Clinical Trial Results Disclosure Synopsis

Short Title: The SPLENDOR study

Name of Sponsor: Takeda Italia S.p.A.
Via Elio Vittorini, 129
00144 Rome, Italy

Title of Study: Effects of Pioglitazone on endothelial progenitor cells in type 2 diabetic patients with vascular complications - The SPLENDOR study

Phase of Development: Phase IV

Name of Active Ingredient: (±)-5-[p-(5-ethyl-2-pyridyl)ethoxy]benzyl]-2,4,thiazolidinedione hydrochloride (pioglitazone)

Name of Finished Product: Pioglitazone

Investigators: 2 principal investigators in Italy enrolled subjects in the double-blind treatment period

Study Site: 2 sites in Italy randomized subjects into the double-blind treatment period

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

Study Period:

Date first subject signed informed consent form: 03 May 2009

Date of last subject's last visit/contact (from the Clinical database): 05 February 2011

Date of last subject's last procedure for collection of data for primary endpoint: 05 February 2011

Objectives:

Primary:

The primary objective of this study was to evaluate the effect of treatment with Pioglitazone on the number of circulating Endothelial Progenitor Cells (EPCs) in type 2 diabetic patients with vascular complications.

Secondary:

The secondary objectives of this study were:

- To evaluate the effects of Pioglitazone on glucose metabolism, lipid metabolism, insulin

sensitivity, markers of inflammation and adipokines (with the aim to explore the mechanisms putatively involved in EPCs modulation);

- To evaluate the effects of Pioglitazone on both endothelium-dependent and endothelium-independent vasomotion;
- To evaluate the safety and tolerability of Pioglitazone.

Methodology: This was a phase IV, 24-week, double blind, randomized, multicentre, parallel-group study.

After a period of about one week from the screening visit, which was necessary to receive the results of laboratory tests carried out to evaluate the inclusion and exclusion criteria, eligible outpatients aged between 40 and 70 years, with type 2 diabetes and prior vascular complications, already on treatment with the maximum tolerated dose of metformin, were randomly assigned to one of the two following treatment arms:

a) Pioglitazone 30 mg/day, or

b) Glibenclamide 10 mg/day.

Five clinic visits in total were planned for this study: screening (Visit 1), randomization (Visit 2), and at 4, 12 and 24 weeks of treatment (Visits 3-5). A follow-up visit, in order to collect adverse events, was performed after about one week from the last visit. An Early Termination Visit was performed in case of discontinuation or withdrawal of a patient from the study, after the randomization.

Number of Subjects:

Planned: 76 subjects

Screened: 39 subjects

Enrolled in the double blind treatment period: 35 subjects

Number of patients (total and for each arm):

	Randomized	**Safety**	**Intent-to-treat (ITT)**	**Completers**
Total	35	35	34	35
Pioglitazone	18	18	17	18
Glibenclamide	17	17	17	17

Diagnosis and Main Criteria for Inclusion: age between 40 and 70 years; males and post-menopausal females; HbA_{1c} >7.5% and <10%; age at onset of type 2 diabetes >35 years; metformin monotherapy up to the maximum tolerated daily dose; normal or only slightly impaired renal function (Modification of Diet in Renal Disease [MDRD] estimated glomerular filtration rate (GFR) >60 ml/min/1.73 m^2); if on antihypertensive, statins or any other

hypolipidemic treatment, these medications had to be initiated at least three months prior to enrolment, with no dose modifications allowed during the study; one or more cardiovascular comorbidity and/or two or more major cardiovascular risk factors; willing and able to give informed consent.

Duration of Treatment: 24 weeks

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	Overencapsulation Lot Number
Pioglitazone	15 mg encapsulated tablets	2 x 15 mg once a day	Oral	2730005S 3730013S	2730005S/CPS1/07A 3730013S/CPS1/09A

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

Study Medication	Product Dose Strength	Study Dosage	Mode of Administration	Drug Product Lot Number	Overencapsulation Lot Number
Glibenclamide	5 mg encapsulated tablets	2 x 5 mg once a day	Oral	B485 C490	B485/CPS1/07A C490 CPS1/2011

Criteria for Evaluation:

Efficacy:

The primary endpoint of the study was the increase of the number of EPCs (CD34+Kinase Domain Receptor [KDR]+) from baseline to a 6- month treatment with pioglitazone, compared with glibenclamide treatment.

The secondary efficacy variables were:

- Integrated markers of cardiovascular risk: circulating progenitor cells (CPCs) (cluster of differentiation [CD]34+, CD133+) and plasminogen activator inhibitor-1 (PAI-1);
- Factors involved in modulation of EPCs recruitment: vascular-endothelial growth factor (VEGF), erythropoietin (EPO) and stromal cell-derived factor-1 (SDF-1);
- Glucose control: glycosylated hemoglobin (HbA_{1c}) and fasting plasma glucose (FPG);
- Lipid parameters: total, low-density lipoprotein (LDL)- and high-density lipoprotein (HDL)-cholesterol, triglycerides, apolipoprotein B and apolipoprotein A1, oxidized LDL, and free fatty acid (FFA);

- Insulin sensitivity: insulin sensitivity indexes by 3-hour oral glucose tolerance test (OGTT) with glucose, insulin and C-peptide estimation;
- Inflammation markers: high sensitive C-Reactive Protein (hs-CRP), interleukin-6 (IL-6), vascular adhesion molecules (soluble intercellular adhesion molecule [sICAM-1], soluble vascular cell adhesion molecule-1 [sVCAM-1]), monocyte chemotactic protein – 1 (MCP-1), tumor necrosis factor-α (TNF-α);
- Adipokines: adiponectin;
- Oxidative stress: MDA (maleic dialdehyde), FRAP (ferric reducing antioxidant power), LOOH (lipid hydroxyperoxide);
- Endothelial vasomotion parameters: Flow Mediated Dilation (FMD) and glyceryl trinitrate (GTN)-induced dilation.

Safety:

Safety variables were: adverse events (AEs), laboratory tests, electrocardiogram (ECG), vital signs and physical examination.

Statistical Methods:

The following population were considered for data analysis: safety population, which included all randomized subjects who received at least one dose of study medication, and intention-to-treat (ITT) population, which included all randomized subjects who took at least one dose of study drug and had the baseline measurement and a measurement recorded during the treatment period. The efficacy summaries and analyses were based on the ITT population and safety parameters were analyzed in the safety population.

Checks on the assumptions underlying the statistical model were restricted to the visual examination of the residuals computed from the analysis of covariance (ANCOVA) model (normal probability plots and plots of residuals against fitted values). Due to an essential violation of ANCOVA assumption, a non-parametric analysis was performed for primary efficacy variable using a two-sided Mann-Whitney-Wilcoxon rank sum test on change from baseline.

Most of secondary parameters showed the same essential violation occurred to the primary efficacy variable. Then, according with the sponsor, analyses regarding all secondary parameters were performed using a two-sided Mann- Whitney-Wilcoxon rank sum test on change from baseline.

Adverse event Investigator terms were assigned to a Lowest Level Term (LLT) and a Preferred Term (PT) and were classified by primary System Organ Class (SOC) according to the Medical Dictionary for Regulatory Activities (MedDRA) thesaurus, Version 13.0.

For each hematology and blood chemistry variable, the change from baseline to each visit after baseline was calculated. A shift table showing blood test values classified as low, normal and high based on reference ranges from baseline to final visit, by treatment group, was also produced.

For ECG interpretation, default frequency tabulation per treatment group by visit was produced. Default summary statistics, by treatment group and visit, were produced for heart rate, RR interval, PR interval, QT interval and QRS interval.

For vital signs (systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate, waist circumference and body mass index (BMI)), default summary statistics for values at each visit and changes form baseline to each post-baseline visit were produced by treatment group.

SUMMARY OF RESULTS:

Baseline Demographics and Other Relevant Characteristics:

The mean age in the ITT population was 62.1 ± 5.6 (mean ± standard deviation (SD)) years in the Pioglitazone group and 60.2 ± 8.7 years in the Glibenclamide group. The Pioglitazone group included 9 males (52.9%) and 8 females (47.1%), and the Glibenclamide group included 10 males (58.8%) and 7 females (41.2%). The mean height in the ITT population was 1.68 ± 0.09 m (range 1.49-1.83) in the Pioglitazone group and 1.69 ± 0.09 m (1.51-1.83) in the Glibenclamide group. The mean weight was 84.8 ± 11.4 (range 62-99) in the Pioglitazone group and 9.04 ± 13.3 kg (range 66-111) in the Glibenclamide group. The mean body mass index (BMI) was 30.1 ± 3.4 kg/m^2 (range 26.3-37.7) in the Pioglitazone group and 31.5 ± 4.1 kg/m^2 (range 24.2-39.3) in the Glibenclamide group. The mean waist circumference in the ITT population was 107.1 ± 8.2 cm (range 90-121) in the Pioglitazone group and 111.9 ± 8.5 cm (97-128) in the Glibenclamide group.

The presence of at least one disease in the medical history was reported in 22 patients in total (10 (58.8%) in the Pioglitazone group vs. 12 (70.6%) Glibenclamide group). Normal findings were reported in the examination of most of body systems. The following abnormalities were reported: abnormalities of eyes were observed in 1 patient (5.9%) patients in the Glibenclamide group; abnormalities of the cardiovascular system were observed in 1 patient (5.9%) in the Pioglitazone group and in 6 (35.3%) in the Glibenclamide group; abnormalities of the extremities were observed in 2 patients (11.8%) in the Pioglitazone group and in 3 (17.6%) in the Glibenclamide group; abnormalities of the musculo-skeletal system were observed in 1 patient (5.9%) patients in the Glibenclamide group; and abnormalities of the nervous system were observed in 1 patient (5.9%) patients in the Glibenclamide group. The number of patients taking previous antidiabetic treatments was 10 (58.8%) in the Pioglitazone group and 8 (47.1%) in the Glibenclamide group. Metformin was the most frequently taken drug, with 6 patients (54.5%) in the Pioglitazone group and 4 (40.0%) in the Glibenclamide group.

Subject Disposition:

Thirty-five patients were randomized to receive the assigned treatment: 18 patients were assigned to treatment with Pioglitazone and 17 were assigned to receive Glibenclamide. All randomized patients in both groups completed the study.

Extent of exposure and compliance:

The mean extent of exposure to study drug was 184.5 ± 8.0 days (range 167-197) in the Pioglitazone group and 184.6 ± 10.2 days (range 172-211) in the Glibenclamide group.

The mean compliance was 96.9 ± 2.7 % (range 90.2-102.9) in the Pioglitazone group and 96.3 ± 5.4 % (range 77.0- 101.1) in the Glibenclamide group. All patients in both groups had a compliance within the range 80-120%, except one patient (5.9%) in the Glibenclamide group, who had a compliance < 80%.

Efficacy Results:

Primary efficacy variable (circulating EPCs CD34+/KDR+):

The increase from baseline to week 24 in mean number of CD34+KDR+ cells/10^6 cytometric events was more marked in the Glibenclamide group (mean change ± SD: 12.24 ± 37.00) than in the Pioglitazone group (mean change ± SD: 5.84 ± 33.83). The comparison between groups did not show statistically significant differences (p = 0.459).

Secondary efficacy variables:

<u>Glucose control and lipid profile:</u>

The mean change (± SD) from baseline to week 24 in fasting plasma glucose was -28.35 ± 14.50 mg/dl in the Pioglitazone group and -9.47 ± 31.15 mg/dl in the Glibenclamide group (p = 0.082 between groups).

The mean change (± SD) from baseline to week 24 in HbA_{1c} was -0.76 ± 0.81 % in the Pioglitazone group and - 0.77 ± 0.75 % in the Glibenclamide group (p = 0.796 between groups).

The mean change (± SD) from baseline to week 24 in total lipids was -9.94 ± 20.29 mg/dl in the Pioglitazone group and -9.76 ± 21.31 mg/dl in the Glibenclamide group (p = 0.959 between groups).

The mean change (± SD) from baseline to week 24 in triglycerides levels was -59.94 ± 80.50 mg/dl in the Pioglitazone group and 2.65 ± 56.22 mg/dl in the Glibenclamide group. The difference between groups was statistically significant (p = 0.008), in favor of the Pioglitazone group.

The mean change (± SD) from baseline to week 24 in LDL cholesterol was -4.47 ± 19.84 mg/dl in the Pioglitazone group and -7.94 ± 15.27 mg/dl in the Glibenclamide group (p = 0.629 between groups).

The mean change (± SD) from baseline to week 24 in HDL cholesterol levels was 5.82 ± 7.66 mg/dl in the Pioglitazone group and -3.53 ± 5.26 mg/dl in the Glibenclamide group. The difference between groups was statistically significant (p=0.0002), in favor of the Pioglitazone group.

The mean change (± SD) from baseline to week 24 in apolipoprotein A1 levels was 0.11 ± 0.18 g/L in the Pioglitazone group and -0.05 ± 0.20 g/L in the Glibenclamide group. The difference between groups was statistically significant (p = 0.029), in favor of the Pioglitazone group.

The mean change (± SD) from baseline to week 24 in apolipoprotein B levels was -0.11 ± 0.14 g/L in the Pioglitazone group and -0.03 ± 0.15 g/L in the Glibenclamide group (p = 0.220 between groups).

The mean change (± SD) from baseline to week 24 in oxidized LDL levels was -17.33 ± 57.11 mU/L in the Pioglitazone group and -0.84 ± 49.55 mU/L in the Glibenclamide group (p = 0.718 between groups).

The mean change (± SD) from baseline to week 24 in free fatty acids levels was 0.00 ± 0.08 g/L in the Pioglitazone group and 0.01 ± 0.07 g/L in the Glibenclamide group (p = 0.719 between groups).

Markers of inflammation and vascular adhesion molecules:

The mean change (± SD) from baseline to week 24 in hs-CRP levels was -20.61 ± 43.71 μg/ml in the Pioglitazone group and 2.74 ± 39.98 μg/ml in the Glibenclamide group (p = 0.084 between groups).

The mean change (± SD) from baseline to week 24 in IL-6 levels was -1.25 ± 5.50 pg/ml in the Pioglitazone group and 0.06 ± 2.15 pg/ml in the Glibenclamide group (p = 0.407 between groups).

The mean change (± SD) from baseline to week 24 in sICAM-1 levels was -7.35 ± 21.20 ng/ml in the Pioglitazone group and -4.97 ± 15.09 ng/ml in the Glibenclamide group (p = 0.471 between groups).

The mean change (± SD) from baseline to week 24 in sVCAM-1 levels was 23.21 ± 199.14 ng/ml in the Pioglitazone group and 45.25 ± 93.22 ng/ml in the Glibenclamide group (p = 0.843 between groups).

The mean change (± SD) from baseline to week 24 in MCP-1 levels was -6.36 ± 56.25 pg/ml in the Pioglitazone group and 26.55 ± 116.28 pg/ml in the Glibenclamide group (p = 0.642 between groups).

The mean change (± SD) from baseline to week 24 in TNFα levels was 0.25 ± 2.92 pg/ml in the Pioglitazone group and -0.43 ± 3.07 pg/ml in the Glibenclamide group (p = 0.408 between groups).

The mean change (± SD) from baseline to week 24 in adiponectin levels was 2506.18 ± 4215.17 pg/ml in the Pioglitazone group and -179.08 ± 1509.17 pg/ml in the Glibenclamide group. The difference between groups was statistically significant (p=0.0005), in favor of the Pioglitazone group.

The mean change (± SD) from baseline to week 24 in MDA levels was 0.39 ± 1.75 μmol/L in the Pioglitazone group and -0.53 ± 1.03 μmol/L in the Glibenclamide group (p = 0.221 between groups).

The mean change (± SD) from baseline to week 24 in FRAP levels was -29.53 ± 109.97 μmol/L in the Pioglitazone group and 503.06 ± 1965.94 μmol/L in the Glibenclamide group (p = 0.102 between groups).

The mean change (± SD) from baseline to week 24 in LOOH levels was 0.44 ± 2.19 μmol/L in the Pioglitazone group and -0.61 ± 1.30 μmol/L in the Glibenclamide group (p = 0.143 between groups).

The mean change (± SD) from baseline to week 24 in PAI-1 levels was -16303.1 ± 37811.41 pg/ml in the Pioglitazone group and 4355.10 ± 74470.18 pg/ml in the Glibenclamide group (p = 0.056 between groups).

The mean change (± SD) from baseline to week 24 in VEGF levels was -3.22 ± 23.80 pg/ml in the Pioglitazone group and -1.90 ± 16.06 pg/ml in the Glibenclamide group (p = 0.471 between groups).

The mean change (± SD) from baseline to week 24 in SDF-1 levels was -2046.21 ± 3795.98 pg/ml in the Pioglitazone group and -870.16 ± 1996.36 pg/ml in the Glibenclamide group (p = 0.640 between groups).

The mean change (± SD) from baseline to week 24 in EPO levels was 0.28 ± 3.37 mIU/ml in the Pioglitazone group and -0.98 ± 3.83 mIU/ml in the Glibenclamide group (p = 0.593 between groups).

<u>CPCs and EPCs parameters:</u>

The mean change (± SD) from baseline to week 24 in number of CD34 cells was -66.81 ± 114.04 in the Pioglitazone group and 201.67 ± 713.93 in the Glibenclamide group. The difference between groups was statistically significant (p = 0.048) including an outlier high value at week 24 in the Glibenclamide group, and was not with the replacement using the week 12 value.

The mean change (± SD) from baseline to week 24 in CD34/CD133 cells ratio was -57.61 ± 101.12 in the Pioglitazone group and -2.10 ± 77.78 in the Glibenclamide group (p = 0.185 between groups).

The mean change (± SD) from baseline to week 24 in CD34/CD133/KDR cells ratio was 4.17 ±

31.49 in the Pioglitazone group and 9.51 ± 34.14 in the Glibenclamide group (p = 0.418 between groups).

OGTT and insulin sensitivity indexes:

The mean change (± SD) from baseline to week 24 in homeostasis assessment model – insulin resistance (HOMA-IR) index was -3.13 ± 2.95 in the Pioglitazone group and -1.05 ± 3.92 in the Glibenclamide group. The difference between groups was statistically significant (p = 0.048), in favor of the Pioglitazone group.

The mean change (± SD) from baseline to week 24 in Quantitative Insulin Sensitivity Check Index (QUICKY) index was 0.01 ± 0.01 in the Pioglitazone group and 0.00 ± 0.01 in the Glibenclamide group. The difference between groups was statistically significant (p = 0.034), in favor of the Pioglitazone group.

The mean change (± SD) from baseline to week 24 in fasting glucose/insulin ratio was -0.01 ± 4.84 in the Pioglitazone group and -1.46 ± 4.74 in the Glibenclamide group (p = 0.517 between groups).

The mean change (± SD) from baseline to week 24 in Insulin Sensitivity Index (ISI) Matsuda was 0.68 ± 0.71 in the Pioglitazone group and - 0.03 ± 0.80 in the Glibenclamide group. The difference between groups was statistically significant (p = 0.022), in favor of the Pioglitazone group.

The mean change (± SD) from baseline to week 24 in Oral Glucose Insulin Sensitivity (OGIS) index was 36.81 ± 47.78 in the Pioglitazone group and 5.07 ± 27.61 in the Glibenclamide group. The difference between groups was statistically significant (p = 0.040), in favor of the Pioglitazone group.

The mean change (± SD) from baseline to week 24 in HOMA-B% index was -5.08 ± 90.19 in the Pioglitazone group and 13.52 ± 45.87 in the Glibenclamide group (p = 0.517 between groups).

The mean change (± SD) from baseline to week 24 in insulinogenic index was -0.03 ± 0.09 in the Pioglitazone group and -0.00 ± 0.06 in the Glibenclamide group (p = 0.305 between groups).

The mean change (± SD) from baseline to week 24 in area under the curve for glucose (AUCgluc) was -8346.88 ± 8635.70 in the Pioglitazone group and -4325.88 ± 6672.67 in the Glibenclamide group (p = 0.105 between groups).

The mean change (± SD) from baseline to week 24 in area under the curve for insulin (AUCins) was -1536.97 ± 8699.34 in the Pioglitazone group and 5330.68 ± 17585.40 in the Glibenclamide group (p = 0.280 between groups).

The mean change (± SD) from baseline to week 24 in AUCins/AUCgluc ratio was 0.04 ± 0.19 in the Pioglitazone group and 0.14 ± 0.35 in the Glibenclamide group (p = 0.940 between groups).

The mean change (± SD) from baseline to week 24 in AUC C-peptide was -18.18 ± 739.74 in the

Pioglitazone group and 363.81 ± 531.99 in the Glibenclamide group (p = 0.113 between groups).

Endothelial vasomotion parameters:

The mean change (± SD) from baseline to week 24 in basal diameter (dependent) was 0.19 ± 0.37 mm in the Pioglitazone group and -0.04 ± 0.34 mm in the Glibenclamide group (p = 0.098 between groups).

The mean change (± SD) from baseline to week 24 in max basal diameter post-ischemia (dependent) was 0.22 ± 0.36 mm in the Pioglitazone group and -0.05 ± 0.34 mm in the Glibenclamide group. The difference between groups was statistically significant (p = 0.034), in favor of the Pioglitazone group.

The mean change (± SD) from baseline to week 24 in FMD was 0.72 ± 3.65 % in the Pioglitazone group and 0.32 ± 3.18 % in the Glibenclamide group (p = 0.564 between groups).

The mean change (± SD) from baseline to week 24 in baseline integral flux velocity was -0.06 ± 0.12 m/sec in both groups (p = 0.910 between groups).

The mean change (± SD) from baseline to week 24 in integral flux velocity post-ischemia was 0.05 ± 0.35 m/sec in the Pioglitazone group and 0.06 ± 0.30 m/sec in the Glibenclamide group (p = 0.850 between groups).

The mean change (± SD) from baseline to week 24 in basal diameter (independent) was 0.24 ± 0.32 mm in the Pioglitazone group and 0.01 ± 0.45 mm in the Glibenclamide group (p = 0.082 between groups).

The mean change (± SD) from baseline to week 24 in max basal diameter post-ischemia (independent) was 0.28 ± 0.28 mm in the Pioglitazone group and -0.24 ± 0.83 mm in the Glibenclamide group. The difference between groups was statistically significant (p = 0.024), in favour of the Pioglitazone group.

The mean change (± SD) from baseline to week 24 in sodium nitroprussiate (SNP) was 0.76 ± 4.11 % in the Pioglitazone group and -0.36 ± 4.15 % in the Glibenclamide group (p = 0.553 between groups).

Safety Results:

Adverse events:

Treatment-emergent adverse events (TEAEs) were reported in none (0.0%) of patients in the Pioglitazone group and in 2 patients (11.8%) in the Glibenclamide group (2 TEAEs in total). No serious TEAEs or TEAE leading to treatment discontinuation were reported in any patient. Treatment-related TEAEs were reported in 1 patient (5.9%) in the Glibenclamide group (hypoglycaemia), which caused dose modification.

Laboratory tests:

The mean red blood cell (RBC) count, hematocrit, hemoglobin and white blood cell (WBC) count slightly decreased from baseline to any post- baseline time point in the Pioglitazone group, compared to negligible changes in the Glibenclamide group. There were no clinically important changes from baseline in blood chemistry parameters and urinary albumin/creatinine ratio in both groups. Furthermore, no individual clinically significant abnormalities in laboratory parameters were reported in both groups.

ECG and vital signs:

The results of ECG did not show no substantial changes from baseline to week 24 in overall results, mean heart rate and measured intervals (RR, PR, QT and QRS) in both groups.

No clinically important changes in vital signs (blood pressure, heart rate, body weight, BMI and waist circumference) from baseline to any post-baseline time point were observed in both groups.

Physical examination:

There were no substantial changes from baseline in both groups.

Conclusions:

The main results of the present study have shown that:

- Treatment with Pioglitazone for 24 weeks in type 2 diabetic patients with vascular complications did not significantly increase the number of circulating EPCs compared to Glibenclamide, both given in addition to the maximum individually tolerated daily dose of metformin.
- Treatment with Pioglitazone was associated with significant improvements vs. Glibenclamide in lipid metabolism, adiponectin levels and insulin sensitivity, and endothelial function.
- Pioglitazone was well tolerated in terms of adverse events, vital signs and safety laboratory parameters.

Study ID Number:

IT-PIO-109

Other Study ID Number(s):

2007-003077-44 [EudraCT Number]

U1111-1114-3045 [UTN Number]

DATE OF DISCLOSURE SYNOPSIS: 31 December 2012