Clinical Trial Results Disclosure Synopsis

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

Title of Study: Effect of Acute Insulin Intervention followed by Pioglitazone and Metformin Treatment on Metabolic Parameters in Type 2 Diabetic Patients with inadequate Metabolic Control

Phase of Development: Phase 2

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

Metformin [1,1-Dimethylbiguanid]

Insulin [Humaninsulin Normal]

Name of Finished Product: Actos® / Metformin Ratiopharm® 850 mg Huminsulin®

Investigator: Prof. Thomas Forst, MD, principal investigator, enrolled subjects in the open-label treatment period.

Study Site: Subjects were enrolled in the open-label treatment period at 1 site in Germany:

Institute for Clinical Research and Development

Parcusstraße 8, 55116 Mainz, Germany

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

Study Period:

Date first subject signed informed consent form: 04 August 2006

Date of last subject's last visit/contact (from the Clinical database): 23 January 2007

Objectives:

Primary:

The primary objective of this study was to investigate the percentage of patients who remain stable on oral therapy with pioglitazone and metformin after an acute intervention with insulin for 2 days. A stable therapy was defined by an improvement in glycosylated haemoglobin

(HbA1c) of at least 0.5% within 4 months.

Secondary:

As secondary objectives various metabolic parameters were measured to evaluate safety and efficacy of treatment described.

Methodology: This clinical study was designed as a single center, prospective, open-label phase II pilot study. All patients were treated with Insulin for 2 days and subsequently with Pioglitazone and Metformin for 4 months.

Number of Subjects:

Planned: 15 subjects

Screened: 23 subjects

Enrolled in the open-label treatment period: 15 subjects

Analyzed: Full Analysis Set: 15 subjects, Per Protocol Set: 14 subjects

Diagnosis and Main Criteria for Inclusion: The trial population consisted of male and female type 2 diabetic individuals aged 30–75 years, pre-treated with metformin in an individually maximal tolerated dose and/or metformin in combination with at least one additional oral antidiabetic drug except peroxisome proliferator-activated receptor (PPAR) γ- agonists and insufficient metabolic control HbA1c ≥ 6.5 % and at least 3 repeated glucose levels > 200 mg/dL within the last two weeks prior to screening) and a fasting C-peptide level > 2 ng/mL.

Duration of Treatment: Oral treatment was planned for 4 months (120 ± 5 days for Metformin and 119 ± 5 days for Pioglitazone) between enrolment (V2) and final visit (V6). Actual mean extent of exposure was for Pioglitazone 111 ± 22 (115) days and for Metformin 112 ± 22 (116) days.

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 tablets	30 mg OD	Oral	9230017B
Metformin	850 mg tablets	850 mg BID	Oral	609995
Insulin	Solution for intravenous infusion	Starting dose 1 IU/hour and then titrated to achieve a stable blood glucose level of 80-60 mg/dL	Continuous Intravenous infusion	556080A

Reference Therapy, Dose and Mode of Administration, and Lot Number: Not applicable

Criteria for Evaluation:

Efficacy:

Primary efficacy variable was the change in HbA1c after 4 months of treatment (V6) compared to baseline (V1).

Secondary efficacy variables were the following parameters

- Change from V2T1 and V2T3 to end of study (V6/ET):
    - Fasting Blood Glucose
    - Fasting Insulin Levels
    - Insulin Sensitivity according to HOMAs
    - Adiponectin
    - Intact Proinsulin
    - High sensitive C-Reactive Protein (hs-CRP)
    - Visfatin
    - Nitrotyrosine
    - Total Cholesterol
    - High-density lipoprotein (HDL)-Cholesterol
    - Low-density lipoprotein (LDL)-Cholesterol
    - Triglycerides

- Change from V1 to end of study (V6/ET):
    - C-Peptide

- Change from V2T1 to V2T2:
    - Amyloid B

Safety:

Safety was addressed by occurrence of adverse events, change in clinical laboratory parameters, and rate of premature withdrawals.

Statistical Methods:

The study was exploratory and was not powered to address any pre-defined hypothesis. Due to the pilot character of this trial, all p-values and the corresponding confidence intervals of inferential statistical methods were interpreted in an exploratory sense.

Primary efficacy variable was the change of HbA1c compared to the screening visit (V1). Patients who terminated treatment prematurely were considered with their last value under investigational medication (last observation carried forward, LOCF). Absolute values and changes from baseline were presented using descriptive summary statistics. Moreover percentage changes from baseline were displayed. Missing values were not replaced.

The primary efficacy variable was analyzed in terms of an exact binomial test based on the Full-Analysis set and the Per-Protocol-Analysis set.

All secondary efficacy parameters were evaluated by using descriptive statistics primarily (absolute values at each time point and if appropriate changes from baseline).

SUMMARY OF RESULTS:

Baseline Demographics and Other Relevant Characteristics:

A total of 15 subjects with type 2 diabetes were enrolled into this study. All subjects (100%) were of Caucasian origin. Nine of the subjects were male (9/15, 60%), and six were female (6/15, 40%). The mean age was 60.8 ± 5.6 (mean ± standard deviation) years in a range of 51 and 71 years. The mean height, weight and BMI of subjects were 169.2cm, 89.7 kg and 31.3 kg/m^2, respectively.

Subject Disposition:

In this study, 23 patients were screened. 7 patients were classified as screening failures. The cohort of successfully screened patients comprises 16 patients. One patient was withdrawn after successful screening but prior to treatment. 15 patients were eligible for treatment and belong to the Full-Analysis-Set of all enrolled and treated patients. One treated patient discontinued the study prematurely after Visit 4 due to repeated fasting glucose > 140 mg/dL. 14 patients completed the study according to the trial protocol (Per Protocol Set).

Efficacy Results:

The percentage of patients who remained stable on the oral therapy as defined by an improvement (reduction) in HbA1c of at least 0.5% within 4 months, is the primary efficacy variable (PEV) of this study. PEV were analyzed in terms of an exact binomial test based on the FAS and the PP data sets. Five patients (PP-Set, 5/14, 35.7%; FA-Set, 5/15, 33.3%) showed a decrease in HbA1c of more than 0.5%. The mean HbA1c value decreases from 7.78 ± 1.03 % at study start to 7.37 ± 1.01 %. The mean individual difference of HbA1c from its baseline value to the final value is –0.41 ± 0.7 %. This reduction is statistical significant (p-value, 0.02, C.I., –0.8207 to -0.0059).

Variation of Efficacy Parameters from screening visit (V1, baseline values) to endpoint (V6)

Variable	Mean	Rel. Mean	STD	C.I. (95%)		t-value	p-value
HbA1c [%]	-0.4133	-5.313	0.7357	-0.8207	-0.005922	-2.176	0.02359
C-Peptide [µg/l]	-0.8467	-23.43	1.009	-1.406	-0.2878	-3.249	0.00291
Glucose [mg/dL]	-30.07	-17.54	30.96	-47.21	-12.92	-3.761	0.001053
HOMA Score	-3.591	-49.6	4.167	-5.899	-1.283	-3.337	0.002442
Insulin [mU/l]	-6.436	-38.57	8.228	-10.99	-1.879	-3.029	0.004505

Variation of Efficacy Parameters from start of insulin intervention (V2T1, baseline values) to endpoint (V6)

Variable	Mean	Rel. Mean	STD	C.I. (95%)		t-value	p-value
Adiponectin [µg/ml]	10.52	128.6	6.226	7.072	13.97	6.544	<0.0001
Glucose [mg/dL]	-28.96	-17.01	28.78	-44.9	-13.02	-3.897	0.00081
HDL [mg/dL]	9.533	23.6	5.817	6.312	12.75	6.347	<0.0001
HOMA Score	-4.196	-53.49	7.201	-8.184	-0.2084	-2.257	0.02026
hs-CRP [mg/l]	-0.9015	-39.85	1.429	-1.765	-0.03827	-2.275	0.02101
Insulin [mU/l]	-6.686	-39.48	12.31	-13.5	0.1316	-2.103	0.027

Variable	Mean	Rel. Mean	STD	C.I. (95%)		t-value	p-value
intact Proinsulin [pM]	-7.289	-39.94	12.1	-13.99	-0.5879	-2.333	0.01754
LDL [mg/dL]	10.27	8.964	23.9	-2.97	23.5	1.664	0.9408
Nitrotyrosine [nmol/l]	124.8	15.41	430.4	-113.5	363.2	1.123	0.8599
Total Cholesterol [mg/dL]	20.13	11.14	27.3	5.015	35.25	2.856	0.9937
Triglycerides [mg/dL]	-10.87	-5.473	79.98	-55.16	33.42	-0.5262	0.3035
Visfatin [ng/mL]	0.5955	1.424	9.032	-4.406	5.597	0.2554	0.5989

Variation of Efficacy Parameters from end of insulin intervention (V2T3) to endpoint (V6)

Variable	Mean	Rel. Mean	STD	C.I. (95%)		t-value	p-value
Adiponectin [µg/ml]	9.181	96.49	5.149	6.33	12.03	6.906	<0.0001
Glucose [mg/dL]	0.5733	0.4073	30.02	-16.05	17.2	0.07397	0.529
HDL [mg/dL]	6.4	14.7	5.926	3.118	9.682	4.183	0.0004603
HOMA Score	0.04832	1.342	1.756	-0.9241	1.021	0.1066	0.5417
hs-CRP [mg/l]	-0.6946	-33.79	1.211	-1.426	0.03723	-2.068	0.03045
Insulin [mU/l]	-0.1443	-1.388	4.863	-2.838	2.549	-0.1149	0.4551
intact Proinsulin [pM]	1.136	11.57	4.936	-1.597	3.87	0.8916	0.8062
LDL [mg/dL]	7	5.942	25.85	-7.314	21.31	1.049	0.844
Nitrotyrosine [nmol/l]	91.27	10.82	476.7	-172.7	355.3	0.7414	0.7647

Variable	Mean	Rel. Mean	STD	C.I. (95%)		t-value	p-value
Total Cholesterol [mg/dL]	15.53	8.381	31.96	-2.166	33.23	1.882	0.9596
Triglycerides [mg/dL]	24	14.66	71.28	-15.47	63.47	1.304	0.8934
Visfatin [ng/mL]	1.111	2.69	9.496	-4.148	6.37	0.453	0.6713

Variation of Amyloid B in the course of insulin intervention (from V2T1 to V2T2)

Variable	Mean	Rel. Mean	STD	C.I. (95%)		t-value	p-value
Amyloid B [pg/ml]	-11.72	-2.645	157.5	-98.92	75.48	-0.2883	0.3887

Safety Results:

There were no serious adverse events, deaths, or early terminations due to adverse events in this study**.** The most frequently reported adverse were nasopharyngitis, hypoglycemia and contusion.

The overall safety evaluation on the marketed medical products investigated in this study revealed no further issues in comparison to each summary of product characteristics in regard of i) the evaluation of adverse events and ii) the safety evaluation as assessed by monitoring of vital signs and safety laboratory. No further risk/benefit assessment deemed necessary in context with this study.

CONCLUSIONS:

This study shows that patients can remain stable on oral therapy with pioglitazone and metformin after an acute short-term insulin intervention. This is displayed by the significant reduction of the HbA1c values. The special study design offers different possibilities to consider the trial success, either for the insulin intervention period, the interval after insulin intervention to endpoint or spanning over the entire treatment phase.

For the complete trial period, significant improvements was shown for insulin-, intact proinsulin- and fasting glucose levels, hs-CRP values, lipid metabolism (assessed by HDL- and adiponectin values) and insulin resistance (HOMA Score). In contrast, no significant improvements were observed for LDL cholesterol, total cholesterol, triglycerides and visfatin. During the insulin intervention, insulin, intact proinsulin, fasting glucose levels, HOMA Score, HDL, triglyderides improved significantly whereas all other parameters investigated did not. In the period following

insulin intervention, significant improvements were shown for adiponectin, hs-CRP and HDL.

These data show that the improvements achieved by short-term insulin intervention can be maintained by oral antidiabetic therapy with pioglitazone and metformin.

It can be stated that the overall safety evaluation on the marketed medical products investigated in this study revealed no further issues in comparison to each summary of product characteristics in regard of the evaluation of adverse events and the safety evaluation as assessed by monitoring of vital signs and safety laboratory. No further risk/benefit assessment is deemed necessary in context with this study.

Significant Changes During Study:

Protocol Amendments:

Amendment 1 : August 24, 2006 : addition of HbA1 assessment at visits 4 and visit 5 and 1.2 mL blood was drawn additionally at both visits to evaluate HbA1c in the laboratory of ikfe GmbH.

Amendment 2: August 09, 2006 : exclusion criteria for sexually active woman of childbearing potential was restricted to hormonal intrauterine devices instead of intrauterine devices.

Amendment 3: January 04, 2007 : exclusion criterion concerning alcohol or drug abuse was constrained to a history of drug and alcohol abuse within the last 5 years prior to study participation.

The following changes in the study conduct were implemented by decision of the principle investigator as Notes to File:

The flow rate of the insulin perfusor was slightly increased to 40 IU Huminsulin® ad 40 ml 0,9% NaCl = 40 IU / 40 ml, 1 U/ml to minimize potential mistakes as well as the risk of blood clotting inside venous catheders.

At visit 4 (or visit 5, respectively) patients received an adequate amount of investigational medication sufficient for a daily intake until visit 5 (or visit 6), respectively. However, in patients with fasting blood glucose levels > 140 mg / dL or worsening of HbA1c > 0,5%, the patients were to return within one week for a follow-up of these parameters and check for a possible withdrawal. In order to avoid returning a high amount of unused investigational medication these patients received only metformin and pioglitazone sufficient for one week and if applicable received the remaining investigational medication at the follow-up visit.

Deviations from drug accountability above 120% were also to be considered as minor protocol violations.

Changes in the planned Analyses:

It was decided to exclude hs-CRP values > 10 mg/L from all statistical analysis as these values were regarded as a sign of a probable acute inflammation process which would bias the efficacy

evaluation of this laboratory parameter.

Study ID Number:

ATS K019/D-Pio-109

Other Study ID Number(s):

EudraCT No: 2006-002354-30

DATE OF SYNOPSIS: 08 April 2008