SYNOPSIS

Name of Sponsor/Company: Astellas Pharma Europe Ltd.		
Name of Finished Product: Advagraf®		
Name of Active Ingredient: Tacrolimus		

Title of Study: A Multicenter, Four Arm, Randomized, Open Label Clinical Study Investigating Optimized Dosing in a Prograf®-/Advagraf®-Based Immunosuppressive Regimen in Kidney Transplant Subjects (OSAKA Study)

Responsible Medical Officer/Coordinating Investigator: Dr. [redacted], Astellas Pharma Europe Limited

Investigators(alphabetically): [redacted]

Study Center(s): In total, 22 countries (110 centers) participated in the study. The countries were: Argentina (2); Austria (3); Belgium (4); Czech Republic (4); France (20); Germany (17); Greece (2); Hungary (2); Ireland (1); Italy (7); Netherlands (1); Norway (1); Poland (6); Portugal (2); Romania (2); Russian Federation (5); Slovakia (2); South Africa (2); Spain (20); Sweden (2); Switzerland (1); United Kingdom (4).

Publication (reference): None to date.

Study Period: **Date of First Enrollment:** 14 May 2008 **Date of Last Evaluation:** 02 March 2010	**Phase of Development:** 3b

Objectives: The primary study objective was to compare the 4 treatment regimens with regard to efficacy failure rate. The secondary objective was to compare the efficacy and safety profiles of the four therapy regimens with each other. Efficacy failure rate was assessed using a composite endpoint consisting of graft loss, biopsy confirmed acute rejection (BCAR) and graft dysfunction.

Study Design: This was a multicenter, randomized, open label 4-arm, parallel group, comparative phase IIIb study. Subjects about to undergo kidney allograft transplantation were randomized to one of the following treatment arms:

Arm 1: Prograf® (0.2mg/kg) + MMF + corticosteroids for 24 weeks
Arm 2: Advagraf® (0.2mg/kg) + MMF + corticosteroids for 24 weeks
Arm 3: Advagraf® (0.3mg/kg) + MMF + corticosteroids for 24 weeks
Arm 4: Advagraf® (0.2mg/kg) + MMF + basiliximab + corticosteroids 1 peri-operative bolus only.

This was a non-inferiority study designed to prove the absence of a meaningful difference between

treatments. Prograf® (Arm 1) was the reference treatment.
Diagnosis and Main Criteria for Inclusion: Adult subjects (> 18 years of age) with end-stage kidney disease about to undergo primary renal transplantation or re-transplantation (unless the previous graft was lost within 12 months) were eligible for enrollment.
Number of Subjects (planned and analyzed): To reach 282 evaluable subjects per treatment arm (for the Per Protocol Set) it was planned to enroll 1200 subjects, 300 subjects per treatment arm and 8 to 48 subjects per participating center. Total enrollment was 1214 in the safety analysis set of which 976 were included in the per protocol set. Details of subject disposition are presented in Table 1.
Test Product, Dose and Mode of Administration: Doses and administration of the study medications as given to subjects in the four treatment groups were as follows: Arm 1: Prograf® initial pre-operative dose of 0.1 mg/kg then 0.2 mg/kg given in 2 doses of 0.1 mg/kg twice daily. Arm 2: Advagraf® initial pre-operative dose of 0.1 mg/kg given in 1 dose then 0.2 mg/kg/day, given preferably in the morning. Arm 3: Advagraf® initial pre-operative dose of 0.15 mg/kg given in 1 dose then 0.3 mg/kg/day, given preferably in the morning. Arm 4: Advagraf® initial pre-operative dose of 0.1 mg/kg given in 1 dose then 0.2 mg/kg/day, given preferably in the morning. Basiliximab 20 mg was given on day 0 followed by a second dose of 20 mg given on day 4. Doses of Prograf® (Arm 1) and Advagraf® (Arms 2, 3, 4) were adjusted on evidence of clinical efficacy and occurrence of adverse events and observing the following targeted tacrolimus trough levels: days 0-14, 10-15 ng/mL; days 15-42, 5-12 ng/mL; days 43-168, 5-10 ng/mL. Doses of mycophenolate mofetil (MMF) in all 4 treatment arms were: MMF 1 g given pre-operatively then 2 g given as 1 g twice daily. Transient changes or suspension in dose due to MMF-related side effects were permitted but not for > 21 days. Corticosteroids were administered in Arms 1, 2 and 3 using the following dosing schedule: day 0, 500 mg; day 1, 125 mg; days 2-14, 20 mg/day; days 15-28, 15 mg/day; days 29-42, 10 mg/day; days 43-84, 5 mg/day; days 85-169, ≤ 5 mg/day. In Arm 4, corticosteroids were given on day 0 (500 mg). Maintenance treatment with corticosteroids was prohibited with the exception of treatment for clinically significant rejection.
Duration of Study and Treatment: Study duration was 24 weeks. Seven study assessment visits took place (baseline, day 1, weeks 1, 2, 4, 12, 24). In subjects with premature study withdrawal, follow-up evaluation was done at 24 weeks to assess subject and graft status.
Criteria for Evaluation: The primary efficacy variable was the occurrence of and time to first incidence of graft loss (defined as re-transplantation, nephrectomy, death, or dialysis ongoing at week 24 or at the time of premature study discontinuation of the subject from the study unless superseded by follow-up information), or biopsy confirmed acute rejection (BCAR), or graft dysfunction at week 24 after transplantation (defined as glomerular filtration rate [eGFR] < 40 mL/min/1.73m^2 estimated by the Modification of Diet in Renal Disease Study Equation (MDRD-4 formula). Secondary efficacy variables were as follows: renal function at week 24 assessed by eGFR using the MDRD formula; renal function at week 24 assessed by calculated creatinine clearance using the Cockcroft-Gault formula; acute rejection (incidence of and time to first acute rejection, incidence of and time to first corticosteroid-resistant acute rejection, overall frequency of acute rejection episodes); BCAR (incidence of and time to first BCAR, incidence of and time to first corticosteroid-resistant BCAR, overall frequency of BCAR, severity of BCAR). Safety variables evaluated were: subject and graft survival; incidence of adverse events; incidence of adverse events of special interest (renal dysfunction, diabetes mellitus, hypercholesteremia, hypertension); standard laboratory parameters.

Other evaluated variables were: subject-reported outcome endpoint (using the EQ-5D health questionnaire); subject compliance questionnaire (using the Morisky Medication Adherence Scale [MMAS]).

Statistical Methods: The safety analysis set (SAF) consisted of all subjects who took at least one dose of study medication. The full analysis set (FAS) included all subjects enrolled in the study who had received at least one dose of study medication and received an allograft transplant. Efficacy analysis of the FAS was done according to the subject's randomized treatment. The per protocol set (PPS) included all subjects from the FAS without a major protocol deviation.

The primary study hypothesis tested the non-inferiority in efficacy failure rate of the 3 Advagraf® arms against the Prograf® arm using a pre-specified non-inferiority margin of -12.5%. Efficacy failure rate at week 24 was estimated using the Kaplan-Meier method. The test of non-inferiority was performed by means of confidence intervals (CI) for the difference in failure rates between each Advagraf® arm and the Prograf® arm. CIs were calculated using the normal approximation with estimates of standard error (SE) according to the Greenwood formula. The Bonferroni-Holm method was applied to simultaneous multiple comparisons to control the family-wise error rate for all hypotheses at level alpha = 5%. Statistical comparisons were performed on the 4 treatment arms using two-sided descriptive P-value and 95% CI. The primary efficacy analysis was performed on the PPS, analyses of efficacy were also performed on the FAS.

RESULTS:

Subject Disposition: Table 1 depicts subject disposition. 16 subjects were not transplanted and were excluded from the FAS. 222 subjects had major protocol violations and were excluded from the PPS; the common reason for exclusion was violation of the initial dose of study medication (172 subjects, 14.2%). 79.0% of the SAF (959 subjects) completed the study. Premature discontinuation was most commonly due to an adverse event (11.0%) and protocol violation (4.9%). Study discontinuation was highest in Arm 4 (26.5%) and ranged from 16-22% in the other treatment arms.

Table 1: Subject disposition, N (%)

	Arm 1 (Prograf 0.2mg/kg)	**Arm 2** (Advagraf 0.2mg/kg)	**Arm 3** (Advagraf 0.3mg/kg)	**Arm 4** (Advagraf 0.2mg/kg+bas)	**Total**
Randomized	320	316	317	298	1251
Safety Analysis Set (SAF)	311 (100.0)	309 (100.0)	307 (100.0)	287 (100.0)	1214 (100.0)
Not transplanted	5 (1.6)	4 (1.3)	3 (1.0)	4 (1.4)	16 (1.3)
Premature study discontinuation	51 (16.4)	68 (22.0)	60 (19.5)	76 (26.5)	255 (21.0)
Completed	260 (83.6)	241 (78.0)	247 (80.5)	211 (73.5)	959 (79.0)
Full Analysis Set (FAS) †	309 (99.4)	302 (97.7)	304 (99.0)	283 (98.6)	1198(98.7)
Excluded from Per Protocol Set	69 (22.2)	42 (13.6)	58 (18.9)	53 (18.5)	222 (18.3)
Per Protocol Set (PPS)	237 (76.2)	263 (85.1)	246 (80.1)	230 (80.1)	976 (80.4)

† Subjects #[redacted], #[redacted] and #[redacted] were randomized to Arm 1; the investigator incorrectly assigned treatment and treated these subjects using the Arm 2 regimen.

Demographics: Treatment arms were similar in general terms regarding baseline characteristics of subjects and donors.

Study Drug Exposure: Exposure to study drug (Prograf® and Advagraf®) was assessed using tacrolimus trough levels. Protocol-recommended tacrolimus trough levels between weeks 7 to 24 were 5-10 ng/mL. At week 24, median tacrolimus trough levels were between 7.7 and 8.3 ng/mL in the 4 study arms (FAS). The rate of subjects with trough levels above recommended range at week 24 ranged from 26.0% in Arm 2 to 21.3% in Arm 4.

Efficacy Results:

Primary efficacy endpoint. Based on results from the PPS, non-inferiority was established for Arm 2: the lower limit of the CI for the difference in efficacy failure is above the pre-specified margin of -12.5% and thus fulfilled the criteria of non-inferiority. Non-inferiority was not established for Arms 3 and 4: in both arms the lower limit of the CI was less than the pre-specified non-inferiority margin of -12.5% (Table 2). Results based on the FAS were consistent with those obtained for the PPS for Arms 2 and 4. Non-inferiority was shown for Arm 3 using the FAS. The difference in incidence of and time to first incidence of efficacy failure rate between Arms 2, 3 and 4 was not significant (P=0.583, Wilcoxon-Gehan test).

In Arm 1 as well as in Arms 2, 3 and 4, the most common factor contributing to efficacy failure was graft dysfunction. Rates were, respectively, 23.6%, 27.4%, 24.4%, and 31.3% (PPS).

Table 2: Incidence of and time to first incidence of efficacy failure

PPS	**Arm 1** (Prograf 0.2mg/kg) (N=237)	**Arm 2** (Advagraf 0.2mg/kg) (N=263)	**Arm 3** (Advagraf 0.3mg/kg) (N=246)	**Arm 4** (Advagraf 0.2mg/kg+bas) (N=230)
Efficacy failure rate†	40.6%	42.2%	44.2%	48.2%
Efficacy failure rate difference (SE) compared to Arm 1	-	-1.6% (4.41%)	-3.5% (4.51%)	-7.1% (4.60%)
95% CI for efficacy failure rate difference‡	-	(-12.2%; 9.0%)	(-13.6%; 6.6%)	(-16.1%; 1.9%)
Kaplan-Meier survival estimate rate§	0.594	0.578	0.558	0.523
95% CI for survival estimate rate¶	(0.531; 0.656)	(0.518; 0.638)	(0.496; 0.621)	(0.458; 0.588)
FAS	(N=309)	(N=302)	(N=304)	(N=283)
Efficacy failure rate†	43.3%	43.7%	44.6%	49.4%
Efficacy failure rate difference (SE) compared to Arm 1	-	-0.4% (4.02%)	-1.3% (4.02%)	-5.7% (4.11%)
95% CI for efficacy failure rate difference‡	-	(-10.0%; 9.3%)	(-10.3%; 7.7%)	(-13.7%; 2.4%)
Kaplan-Meier survival estimate rate§	0.567	0.563	0.554	0.51
95% CI for survival estimate rate¶	(0.511; 0.622)	(0.507; 0.619)	(0.498; 0.610)	(0.451; 0.568)

SE=standard error. CI=confidence interval. † Incidence of and time to first incidence of one of the following events within the first 24 weeks after transplantation: death, graft loss, BCAR, or graft dysfunction at week 24. ‡ Confidence intervals using Bonferroni-Holm adjustment for multiplicity testing to preserve a 5% family-wise alpha error. § Incidence of and time to first incidence of efficacy failure calculated using Kaplan-Meier survival estimate. ¶ Survival without experiencing efficacy failure.

Secondary efficacy endpoints. The FAS is used for the presentation of secondary efficacy endpoint results. Last observation carried forward procedure was used for assessment of eGFR and creatinine clearance in subjects prematurely withdrawn from the study. Both eGFR and calculated creatinine clearance at week 24 were significantly lower in Arm 4 compared with Arm 1 and similar between Arm 2 and Arm 1 and between Arm 3 and Arm 1 (Table 3). There was no difference between treatment arms in the incidence of and time to first incidence of BCAR or corticosteroid resistant BCAR (Table 3). The severity grade of BCAR was comparable across treatment arms. Rates of acute rejection ranged from 19.5-27.0% with no significant difference between treatment arms in the incidence of and time to first incidence of acute rejection (P=0.095, Wilcoxon-Gehan test) or corticosteroid-resistant acute rejection (P=0.521, Wilcoxon-Gehan test).

Table 3: Renal function and biopsy confirmed acute rejection (BCAR) at week 24

	Arm 1 (Prograf 0.2mg/kg) (N=309)	**Arm 2** (Advagraf 0.2mg/kg) (N=302)	**Arm 3** (Advagraf 0.3mg/kg) (N=304)	**Arm 4** (Advagraf 0.2mg/kg+bas) (N=283)	P value
eGFR					
LS Mean (SE)	48.3 (1.09)	45.7 (1.10)	45.9 (1.09)	41.7 (1.13)	<0.001†
95% CI	(46.12; 50.40)	(43.50; 47.82)	(43.80; 48.06)	(39.45; 43.88)	
Creatinine clearance					
LS Mean (SE)	57.0 (1.26)	55.1 (1.27)	53.7 (1.25)	49.8 (1.3)	0.001†
95% CI	(54.53; 59.46)	(52.61; 57.58)	(51.22; 56.12)	(47.23; 52.33)	
BCAR, n (%)	42 (13.6%)	31 (10.3%)	49 (16.1%)	36 (12.7%)	
Kaplan-Meier survival estimate rate	0.859	0.892	0.831	0.865	0.244‡
95% CI for survival estimate rate	(0.820; 0.899)	(0.856; 0.928)	(0.788; 0.875)	(0.823; 0.906)	
BCAR, corticosteroid resistant, n (%)	16 (5.2%)	14 (4.6%)	19 (6.3%)	15 (5.3%)	
Kaplan-Meier survival estimate rate	0.947	0.951	0.934	0.945	0.869‡
95% CI for survival estimate rate	(0.922; 0.972)	(0.925; 0.976)	(0.905; 0.963)	(0.918; 0.972)	

FAS. LS=least square. SE=standard error. Bas=basiliximab. eGFR measured using the MDRD-4 formula. Creatinine clearance calculated using the Cockcroft-Gault method. † ANCOVA model for GFR/creatinine clearance at week 24 with treatment group and center as fixed factors and age as the covariate: P value applies to difference between Arm 4 and Arm 1. ‡ Wilcoxon-Gehan test.

Safety Results: The SAF is used for the presentation of safety results. In total, 24 deaths were reported: 15 during the study and 9 following premature study withdrawal. Most commonly, deaths were due to infectious complications and cardiovascular adverse events. Subject death rates were 1.9%, 2.6%, 2.3%, and 1.0% in Arms 1, 2, 3, and 4, respectively. The overall rate of graft loss was 7.5% (90 cases), most commonly due to early surgical complications. Acute rejection was reported as a cause of graft loss in 5 cases. Rates of graft loss were 5.9%, 9.5%, 8.0%, and 7.1% in treatment arms 1, 2, 3 and 4. There was no difference of statistical significance between the treatment arms in Kaplan-Meier estimates of subject and graft survival.

Most subjects (93.5-96.1% across treatment arms) experienced ≥ 1 treatment-emergent adverse event. Anemia was the most commonly occurring adverse event in all arms (Table 4). Over half of subjects (55-62.7% across treatment arms) experienced a serious treatment-emergent adverse event. Immune system disorders, including kidney transplant rejection, were the most common serious adverse events in all 4 treatment arms and had an incidence of 19.4-26.7% The overall rate of treatment emergent adverse events leading to discontinuation of study drug ranged from 8.7% of subjects in Arm 1 to 17.4% of subjects in Arm 4. Most commonly, thrombosis of the renal vein/artery and renal transplant rejection lead to study drug discontinuation: incidences ranged from 0.7-4.5% (renal vein thrombosis/renal artery thrombosis) and from 0.7-4.5% (renal transplant rejection).

Table 4: Adverse events occurring in >10% of subjects and adverse events of special interest occurring in >3% of subjects, N (%)

MedDRA Preferred Term	Arm 1 (Prograf 0.2mg/kg) (N=311)	Arm 2 (Advagraf 0.2mg/kg) (N=309)	Arm 3 (Advagraf 0.3mg/kg) (N=307)	Arm 4 (Advagraf 0.2mg/kg+bas) (N=287)
Most common adverse events †				
Anemia	100 (32.2%)	105 (34%)	98 (31.9%)	93 (32.4%)
Urinary tract infection bacterial	83 (26.7%)	72 (23.3%)	96 (31.3%)	79 (27.5%)
Diarrhea	70 (22.5%)	69 (22.3%)	77 (25.1%)	68 (23.7%)
Kidney transplant rejection	58 (18.6%)	57 (18.4%)	79 (25.7%)	65 (22.6%)
Complications of transplanted kidney	44 (14.1%)	50 (16.2%)	35 (11.4%)	54 (18.8%)
Peripheral edema	52 (16.7%)	55 (17.8%)	43 (14%)	36 (12.5%)
Blood creatinine, increased	45 (14.5%)	47 (15.2%)	45 (14.7%)	37 (12.9%)
Constipation	40 (12.9%)	48 (15.5%)	51 (16.6%)	30 (10.5%)
Hyperkalemia	51 (16.4%)	36 (11.7%)	36 (11.7%)	52 (18.1%)
Hyperglycemia	37 (11.9%)	41 (13.3%)	45 (14.7%)	38 (13.2%)
Hypertension	45 (14.5%)	46 (14.9%)	34 (11.1%)	37 (12.9%)
Renal impairment	37 (11.9%)	33 (10.7%)	49 (16%)	34 (11.8%)
Leukopenia	27 (8.7%)	24 (7.8%)	41 (13.4%)	37 (12.9%)
Tremor	37 (11.9%)	37 (12%)	32 (10.4%)	28 (9.8%)
Insomnia	31 (10%)	31 (10%)	31 (10.1%)	32 (11.1%)
Vomiting	28 (9%)	29 (9.4%)	29 (9.4%)	34 (11.8%)
Hypokalemia	27 (8.7%)	24 (7.8%)	33 (10.7%)	31 (10.8%)
Cytomegalovirus infection	26 (8.4%)	28 (9.1%)	34 (11.1%)	22 (7.7%)
Nausea	27 (8.7%)	21 (6.8%)	29 (9.4%)	32 (11.1%)
Hyperlipidemia	35 (11.3%)	22 (7.1%)	22 (7.2%)	15 (5.2%)
Adverse events of special interest ‡				
Renal impairment	37 (11.9)	33 (10.7)	49 (16.0)	34 (11.8)
Renal failure, acute	1 (0.3)	5 (1.6)	7 (2.3)	9 (3.1)
Renal tubular necrosis	24 (7.7)	23 (7.4)	22 (7.2)	21 (7.3)
Nephropathy, toxic	12 (3.9)	10 (3.2)	17 (5.5)	24 (8.4)
Proteinuria	10 (3.2)	112 (3.9)	13 (4.2)	6 (2.1)
Blood creatinine, increased	45 (14.5)	47 (15.2)	45 (14.7)	37 (12.9)
Diabetes mellitus	24 (7.7)	23 (7.4)	23 (7.5)	11 (3.8)
Type 2 diabetes mellitus	13 (4.2)	14 (4.5)	14 (4.6)	7 (2.4)
Type 1 diabetes mellitus	10 (3.2)	10 (3.2)	12 (3.9)	4 (1.4)
Insulin-requiring type 2 diabetes mellitus	7 (2.3)	10 (3.2)	10 (3.3)	2 (0.7)
Hypercholesterolemia	14 (4.5)	12 (3.9)	9 (2.9)	7 (2.4)
Hypertension	45 (14.5)	46 (14.9)	34 (11.1)	37 (12.9)

SAF. † Incidence rate >10% in any treatment arm by MedDRA preferred term. ‡ Incidence rate >3% in any treatment arm by MedDRA preferred term.

Changes from baseline to week 24 in the serum lipid parameters cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides were assessed. Using LS mean to analyze a comparison of the changes in serum lipids showed a significant increase in total cholesterol in Arms 1, 2 and 3 and a decrease in Arm 4. HDL-cholesterol significantly increased in Arms 1, 2 and 3 and remained unchanged in Arm 4. LDL-cholesterol showed a significant increase in treatment arms 1 and 2. Triglycerides significantly decreased in all treatment arms.

Other evaluated variables:

Subject reported outcome (EQ-5D health questionnaire). Based on results from the FAS, there was no meaningful difference between treatment arms in the mean change from baseline in EQ-5D scores. Using a visual analog scale to self-rate the state of health (0= worst state, 100=best state), health state improved by approximately 11% in Arms 1, 2 and 3 and by 9% in Arm 4.

Morisky Medication Adherence Scale. High adherence to taking the prescribed medication regimen was reported by 60-63% of subjects in Arms 1, 2 and 3 and by slightly fewer, 56%, in Arm 4.

CONCLUSIONS:

- Using the PPS, non-inferiority was established for Arm 2: the lower limit of the CI for the non-inferiority margin was -12.2% which was above the pre-specified non-inferiority margin of -12.5%. The mean difference in efficacy failure rate between Arm 2 and Arm 1 was -1.6%. Results did not prove non-inferiority of Arms 3 and 4 against Arm 1. Using the FAS, results were consistent with those obtained for Arms 2 and 4 using the PPS; results for Arm 3, however, showed non-inferiority of Arm 3 against Arm 1. Of the 4 events comprising efficacy failure, graft dysfunction was the major contributor to efficacy failure in all 4 treatment arms.
- Estimated glomerular filtration rates (MDRD-4 formula) and creatinine clearance (Cockcroft-Gault method) showed comparable results between Arms 1, 2 and 3. Results of both measurements were significantly lower in Arm 4 compared to results in Arm 1.
- The different treatment regimens had no significant impact on the incidence of and time to first incidence of acute rejection, biopsy confirmed acute rejection, or corticosteroid resistant rejection across treatment arms.
- There were 24 subject deaths with no differences across treatment arms in rates of death. The overall rate of graft loss was 7.5%; rates were similar across treatment arms. Kaplan-Meier estimates of subject and graft survival were comparable for the 4 treatment arms.
- Adverse events were reported for a majority of subjects with comparable rates of incidence across treatment arms.
- Total cholesterol showed a significant increase from baseline in Arms 1, 2 and 3 while a significant decrease was shown in Arm 4. Triglycerides significantly decreased in all treatment arms.
- Subject-rated state of health showed comparable however small improvement in all treatment arms. Adherence to the prescribed medication regimen was rated as high by a majority of subjects across treatment arms.

Date of Report: November 29, 2010