

Triolex[®] Clinical Development Backgrounder

for Obesity-induced Insulin Resistance and Type-2 Diabetes Mellitus

Harbor Biosciences is developing its proprietary compound Triolex, which is a synthetic derivative of a naturally occurring steroid in humans and is known to possess anti-inflammatory properties without immune-suppressive side effects. To date, four clinical studies have been conducted. Three are complete and one is ongoing. Of these, one was in healthy volunteers and two were in obese, insulin-resistant subjects. The fourth study was in type 2 diabetes mellitus (T2DM) subjects conducted in two stages: an exploratory trial in patients on a stable dose of metformin and a confirmatory trial that was conducted in drug-naïve patients. In the most recently completed study, a three-month clinical trial of patients with type 2 diabetes, those who were obese and took Triolex showed significantly-improved blood levels of hemoglobin A1c (HbA1c).

Triolex Phase I Single Dose Safety, Tolerability and Pharmacokinetics: HE3286-100

A single-dose, dose-escalation Phase I clinical trial was conducted during 2007 and demonstrated that Triolex is orally bioavailable in humans, with significant drug concentrations detected in the blood at all doses tested. The findings also showed that in healthy volunteers, all Triolex doses tested appeared to be safe and well-tolerated with no drug-related serious adverse side effects reported. Plasma concentrations of Triolex were dose proportional in the 10 to 100 mg dose range with an approximate 8-hour plasma half-life.

Triolex Phase I/II 28-Day Dose Escalation in Healthy Insulin-Resistant Obese Subjects: HE3286-0102

HE3286-0102 was a 4-week, multi-center, randomized, double-blind, parallel group, placebo-controlled Phase 1 clinical trial that evaluated the safety, tolerability and early signs of activity using either placebo or a 5 mg daily or twice-daily 2, 5 or 10 mg Triolex dose in obese, insulin resistant patients. The primary objectives for the trial were to evaluate safety, tolerability and its effects on insulin resistance. The trial was conducted using hyperinsulinemic euglycemic clamp technology to assess changes in insulin resistance after treatment. A total of 36 patients were enrolled in the study. In addition, six patients with T2DM were enrolled in an open-label cohort and were treated with 10 mg

(5 mg twice-daily) Triolex for 28 days. This study is now complete and the data have been analyzed.

Euglycemic/hyperinsulinemic clamp studies were performed on 36 subjects and the results in 34 cases were of adequate quality for interpretation. Although the entry criteria selected for obese subjects was impaired glucose tolerance, baseline clamp studies indicated that only 23 of the 34 were insulin resistant with an impaired insulin-stimulated glucose utilization rate (defined as $M < 5$ at baseline) and 11 of the 34 were the so-called “healthy obese” subjects ($M > 5$ at baseline).

Triolex significantly improved insulin sensitivity (Clamp M value) in all treated subjects. The effect was greater in insulin-resistant subjects ($M < 5$). At the two highest dose levels, Triolex increased insulin sensitivity compared to placebo (M value increase = + 2, $p < 0.001$). Triolex lowered day-28 fasting blood glucose in insulin-resistant subjects but without statistical significance compared to the placebo group (- 11 mg/dL, $p = 0.103$).

Elevated levels of C-reactive protein (CRP) and retinol binding protein-4 (RBP-4) are correlated with cardiovascular risk in T2DM. Serum RBP-4 was decreased significantly on day 29 by Triolex compared to insulin-sensitive subjects (day 29, -226,000 ng/mL, $p = 0.012$), and CRP was decreased on day 28 in insulin-resistant subjects (- 1.6 mg/L, $p = 0.14$) but the change from placebo did not reach statistical significance.

In concordance with the hypothesis of inflammation-induced insulin resistance, the insulin-resistant subjects had higher baseline LPS-stimulated PBMC inflammatory responses compared to insulin-sensitive subjects (MCP-1, + 2,100 pg/mL, $p = 0.007$; IL-6, + 8,600 pg/mL, $p = 0.048$; IL-1 β , + 2,800 pg/mL, $p = 0.053$; and TNF α , + 800 pg/mL, $p = 0.018$). Following 28 days of treatment, Triolex substantially reduced LPS-stimulated PBMC cytokines in insulin-resistant subjects compared to insulin-sensitive subjects (day 42, MCP-1, - 43,000 pg/mL; IL-6, -17,000 pg/mL; IL-1 β , -3,600 pg/mL; TNF α , -5,300 pg/mL), compared to placebo and (MCP-1, -43,000 pg/mL; IL-6, -16,300 pg/mL; IL-1 β , -2,400 pg/mL; TNF α , -1,700 pg/mL) compared to insulin-sensitive subjects. However, these analyses did not reach statistical significance.

Triolex was safe and well tolerated in both obese insulin-resistant and T2DM subjects at all dose levels tested. Of all adverse events reported, 23% were in the placebo group and an average of 15% was in the treated groups (range 6 – 21%). Of these adverse events,

15% reported in the placebo group were assessed as possibly related to study treatment as compared to an average of 15.8 % in the treated groups (range 0-35 %).

Triolex Phase IIa Insulin Resistance in Type 2 Diabetes: HE3286-0401

During 2008, Harbor BioSciences initiated a two-stage clinical trial in the U.S. with Triolex in T2DM patients. HE3286-0401 was a 12-week, multi-center, randomized, double-blind, parallel group, placebo-controlled Phase IIa trial that evaluated the safety, tolerability and early signs of activity using a twice-daily 5 mg dose of Triolex dose placebo in patients with T2DM. The intent to treat population consisted of 164 patients randomized in a 1:1 ratio (95 to the exploratory stage and 69 to the confirmatory stage). The primary objectives for the trial were to evaluate safety, tolerability and effects on HbA1c. Stage 1 was exploratory and conducted in patients on a stable dose of metformin treatment only, the current first-line therapy for type 2 diabetes; Stage 2 was confirmatory and conducted as a single agent in drug naïve patients.

Stage I

Stage I of this trial enrolled 95 patients who were on a stable dose of metformin with an HbA1c level in excess of 7.5% (mean 8.7 % for both groups). The primary objectives of the trial were to evaluate the safety and tolerance of Triolex 10 mg per day (5 mg BID) and the change in HbA1c in the treated group compared to placebo. The initial entry criteria did not specify limitations on BMI, but upon interim analysis was amended to accept only individuals that were overweight (BMI \geq 26.0).

As previously reported, an interim analysis of Stage I of the trial failed to show beneficial changes for day 84 HbA1c in the entire Triolex-treated study population when compared to the placebo-treated subjects. On completion of the study, a final analysis on all subjects (72 patients) who completed 12 weeks of dosing again revealed no statistical difference between treatment and placebo groups for changes in HbA1c in the overall patient population.

A retrospective analysis was performed on a subpopulation of patients that represented the inflamed, obese, insulin-resistant diabetic population, which is similar to the insulin-resistant subjects who responded to treatment in the prior healthy insulin-resistant obese Phase I study (HE3286-0102). That analysis included 31 patients that responded to Triolex who had a baseline body mass index (BMI) greater than or equal to 28; fasting plasma insulin levels greater than or equal to 4 μ U/mL; fasting plasma C-peptide levels greater

than or equal to 2 ng/mL; and serum monocyte chemotactic protein-1 (MCP-1) levels greater than or equal to 400 pg/mL. This phenotype represented 35% of all subjects evaluable at baseline (89 patients). Twenty-two of these 31 individuals completed 12 weeks of treatment. Those treated with Triolex (10 patients) showed improvement in a variety of relevant clinical parameters when compared to placebo (12 patients). Statistical trends were found for a decrease in day 84 HbA1c (-0.53%, p=0.08) and fasting plasma glucose (-28.75 mg/dL, p=0.09). Triolex decreased HbA1c compared to placebo at least -0.5% in 4/10 patients compared to 1/12 patients at day 84 and 3/10 patients compared to 2/12 patients at day 112. Fasting plasma C-peptide (-0.43 ng/mL), fasting plasma insulin (-0.48 μ U/mL), fructosamine (-25.75 μ mol/L) and HOMA2 insulin resistance (-0.65 IR) all decreased and HOMA2 insulin sensitivity (11.3 % S) and HOMA2 beta cell function (17.95 % B) both increased but none with significance. The observed changes in these secondary indicators of activity are all consistent with the observed change in HbA1c and glucose in this obese, inflamed, diabetic subpopulation.

Stage II

In 2009, Harbor BioSciences initiated a Stage II follow-on study in drug-naïve T2DM subjects using the Stage I responder phenotype as the inclusion criteria. This study is now complete, with 69 patients enrolled, and the data have been analyzed. There was no significant difference in the overall Triolex-treated patients on day 84- or day 112- HbA1c when compared to placebo-treated patients.

In a post-hoc analysis, Triolex benefit was found in obese subjects with a BMI \geq 31.3. A significant time-dependent decrease in HbA1c was observed with a change from baseline of -0.55% at day 84 and of -1.1% at day 112 (p=0.041). In this obese subgroup, Triolex decreased HbA1c at least -0.5 % in 5/9 (56 %) patients compared to 3/14 (21 %) in placebo patients at day 84 and 7/9 (78 %) compared to 3/13 (23 %) in placebo patients at day 112. Furthermore, Triolex reduced HbA1c at least -1.0 % in 5/9 (56 %) compared to 2/11 (18 %) in the placebo group on day 112. Fasting blood glucose and fructosamine levels were decreased but did not reach statistical significance when compared to placebo. The treatment effect on HbA1c plasma concentration did not correspond to an effect on fasting plasma glucose.

This observation lead to measurement of the treatment effects on post-prandial glucose using the GlycoMark assay, which measures post-prandial glucose excursions using the 1,

5-anhydroglucitol biomarker. Results showed that the Triolex-treated responder phenotype had a decrease in post-prandial hyperglycemic exposure, whereas the placebo subset had no change. The anti-inflammatory activity of Triolex was evaluated and there was a statistical trend for decreased CRP (p=0.053); day 84, (p=0.09), and a non-significant loss of 1.5 kg in Triolex-treated subjects when compared to placebo on day 84.

HE3286-0401 Meta Analysis

At the conclusion of the Phase IIa trial, a meta-analysis was conducted of the responding subgroups from Stage I and Stage II to increase patient numbers and statistical power for the secondary variables and biomarkers of inflammation. The baseline parameters were the same for both subgroups except for BMI. The overweight metformin-treated patients with BMI ≥ 28 kg/m² responded (Stage I), whereas the Stage II drug-naïve subjects (no metformin) did not respond unless they were obese, BMI ≥ 31.3 kg/m². The reason for this difference between the two groups is unknown, but it is believed to be related to additive beneficial effects of Triolex and metformin in the overweight group.

In the analysis, Triolex progressively decreased HbA1c to - 0.5 % HbA1c at day 84 (n=19, p=0.04), compared to placebo, + 0.05 % HbA1c (n = 26). Overall, Triolex decreased HbA1c at least -0.5 % in 9/19 patients compared to 4/26 in placebo patients at day 84 (p< 0.04) and 10/19 compared to 5/25 in placebo patients at day 112 (p< 0.03). Triolex reduced HbA1c at least -1.0 % in 6/19 patients (32 %) compared to 2/25 (8 %) in the placebo group on day 112. Triolex showed a non-significant decrease in day 84 blood glucose (-10 mg/dL, p = 0.105), compared to placebo, which showed essentially no change (-0.75 mg/dL).

The GlycoMark test for post-prandial excursions showed Triolex significantly improved the urinary biomarker reabsorption on day 84 (+1.8 μ g/mL, p=0.038), indicating a major effect on post-prandial glucose control compared to placebo. The placebo showed essentially no change - 0.1 μ g/mL). In addition, Triolex showed a significant increase in HOMA2 insulin sensitivity (+ 4.2 HOMA2 %S, p=0.025), and a significant decrease in insulin resistance (-0.43 HOMA2 %IR, p=0.03) on day 57.

These results suggest that Triolex decreased obesity-induced inflammation that otherwise leads to insulin resistance in T2DM.

Triolex Clinical Safety Summary to Date

In the three completed studies, Triolex was found to be safe and well tolerated when administered orally for up to 84 days in normal; obese insulin-resistant; and T2DM subjects. There were no trends in any adverse event with Triolex compared to placebo-treated subjects. Specifically, there were no trends in laboratory values or physical examination, including electrocardiograms.

A maximum-tolerated dose has not been established. The 10 mg dose was used in these studies to explore translation of activity from rodent models into type 2 diabetics. The dose has an estimated margin of safety greater than 80 based on plasma drug exposure estimates. Accordingly, Triolex was found to be safe and well tolerated in subjects with T2DM, both in combination with metformin and treatment-naïve subjects. Seventy one percent of placebo and Triolex-treated subjects reported adverse events. Most of these events were mild to moderate in severity with 29% in the placebo group assessed as possibly or probably related to study drug in contrast to 19 % in the Triolex treated group. One SAE was reported as possibly related to Triolex, which was an elevated amylase.

Triolex Site-of-Action in Obese, Insulin-Resistant Subjects: HE3286-0103

In 2009, a study was initiated to identify the Triolex target organs in obese, impaired glucose tolerant subjects. This study is in progress and is designed to study the drug effects on liver glucose output and muscle glucose uptake in response to insulin.

Insulin Resistance

Insulin resistance (IR) is a condition in which body cells become less sensitive to the glucose-lowering effects of the hormone insulin. Fat and muscle cells require insulin to utilize glucose. The liver does not require insulin to absorb glucose, but responds to insulin by reducing glucose output. In most cases of IR in humans, normal blood levels of insulin become inadequate to keep blood glucose within a normal range.

Insulin resistance in muscle and fat cells reduces glucose uptake (and increases local storage of energy as glycogen and triglycerides, respectively), whereas insulin resistance in liver cells results in reduced glycogen synthesis and storage, and a failure to suppress glucose production and release into the blood. Insulin resistance normally refers to the reduced glucose-lowering effects of insulin. However, other functions of insulin can also be affected. For example, insulin resistance in fat cells reduces the normal effects of insulin on lipids and results in reduced uptake of circulating lipids and increased hydrolysis of stored triglycerides. Increased mobilization of stored lipids in these cells elevates free fatty

acids in the blood plasma. Elevated blood fatty-acid concentrations (associated with insulin resistance and T2DM), reduced muscle glucose uptake, and increased liver glucose production all contribute to elevated blood glucose levels. High plasma levels of insulin and glucose due to insulin resistance are a major component of the metabolic syndrome. If insulin resistance exists, more insulin needs to be secreted by the pancreas. If this compensatory increase does not occur, blood glucose concentrations increase to initiate the disease T2DM.

Inflammation and Insulin Resistance

Chronic activation of intracellular proinflammatory pathways within insulin-target cells can lead to obesity-related insulin resistance. Several cytokines and chemokines, such as CCL2, IL-6, IL-1 β , macrophage inhibitory factor (MIF) and TNF- α can be released by both adipocytes and macrophages. Consistent with this, elevated proinflammatory cytokines TNF-alpha and IL-6 have been shown in individuals with insulin resistance and diabetes.

Triolex Mechanism of Action

It is believed that the mechanism of action for Triolex may be the regulation of the MAP kinase, NFkappaB and other proinflammatory pathways, particularly when these are stimulated through the TLR-4 receptor. TLR-4 is a receptor expressed in the cell surface of macrophages and other cells that is stimulated by certain pathogens such as bacteria and viruses, or certain nutrients such as dietary fatty acids. Upon stimulation of the TLR-4 receptor, a cascade of proinflammatory kinases that include IKK, JNK and p38 is activated, setting off a complex network of signaling pathways, which culminate with the activation of NFkappaB and a number of genes involved in the inflammatory and cell stress response. Stimulation of the inflammatory kinases JNK, p38 and IKK can also impair insulin signaling by inhibiting the biological function of IRS-1, a protein that acts as a major mediator of insulin action in target cells. This inflammatory pathway that leads to insulin resistance is now well characterized in the scientific literature (“TLR4 Links Immunity and Fatty Acid-Induced Insulin Resistance,” *The Journal of Clinical Investigation*, Volume 116, Number 11, pp 3015-25). In addition, activation of NFkappaB due to inflammatory mediators or oxidative stress leads to a feed-forward cycle of increased production of inflammatory cytokines such as TNF-alpha, IL-6, IL-1beta and MCP-1. In experiments with macrophages *in vitro*, Triolex appears to act upstream of

NFkappaB, attenuating the response of IKK, JNK and p38 to endotoxin (LPS) or fatty acid stimulation, restoring the function of IRS-1.

Triolex also appears to act independently of the PPAR-gamma pathway and thereby may avoid the side effects associated with the current glitazone class of insulin-sensitizing agents, such as Avandia® and Actos®, which work through the PPAR-gamma pathway. Side effects reported to date with the glitazone class of drugs include weight gain, edema and increased cardiovascular events. Experiments *in vitro* have shown no evidence that Triolex directly binds and/or activates (transactivates) the PPAR-gamma receptor. Unlike the glitazones, Triolex does not cause body weight gain when administered to mice or rats. These and other observations are consistent with Triolex acting through a different pathway than the PPAR-gamma receptor. These data, together with results of Triolex in the standard *in vivo* mouse models of insulin resistance, have recently been published in the peer reviewed scientific literature. (“Amelioration of glucose intolerance by the synthetic androstene HE3286: link to inflammatory pathways,” Wang T, Villegas S, Huang Y, White SK, Ahlem C, Lu M, Olefsky JM, Reading C, Frincke JM, Alleva D, Flores-Riveros J. J Pharmacol Exp Ther. 2010 Apr;333(1):70-80).

In the ZDF model, animals typically exhibit hyperphagic behavior (overeating) due to a genetic lesion causing them to become obese, which over time contributes to progressive deterioration of glucose homeostasis resulting from insulin resistance and defective beta-cell function. Treatment of ZDF rats with Triolex is associated with complete normalization of fasting and fed blood glucose levels throughout the study. Glucose tolerance tests have demonstrated that insulin sensitivity was improved by treatment with Triolex as indicated by markedly blunted blood glucose elevation after an oral glucose load. In addition, Triolex reduced the concentration of pyruvate and glycerol in the blood, two important precursor molecules that the liver normally utilizes to make more glucose, a process called gluconeogenesis. Since excessive glucose production by the liver is a major contributor to fasting hyperglycemia in the diabetic state, the observation that Triolex blocks hepatic glucose output in ZDF rats provides an additional piece of information to understand the physiological mechanism of action of the compound. In these animals, there was also a decrease in the concentration of free fatty acids in the blood after treatment with Triolex, which when considered together with the observed reduction in

glycerol levels, suggests that Triolex may suppress excessive lipolysis in adipose tissue, an effect consistent with a drug-induced improvement in insulin sensitivity in fat tissue.

In addition to the beneficial effects of Triolex on glucose homeostasis, liver cholesterol and triglyceride content were also reduced after drug treatment, causing the activation of a physiological feedback loop whereby the expression levels of the LDL receptor are elevated, thereby facilitating cholesterol uptake by the liver. Accordingly, treatment with Triolex caused a marked decrease in total serum cholesterol. Previously reported preclinical work *in vitro* and in other animal models indicated that Triolex attenuates proinflammatory pathways in macrophages, leading to the hypothesis that Triolex improves insulin action as a result of an anti-inflammatory effect which reduces the negative impact of obesity-induced inflammation on insulin signaling. Consistent with this hypothesis, treatment of ZDF rats with Triolex down-regulates expression of a number of cytokines and other inflammatory effectors in liver and adipose tissue, including MCP-1, which is involved in macrophage infiltration in fat. Accordingly, Triolex reduced the degree of macrophage infiltration in adipose tissue, which is consistent with an anti-inflammatory effect that curbs the contribution that macrophage inflammatory pathways are thought to have in causing insulin resistance. These results have recently been published in the peer reviewed scientific literature (“A new antidiabetic compound attenuates inflammation and insulin resistance in Zucker diabetic fatty rats,” Lu M, Patsouris D, Li P, Flores-Riveros J, Frincke JM, Watkins S, Schenk S, Olefsky JM., *Am J Physiol Endocrinol Metab.* 2010 May; 298(5)).

Measurements Used in Diabetes Research

Hyperinsulinemic Euglycemic Clamp

The gold standard for investigating and quantifying insulin resistance is the “hyperinsulinemic euglycemic clamp,” so-called because it measures the amount of glucose necessary to compensate for an increased insulin level without causing hypoglycemia. The test is used in medical research to assess the effects of different medications on the rate of glucose infusion and is commonly referred to in diabetes literature as the “M” value.

GlycoMark, and the 1, 5-Anhydroglucitol Biomarker

The GlycoMark assay measures blood levels of 1,5-anhydroglucitol (1,5-AG). It is found in nearly all foods and is ingested during the course of a regular diet. Once ingested,

1,5-AG is nearly 100% non-metabolized and remains in a relatively constant amount in the blood and tissues. A small amount (equal to the amount ingested) of 1,5-AG is released in the urine to maintain a constant amount in the blood and tissue. This process occurs in non-diabetic people as well as people with diabetes who do not have their blood glucose values rising over 180 mg/dL.

When a diabetic person's blood glucose exceeds 180 mg/dL for any period of time, the kidney attempts to re-absorb glucose back into the blood. Any glucose that cannot be re-absorbed is excreted in the urine in a process known as glycosuria. During times of glycosuria, the additional amount of glucose in the kidney blocks 1,5-AG from being re-absorbed into the blood and 1,5-AG is excreted in the urine at a higher rate than normal. Due to the lack of 1,5-AG being re-absorbed, blood levels of 1,5-AG decrease and continue to decrease until glucose values go below 180 mg/dL. Once hyperglycemia is corrected, 1,5-AG begins to be re-absorbed from the kidney back into the blood at a steady rate. If a person's glucose levels remain below 180 mg/dL for approximately 4 weeks, 1,5-AG will return to its normal levels. It is this competitive inhibition of 1,5-AG by glucose that allows GlycoMark to accurately reflect time integrated hyperglycemic episodes over 180 mg/dL.

HbA1c

A form of hemoglobin, HbA1c indicates the average plasma glucose concentration over a period of approximately three months. Glycated hemoglobin is a form of hemoglobin used primarily to identify the average plasma glucose concentration over prolonged periods of time. It is formed irreversibly in a non-enzymatic pathway by hemoglobin's normal exposure to high plasma levels of glucose. Glycation of hemoglobin has been associated with cardiovascular disease, nephropathy and retinopathy in diabetes mellitus.

About Triolex

Triolex is a chemically modified version of a naturally occurring hormone found in the human body, a 7 β -hydroxylated metabolite of the well-known 5-androstenediol. The compound has been studied in diverse rodent models of inflammation and is known to possess anti-inflammatory properties without immune-suppressive side effects. Harbor BioSciences believes that one of the key functions of the natural hormone in the human body is to restore immunological and metabolic homeostasis under conditions of

unproductive chronic inflammation such as that associated with a variety of autoimmune diseases and insulin resistance and glucose metabolism.

About Type 2 Diabetes Mellitus

Diabetes is characterized by high levels of blood glucose caused by insufficient action of insulin and inadequate production of glucose by the liver. It can lead to serious medical complications and death. In the United States, more than 10% of adults over the age of 40 have diagnosed or undiagnosed diabetes, and rates are anticipated to increase in the coming years. Type 2 diabetes is the predominant form of diabetes, accounting for 90 to 95% of diagnosed cases.

About Metformin

Metformin is an oral anti-diabetic drug in the biguanide class. It is the first-line drug of choice for the treatment of T2DM, particularly in overweight and obese people and those with normal kidney function.

About Harbor Biosciences

Harbor BioSciences is a development-stage company with two product candidates in clinical trials: Apoptone® in the cohort expansion portion of a Phase I/IIa trial of patients with late-stage prostate cancer; and Triolex® in a Phase IIa trial in obese type 2 diabetes mellitus patients. The company is using its novel, proprietary C-19 steroid human metabolome-based chemical platform to develop potent and effective medicines to treat inflammation by harnessing the therapeutic potential of newly discovered components of the human metabolome.

Apoptone and Triolex represent the lead candidates from Harbor BioSciences' small molecule platform based on metabolites or synthetic analogs of endogenous steroid hormones.