Design: This was an exploratory, open, multicentre, randomised, 2-arm, parallel group comparative phase III study, in patients receiving primary suboptimal liver allograft transplantation. The patients were randomised to one of the following treatment arms:

Arm 1: steroid bolus (intraoperative), monoclonal anti-IL2R antibodies (daclizumab) induction, tacrolimus

Arm 2: steroid bolus (intraoperative), tacrolimus, steroids

Diagnosis and Main Criteria for Inclusion:

1. Male or female patients aged ≥ 18 years that were receiving a primary cadaveric orthotopic liver allograft transplantation with compatible AB0 blood type, assigned according to the local allocation policy.
2. Patient had to be capable of understanding the purpose and risks of the study, had been fully informed and had given written informed consent to participate in the study. Patient unable to write and/or read but who fully understood the oral information given by the investigator (or nominated representative) had given oral informed consent witnessed by an independent person.
3. Female patients of childbearing potential agree to maintain effective birth control practice during the study and must have had a negative pregnancy test at baseline.
4. Donor is older than 65 years or/and had liver macrosteathosis >15%.
5. Maximum cold ischemia time less than 10 hours

Number of Subjects (planned and analyzed): A total of 100 patients (50 patients per treatment arm) in 8-10 centers were to be included in the study. 101 patients were actually included, 50 in arm 1 and 51 in arm 2, in 8 centres.

Test Product, Dose And Mode of Administration: The initial daily dose of tacrolimus was 0.10 to 0.15 mg/kg given orally in two doses (equals 0.05 or 0.075 mg/kg twice daily), the first dose was given within 12 hours after closure of the skin. If the patient suffered of renal insufficiency, defined as:
pre-transplantation: < 50% of normal creatinine clearance
post-transplantation: < 1 mL/kg/hour urine output for 12 hours
The recommended initial daily starting dose of tacrolimus was 0.10 mg/kg/day p.o. (0.05 mg/kg/b.i.d.) and was given latest within 12 hours after reperfusion.
Subsequent oral tacrolimus doses were adjusted on the basis of clinical evidence of efficacy and occurrence of adverse events, and observing the recommended whole blood trough level range 5-15 ng/mL The patient's status regarding rejection and toxicity always took precedence over whole blood trough levels when assessing the appropriate dose. As tacrolimus requires dosing defined for individual patients, the optimal whole blood trough level could be outside the recommended ranges.
The investigator could adjust the patient's dose and modify the tacrolimus dose regimen as deemed necessary to minimise adverse events and maintain effective immunosuppression. Due to a long half life of tacrolimus (approximately 16 hrs), it was recommended that dosing adjustments be limited to a maximum of 2 times per week as changes in trough blood levels occur slowly, usually only 48 to 72 hours after dose adjustment. Changes in tacrolimus dose were to be made in steps of 25% of the current dose. Temporary tacrolimus dose interruption could be considered if unacceptable drug-related side effects were observed. However, all attempts were made to maintain the patients on the allocated therapy for the duration of the study.
The first dose of daclizumab of 2.0 mg/kg had to be administered intravenously during the anhepatic phase. It was recommendable to start the infusion after the hepatectomy was completed and the hemostasis of the entire retroperitoneal zone was guaranteed but in any case prior to reperfusion. The second dose of daclizumab of 1.0 mg/kg had to be given intravenously on postoperative day 7. Corticosteroids for treatment arm 1: perioperative administered intravenous methylprednisolone had not to exceed 1000 mg. Corticosteroids for treatment arm 2: perioperative administered intravenous methylprednisolone had not to exceed 1000 mg; oral steroid regimen had to be tapered from 20 mg/day to 5 mg day in 6 weeks and continued throughout the study.Oral administration had to be as a single dose per day.

Lot Numbers:

Duration of Study and Treatment:
The duration of patient participation in the study was 3 months. A total of 9 assessment visits were scheduled during the 3-month individual patient study period. Serious adverse events, as defined in the protocol, were recorded for each patient for an additional 28-day period after study end or study withdrawal.

Criteria for Evaluation:
The primary endpoint was the incidence of and time to first biopsy-proven and treatment requiring acute rejection within the first 3 months following transplantation.
The secondary Safety Endpoints were the incidence of post-transplant diabetes (PTD), defined by long term insulin or oral antidiabetic drug treatment (> 30 days), at month 3; the incidence of adverse events; the incidence of documented infections (confirmed by culture, biopsy or serology); the lipid profile (Cholesterol levels) and the vital signs (systolic/diastolic blood pressure).
The secondary Efficacy Endpoints were the incidences of acute rejections categorised as in section 14.3.3; the overall frequency of and time to biopsy proven acute rejection episodes (for Histological Grading see Appendix 1); the incidence of and time to first acute rejection (not necessarily biopsy proven and treatment requiring); the incidence of and time to first steroid resistant rejection; the severity of biopsy proven acute rejections (see appendix 1); the graft survival and patient survival at month 3.
Moreover, the bile composition: in a subgroup of 30 patients (15 patients per arm) in selected centres, bile composition was to be assessed, including the profile of individual bile salts (i.e. cholic, chenodeoxycholic, deoxycholic, lithocholic acids and other more) and lipids (i.e. bile cholesterol and

total lecithin) on days 3, 5, 10 and 15, but foreseen number was not achieved (total patients were 25 only, 14 in arm 1 and 11 in arm 2) and data collected were heavily incomplete in many patients, hence they have not jet analysed, as no significant results are foreseen.

Statistical Methods:

Although two populations for analysis were defined, the difference between Full Analysis Set (FAS) and Per Protocol Set (PPS) was negligible, therefore the Full Analysis Set was considered for analyses. All safety analyses were done with the Safety Analysis Set.

A patient listing was produced, containing assignment to the analysis set(s), and reasons for exclusion from each analysis set.

According to the exploratory character of the study the following hypotheses for primary endpoint were tested for exploratory purposes on significance level $\alpha = 0.05$:

Primary endpoint:

H_{0A}: $\pi_1 = \pi_2$ versus H_{1A}: $\pi_1 \neq \pi_2$

With π_i = rate of patients free from biopsy-proven acute rejection at month 3 (Kaplan-Meier estimation) in treatment arm i (i = 1, 2).

The hypothesis were tested using the Wilcoxon-Gehan test.

All secondary endpoints were summarized per treatment group using appropriate descriptive statistics, i.e. number and percentage of patients for categorical variables, and mean, standard deviation, median, minimum, maximum for continuous variables. Differences between treatment arms in the incidence of post-transplant diabetes (PDT) and of all cases of glucose metabolism disorders were tested by means of Cochran-Mantel-Haenszel test controlling for centre.

Incidences of adverse events during treatment were summarized separately for each treatment group. All adverse events started on or after the day of first study medication intake (tacrolimus, MAB or steroids), hence all adverse events were analyzed. The overall incidence of adverse events were compared using descriptive p-values from Fisher's exact test.

RESULTS:

Analysis Sets and Subject Disposition:

Of the 101 patients randomized to receive treatment, all underwent transplantation and received at least one dose of study medication, and thus were eligible for FAS. A total of 19 FAS patients were excluded from the PPS, 12 and 7 in the 2 arms respectively.

Table 4: Populations for Analysis

	Number of Patients		
	Tacrolimus/ Daclizumab (arm 1)	**Tacrolimus/ steroids** (arm 2)	**Total**
Patients enrolled	51	50	101
Not randomized	0	0	0
Randomized to treatment	51	50	101
Excluded from full analysis set	0	0	0
Not transplanted, no study med. received	0	0	0
Full Analysis Set	51	50	101
Excluded from Per-protocol Analysis Set	12	7	19
Per-protocol Analysis	39	43	82

Table 5: Patient disposition - FAS

	Number of Patients (%)		
	Tacrolimus/ Daclizumab (arm 1)	**Tacrolimus/ steroids** (arm 2)	**Total N = 101**
Completed	39 (76.5)	43 (85.7)	81(81.2)
Total deaths	2 (3.9)	2 (4.0)	4(4.0)
During study	2 (3.9)	2 (4.0)	4 (4.0)
After withdrawal/EOS	0 (0.0)	0 (0.0)	0 (0.0)
Withdrawn †	10 (19.6)	5 (10.0)	15(14.9)

Graft loss	2 (3.9)	2 (4.0)	4 (4.0)
Investigator feels it is in patient's best interest toAE	5 (9.8)	0 (0.0)	5 (5.0)
Lost to follow-up	0 (0.0)	0 (0.0)	
Protocol violation	3 (5.9)	2 (4.0)	5 (5.0)
Prohibited medication	3 (5.9)	2 (4.0)	5 (5.0)
Pregnancy	0 (0.0)	0 (0.0)	0 (0.0)
Tacrolimus stopped §	0 (0.0)	0 (0.0)	0 (0.0)
MAB not or erroneously given	0 (0.0)	0 (0.0)	0 (0.0)
Other*	0 (0.0)	1 (1.0)	1(1.0)

Demographics:

Table 6: Summary of Patient Demographics - FAS

	Number of Patients (%)		
	Tacrolimus/ Daclizumab (arm 1) N=51	**Tacrolimus/ steroids** (arm 2) N=50	**p-value**
Male n (%)	45 (88.2)	41 (82.0)	0.378†
Female n (%)	6 (11.8)	9 (18.0)	
Age (years) n (%)			
≤19	0 (0.0)	0 (0.0)	
20-29	0 (0.0)	0 (0.0)	
30-39	0 (0.0)	4 (8.0)	
40-49	10(19.6)	6 (12.0)	
50-59	21(41.2)	24(48.0)	
≥60	20(39.2)	16(32.0)	
Mean (SD) Age	56.1(6.3)	54.8(8.4)	0.398‡
Height (cm)			
Mean (SD)	171.5 (8.6)	171.4 (7.3)	0.965‡
Median (range)	172.0 (153-194)	172.5 (153-186)	
Weight (kg)			
Mean (SD)	75.7 (12.4)	76.2 (12.6)	0.843‡
Median (range)	76.0 (44.0-101.0)	75.0 (47.0-103.0)	

Source: Tables 12.3.1 ; 12.3.2
† Chi-squared test; ‡ Student's t-test; § Fisher's exact test;

Study Drug Exposure:

One patient in arm 1 received no tacrolimus. The mean total daily dose of tacrolimus was similar for both treatment arms for the duration of the study (Table 11).The mean total daily dose of tacrolimus was highest in week 3 and 4 for both treatment arms.

Table 11: Tacrolimus administration : total daily dose (mg/kg) – FAS

	Tacrolimus/ Steroid (arm 2) N=50					**Tacrolimus/ Daclizumab (Arm 1) N=50***			
Time	N	Median	Mean	SD		N	Median	Mean	SD
Week 1 ~	50	0.0538	0.0584	0.0344		50	0.0500	0.0577	0.0331
Week 2 ~	43	0.0600	0.0689	0.0447		43	0.0724	0.0859	0.0525
Week 3 ~	27	0.0667	0.0865	0.0484		27	0.0552	0.0920	0.0848
Week 4 ~	26	0.0546	0.0645	0.0424		22	0.0704	0.0962	0.0849
Month 2 ~	33	0.0610	0.0646	0.0412		30	0.0507	0.0674	0.0580
Month 3 ~	21	0.0438	0.0514	0.0355		22	0.0613	0.0722	0.0408

~ Mean during time period
*One patient never received 1st dose of Tacrolimus

In arm 1, (results for FAS, all patients) the mean dose of corticosteroid ranged from 13.3 mg/kg (bolus, peri-operatively) then was reduced to 0.27 mg/kg at Week 2 and 0.004 mg/kg at Month 3. In treatment arm 2 where corticosteroid therapy was continued in the patients for the course of the study, the mean dose of corticosteroid ranged from 13.6 mg/kg ± 7.88 (bolus, peri-operatively) to 0.29 mg/kg in Week 2 and 0.097 mg/kg in Month 3.
The introduction of MMF was allowed after reduction or interruption of tacrolimus if are related to an Adverse Event and after occurrence of BPAR is allowed and left to the clinical judgment of the Investigator. The administration of MMF occurred in 14 patients in arm 1 and 23 patients in arm 2 .

Efficacy Results:

Table 14: Overall frequency of rejection

	Tacrolimus/ Steroid (arm 2) N=50			Tacrolimus/ Daclizumab (arm 1) N=51			
	Subjects		Episodes	Subjects		Episodes	
	N	(%)	N	N	(%)	N	p-value
Acute rejections	16	(32.0)	17	8	(15.7)	8	0.0541>
Spontaneously resolving acute rejections ~	2	(4.0)	2	3	(5.9)	3	0.6628>
Corticosteroid sensitive acute rejections+	14	(28.0)	15	5	(9.8)	5	0.0193>
Corticosteroid resistant acute rejections #	0	(0.0)	0	0	(0.0)	0	NA
Suspected acute rejections *	1	(2.0)	01	1	(2.0)	1	0.9887>
Supected chronic rejections*	0	(0.0)	0	1	(2.0)	0	1.000<

Note: As all rejection episodes were classified into one of the above categories no additional categories were be considered
~ A spontaneously resolving acute rejection is defined as a rejection episode which was not treated with new or increasing corticosteroid medication, antibodies or any other medication and resolved irrespective of any tacrolimus dose changes
+ A corticosteroid sensitive acute rejection is defined as a rejection episode which was treated with new or increasing corticosteroid medication only and resolved, irrespective of any tacrolimus dose changes
# A corticosteroid-resistant acute rejection is defined as a rejection episode, which did not resolve following treatment with corticosteroids. In the case that a rejection episode was not treated with corticosteroids first but only with antibodies, it was to be included in this category. *Note* [redacted] *had an acute rejection episode first contemporarily treated with antibodies and steroids, and classified as steroid sensitive by the Investigator; this episode is classified in steroid sensitive acute rejection episodes.*
* A suspected acute or chronic rejection is defined as a rejection, which was not histologically confirmed
> Chi-square test comparing the numbers of subjects
< Fisher's exact test comparing the numbers of subjects

Overall 24 pts experienced acute rejection (arm2: 16; arm 1: 8); most were corticosteroids sensitive (arm 2: 14; arm1: 5), showing a statistical significant association with treatment (p=0.0193, Fisher exact test). A similar pattern was confirmed when considering only biopsy proven acute rejections: (arm2: 15; arm 1: 8) most were corticosteroids sensitive (arm 2: 13; arm1: 5), showing a statistical significant association with treatment (p=0.0335, Fisher exact test). No difference in rejection rates

between arms was detected among center.

In all the analyses, Tacrolimus/Daclizumab resulted in a higher 3 month rejection free estimate: the absolute difference was about 10%. However no statistically significant differences were detected. According to the worst histological grade of rejection detected, the proportion of subjects experiencing mild/moderate grade was 28% in arm 2 vs 13.7% in arm 1.

Table 16 reports survival estimates for graft lost free, failure free and overall survival. Two patients in arm 2 and 3 in arm 1 experienced graft lost and death. Three months graft survival rate was 0.9587 and 0.9572 in arm 2 and 1 respectively. Treatment failure free rate was 0.9166 vs. 0.82 in arm 2 and arm 1 respectively, while survival was 0.9560 and 0.9545 in the two groups respectively.

Safety Results:

Percentage of adverse events was similar in the two groups (94% in arm 2, 96.1% in arm 1), although serious causally related adverse events, as assessed by investigators, were 18% in arm 2 compared to 23.5% in arm 1.

Five patients (2 in arm 2, 3 in arm 1) experienced death. Four deaths occurred during study (2 in arm 2 and 2 in arm 1) and 1 in arm1 after withdrawal. Causes of death where: cardiac arrest (1 in arm 1) respiratory failure (2: 1 in arm 2, 1 in arm 1), septic shock (2: 1 in arm 2, 1 in arm 1, after withdrawal).

Overall incidence of the most frequently^ reported serious adverse events assessed by the investigator as being causally related+ to study medication

	Tacrolimus/ Steroid (arm 2) N=50			Tacrolimus/ Daclizumab (arm 1) N=51			
	Subject		Events	Subjects		Events	
Adverse event~	N	(%)	N	N	(%)	N	p-value*
Blood creatinine increased	1	(2.0)	1	3	(5.9)	3	0.6175
HCV recurrence	1	(2.0)	1	2	(3.9)	3	1.0000
Biliary tract infection, bacterial	1	(2.0)	1	1	(2.0)	1	1.0000
Pneumonia, bacterial	2	(4.0)	2	0	(0.0)	0	0.2426
Renal impairment	1	(2.0)	1	1	(2.0)	1	1.0000
Cholestasis	0	(0.0)	0	1	(2.0)	1	1.0000
CMV infection	0	(0.0)	0	1	(2.0)	1	1.0000
Dysarthria	0	(0.0)	0	1	(2.0)	1	1.0000
Encephalopathy	1	(2.0)	1	0	(0.0)	0	0.4950
Lung infection	0	(0.0)	0	1	(2.0)	1	1.0000
Pneumonia, fungal	1	(2.0)	1	0	(0.0)	0	0.4950
Septic shock	1	(2.0)	1	0	(0.0)	0	0.4950
Vomiting	0	(0.0)	0	1	(2.0)	1	1.0000
Worsening of renal impairment	0	(0.0)	0	1	(2.0)	1	1.0000

~ Coded using modified MedDRA
^ All causally related serious adverse events are reported in this Table
+ Causally-related is defined as a highly probable, probable and possible as assessed by the investigator
* Fisher's exact test comparing the number of subjects

Adverse events resulting in patient premature discontinuation

	Tacrolimus/ Steroid (arm 2) N=50			Tacrolimus/ Daclizumab (arm 1) N=51			
	Subjects		Events	Subjects		Events	
Adverse event~	N	(%)	N	N	(%)	N	p-value+
Liver graft function delayed	0	(0.0)	0	2	(16.7)	2	0.5088

Renal impairment	0	(0.0)	0	2	(16.7)	2	0.5088
Respiratory failure	1	(14.3)	1	1	(8.3)	1	1.0000
Liver graft dysfunction	1	(14.3)	1	0	(0.0)	0	0.3684
Acute liver transplant rejection	0	(0.0)	0	1	(8.3)	1	1.0000
Hepatic hemorrhage	1	(14.3)	1	0	(0.0)	0	0.3684
Hepatic necrosis	0	(0.0)	0	1	(8.3)	1	1.0000
Dysarthria	0	(0.0)	0	1	(8.3)	1	1.0000
Septic shock	1	(14.3)	1	0	(0.0)	0	0.3684
Cardiac arrest	0	(0.0)	0	1	(8.3)	1	1.0000
Total	4	(8.0)	4	9	(17.6)	9	

~ Coded using modified MedDRA
+ Fisher's exact test comparing the number of subjects

The most frequent type of infection was due to bacterial (26%) and viral (23%) agents. Al type of infection were similar between the groups, with the exception of CMV infection affected 14% of patients in arm 2 but only 3.% in arm 1. No evidence of significant difference in distribution between the two arms was detected. Overall 5 pts (9.8%) were affected by cardiac AEs, 4 in arm 1 and 1 in arm 2. No evidence of significant difference in distribution between the two arms was detected. Eight patients experienced hypertension (5 (10%) in arm 2 and 3 (3.9%) in arm 1. The number of patients receiving antihyperthensive treatment at any time during study was 15 (30%) in arm 2 and 11 (21.6%) in arm 1. No evidence of significant difference in distribution between the two arms was detected. Overall 15 pts in both arms experienced AEs related to nephrological disorders. In particular, renal impairment occurred in 9 and 11 pts in arm 2 and 1 respectively. No evidence of significant difference in distribution between the two arms was detected. Overall 23 pts in arm 2 and 11 pts in arm 1 experienced glucose metabolism disorders (Diabetes mellitus, Glucose tolerance decreased, hyperglycemia). In particular, post transplant glucose metabolism disorders were statistically associated with steroids intake in arm 2. 20 patients (57.1%) in arm 2 vs 9 (24.3%) in arm 1; p=0.0077). Overall 4 patients (1 in arm 2 and 3 in arm 1) experienced gastrointestinal disorders. No evidence of significant difference in distribution between the two arms was detected.

Overall no difference between the two arms was detected for any of the haematological parameters, SGOT, GPT. Creatinine levels showed a tendency to increase particularly evident at week 2, month 1, month 3 and End of Study. Increases seem more evident in arm 1. An increase of cholesterol levels was noted after the first two weeks of study and persisted until study end. Arm 2 seemed to show higher level after the first two weeks although not statistically significant.

Date of Report: 30 April 2010