Clinical Trial Results Disclosure Synopsis

Name of Sponsor: Takeda Pharma Vertieb GmbH & Co. KG
Jägerstr.27, 10117 Berlin, Germany

Title of Study: Pilot trial studying the effects of Pioglitazone in comparison to Placebo on myocardial function and oxidative stress in patients with type-II-diabetes and insulin resistance undergoing elective Percutaneous Transluminal Coronary Angioplasty (PTCA). A randomized, double-blinded phase II study.

Phase of Development: Phase II

Name of Active Ingredient: [(±)-5-[[4-[2-(5-ethyl-2-pyridinyl) ethoxy] phenyl] methyl]-2,4-] (pioglitazone)

Name of Finished Product: Actos®

Investigators: 5 principal investigators enrolled subjects into the double-blind treatment period.

Study Sites: 5 sites in Germany enrolled subjects into the double-blind treatment period.

Study Site 01: Ikfe GmbH,55116 Mainz
Study Site 02: Cardioclinic Mainz, 55116 Mainz
Study Site 03: Clinic for Diagnostics, 65191 Wiesbaden
Study Site 04: University Hamburg, 22527 Hamburg
Study Site 05: Heart-Centre Kassel, 34121 Kassel
Study Site 06: Kerckhoff-Clinic, 61231 Bad Nauheim
Study Site 07: Helios Clinic – Cardiology, 42117 Wuppertal
Study Site 08: Cardio-Centre, 61348 Bad Homburg
Study Site 09: Cardio-Fit, 60594 Frankfurt
Study Site 10: University Jena, 07743 Jena

The study centres 02, 03, 04, 06 and 08 did not enrol any own study patients.

Publications Based on the Study (Citations) at Time of Study Completion: None

Study Period:

Date first subject signed informed consent form: 02 November 2006

Date of last subject's last visit/contact (from the Clinical database): 25 January 2010

Objectives:

The aim of this study was to determine the effect of Pioglitazone compared to Placebo on potential myocardial damage in type-II-diabetic patients after elective percutaneous coronary intervention (with stent implantation) (stent-PCI) by investigating myocardial and endothelial oxidative stress parameters like cardiac troponin I, creatine kinase (-myocard-type) (CK-MB), high sensitive C-Reactive Peptide (hs-CRP), nitrotyrosin, asymmetric dimethylarginin (ADMA), E-selectin, myoglobin, intact proinsulin, adiponectin, and visfatin.

Methodology: Prospective, double-blind, multicentre, randomized, exploratory, parallel study

Number of Subjects:

Planned: maximum 300 patients screened to achieve 56 randomized cases (28 per group)
Screened and Enrolled: 98 patients
Randomized and Treated in Double-Blind Treatment Period: 95 patients
Analyzed: Safety Set: 95 patients; Full-Analysis Set: 77 patients; Per-Protocol Set: 76 patients

Diagnosis and Main Criteria for Inclusion: Male and female patients at an age of 18-75 years with stable coronary artery disease, planned for elective stent-PCI, and showing type-II-diabetes treated with oral antidiabetics with exception of peroxisome proliferator-activated receptor (gamma) PPAR γ agonists and/or insulin resistance measured by measure for insulin resistance and increased vascular risk (score) (IRIS)-II-score ≥ 50. A signed written consent had to be available prior to any study related procedure.

Duration of Treatment: The duration of study participation for patients completing the study was planned to be about 20-30 days. The estimated duration of the total study period was 9 months.

Test Product, Dose and Mode of Administration, and Lot Number:

Study Medication	Product Dose Strength and Form	Study Dosage	Mode of Administration	Drug Product Lot Number
Pioglitazone	30 mg tablet	30 mg QD	Oral	N/A
Pioglitazone	45 mg tablet	45 mg QD	Oral	N/A

Reference Therapy, Dose and Mode of Administration, and Lot Number:

Study Medication	Product Dose Strength	Study Dosage	Mode of Administration	Drug Product Lot Number
Placebo to Pioglitazone 30mg	Tablet	N/A	Oral	N/A
Placebo to Pioglitazone 45mg	Tablet	N/A	Oral	N/A

Criteria for Evaluation:

Efficacy:

Primary: The primary efficacy variable was the incidence of cardiac troponin I elevation (>1 upper limit of normal (ULN)) at the time of 24 hours after the stent-PCI.

Secondary: Investigations on the effect of Pioglitazone compared to Placebo therapy over at least 1 week on several myocardial and endothelial oxidative stress parameters like cardiac troponin I, CK-MB, hs-CRP, nitrotyrosin, asymmetric dimethylarginin (ADMA), e-selectin, myoglobin, intact proinsulin, adiponectin, visfatin, and the frequency of Doppler-detected microembolisms high intensity transient signals (HITS) during PCI which were to be determined at one centre only (Jena).

Safety:

Incidence of adverse events; changes in safety laboratory parameters, physical examination and vital signs; rate of premature withdrawals.

Statistical Methods:

Data were summarized with respect to demographic and baseline characteristics, efficacy and safety observations and measurements. Standard descriptive summary statistics were done for continuous variables (i.e. arithmetic mean, standard deviation, minimum/maximum value, lower/median/upper quartile, number of non-missing values). Categorical data were displayed in frequency tables using counts and percentages. Individual patient data listings were presented parameterwise and were sorted by treatment group, centre, patient number and visit. Summary tables were displayed by treatment group and for the total of the sample. The safety analyses were done for the all patients treated set, efficacy analyses were conducted for the full analysis set (primary analysis set) and the per-protocol analysis set.

Since no reliable clinical assumptions were available for the primary parameter, no pre-defined confirmatory statistical hypotheses applied, and hence a sample size of 50 evaluable patients (25 per group) was deemed sufficient for the full analysis set. A rate of about 80% patients not evaluable for efficacy due to procedural reasons was assumed, thus leading to a maximum of 300 patients planned to be enrolled. Moreover, in a sub-study at the centre in Jena a number of 50 cases had to be enrolled for an additional measurement of microembolizations during PCI. Therefore, two exploratory analyses were carried out with a first one when 56 patients with stent-PCI completed or terminated the study prematurely. The second analysis was to be done when 50 patients in Jena completed the study or dropped out due to early termination.

The primary statistical analysis was to test for superior efficacy of Pioglitazone compared to Placebo with respect to the incidence of cardiac troponin I elevation at 24 hours after stent- PCI using Fisher's exact test at a one-sided significance level of $\alpha = 0.025$ and the Cochran-Mantel-Haenszel test adjusted for centre. All other analyses were performed primarily with standard descriptive statistical methods interpreting all p-values and confidence intervals of possible further inferential statistical methods in an exploratory sense only.

SUMMARY OF RESULTS:

Baseline Demographics and Other Relevant Characteristics:

When the two treatment groups are compared in this section, the order Pioglitazone versus Placebo always applies. The average age overall was 65.5 (7.2) (mean ± standard deviation (SD)) and was comparable across both treatment groups (65.8 (7.4) vs. 65.0 (7.1)). Seventy-five (75) patients were male (41 vs. 34) and 20 were female (13 vs. 7). Height, weight and body mass index (BMI) were also comparable across the two treatment groups (height: 171.8 (8.3) vs. 171.6 (8.5); weight: 88.0 (14.9) vs. 92.5 (15.2); BMI: 29.8 (4.0) vs. 31.4 (4.4).

Prior medications were recorded at least once in all 95 patients with the most frequently listed preparations (i.e. in > 10% of patients) predominantly corresponding with the pre-defined study indication of type II diabetes with confirmed insulin resistance and findings in medical history. Most frequent findings from physical examination at screening concerned extremities in 13/95 (13.7%; 4 vs. 9), the cardiovascular system in 11/95 (11.6%, 4 vs. 7), and other (mainly obesity) in 8/95 (8.4%; 5 vs. 3) patients. In 86/95 patients (90.5%; 50 vs. 36) a concurrent disease was recorded with hyperlipidaemia/ hypercholesterolaemia as by far the most frequently mentioned pathological findings beyond diabetes type II which was indication for inclusion in each study patient.

Subject Disposition:

A total of 98 patients were screened and enrolled by 5 participating German study centres. Thereof, 95 (54 Pioglitazone vs. 41 Placebo) patients were randomized and treated with at least one dose of study medication yielding the safety set. Eighteen (18) patients were not suitable for the main efficacy analysis as they failed to provide at least one of the criteria of a performed stent-PCI, a troponin I value ≤ 1 ULN at V4 (0 hours), or a troponin I result 24 hours after stent-PCI, thus yielding a full-analysis set of 77 (40 Pioglitazone vs. 37 Placebo) patients. Moreover, 18 patients with major protocol violations and one patient with a minor violation could not be considered for further efficacy analyses, leading to 76 (39 Pioglitazone vs. 37 Placebo) patients allocated to the per-protocol analysis set. A total of 8 patients (4.4%; 7 Pioglitazone vs. 1 Placebo) discontinued the study prematurely.

Efficacy Results (Full Analysis Set: n=77, 40 vs. 37):

Primary Efficacy Parameter:

The primary efficacy variable was the incidence of cardiac troponin I elevation (> 1 ULN) at the time of 24 hours after stent-PCI. This was higher for treatment with Pioglitazone compared to Placebo showing an elevation in 20/40 (50.0%) vs. 12/37 (32.4%) patients. The corresponding odds ratio indicates that there was a 2.08-fold higher risk of having a troponin I elevation 24 hours after stent-PCI for treatment with Pioglitazone as compared to Placebo.

Secondary Efficacy Parameters:

Results of quantitative secondary efficacy variables are presented below comparing the two treatment groups in terms of changes between baseline (V2) and the individual final visit (last

observation carried forward (LOCF)/V8 or other) by providing means, SD, medians, and the corresponding ANCOVA p-values for each secondary parameter in all patients evaluable for efficacy (n=77):

Parameter (changes V2 vs. LOCF/V5 or other)	Unit	n;	Pioglitazone; n=40 Mean ± SD (Median)	n;	Placebo; n=37 Mean ± SD (Median)	Between Group Comp.
Troponin I	µg/L	38	0.03 ± 0.22 (0.00)	33	-0.08 ± 0.46 (0.00)	0.9635
CK-MB	U/L	39	-0.03 ± 0.87 (0.00)	34	-0.29 ± 3.20 (0.00)	0.3015
hs-CRP	mg/L	39	-0.25 ± 21.79 (0.48)	34	4.56 ± 11.56 (0.94)	0.2309
hs-CRP (≤ 10 mg/L)	mg/L	36	-0.03 ± 2.90 (0.35)	27	0.73 ± 2.79 (0.81)	0.3631
Adiponectin	µg/mL	39	11.61 ± 8.21 (10.95)	34	-0.28 ± 2.22 (0.00)	<.0001
Proinsulin intact	pmol/mL	39	-1.93 ± 8.62 (-2.37)	34	-1.69 ± 6.67 (0.21)	0.1750
Nitrotyrosine	nmol/L	38	-37.26 ± 290.32 (-13.47)	34	129.21 ± 483.79 (18.52)	0.2561
ADMA	µmol/L	39	-0.00 ± 0.06 (-0.01)	34	0.01 ± 0.08 (0.00)	0.2740
E-selectin	ng/mL	39	-6.47 ± 7.36 (-4.99)	34	-0.58 ± 8.36 (0.00)	0.0002
Myoglobin	µg/L	36	-8.86 ± 28.85 (-5.00)	30	-0.87 ± 10.21 (-1.00)	0.5589
Visfatin	ng/mL	39	-0.23 ± 5.03 (-0.02)	34	2.06 ± 4.31 (2.07)	0.0906
Fasting glucose	mg/dL	39	-9.31 ± 34.13 (-12.00)	34	-2.53 ± 18.39 (-3.00)	0.0482
Fasting insulin	mU/L	39	2.81 ± 20.19 (-1.87)	34	-0.27 ± 15.05 (0.79)	0.0710
HOMA-S	mU x mmol/L^2	39	0.78 ± 7.21 (-0.95)	34	-0.16 ± 4.94 (0.26)	0.0689
Cholesterol	mg/dL	38	-5.53 ± 28.45 (-3.00)	34	-13.00 ± 30.08 (-8.00)	0.4948
HDL cholesterol	mg/dL	38	-1.11 ± 10.35 (-0.50)	34	-1.71 ± 8.64 (-2.50)	0.2206
LDL cholesterol	mg/dL	38	-1.89 ± 20.24 (1.00)	33	-9.15 ± 27.64 (-3.00)	0.5527

Parameter (changes V2 vs. LOCF/V5 or other)	Unit	n;	Pioglitazone; n=40 Mean ± SD (Median)	n;	Placebo; n=37 Mean ± SD (Median)	Between Group Comp.

Triglycerides	mg/dL	37	-14.78 ± 71.33 (-6.00)	34	-9.32 ± 89.16 (-20.50)	0.4967
n: number of patients; SD: standard deviation; HDL: high density lipoprotein; LDL low density lipoprotein; HOMA-S: Homeostatic Model Assessment – Sensitivity						

An increase of CK-MB (> 1 ULN) 24 hours after stent-PCI occurred in only one patient of the Pioglitazone group (1/40 vs. 0/37). Between-group differences with more increase for Placebo treatment were seen in hs-CRP (both original and ≤ 10 mg/L]), nitrotyrosine, visfatin, fasting glucose, E- selectin, myoglobin, and triglycerides whereas more increase under Pioglitazone occurred in adiponectin, fasting insulin, HOMA-S, cholesterol and LDL cholesterol. Statistical significance was secured only for adiponectin (more elevation for Pioglitazone treatment), and E-selectin with more increase in the Placebo group.

Per-protocol analysis (PP-Set: n=76, 39 vs. 37):

Since the 'per-protocol analysis set' did not differ relevantly from the 'full analysis set' (76 vs. 77 patients) clinically clear deviations regarding the per-protocol efficacy results did not occur.

Multicentre analyses (Full Analysis Set: n=77; 40 vs. 37):

To compare the treatment groups while controlling for centre effects an exploratory analysis of the primary efficacy variable was performed with the Cochran-Mantel-Haenszel test using the study centres as strata. The three centres with the smallest number of evaluable patients (i.e. the cardiologic sites at Frankfurt, Mainz, and Wuppertal) were pooled for these analyses. The incidence of troponin I elevation (> 1 ULN) 24 hours after stent-PCI was slightly lower in the Pioglitazone group than in the Placebo group only at the cardiologic site in Kassel (4/8; 50.0% vs. 7/12; 58.3% patients) but not for Jena and the pooled 3-site centre. The one-sided p-value obtained from the Cochran-Mantel-Haenszel test for testing superior efficacy of Pioglitazone compared to Placebo based on the stratified samples was p=0.9628. This result corresponds to Fisher's exact test where a one-sided p-value of p=0.9641 was calculated indicating that no relevant difference between centre-adjusted and not-adjusted analysis occurred.

Subgroup analyses (full analysis set: n=77; 40 vs. 37):

From the predetermined subgroups analysed (treatment group, gender, age, BMI, severity of coronary artery disease (CAD), location of target lesion, with/without pre-dilatation, with/without after-dilatation, diameter of stenosis (DS), diameter of stent, maximal inflation pressure, duration of inflation, additional stent used, complexity score, outlier/non-outlier, complexity score in outliers/non-outliers) only 'DS' was identified as relevant prognostic factor besides 'treatment group'. For the subset of patients in Jena (n=49), clinically relevant differences compared to the results for all patients did not occur including the evaluation of HITS.

Safety results (All-Patients-Treated Set: n=95; 54 vs. 41):

Adverse events (AEs) were documented in 34/95 (35.8%; 20 Pioglitazone vs. 14 Placebo) treated patients showing 57 (38 vs. 19) individual events classified as treatment emergent adverse events (TEAEs). Frequently reported events, i.e. in more than one of the 95 treated patients were vertigo in 6/95 patients (6.3%; 5 vs. 1), nasopharyngitis in 4 patients (4.2%; 2 vs. 2), angina pectoris in 3 patients (3.2%; 2 vs. 1), headache, diarrhoea, and peripheral oedema in 2 patients (2.1%; 2 vs. 0), and constipation, haematoma, cough, and acute myocardial infarction in also 2 patients (2.1%; 1 vs. 1). 36 of 57 events (24 vs. 12) were assessed as mild, 16 (11 vs. 5) as moderate, and 5 as

severe (3 vs. 2; presyncope, asthenia, and angina pectoris vs. vascular access complication and myocardial infarction).

Relationship to study drug intake was rated as unlikely/not related in 40 (23 vs. 17), as possibly related in 13 (11 vs. 2), as probably related in 3 events (3 vs. 0; diarrhoea, vertigo, angina pectoris), and as definitely related in one event (1 vs. 0; chest discomfort), which were mainly classified as recovered during the study period (43/57 events; 30 vs. 13).

In 6/95 patients (6.3%; 4 vs. 2) a total number of 9 (7 vs. 2) events referring to TEAEs were documented as treatment emergent serious adverse events (TESAEs) based on hospitalization in all cases. Thus, 48 (31 vs. 17) events were non-serious. The serious adverse events (SAEs) for Pioglitazone were described as single episodes derived from general disorders in two, and from cardiac, gastrointestinal, nervous system, and renal/urinary disorders in one patient each. For the two Placebo patients an acute myocardial infarction (cardiac disorders) and vascular access problems (injury, poisoning, and procedural complications) were rated as TESAEs. All SAEs were assessed as not related to the administration of study medication. Premature discontinuation of the study due to an adverse event according to the entries in the appropriate AE-form was reported in only one patient of the Pioglitazone group (urinary retention as the leading AE). Cases of death did not occur during the entire study period.

Concerning safety laboratory a clear trend towards a study therapy related influence on specific parameters cannot be derived, and the evaluation of vital signs did not reveal any clinically relevant changes both during the study course and between the treatment groups when comparing baseline with final measurements.

Overall conclusions:

A protective effect of Pioglitazone in comparison to Placebo could not be demonstrated since troponin I elevation after stent-PCI was clearly lower in the subgroup of patients treated with Placebo. Pioglitazone treated patients showed, however, higher adiponectin and lower E-selectin values compared to Placebo. Because adiponectin influences the response to insulin and has an antiinflammatory effect on the cell-lining blood vessels, higher adiponectin levels have shown to be associated with a decreased risk of diabetes mellitus and heart attack. E-selectin on the other hand is increased during inflammatory response and a causative agent of greater microvascular permeability. Thus, Pioglitazone seems to be able to positively influence both adiponectin and E-selectin levels but this effect is not reflected by a troponin I release that was higher in Pioglitazone treated patients as compared to Placebo and therefore might indicate greater myocardial injury under verum during stent- PCI.

With regard to safety the study did not reveal any potential new or unexpected sign or symptom allocated to the study drug in comparison to the known range of thiazolidinediones specific adverse reactions. Observations such as vertigo, diarrhoea, peripheral oedema, headache, gastrointestinal problems, and fatigue are consistent with the expected safety profile of the tested investigational medicinal product (IMP). Findings like changes in blood glucose, angina pectoris, or myocardial infarction can be rated as expectable for a study considering a patient collective

suffering from diabetes with insulin resistance and a clearly increased risk for vascular complications. The evaluation of laboratory tests and vital signs did not show a clear trend towards a study therapy induced pathologic influence on specific parameters.

Significant Changes During Study:

Changes to the study protocol or the conduct of the clinical trial were carried out according to four officially approved substantial amendments which are summarized in the following:

Amendment No. 1 of 06 December 2006 implemented new study sites and changed the second inclusion criterion for the IRIS score from >70 to >50 score points as it was too restrictive to permit an adequate recruitment of study patients (only 2 patients enrolled within 12 weeks). Most of patients with an IRIS score >70 already show a manifest type-II-diabetes. Using a lower limit patients with moderate insulin resistance could be included.
As a common practice in interventional cardiology, the definite decision whether a stent-PCI is indicated or not is usually done during catheterisation according to angiographic findings. Thus, it was possible that up to 80% of the included and randomized patients with planned PCI would not undergo the initially intended intervention due to medical reasons (no findings or findings that require other treatment). Therefore, the number of randomized patients was increased to a maximum of 300 patients in order to realize 56 patients with performed PCI. Furthermore, there were minor differences in the treatment regime between the single centres leading to a redefinition for prescription of clopidogrel and ASS medication.

Amendment No. 2 of 14 May 2007 also implemented new study sites due to low recruitment.

Amendment No. 3 of 06 September 2007 implemented the specific new study site in Jena for conduct of a sub-study featuring additional measurement of microembolic events (HITS).

Amendment No. 4 of 13 May 2008 changed the exclusion criterion No. 15 in order to allow insulin treatment and to accelerate recruitment because the contraindication of insulin treatment was cancelled during the clinical phase of the study.

Study ID Number:

ATS K021 / D-PIO-111

Other Study ID Number(s):

2006-002237-20 [EudraCT Number]

D-PIO-111 [Takeda ID]

U1111-1115-9160 [Registry ID: WHO]

DATE OF DISCLOSURE SYNOPSIS: 13 June 2012