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Lilly-Sponsored Controlled Clinical Trials of GH Treatment of Short Stature in Turner Syndrome

1. Introduction

Humatrope (somatropin) is a recombinant DNA-derived human growth hormone, identical in amino acid sequence to the 22 kilodalton native human growth hormone. In 1987, Humatrope was approved (NDA 19-640) for use as replacement therapy "for the long-term treatment of children who have growth failure due to an inadequate secretion of normal endogenous growth hormone". It has also been approved for the treatment of short stature due to Turner syndrome in 32 countries (Europe and Japan). This briefing document is submitted to the Endocrine Advisory Committee of the Food and Drug Administration in support of the application by Eli Lilly and Company, for approval of Humatrope (recombinant human growth hormone; somatropin) for the indication of treatment of short stature and growth failure due to Turner syndrome.

2. Overview of Lilly-Sponsored North American Clinical Trials

The three Lilly-sponsored randomized, controlled clinical studies in progress in North America were commenced in 1987 (USA, B9R-MA-GDCI), 1988 (USA, B9R-MA-GDCK), and 1989 (Canada, B9R-CA-GDCT). Each study was designed, after final amendments, to evaluate the safety and efficacy of Humatrope treatment given to patients with Turner syndrome with the intent of treating to final height. Two of these studies (B9R-MA-GDCI and B9R-CA-GDCT) form the basis of this submission. The third study (B9R-MA-GDCK) has not yet completed enrollment.

The Canadian study (B9R-CA-GDCT), referred to throughout this document as GDCT, which is an ongoing randomized, open-label, concurrent non-treatment control study was designed to determine the effect of recombinant human growth hormone (rhGH; Humatrope) on final height of patients with Turner syndrome. Enrollment for this study, which is being conducted by 13 principal investigators at 13 study centers, has closed. Forty-six of the 154 patients have now completed the study. The analysis of final height data reveals a significant increase of 5.4 cm in final height of GH-treated versus control patients with Turner syndrome. Apart from an increase in the frequency of otitis media (a condition that occurs commonly in Turner syndrome) in GH-treated patients, there were no new safety concerns regarding the use of GH in this patient population. These data support the efficacy and safety of GH treatment in increasing the final height of patients with Turner syndrome.

The multicenter study nearing completion in the United States (B9R-MC-GDCI), referred to throughout this document as GDCI, is a randomized, placebo-controlled (for

18 months), dose-response study evaluating the effect of two different doses of GH, (with or without early introduction of low dose estrogen), on linear growth in patients with Turner syndrome. Patient enrollment for this study, which is being conducted at 50 centers, is complete. At the time of the present analysis, 31 of the 232 patients enrolled had completed the study, 63 patients remained active and 138 patients had discontinued early, primarily due to patient satisfaction with height attained. Interim analysis of this study indicates that patients in both of the GH dosage groups have had a significant increase in height relative to the height standards for the normal female population or for patients with Turner syndrome evidenced by increases in height standard deviation (SD) scores between baseline and protocol completion. There is evidence of a mild dose-response effect, since patients in the higher dosage group had a greater increase in mean height SD score than those in the lower dosage group. No new safety concerns have arisen during this study. These results support the conclusion of the Canadian study (GDCT) that GH is effective in significantly increasing the height of patients with short stature due to Turner syndrome.

The studies described above were designed to evaluate in a prospectively controlled fashion issues that had not been addressed fully by previous uncontrolled studies, including the effect of pharmacologic treatment with recombinant human growth hormone on final height and the relationship of growth hormone dose to outcome. In addition, these studies were designed to be of long duration, and to include large patient numbers. The status of the Lilly-sponsored studies detailed in this report is summarized in Table 1.

Table 1. Lilly-Sponsored Studies of Growth Hormone Treatment of Short Stature in Turner Syndrome

	Study B9R-CA-GDCT	Study B9R-MC-GDCI
Location	Canada	U.S.
Design	Randomized Open-label Non-treatment concurrent control	Randomized Placebo-controlled Dose-response
Enrollment	154 Closed	232 Closed
Completed Protocol*	46	31
Key Outcomes	Final Height Safety	Dose Response Final Height Early Low Dose Estrogen Effect Safety

*Protocol completion defined as: GDCT - bone age ≥ 14 years; growth velocity < 2 cm per year
GDCI - bone age ≥ 15 years; growth velocity < 2 cm per year

Efficacy Analyses: Canadian Study, B9R-CA-GDCT

Study B9R-CA-GDCT is an ongoing, randomized, parallel, open-label study to final height in patients with Turner syndrome, being conducted at 13 study centers by 13 principal investigators. The primary objective of this study is to determine the efficacy of GH in promoting an increase in final height in patients with Turner syndrome. The study design evaluates the effect of recombinant human growth hormone (rhGH; Humatrope®; hereafter referred to as GH) by comparison of GH-treated with a concurrent non-GH treated Control group. The second objective of this study is to determine the antigenicity and other variables of clinical safety of GH in these patients.

1. Population

Patients were included in the study if they fulfilled the following criteria: karyotypically-proven Turner syndrome; age greater than 7 years and less than 13 years; growth velocity <6 cm/year; height ≤10th percentile of the normal female standard [NCHS Growth Charts]; prepubertal status; absence of diabetes or other significant chronic disease. Before a patient was enrolled in the study, informed consent from a parent or guardian was obtained.

At closure of enrollment in this study, there were 154 patients, 76 in the GH-treated group and 78 in the non-GH-treated Control group. Of these, there are baseline data available for 140 patients, 75 in the GH-treated group and 65 in the Control group.

2. Study Design

At enrollment, patients were stratified for height into 3 groups according to a scheme based on the height data for untreated patients with Turner syndrome from the study of Lyon et al. (Lyon et at. 1985). There were three designated stature strata (Lower, Middle, and Upper), each including approximately one-third of the enrolled patients. To ensure that there would be no bias in the assignment of patients to treatment and control groups, patients were randomly assigned, after stratification, to one of the two following groups:

1) GH-treated (Humatrope®) - also referred to in the documents submitted to the FDA as the Humatrope group or GH05 group. These patients received GH 0.05 mg/kg/dose, 6 times per week (0.30 mg/kg/week) by subcutaneous injection.

2) Non-GH-treated control - also referred to in documents submitted to the FDA as Untreated. This group received no GH therapy. Hereafter this will be referred to as the Control group.

This method of stratification and randomization ensured homogeneity between treatment groups and allowed for adjustment for baseline stature stratum in the assessment of efficacy.

After 12 months in the study, all patients at least 13 years old in both the GH-treated and Control groups were prescribed ethinyl estradiol. The ethinyl estradiol dosage was controlled across the study, beginning at a low dose of 2.5 μg daily and increasing between 13 and 15 years of age, as detailed below. Medroxyprogesterone acetate was added after one year of ethinyl estradiol therapy, and cyclic therapy introduced. Details of the estradiol and progesterone treatment in this study are as follows:

- No ethinyl estradiol or medroxyprogesterone acetate are administered to patients less than 13 years old.
- After completion of a minimum of 12 months in the study, patients in each group at least 13 years old receive 2.5 μg of ethinyl estradiol daily (half of a 5 μg tablet).
- Patients in each group at least 14 years old but not yet 15 years old receive 5.0 μg of ethinyl estradiol daily.
- After one year of treatment with 5 μg ethinyl estradiol daily, the dose is increased for patients at least 15 years of age to 20 μg of ethinyl estradiol per day from the first day of the month for 24 days. For the last 10 days of ethinyl estradiol therapy, (Days 15-24), 10 mg of medroxyprogesterone is given orally. On Day 24 both the ethinyl estradiol and medroxyprogesterone are suspended. On the first day of the following month, ethinyl estradiol is restarted and the cycle repeated as above.

3. Patient Accountability

At closure of enrollment in Study GDCT there were 154 patients randomized, 76 to the GH-treated group and 78 to the Control group. Fourteen of the 154 randomized patients were not included in any data analyses. Twelve of these 14 patients withdrew without completing Visit 1; two patients completed Visit 1, but their data have not been received by the sponsor. Thirteen of these 14 patients had been randomized to the Control group. Therefore, of the 154 patients initially randomized, there are baseline data available for 140 patients, 75 in the GH-treated group and 65 in the Control group. At the time of this analysis, 46 case report forms had been received by Lilly, designated as "protocol complete."

For analysis of the safety of GH treatment in this study, the ***safety population*** is defined as those patients who were randomized, and either received any study medication (in the GH-treated group) or had post-baseline safety data (in the Control group). The safety population comprises 136 patients (97% of the 140 randomized

patients with baseline data). Of the four randomized patients not included in the safety analysis, two decided not to continue shortly after being randomized and had no post-baseline data, and two violated the entry criteria.

The ***population with ≥ 6 months efficacy data*** is comprised of those patients who were randomized and have efficacy data at Visit 3 (180 days after randomization) or beyond; 134 patients (96% of the patients randomized with baseline data) comprise this population. Six patients were not included in this population; four were those excluded from the safety population, one additional patient decided to leave the study before Visit 3, and one violated the entry criteria.

The group analyzed as ***protocol completers*** comprises those patients designated by the investigators as such. In most cases these patients fulfilled study criteria for completion, however, there were a number of patients who were found, upon analysis of their data, to have not quite met the criteria for completion. Never the less, as they had been discontinued as protocol complete, they were included in the analyses. At the closure of data collection for this interim analysis (February, 1996), 46 (33%) of the 140 randomized patients with baseline data had completed the study, having achieved final height according to the investigators. Twenty-five patients (18%) were discontinued from the study for a variety of reasons, and 69 (49%) remained in the study. Table 2 summarizes patient accountability for Study GDCT.

Table 2. Summary of Patient Accountability: Study GDCT

Patient Disposition	Overall	Control	GH-Treated
All Randomized	154	78	76
Discontinued without Data	14	13	1
Randomized with Data	140	65	75
Safety Population	136 (97%)	62 (95%)	74 (99%)
Population With ≥ 6 Months Efficacy Data	134 (96%)	60 (92%)	74 (99%)
Protocol Complete	46 (33%)	19 (24%)	27 (36%)
Discontinued	25 (18%)	17 (26%)	8 (11%)
Ongoing	69 (49%)	29 (45%)	40 (53%)

Percentages relative to number of randomized patients with data.

4. Baseline Characteristics

A summary of ethnic origin and stature stratification at baseline by treatment group for the 134 patients in the population with at least 6 months efficacy data is presented in Table 3. Baseline age and height data are presented for all patients who had at least 6 months efficacy data in Table 4 and for the subgroup that completed the protocol, in Table 5.

Table 3. Ethnic Origin and Baseline Stature Strata - All Patients With ≥ 6 Months Efficacy Data - Study GDCT

Characteristic	Control (n=60)	GH-Treated (n=74)	p-value[1]
Ethnic Origin			
Caucasian	48 (80.0%)	65 (88%)	0.287[2]
All Other	12 (20%)	9 (12%)	
Hispanic	0	2	
Native American	1	1	
Asian	7	6	
Other	4	0	
Stature Strata			
Lower	16 (27%)	18 (24%)	0.490
Middle	25 (42%)	26 (35%)	
Upper	19 (32%)	30 (41%)	

* Statistically significant at $p < 0.050$.
[1] p-value tests proportions of patients for homogeneity between the treatment groups.
[2] p-value for origin is based on testing Caucasian relative to all other origins combined.
Note: Percentages relative to number of patients in the population with ≥6 months efficacy data

There was no significant difference between the GH-treated and Control groups for ethnic origin or proportions of patients within each stature stratum.

Of the 134 patients comprising the population with ≥ 6 months of efficacy data, 60% of each group (GH-treated and Control) had 45,X karyotypes. The predominance of patients with 45,X karyotype was also seen in the subgroup of patients who had completed the protocol, with a slightly higher percentage in the GH-treated group than the Control group (63% vs 53%).

Table 4. Baseline Data – All Patients With ≥ 6 Months Efficacy Data - Study GDCT

Characteristic Mean ± SD	Control (n=60)	GH-Treated (n=74)	p-value[1]
Age (y)	10.5 ± 1.8	10.4 ± 1.8	0.709
Bone Age (y)	8.6 ± 1.5 (n=58)	8.8 ± 1.4 (n=73)	0.272
Height (cm)	119.6 ± 8.4	119.9 ± 8.5	0.726
Height SDS [NCHS][2]	-3.3 ± 0.8	-3.2 ± 0.8	0.450
Height SDS [Lyon][3]	-0.2 ± 0.9	-0.1 ± 0.9	0.258
Midparental Height (cm)	159.4 ± 6.0 (n=58)	161.4 ± 6.2 (n=71)	0.041*
Pretreatment Growth Velocity (cm/y)	4.1 ± 1.0	4.2 ± 1.1	0.360

* Statistically significant at p≤0.050.
[1] p-value is for comparison of means between treatment groups.
[2] Normal female reference standard.
[3] Turner syndrome reference standard.

There was no significant difference between the GH-treated and Control groups for baseline variables including age, bone age, height, height standard deviation score (SDS) [NCHS], height SDS [Lyon], and pretreatment growth velocity (cm/yr). However, a difference between the GH-treated and Control groups was observed for midparental height, which was on average 2 cm greater in the GH-treated group (p=0.041; Table 4).

Table 5. Baseline Data – Protocol Completers - Study GDCT

Characteristic Mean±SD	Control (n=19)	GH-Treated (n=27)
Age (y)	11.7 ± 1.3	11.7 ± 1.1
Bone Age (y)	9.7 ± 1.0	9.8 ± 1.1
Height (cm)	126.3 ± 6.2	123.8 ± 6.9
Height SDS [NCHS][1]	-3.2 ± 0.9	-3.5 ± 0.8
Height SDS [Lyon][2]	0.2 ± 0.7	-0.3 ± 0.9
Midparental Height (cm)	158.8 ± 6.0 (n=18)	160.6 ± 7.0 (n=27)
Pretreatment Growth Velocity (cm/y)	4.1 ± 1.1	3.9 ± 0.6

[1] Normal female reference standard.
[2] Turner syndrome reference standard [Lyon].

Table 5 summarizes baseline data for the 46 patients analyzed as protocol completers. The groups were fairly similar for most parameters. Although the Control patients had mean height approximately 2.7 cm greater than the GH-treated patients, this difference was not statistically significant.

5. Efficacy Results

Final Height of Protocol Completers

Criteria for achievement of final height were bone age ≥14 years and growth velocity <2 cm/year. Of the 134 patients included in the population with ≥ 6 months of efficacy data, 46 patients were considered to have completed the protocol: 27/74 (37%) patients in the GH-treated group and 19/60 (32%) patients in the Control group. During the present analysis it was determined that 14 of the patients declared as having completed the protocol did not completely meet the protocol criteria for achievement of final height: all had bone ages ≥13.5 years and all but one had growth velocities of ≤3 cm/year. Nevertheless, as these patients were felt by the investigators to have achieved close to their final heights, the group was analyzed as a whole. To account for any potential baseline imbalances between treatment groups, an ANCOVA analysis was performed with adjustments for midparental height, stature strata, and geographically pooled sites.

Final height data for the group analyzed as protocol complete are summarized in Table 6 and Figure 1

By ANCOVA analysis the mean height of the GH-treated patient group was 5.4 cm greater than that of the Control group. This difference was highly significant ($p<0.001$).

Following submission of the Lilly supplemental NDA, one patient in the non-GH-treated Control group was identified as having not been included in the ANCOVA analysis for height gain due to missing midparental height data. Since this patient was a tall control patient , an estimated correction was made for the missing data and the patient was included for a reanalysis by ANCOVA. In this analysis, the height gain for treated versus non-treated patients was 4.9 cm. In addition, at the request of the FDA, an analysis of the efficacy data for the 38 patients who met the strict criteria for protocol completion was undertaken. In an ANCOVA analysis that corrects for the one missing midparental height, the mean height of the GH-treated group was 6.4 cm greater than that of the Control group for the patients who met the strict definition of protocol completion. These supplemental data are summarized in Appendix C. We conclude that, with the most conservative analysis, GH-treated patients who begin therapy on average by 11 years of age demonstrate a mean height gain of 5 cm as compared to non-GH-treated patients.

As supporting evidence for the efficacy of GH treatment of the short stature in Turner syndrome, final height was also evaluated by analyzing standard deviation scores (SDS) for height (Table 6). The height SDS (also known as Z-scores) were derived by subtracting the age-matched population mean height value from the patient's height and dividing the value obtained, by the age-matched population standard deviation. The SDS were calculated using height data both for the US general female population (National Center for Health Statistics; [NCHS]) and for a Turner syndrome reference population (Lyon et al. 1985). These data were also subjected to ANCOVA analysis with adjustment for midparental height, stature strata, and geographically pooled sites.

The mean final height of the GH-treated group increased by 1.0 SD score, from -3.5 SDS [NCHS] at baseline to -2.5 at final height, bringing the mean final height of this group close to the lower end of the normal female range. In contrast, the mean height SDS of the Control group was essentially unchanged (-3.2 at baseline, -3.1 at protocol completion). The difference between groups for this change was significant ($p=0.013$). The change in height was also significantly greater for the GH-treated group than the Control group when analyzed on the basis of the Lyon standards: the GH-treated group had 1.3 SDS increase compared with 0.3 SDS for the control group ($p<0.001$).

Table 6. Final Height - Protocol Completers - Study GDCT

	Control (n=19) Mean ± SD	GH-Treated (n=27) Mean ± SD	Mean GH Effect (ANCOVA) [a] Mean ± SE	p-value (ANCOVA) [a]
Duration on Study (y)	4.6 ± 0.8	4.7 ± 0.9	NA	NA
Age (y)	16.3 ± 1.2	16.3 ± 0.7	NA	NA
Bone Age (y)	14.4 ± 0.6	14.4 ± 0.6	-0.1 ± 0.2	0.547
Height (cm)	142.1 ± 4.8	146.0 ± 6.2	5.4 ± 1.3	< 0.001
Height SDS (NCHS)	-3.1 ± 0.9	-2.5 ± 1.0	0.8 ± 0.2	0.004
Height SDS (Lyon)	0.5 ± 0.8	1.0 ± 1.0	0.9 ± 0.2	< 0.001
Δ Height (cm)	15.8 ± 4.6	22.1 ± 5.4	7.7 ± 1.8	< 0.001
Δ Height SDS (NCHS)	0.0 ± 1.1	1.0 ± 0.9	0.8 ± 0.3	0.013
Δ Height SDS (Lyon)	0.3 ± 0.4	1.3 ± 0.4	1.1 ± 0.1	<0.001

[a] ANCOVA accounts for stature strata, geographical site, and midparental height

In summary, as shown in Table 6, by ANCOVA analysis the GH-treated patients had mean final height 5.4 cm greater than that of the Control patients ($p<0.001$). In addition, the change in height across the duration of the study was 7.7 cm greater for the GH-treated group than the Control group by ANCOVA analysis. Absolute height SD scores and changes in height SD scores were also significantly greater for the GH-treated patients than the Controls. Notably, the GH-treated patients had a height SD score of -2.5 by NCHS standards at completion, placing them on average at the lower end of the normal female height range, while the Control patients remained significantly further below the normal range.

GH Effect on Height
GDCT Protocol Completers

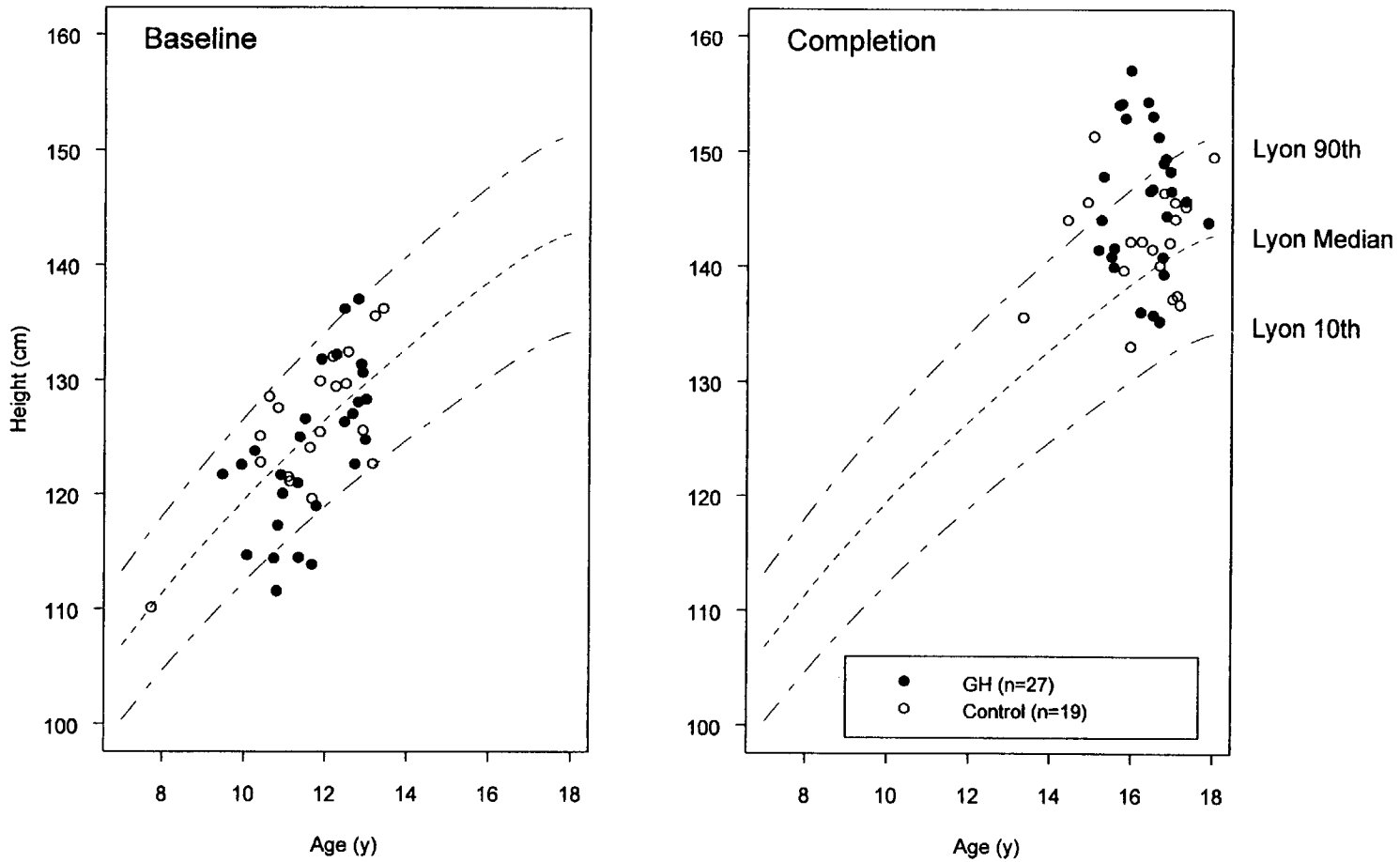

Figure 1. **Final Height for GDCT Protocol Completers**

Figure 1 demonstrates the change in height of GH-treated versus Control patients in Study GDCT. The left panel shows the similarity of heights of GH-treated and Control patients at baseline, which are distributed fairly evenly around the mean of the Turner syndrome-specific Lyon growth curve. The right panel shows heights at protocol completion, showing an upward shift of the height distribution of treated versus non-treated patients.

Supporting Analyses for Efficacy of GH in Study GDCT

In support of the data demonstrating a significant difference in final height between GH-treated and control patents, analyses of height and height SDS at most recent visit were performed for all patients who had efficacy data for at least 6 months. An analysis of these data provides a broader overview of the effectiveness of GH treatment in a larger sample of patients. For this analysis, "Most Recent Visit" refers to the most recent available visit for all patients with at least 6 months of efficacy data, including the protocol completers, the patients who discontinued early, and those still actively participating in the study.

Further supporting analyses that utilize the explanatory power of individual baseline height and baseline age information are presented in Appendix C.

Height and Height SDS at Most Recent Visit for All Patients with ≥ 6 Months Efficacy Data

By ANCOVA analysis the mean height of the GH-treated patients at most recent visit in the population with at least 6 months was 9.1 cm greater than that of the Control patients. Not all patients had a bone age X-ray performed at the most recent visit, therefore, to adjust for any inter-group bone age differences and the potential effects of such differences on height, an ANCOVA analysis was performed comparing the mean heights of the groups at the most recent visit at which a bone age X-ray was obtained. By ANCOVA analysis with adjustments for bone age (which accounts for the slightly more advanced bone age in the treated patients), midparental height, stature strata, and geographically pooled sites, the mean height of the GH-treated group was 6.6 cm greater than that of the Control group at most recent visit. This difference was highly significant ($p<0.001$). In addition to the significantly greater height gains made by the GH-treated patients analyzed at their most recent visit, this group was also continuing to grow more rapidly than the Control patients ($p<0.001$ for growth velocity SD score according to the standards of Ranke). These data are summarized in Table 7.

Table 7. Most Recent Visit - All Patients With ≥ 6 Months Efficacy Data

	Control (n=60) Mean ± SD	GH-Treated (n=74) Mean ± SD	Mean GH Effect (ANCOVA) Mean ± SE	p-value (ANCOVA)
Baseline Age (y)	10.5 ± 1.8	10.4 ± 1.8	NA	NA
Baseline Height (cm)	119.6 ± 8.4	119.9 ± 8.5	NA	NA
Study Duration (y)	3.8 ± 1.5	4.1 ± 1.5	NA	NA
Most Recent Age (y)	14.4 ± 2.3	14.5 ± 2.3	NA	NA
Bone Age (y)	12.2 ± 2.4 (n=55)	12.8 ± 2.1 (n=71)	1.0 ± 0.4 [a]	0.020[a]
Height (cm)	133.5 ± 10.1	142.6 ± 9.0	6.6 ± 1.0 [b]	< 0.001[b]
Height SDS [NCHS]	-3.7 ± 1.2	-2.4 ± 1.0	1.2 ± 0.2 [b]	< 0.001[b]
Height SDS [Lyon]	0.0 ± 1.0	1.4 ± 0.9	1.3 ± 0.1 [b]	< 0.001[b]
Δ Height (cm)	13.9 ± 5.9	22.7 ± 8.1	8.1 ± 1.2 [b]	< 0.001[b]
Δ Height SDS [NCHS]	-0.4 ± 1.0	0.9 ± 0.8	1.2 ± 0.2 [b]	< 0.001[b]
Δ Height SDS [Lyon]	0.2 ± 0.4	1.5 ± 0.6	1.3 ± 0.1 [b]	< 0.001[b]
Growth Velocity (cm/y)	2.9 ± 1.4	3.7 ± 2.2	1.1 ± 0.3 [b]	< 0.001[b]
Growth Velocity SDS [Ranke]	0.3 ± 1.0 (n=38)	1.0 ± 1.3 (n=52)	0.8 ± 0.2 [b]	< 0.001[b]

[a] ANCOVA accounts for stature strata, geographical site, and midparental height.
[b] ANCOVA accounts for bone age, stature strata, geographical site, and midparental height. Analysis based on n=121 patients having both midparental height and recent bone age at most recent visit.
[c] Turner syndrome reference standard for growth velocity (Ranke).

Analyses of the height SD scores for GH-treated and Control patients in the group with at least 6 months efficacy data provide additional evidence of efficacy. The GH-treated and Control groups had similar height at baseline, which was more than 3 SDS below the mean of the normal female reference standard (Table 4). In contrast, at most recent visit, height of the GH-treated group was 1.2 SDS greater than that of the Control group by NCHS standards ($p<0.001$ by ANCOVA analysis) and 1.3 SDS greater by Lyon standards ($p<0.001$). By ANCOVA analysis the change in height SDS

between baseline and most recent visit was 1.3 SDS greater for the GH-treated patients than the Control patients ($p<0.001$; Table 7).

Supplemental Analyses

Supplemental analyses of efficacy are presented in Appendix C. When data of patients strictly meeting protocol completion criteria are analyzed, and when the ANCOVA incorporates all of the explanatory power of individual baseline height and baseline age, the estimate of height increase due to GH therapy in study GDCT is 8.1 cm (Table C.3.2 in Appendix C).

6. Summary of Efficacy - Study GDCT

Protocol Completers

Final Height: The GH-treated group achieved mean final height that was 5.4 cm greater than that of the Control group by ANCOVA analysis. This difference was statistically significant ($p<0.001$).

Final Height SDS and Change in Height SDS: The mean final height SDS [NCHS] of patients treated with GH was significantly greater than that of the Control patients (-2.5 SDS vs. -3.1 SDS; $p=0.001$). This result indicates that GH-treated patients achieved final height significantly closer to the lower end of the normal female reference range than Control patients. The GH-treated patients had a mean increase of 1.0 SD score from baseline to protocol completion, while the Control group demonstrated no change (0.0 SDS), and the difference between groups was significant ($p=0.013$).

All Patients With ≥ 6 Months Efficacy Data

Height and Height SDS at Most Recent Visit: The mean height at the last visit at which a bone age X-ray was obtained was significantly greater for the GH-treated group than for the Control group. By ANCOVA analysis with adjustment for bone age, the mean height of the GH-treated group was 6.6 cm greater than that of the Control group ($p<0.001$).

Mean height SDS of the GH-treated patients in this population increased significantly from baseline to most recent visit, both for SDS according to the NCHS (normal female) standard and for the Lyon (Turner syndrome) standard. Mean height of the GH-treated patients at most recent visit was more than 1 SD closer to the mean of the normal population than that of the Control patients (-2.4 SDS versus -3.7 SDS), indicating that GH-treated patients had mean height at the lower end of the normal female height standards, while mean height of the Control group was significantly below this. The difference between the groups was significant for analyses by either the normal female or the Turner syndrome standards ($p<0.001$).

Growth Velocity: The GH-treated patients grew more rapidly than the Control patients throughout the study and were growing significantly faster at the most recent visit (1.0 SDS vs 0.3 SDS by analysis according to Ranke growth velocity standards; $p<0.001$).

Efficacy Data Study B9R-MC-GDCI

The primary objective of this study is to determine the efficacy of recombinant GH alone and in combination with low dose estrogen given at an early age, in promoting linear growth in patients with Turner syndrome. The secondary objective of this study is to determine the antigenicity and other variables of clinical safety of GH in these patients. The study involves 58 principal investigators at 50 study centers (eight investigators did not enroll any patients); enrollment in this study has closed.

1. Population

Patients were included in the study if they fulfilled the following criteria: karyotypically-proven Turner syndrome with absence of Y chromatin; age at least 5 years and bone age no greater than 12 years; prepubertal status; height ≤10th percentile of the normal female reference standard [NCHS]; growth velocity <6 cm/year; absence of diabetes or other significant chronic disease. Patients were excluded from the study if they had received growth hormone, androgen or estrogen therapy within 3 months of the study or for more than 12 months total. Informed consent from a parent or guardian was obtained at enrollment.

2. Study Design

This study is an ongoing double-blind, randomized, placebo-controlled, parallel study of treatment with GH and low dose estrogen in patients with Turner syndrome. After enrollment, the patients were stratified into four groups by age category (5, 6, and 7 years; 8 and 9 years; 10 and 11 years; 12 years and older). Patients were then randomly assigned to one of five treatment groups described below, such that each age was balanced with respect to treatment groups. The five treatment arms included two 0.36 mg/kg/week GH arms with either low dose estrogen or placebo tablets; two 0.27 mg/kg/week GH with either low dose estrogen or placebo tablets; and a fifth arm receiving placebo injections and placebo tablets. At closure of enrollment in the study, 232 patients had been randomized; there were between 43 and 49 patients in each of the 5 treatment groups (see Summary of Patient Accountability).

The treatment received by original five groups was as follows (summarized in Table 8):

1. GH 0.27 mg/kg/week (0.09 mg/kg/dose, three times per week), with low dose ethinyl estradiol; designated GH0.27/LDE
2. GH 0.27 mg/kg/week (0.09 mg/kg/dose, three times per week) with placebo tablets; designated GH0.27/Placebo

3. GH 0.36 mg/kg/week (0.12 mg/kg/dose, three times per week), with low dose ethinyl estradiol; designated GH0.36/LDE)
4. GH 0.36 mg/kg/week (0.12 mg/kg/dose, three times per week), with placebo tablets; designated GH0.36/Placebo
5. placebo injections with placebo tablets (designated Placebo/Placebo).

After completion of the initial 18-month treatment period, patients were allowed to enter an extension phase. During the extension period, the therapy remained unchanged for four treatment groups which were found, upon blinded interim analysis, to be more responsive than the fifth group. Patients in the least responsive treatment group with respect to growth velocity (determined at the time of unblinding for the current report to be the group receiving placebo injections and placebo oral medication) were reassigned to the treatment group receiving 0.36 mg/kg/week GH with placebo tablets. This reassignment of one treatment group required that all patients in the trial receive new drug kit numbers at visit 7 so that neither patients nor investigators were unblinded.

The prescribed dose of injectable study drug (GH or placebo) was injected subcutaneously three times per week up to and including Visit 25 (72 months); thereafter, the dose of GH was halved and injected six times per week.

Table 8. Summary of Study Design - Study GDCI

Start		At 18 Months
GH0.27/LDE	→	GH0.27/LDE
GH0.27/Placebo	→	GH0.27/Placebo
GH0.36/LDE	→	GH0.36/LDE
GH0.36/Placebo	→	GH0.36/Placebo
Placebo/Placebo	↗	

Since the aim of the current application is to address the efficacy of GH treatment in Turner syndrome, the estrogen treatment regimen and analyses are not addressed in the body of this document but are provided in Appendix D.

3. Patient Accountability

For analyses of the safety of GH and low dose estrogen therapy in Turner syndrome in this study, the ***safety population*** is defined as those patients who were randomized and took any study medication. Two hundred and thirty two patients were initially

randomized. Two hundred and thirty patients (99%) are included in the safety population. Two of the randomized patients were excluded from the safety population due to the fact that they had no documentation regarding taking study medication.

The ***population with ≥ 6 months efficacy data*** comprises all randomized patients who had efficacy data at Visit 3 or beyond (180 days after randomization). If randomized to the group receiving placebo injections and placebo tablets, efficacy data were required at Visit 9 (180 days after reassignment to their new treatment) or beyond. There are 224 patients (97% of those randomized) in the population with ≥ 6 months efficacy data. Eight of the 232 randomized patients were excluded from this population; two were those excluded from the safety population and a further six were excluded due to lack of height data after 180 days of GH treatment.

In 1993 an amendment was made to the study to include a definition of final height for patients nearing protocol completion. Patients were to be considered protocol complete when growth velocity was less than 2 cm per year and bone age was at least 15 years. As of the 8 February 1996 data cutoff date, 31 of the 232 randomized patients (13%) had completed the study. These patients were designated as ***protocol completers***. Sixty-three patients (27%) remained active, and 138 patients (60%) had been discontinued from the study.

Of the 138 patients who discontinued the study, 90 (66%) discontinued due to patient decision. The investigator or study coordinator comments on each patient's final summary report suggest that approximately 40% of the patient decisions were attributed to satisfaction with attained height whereas lack of efficacy (about 20%) and complaints regarding injections or the protocol (about 10% and 3%, respectively) were less common. Investigators discontinued 16 (12%) patients. Comments made regarding physician decision suggested that physicians were more likely to discontinue patients for reasons associated with poor response (about 30%) than for satisfaction with efficacy of treatment (about 25%). Twelve patients (9%) were discontinued due to protocol violations with most being noncompliant with respect to either study drug administration or visit schedules. Seven patients (5%) were discontinued for lack of efficacy, five patients (4%) were lost to follow-up, four patients (3%) were discontinued because of adverse events (and are discussed in more detail in Table 17), and two patients (1%) were discontinued by the sponsor. Table 9 summarizes patient accountability overall and by treatment group for Study GDCI.

Table 9. Patient Accountability - Study GDCI

Patient Disposition	Overall	GH0.36/ LDE	GH0.36/ Pla	GH0.27/ LDE	GH0.27/ Pla	Pla/Pla
Randomized	232	43	49	48	46	46
Safety Population	230 (99%)	42 (98%)	49 (100%)	47 (98%)	46 (100%)	46 (100%)
Population With ≥ 6 Months Efficacy Data	224 (97%)	42 (98%)	49 (100%)	47 (98%)	45 (98%)	41 (89%)
Completed	31 (13%)	8 (19%)	7 (14%)	6 (13%)	5 (11%)	5 (11%)
Discontinued	138 (60%)	24 (56%)	26 (53%)	31 (65%)	30 (65%)	27 (59%)
Ongoing	63 (27%)	11 (26%)	16 (33%)	11 (23%)	11 (24%)	14 (30%)

Percentages are relative to number of randomized patients.

4. Baseline Characteristics

Tables 10 and 11 summarize patient characteristics at study entry for ethnic origin, age strata, age, bone age, height, height SDS [NCHS], height SDS [Lyon], and midparental height for the 224 patients with ≥ 6 months of efficacy data, overall and by GH dosage group.

Table 10. Ethnic Origin and Age Strata - All Patients With ≥ 6 Months Efficacy Data

Mean ± SD	GH0.36 (n=132) n (%)	GH0.27 (n=92) n (%)
Ethnic Origin		
Caucasian	101 (77)	73 (79)
All Others	31 (23)	19 (21)
Age Stratum		
< 8	42 (32)	32 (35)
8 to < 10	28 (21)	17 (19)
10 to < 12	32 (24)	21 (23)
≥ 12	30 (23)	22 (24)

Table 11. Baseline Data - All Patients With ≥ 6 Months Efficacy Data

Mean ± SD	GH0.36 (n=132)	GH0.27 (n=92)
Age (y)	9.7 ± 2.8	9.6 ± 2.7
Bone Age (y)	7.8 ± 2.3	7.8 ± 2.2
Height (cm)	118.0 ± 12.7	117.9 ± 12.8
Height SDS [NCHS]	-3.0 ± 0.9	-3.0 ± 1.1
Height SDS [Lyon]	0.1 ± 0.9	0.1 ± 1.0
Pretreatment Growth Velocity (cm/y)	4.0 ± 1.1	3.9 ± 1.4
Midparental Height (cm)	163.1 ± 5.5 (n=127)	163.0 ± 6.3 (n=87)

At baseline there were no significant differences between the treatment groups for mean values for chronological age, bone age, height, weight (not shown), height SDS (by NCHS or Lyon standards), pretreatment growth velocity, or midparental height.

One hundred and fifty-one (67%) of the 224 patients with ≥ 6 months of efficacy data had karyotype 45,X. There was no statistically significant difference among treatment groups for the proportion of patients with the 45,X karyotype compared with all other karyotypes.

5. Efficacy Results

In this study, patients received one of three possible GH dosages: 0.36 mg/kg/week, 0.27 mg/kg/week, or 0.0 mg/kg/week (placebo). At 18 months of study, patients receiving placebo injections were reassigned (maintaining the double blind study design) to the group receiving 0.36 mg/kg/week and oral placebo. For purposes of this report regarding the dose-response effect of GH and its efficacy with respect to final height, all patients who received 0.36 mg/kg/week, including those originally receiving placebo injections, are pooled for analysis into a single group designated GH0.36, and those who received 0.27 mg/kg/week are pooled as a single group designated GH0.27. Data addressing the effect of low dose estrogen in this study are provided in Appendix D.

Final Height of Protocol Completers

Thirty one of the 224 patients with ≥ 6 months of efficacy data (14%) had completed the protocol by the February, 1996 data cutoff date. The criteria for protocol completion were bone age ≥15 years and growth velocity <2 cm/year. Upon analysis of the data for the patients designated by the investigators as protocol completers it was found that 7 of the 31 patients did not completely meet the criteria for achievement of final height: all had bone ages ≥ 13.5 years and growth velocities of <3 cm/year. Nevertheless, as these patients were felt by the investigators to have achieved close to final height, the group was analyzed as a whole for the purpose of evaluating GH effect on final height. Of the 31 protocol completers, twenty patients had received GH at the 0.36 mg/kg/week dose (GH0.36 group) and 11 at the 0.27 mg/kg/week dose (GH0.27 group). Fourteen of the 31 protocol completers were in the treatment groups randomized to receive low dose estrogen from 8 years of age, according to the regimen provided in Appendix D.

The baseline and final heights of the 31 designated protocol completers in this study are shown in Figure 2. These figures demonstrate the substantial change in distribution of patients heights from baseline to protocol completion. The heights are distributed fairly symmetrically about the mean of the Lyon curve at baseline. In contrast, at protocol completion, 29 of the 31 patients have heights at or above the mean, providing evidence for the efficacy of GH in this patient population, as compared to a historical reference population.

GH Effect on Height
GDCI Protocol Completers

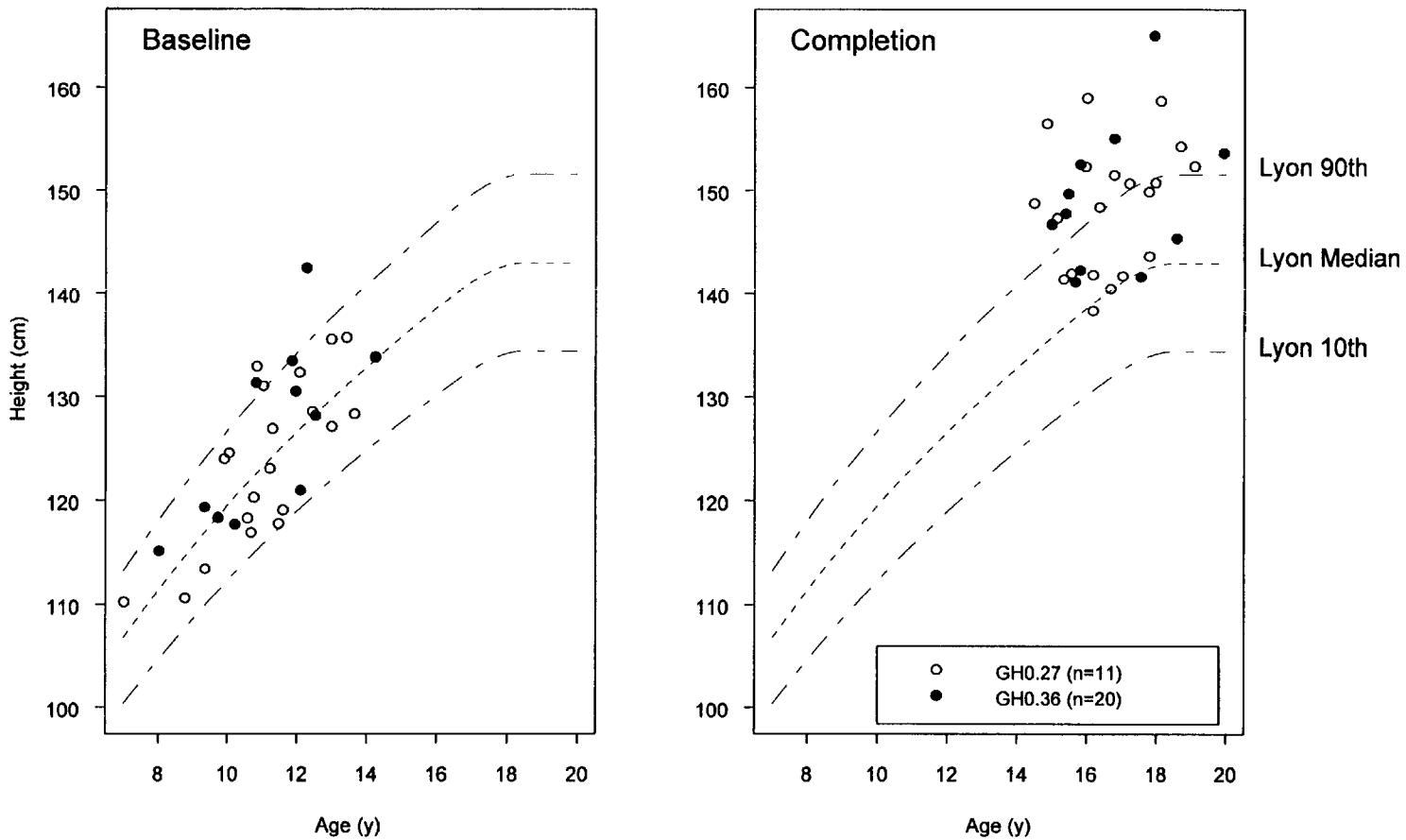

Figure 2. **Baseline and final height for GDCI protocol completers**

Table 12. Summary Data at Baseline and Protocol Completion by GH Dosage Group - Study GDCI

Mean ± SD [a]	GH0.36 group (n=20)	GH0.27 group (n=11)
At Baseline		
Age (y)	11.1 ± 1.6	11.2 ± 1.8
Bone Age (y)	8.5 ± 1.3	9.3 ± 1.4
Height (cm)	123.8 ± 7.8	126.5 ± 8.7
Height SDS [NCHS]	-3.1 ± 1.1	-2.9 ± 0.9
Height SDS [Lyon]	0.1 ± 0.9	0.5 ± 1.0
Midparental Height (cm)	162.2 ± 4.4	165.3 ± 7.0
At Protocol Completion		
Age (y)	16.7 ± 1.3	16.4 ± 1.6
Bone Age (y)	15.5 ± 0.9	15.7 ± 0.6
Duration of GH Therapy (y)	4.5 ± 1.6	4.6 ± 1.8
Height (cm)	148.5 ± 6.3	149.2 ± 7.3
Height SDS [NCHS]	-2.3 ± 1.0	-2.3 ± 1.2
Height SDS [Lyon]	1.3 ± 1.0	1.4 ± 1.0
Δ Height (cm)	24.7 ± 7.2	22.7 ± 6.7
Δ Height SDS [NCHS]	0.8 ± 0.8	0.7 ± 1.0
Δ Height SDS [Lyon]	1.2 ± 0.6	0.9 ± 0.6

[a] There are no statistically significant differences between the two dosage levels among these variables, at the 0.05 level.

Table 12 summarizes the data at baseline and at protocol completion for the subgroup of patients in this study who completed the protocol, by GH dosage group. There were no significant differences between the groups for any of the parameters listed in the table, either at baseline or at protocol completion. Although the group receiving the lower dose of GH (0.27 mg/kg/week) had 2.7 cm greater mean height at baseline, this was not statistically significant. Mean final height of the 20 patients in the group receiving 0.36 mg/kg/week was 148.5

cm; and was 149.2 cm for the 11 patients in the group receiving 0.27 mg/kg. Since there was no significant difference between the groups, the two GH dosage groups were pooled for analysis of final height: in this analysis the mean final height of all patients who completed the protocol was 148.7 ± 6.5 cm. Summary data for the whole group of protocol completers, irrespective of GH dose, are provided in Table 13.

Table 13. Summary Data for Protocol Completers - Study GDCI

Mean ± SD	At Baseline (n=31)	At Completion (n=31)
Age (y)	11.1 ± 1.7	16.7 ± 1.4
Bone Age (y)	8.8 ± 1.4	15.6 ± 0.8
Height (cm)	124.7 ± 8.1	148.7 ± 6.5
Height SDS [NCHS]	-3.1 ± 1.0	-2.3 ± 1.1
Height SDS [Lyon]	0.3 ± 0.9	1.4 ± 1.0
Duration of GH Therapy (y)	NA	5.3 ± 1.1
Δ Height (cm)	NA	24.0 ± 7.0
Δ Height SDS [NCHS]	NA	0.8 ± 0.9
Δ Height SDS [Lyon]	NA	1.1 ± 0.6

As shown in Table 13, the patients who completed the protocol had baseline height that was more than 3 SD below the mean of the general female population. However, after approximately 5.3 years of GH treatment, these patients had final height of 148.7 cm, which is considerably closer to the normal range, being approximately 2.3 SD below the mean for the general female population, and 1.4 SD above the mean for the Turner syndrome population.

In summary, at protocol completion patients receiving either dosage of GH in this study were taller than expected for untreated patients with Turner syndrome, achieving mean height of 148.7 ± 6.5 cm (n=31). Both groups had height SDS [NCHS] at the lower end of the normal female range, a significant improvement from their pretreatment position. Fifty-eight percent (18 of 31 patients) in fact had final heights that fell within the normal female range as they were taller than -2.5 SDS [NCHS].

Supporting Analyses for Efficacy of GH in Study GDCI

Data at Most Recent Visit for All Patients With ≥ 6 Months Efficacy Data

Height

As supporting evidence of the efficacy of GH treatment in improving growth in Turner syndrome, an analysis was performed to evaluate the increase in height of all patients who had received GH treatment for at least 6 months. The height measurements in this analysis do not represent the patients' final heights, since many patients have not yet attained protocol completion. Mean chronological age at the time of this analysis was approximately 14.0 years and bone age was 12.7 years. By ANCOVA analysis with adjustments for midparental height, low dose estrogen, baseline age strata, geographically pooled sites and bone age, the mean height of the GH0.36 group was 1.6 cm greater than that of the GH0.27 group, the difference showing a statistical trend (p=0.065), suggesting a dose-response effect.

Table 14. Height SDS and Growth Velocity - All Patients With ≥ 6 Months Efficacy Data

Mean ± SD	GH0.36 (n=132)	GH0.27 (n=92)	p-value
At Baseline			
Height (cm)	118.0 ± 12.7	117.9 ± 12.8	0.980[a]
Height SDS [NCHS]	-3.0 ± 0.9	-3.0 ± 1.1	0.604[a]
Height SDS [Lyon]	0.1 ± 0.9	0.1 ± 1.0	0.876[a]
Growth Velocity (cm/y)	4.0 ± 1.1	3.9 ± 1.4	0.448[a]
At Most Recent Visit			
Height (cm)	143.8 ± 11.2	140.2 ± 11.6	0.021[b]
Height SDS [NCHS]	-2.3 ± 1.1	-2.6 ± 1.1	0.087[b,c]
Height SDS [Lyon]	1.5 ± 1.1	1.3 ± 1.1	0.291[b]
Δ Height (cm)	25.9 ± 11.1	22.3 ± 11.0	0.005[b]
Δ Height SDS [NCHS]	0.7 ± 0.9	0.4 ± 0.9	0.012[b]
Δ Height SDS [Lyon]	1.4 ± 0.7	1.1 ± 0.7	0.008[b]
Growth Velocity (cm/y)	3.4 ± 1.6	3.2 ± 1.7	0.268[b]
Growth Velocity SDS [Ranke]	0.9 ±1.2 (n=79)	0.3 ± 1.2 (n=68)	0.017[b]

[a] p-value by ANOVA analysis including effect for geographical site

[b] ANCOVA analysis includes effects for geographical site, age strata at baseline, and low dose estrogen regimen

[c] p-value for ANCOVA analysis of treatment group means additionally adjusted for midparental height is 0.121

Height SDS

Comparison of the height SDS at baseline and most recent visit provides further evidence for GH efficacy in the large population of Study GDCI who had received at least 6 months of GH treatment (Table 14). The term 'most recent visit' as used in this analysis refers to the visit that occurred closest to the February, 1996 cut-off date for this analysis and includes patients who were still active in

the study at that time, in addition to those who had completed the protocol or had discontinued after 6 months in the study. This analysis does not evaluate final height, since many of the 224 patients analyzed are still active in the study. Patients receiving GH 0.36 mg/kg/week (GH0.36 group) are compared with those receiving 0.27 mg/kg/week (GH0.27 group).

At baseline, the mean height of both GH dosage groups was approximately 3 SDS below the mean of the general female population [NCHS]. At most recent visit, mean height SDS had improved to -2.3 for the GH0.36 group and -2.6 for the GH0.27 group. The change in height SDS [NCHS] between baseline and most recent visit was significantly greater for the treatment group receiving 0.36 mg/kg/week, than for the group receiving 0.27 mg/kg/week, with increases of +0.7 SDS and +0.4 SDS, respectively (p=0.012).

Growth Velocity

Patients with ≥ 6 months of efficacy data who received GH at 0.36 mg/kg/week grew more rapidly than those receiving 0.27 mg/kg/week during the study and were growing more rapidly at last visit (0.9 SDS vs 0.3 SDS according to the standards of Ranke; p=0.017). The Ranke reference only accounts for patients younger than 16 years and who therefore are in more active phases of growth.

Evidence for Dose Response Effect of GH Therapy in Turner Syndrome

This study provides the following evidence for a dose-response effect of GH treatment of short stature due to Turner syndrome:

1. The change in height SDS (by both NCHS and Lyon standards) between baseline and most recent visit for all patients with ≥ 6 months efficacy data was significantly greater for the patients receiving 0.36 mg/kg/week than for those receiving 0.27 mg/kg/week.
2. Patients with ≥ 6 months of efficacy data who received GH at 0.36 mg/kg/week grew more rapidly than those receiving 0.27 mg/kg/week. At most recent visit there was a significant difference between groups for growth velocity SDS according to the standards of Ranke (p=0.017).

Summary of Efficacy - Study GDCI

Final Height for Protocol Completers: The mean final height of the 31 patients in this study who completed the protocol was 148.7 ± 6.5 cm. Height improved with respect to the general population standards, the mean height increasing from 3.0 SDS below the normal population mean at baseline, to -2.3 SDS at protocol completion, which is at the lower end of the normal female range.

Height, Height SDS and Growth Velocity at Most Recent Visit for All Patients With ≥ 6 Months Efficacy Data: The height SDS [NCHS] of the GH0.36 and GH0.27 groups improved from approximately -3.0 SDS at baseline to -2.3 and -2.6 SDS, respectively at most recent visit. This finding indicates that the age-adjusted mean heights of both groups were close to the lower end of the normal female reference range. Change in height SDS [NCHS] from baseline to most recent visit was significantly greater for the GH0.36 group than the GH0.27 group of this population, suggesting of a mild dose response effect of GH in this study. GH treatment increased growth velocity in both treatment groups, with growth rates after 1 year of treatment approximately three fold greater than those before treatment. At most recent visit the patients receiving GH 0.36 mg/kg/week were growing significantly faster than the patients receiving 0.27 mg/kg/week (0.9 SDS vs 0.3 SDS by Ranke growth velocity standards; p=0.017).

Summary of Efficacy - Lilly North American Studies

Figure 3 demonstrates the change in height of GH-treated versus non-GH-treated patients in both the North American studies, GDCT and GDCI. The GH treated group includes the GH0.27 and GH0.36 groups from Study GDCI and GH-Treated group from Study GDCT. The non-GH group is the Control group from Study GDCT which did not receive any GH therapy. The left panel shows the similarity of heights of GH-treated and Control at baseline, which are distributed fairly evenly around the mean of the Turner syndrome-specific Lyon growth curve, ranging between the 10^{th} and 90^{th} percentile for girls with Turner syndrome. The right panel displays heights at protocol completion, showing an upward shift of the height distribution of GH-treated versus non-GH-treated patients.

GH Effect on Height
GDCT and GDCI Protocol Completers

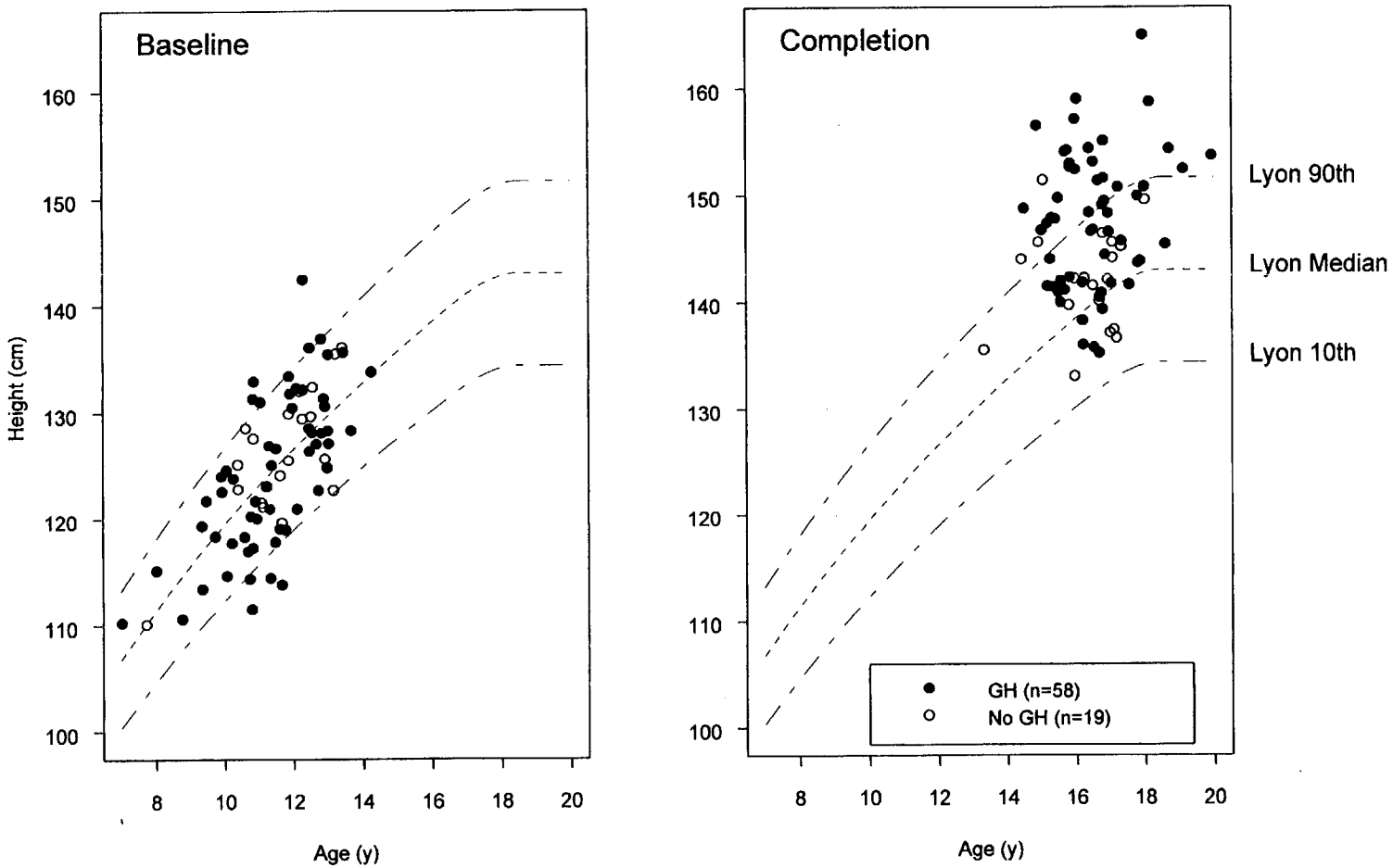

Figure 3. **Height at baseline and at protocol completion – GDCI and GDCT**

Safety of GH Therapy in Turner Syndrome

1. Potential Risks

A number of disorders in a variety of organ systems occur with increased frequency in patients with Turner syndrome. The potential risks of growth hormone therapy must be considered in the light of any underlying disorder present in these patients. The most serious health problems relate to congenital left-sided cardiovascular defects present in up to one third of patients with Turner syndrome (Mazzanti L et al.1988). These defects include coarctation of the aorta, bicuspid aortic valve, and ventricular septal defect. Structural abnormalities of the renal tract occur in 35% to 70% of patients (Lippe B 1991). Additionally, some patients have hypertension without evidence of coarctation of the aorta or renal abnormalities, the etiology of which remains unclear (Virdis R et al.1986). There is no evidence that treatment with human growth hormone increases the occurrence rate or severity of cardiovascular disorders in Turner syndrome.

Patients with Turner syndrome have an increased incidence of autoantibodies to endocrine organs (Nienhuis HE et al.1993) and are at risk for development of autoimmune disorders, including thyroiditis (approximately 30% of patients (Lippe B 1991) and inflammatory bowel disease, especially Crohn disease (Lippe B 1991). Since growth hormone has immunomodulatory effects, its long-term use in patients with underlying immune dysfunction raises questions regarding its potential to exacerbate or ameliorate such disorders. A number of studies have addressed this issue. Nienhuis et al. (Nienhuis HE et al.1993) and Rongen-Westerlaken et al.(Rongen-Westerlaken C et al.1991) examined the effect of administration of somatropin on immune function in patients with Turner syndrome and found only minor changes in some immunologic parameters that were not associated with any clinical disturbance of immune function. As exogenously administered human growth hormone represents introduction of a foreign protein, there is the potential for stimulation of anti-growth hormone antibodies.

A proportion of patients with Turner syndrome have preexisting insulin resistance, and a smaller subgroup have carbohydrate intolerance or frank non-insulin dependent diabetes mellitus (Caprio S et al.1991). Growth hormone is known to antagonize insulin action, reduce insulin sensitivity and induce compensatory hyperinsulinemia both in short non-growth hormone deficient children (Walker J et al.1989) and in those with Turner syndrome (Caprio S et al.1992). Therefore, the effects of growth hormone therapy on carbohydrate metabolism were an important safety concern, since the effect of long-term administration of growth hormone was unknown at the time these studies began.

2. Study Design: Safety Considerations

Human growth hormone has been administered to patients with Turner syndrome since 1960. The numbers of patients treated were initially small, so data evaluating the effects of long-term exposure to GH were lacking. The North American studies detailed in this submission were designed to include careful monitoring for adverse events. Special attention was given to laboratory assessments to monitor for potential development of glucose intolerance, thyroid dysfunction and antibodies to GH. Because patients with Turner Syndrome are at increased risk for insulin resistance, and growth hormone may reduce insulin sensitivity, all patients in the North American studies had fasting glucose concentrations and glycosylated hemoglobin or hemoglobin A_1C measured at 6 monthly intervals. In addition, some patients in study GDCT and almost all in Study GDCI underwent a modified 2-hour glucose tolerance test. Serum IGF-I concentrations were measured at least annually in all patients in Study GDCI.

3. Safety Results

This summary of safety events discusses the North American Studies GDCT and GDCI and, in addition, the world-wide experience for deaths, serious adverse events and other adverse events possibly related to GH therapy. Data analyzed include rates of occurrence of adverse events as well as laboratory data collected under randomized, controlled conditions.

Deaths

Across all Lilly Turner syndrome GH treatment experience worldwide, including controlled studies and spontaneous reports, three deaths were reported; one patient was in a non-GH treated group and two patients were receiving GH. All deaths were due to catastrophic cardiovascular events related to known (coarctation of the aorta) or presumed (cerebrovascular aneurysm) congenital defects. Relationship to GH is considered unlikely.

Unexpected and Possibly Related Serious Adverse Events

Table 15 provides a listing, by patient, of serious adverse events which were considered unexpected and possibly related to treatment with study drug. Among the North American studies, a total of eleven patients out of 304 (3.6%) patients in the safety population of Studies GDCT and GDCI had such an event. There were five patients in Study GDCT and five patients in Study GDCI who had serious adverse events which were considered unexpected and possibly related to treatment with GH. One patient in the Control group of Study GDCT (Patient 116-2001) also had a serious adverse event (thrombocytopenic purpura) prior to the fatal event described in the previous section. In this case, the study

drug was ethinyl estradiol alone (this patient did not receive GH). Treatment with ethinyl estradiol may have also been responsible for the hypochromic microcytic anemia observed in Patient 104-2500 of Study GDCT, according to the investigator.

Table 15. Patients in Studies GDCT and GDCI with Serious Adverse Events Classified as Unexpected and Possibly Related to Study Medication

Study Patient No.	Treatment Group	Age	Event Classification Term	Event Description	Days in Study
B9-CA-GDCT					
104-2500	GH	13	Hypochromic Microcytic Anemia	Hypochromic Microcytic Anemia	1579
105-2408	GH	10	Dyspnea	Shortness of Breath	302
106-2303	GH	13	Psoriasis	Pustular Rash	1139
114-2712	GH	14	Gastrointestinal Disorder	Stomach Flu	614
		14	SGOT Increase	SGOT Elevation	586
116-2001	Control	13	Thrombocytopenic Purpura	Idiopathic Thrombocytopenic Purpura	392
		13	Vascular Disorder	Ruptured Aortic Aneurysm	438
116-2210	GH	7	Surgical Procedure	Valve Replacement	515
B9R-MC-GDCI					
021-1171	GH0.27/Pla	15	Surgical Procedure	Repair of Aortic Aneurysm	1825
026-1242	GH0.36/LDE	15	Hypertension	Hypertension	1800
040-1568	Pla/Pla	8	Hypertension	Hypertension	730
045-1386	GH0.36/LDE	14	Surgical Procedure	Osteotomy and Bunionectomy	2299
059-1502	Pla/Pla	16	Bone Disorder	Scoliosis	635

All Serious Adverse Events

Table 16 provides a listing of the number of patients in each study for whom a serious adverse event was reported (regardless of relationship to study medication), as of the 8 February 1996 cutoff date.

Table 16. Summary of Serious Adverse Events in Clinical Trials of GH for Turner Syndrome

Study	Country	GH Doses	Drug Exposure	Number of Patients		
				Enrolled	SAEs	Deaths
B9R-MC-GDCI	US	0.27 mg/kg/wk 0.36 mg/kg /wk	>8 years	232	48	0
B9R-CA-GDCT	Canada	0.30 mg/kg /wk	>7 years	154	28	1

In these two studies, among patients who were receiving GH at the time of their serious adverse event, surgical procedure was the most common adverse event (serious or nonserious) which was listed on the serious adverse event reports (22 events reported among 11 patients in Study GDCT, and 34 events reported among 26 patients in Study GDCI). Many of these surgical procedures were for conditions associated with Turner syndrome (e.g., surgery for webbed neck, repair of coarctation of aorta). Bone fractures and ear problems were also commonly reported in these North American studies, some of which required surgical procedures

Discontinuations Due to Adverse Events

In studies GDCT and GDCI, 7 (2%) of patients prematurely discontinued from the study due to an adverse event. Table 17 provides a listing of these individual patients, their treatment group, and the event leading to discontinuation.

Table 17. Patients Discontinued Due to Adverse Events

Study Patient	Treatment Group	Age	Origin	Visit	Days in Study	Event Classification Term
B9R-CA-GDCT						
114-2712	GH	14	Native American	8	586	SGOT Increased
116-2001	Control	13	Caucasian	20	438	Vascular Disorder
116-2210	GH	7	Caucasian	7	515	Intracranial Hypertension
B9R-MC-GDCI						
012-1328	GH/LDE	14	Caucasian	13	1000	Migraine
021-1171	GH0.27/Pla	15	Caucasian	18	1825	Vascular Disorder
021-1176	GH0.27/Pla	9	Caucasian	3	62	Gastrointestinal Disorder
059-1502	Pla/Pla	16	Caucasian	15	635	Bone Disorder

Treatment-Emergent Events

Almost all patients in both the GH and Control groups of study GDCT and in both dosage groups in study GDCI reported at least one treatment-emergent event. In Study GDCT, statistical analyses were performed to compare the proportion of patients in the GH-treated versus the Control group, in whom treatment-emergent events occurred. No statistical analyses were performed for treatment-emergent events in Study GDCI.

Treatment Emergent Events With Significant Difference Between GH-Treated and Control Groups - Study GDCT

Surgical procedure, otitis media, ear disorder, and accidental overdose were the only treatment-emergent events for which the difference between the GH-treated and the Control group was statistically significant ($p \leq 0.050$; Table 18.). Each of these events was observed in a higher proportion of patients in the GH-treated group than in the Control group.

Table 18. Treatment-Emergent Events with Significant Difference Between Groups - Study GDCT

	Control (n=74) number of patients reported with event (%)	GH-Treated (n=62) number of patients reported with event (%)
Surgical Procedure	17 (27)	33 (45)
Otitis Media	16 (26)	32 (43)
"Ear Disorder"	3 (5)	13 (17)
Accidental Overdose	0	8 (10)

Treatment Emergent Events of Special Interest

Treatment-emergent events of special interest were identified for the major North American studies because of concern that development or worsening of some events is potentially causally related to treatment with GH. Tables 19 and 20 present comparisons of these events in all patients from each study who received GH. Table 19 compares the GH-treated and Control groups of study GDCT; Table 20 compares the two GH dosage groups of study GDCI. These tables report the number and percent of patients within each group for whom the event of interest was reported.

Table 19. Treatment-Emergent Events of Special Interest - Study GDCT

	Control (n= 62) n (%)	GH-Treated (n=74) n (%)
Bone Disorder (includes scoliosis)	7 (11)	6 (8)
Edemas	3 (5)	8 (11)
Hypothyroidism	5 (8)	10 (14)
Increased Nevi	2 (3)	8 (11)
Hyperglycemia	0	0
Lymphedema	0	0

Although edemas and hypothyroidism were reported somewhat more frequently in the GH-treated than the Control group, there was no significant difference between groups for any of the treatment emergent events of special interest reported above.

Table 20. Treatment-Emergent Events of Special Interest - Study GDCI

	GH 0.36 mg/kg/wk (n=137) n (%)	GH 0.27 mg/kg/wk (n=93) n (%)
Hypothyroidism	27 (20)	13 (14)
Edemas	16 (11)	9 (10)
Increased Nevi	10 (7)	4 (4)
Hypertension	8 (6)	5 (5)
Bone Disorder (includes scoliosis)	8 (6)	9 (10)
Hyperglycemia	1 (1)	0

Hypothyroidism and increased nevi were reported somewhat more frequently in the patient group receiving GH at the 0.36 mg/kg/wk dose, while bone disorders were reported somewhat more frequently in the group receiving 0.27 mg/kg/wk. These differences were not statistically significant.

Treatment Emergent Events During Placebo-Controlled Period - Study GDCI

During the first 18 months of study GDCI, a number of treatment-emergent events were reported more commonly in GH-treated patients than in those receiving placebo injections. These included otitis media and ear disorders, increased cough and gastrointestinal complaints including gastroenteritis and dyspepsia. In contrast, rash and surgical procedures were reported more frequently for placebo-injected patients than for those receiving GH. It is notable that none of the treatment emergent events of special interest described above, other than hypothyroidism, was reported with a frequency greater than 5%. There was no difference in reported frequency of hypothyroidism between GH-treated and placebo-treated patients.

Clinical Laboratory Evaluation

The clinical laboratory evaluation in studies GDCT and GDCI included assessments of blood chemistry, hematology, urinalysis, thyroid function, glucose homeostasis

(including fasting glucose and hemoglobin A_{1C} in both studies, fasting and postprandial insulin and postprandial glucose every 6 months for the first 36 months and yearly thereafter in GDCI, and as clinically indicated in GDCT), IGF-I concentration (GDCI), lipids, and antibodies to rhGH and *Escherischia coli* polypeptide. Comparisons were made between treatment groups for each of these parameters at baseline and at most recent visit. In addition, in order to detect patients who had potentially significant increases of laboratory variables, an analysis was performed to identify any patient who had values that fell above specific numerical cutpoints at any time in the study (including baseline).

Serum Chemistry and Cholesterol

As shown in Table 21, there was a greater proportion of GH-treated than Control patients in study GDCT who had alkaline phosphatase and creatine kinase concentrations above the designated cutpoints at some time in the study. In contrast, the Control group had a somewhat higher proportion of patients with serum cholesterol above the designated cutpoint at some time in the study.

Table 21. Selected Laboratory Data: Patients with at Least One Value Above Designated Cutpoints - Study GDCT

	Control (n=62) n (%)	GH-Treated (n=74) n (%)
Alkaline phosphatase >312 U/L	10 (16)	28 (38)
Creatine Kinase >370 U/L	3 (5)	13 (18)
Cholesterol >5 mMol/L	44 (71)	45 (60)

Table 22. Selected Laboratory Data: Patients with at Least One Value Above Designated Cutpoints - Study GDCI

Analyte	Treatment Group: GH 0.36mg/kg/wk (n=136) n (%)	Treatment Group: GH 0.27mg/kg/wk (n=93) n (%)	Age Range (Years)	Designated Cut Point (U/L)
Alkaline Phosphatase	40 (29)	23 (25)	0-10	312
			10-15	300
			15-20	110
Creatine Kinase	26 (19)	16 (17)	All Ages	338

In study GCDI there were no differences between GH dosage groups for proportions of patients with alkaline phosphatase or creatine kinase above the designated cutpoints at any time in the study. The lower proportion of patients in this study than in study GDCT with alkaline phosphatase above the designated cutpoints, likely reflects the more conservative cutpoints utilized in study GDCI.

Thyroid Function

Concern has existed regarding the potential for GH treatment to induce hypothyroidism, particularly in patients with Turner syndrome who have a higher baseline incidence of autoimmune thyroid disease and hypothyroidism than the normal population. Therefore, thyroid function tests were performed regularly in both studies.

Table 23. Thyroid Function Tests: Patients with at Least One Value Outside Designated Cut Points - Study GDCT

	Control (n=62) n (%)	GH-Treated (n=74) n (%)
T4 < 93 nMol/L	38 (61)	54 (73)
TSH > 10 mU/L	10 (16)	3 (4)

Although there was a high frequency of patients with thyroxine (T4) values below the designated cutpoint, this was similar for both the GH-treated and the Control groups.

Furthermore, these T4 values were not indicative of primary hypothyroidism in most cases, evidenced by absence of TSH elevation. In fact, the proportion of patients with TSH values above the cutpoint was greater for the Control than for the GH-treated group.

Table 24. Thyroid Function Tests: Patients with at Least One Value Outside Designated Cut Points - Study GDCI

	GH 0.36 mg/kg/wk (n=136) n (%)	GH 0.27 mg/kg/wk (n=93) n (%)
T4 < 94 nmol/L (1-6y) < 82 nmol/L (6-11y) < 72 nmol/L (11-16y) < 54 nmol/L (16-21y)	41 (30)	17 (18)
TSH > 10 mU/L	17 (13)	4 (4)

In study GDCI, the proportion of patients with T4 values below the designated cutpoints was lower than that seen in study GDCT, likely due to the difference between the studies with respect to the cutpoints utilized. In this study there was a greater proportion of patients in the treatment group receiving 0.36 mg/kg/wk GH than in the group receiving 0.27 mg/kg/wk who had TSH above the designated cutpoint of 10 mU/L at some time in the study. However, this proportion was similar to that seen for Control patients in study GDCT.

In summary, there was no evidence in either of these studies for an increase in the rate of occurrence of primary hypothyroidism in GH-treated patients, compared with that seen in Control patients in study GDCT, or with the expected rate of hypothyroidism in this population.

Glucose Homeostasis

There is evidence from other studies that treatment with GH induces a state of insulin resistance in some patients, although normal carbohydrate tolerance usually is maintained. In addition, patients with Turner syndrome have an increased rate of occurrence of diabetes mellitus. Therefore, the effects of GH treatment on glucose homeostasis were an important safety concern, and were examined in both of these studies.

Table 25. Glucose Tolerance: Patients with at Least One Value Above Designated Cut Points - Study GDCT

	Control n (%)	GH-Treated n (%)
Fasting Glucose > 6.4 mMol/L	1 (2) n=61	3 (4) n=71
2-hr Post-Prandial Insulin > 400 pMol/L	1 (6) n=17	3 (18) n=17

Modified glucose tolerance tests conducted as a follow-up to the finding of increased fasting blood glucose concentrations in 17 GH-treated patients and 17 Controls, revealed no difference in frequency of insulin values > 400 pMol/l between GH-treated and Controls. In addition, during the GDCT study, no meaningful differences between treatment groups were observed in the numbers of patients with values above the designated cut points for any of the glucose homeostasis variables (Table 25). In this study, no patient had HbA1c greater than 6.8%.

Table 26. Glucose Tolerance: Patients with at Least One Value Above Designated Cut Points - Study GDCI

	0.36mg/kg/wk n (%)	0.27 mg/kg/wk n (%)
Fasting Glucose		
> 6.4 mMol/L	12 (9)	3 (3)
	n=136	n=93
Fasting Insulin		
> 251 pmol/L	17 (13)	10 (11)
	n=136	n=92
2-hr Post-Prandial Glucose		
> 8.3 mMol/L	22 (16)	14 (15)
	n=135	n=92
2-hr Post-Prandial Insulin		
> 400 pMol/L	75 (56)	55 (60)
	n=135	n=91
HbA1c		
> 6.8%	10 (7)	9 (10)
	n=136	n=93

The numbers and percentages of patients with values for glucose, insulin, and hemoglobin A_{1C} above the assigned cut points for Study GDCI are shown in Table 26. The two pooled treatment groups were similar with respect to the percentage of patients who had at least one value above the designated cut point for each of the variables analyzed. Approximately half of the patients in each pooled treatment group (75 patients or 56% in the group receiving 0.36 mg/kg/wk GH and 55 patients or 60% in the group receiving 0.27 mg/kg/wk) had at least one value above the designated cut point for 2-hour postprandial insulin concentration. However, in almost all cases these were sporadic events, and in some cases the elevated postprandial insulin concentrations were present at baseline.

In addition to the results tabulated above, review of data from both studies revealed no meaningful difference between GH groups as a whole (including the Control group), and no relevant changes from baseline to most recent visit, with respect to mean and median fasting, and 2-hour postprandial, glucose concentrations, fasting insulin concentrations, or hemoglobin A_{1C}. In summary, neither study revealed a significant effect of GH upon glucose homeostasis.

IGF-I Concentration

In study GDCI IGF-I (somatomedin-C) concentrations were analyzed for both growth hormone dosage groups (GH0.36 and GH0.27). IGF-I concentrations were not assayed routinely in study GDCT. Mean and median IGF-I concentrations were normal at baseline and most recent visit in both GH dosage groups of study GDCI. Mean and

median increases in IGF-I concentration from baseline to most recent visit were greater for the group receiving the higher GH dosage, but this difference was of marginal statistical significance (p = 0.061).

Table 27. IGF-I Concentration (ng/mL) - Safety Population Study GDCI

		Group		
	Statistic	GH0.36	GH0.27	p-value[1]
Baseline	n	124	81	
	Mean ± SD	142 ± 89	136 ± 76	NA
	Median	128	118	
	Minimum	12	20	
	Maximum	576	390	
Most Recent Visit	n	136	92	
	Mean + SD	374 ± 224	333 ± 169	NA
	Median	325	297	
	Minimum	0	45	
	Maximum	1381.0	958	
Change	n	124	80	
	Mean	241 ± 239	188 ± 165	0.061
	Median	191	163	
	Minimum	-285	-115	
	Maximum	1284	715	

A greater proportion of patients in the group receiving GH 0.36 mg/kg/week (62 patients; 46%) than in the GH 0.27 group (35 patients or 38%) had at least one value for IGF-I concentration greater than 455 ng/ml. This finding, in addition to the greater mean change from baseline for IGF-I for the higher GH dosage group, likely reflects dose-related effects of GH upon IGF-I generation. Inspection of the individual values indicates that those above 455 ng/ml were sporadic events for individual patients. The relationship between blood sampling time and last GH injection is unknown, but may have influenced the variability of results within individuals.

General Safety Conclusions

In Study GDCT, the only adverse events that occurred with a statistically significant increase in frequency in GH-treated patients compared with Control patients were otitis media and a more general category termed "ear disorders", which includes such problems as ears feeling clogged and ear fullness. In the dose-response study, GDCI,

no notable differences in adverse events occurred between patients treated with a weekly GH dose of 0.27 mg/kg or 0.36 mg/kg.

Glucose homeostasis was not adversely affected by GH treatment in these studies, as evidenced by the absence of significant change in fasting glucose, hemoglobinA_{1c} and 2-hour postprandial glucose concentrations. However, approximately one-third of the patients had increased 2-hour postprandial insulin concentrations intermittently during the study. Notably, in some patients the postprandial hyperinsulinemia was present at baseline.

As expected in a group of patients with Turner syndrome, between 8 and 20% of patients were reported to have developed hypothyroidism during the course of the studies. However, the clinical laboratory monitoring performed throughout these studies revealed that TSH concentrations greater than 10 mU/L were found at least as frequently in Control patients as in GH-treated patients, indicating that the development of primary hypothyroidism was unlikely to have been GH related.

In summary, apart from a greater frequency of otitis media in GH-treated patients than controls, there were no unexpected safety concerns related to the use of GH in this patient population.

Benefit/Risk Relationship for Treatment of Short Stature Due to Turner Syndrome

Turner syndrome is the most common chromosomal disorder in females, and is estimated to be present in approximately 7,500 pediatric patients in the United States. The disorder, which results from deficiency of X-chromosomal genetic material (X chromosome monosomy and variants), is characterized primarily by short stature and slow linear growth during childhood, and by delayed, limited, or nonexistent pubertal development during adolescence, the latter being the result of gonadal dysgenesis. Additionally, some patients with Turner syndrome have noticeable phenotypic abnormalities, such as webbed neck, lymphedema, ptosis, broad (shield) chest, increased carrying angle at the elbows, shortened metacarpals or metatarsals, and abnormalities of the finger nails. For a minority of patients, one or more of the following significant additional medical concerns may exist: cardiovascular disease, renal malformation or dysfunction, hypertension, frequent otitis media, hearing loss, dental malocclusion, thyroiditis, Crohn disease, insulin resistance or frank glucose intolerance, and an increased bone fracture rate. The diagnosis of Turner syndrome is generally confirmed by lymphocyte karyotype, although occasionally (usually in situations of mosaicism) a karyotype analysis of skin fibroblasts or an ovarian biopsy may be required. In many cases, the short stature, physical anomalies, and failure of pubertal maturation that characterize Turner syndrome, induce in these girls feelings of difference from their peers. The growth failure that occurs in almost 100% of patients with Turner syndrome begins in the pre-school years, and becomes most evident during late childhood. This submission addresses treatment of the most prevalent , and to many affected individuals the most handicapping, feature of Turner syndrome, short stature.

This supplemental submission provides the scientific information that justifies an acceptable benefit/risk ratio for the use of GH for pharmacologic treatment of short stature in pediatric patients with Turner syndrome. The required evidence for a beneficial effect on final height is convincingly provided in this submission. Safety concerns which were considered at the time the protocols were initiated, are addressed and world-wide experience from adverse event reporting is summarized.

There are presently 32 countries that have approved the use of GH for the treatment of short stature in patients with Turner syndrome. Benefits identified in the present submission are the following:

Final Height

In Study GDCT a randomized group of GH-treated patients was compared at final height with a group of non-GH treated patients followed concurrently in the same study. At the beginning of the study, both treatment groups were balanced for age, race, karyotype, height, weight, bone age, height standard deviation score (SDS) (based on the National Center for Health Statistics normal female standard), and pretreatment growth velocity. However, during the statistical analyses of the study data, it was determined that there was an imbalance between groups for midparental height. GH-treated patients, who received a weekly dose of 0.3 mg/kg GH, had mean final height 5.4 cm greater than patients in the concurrent, non-GH treated control group, when ANCOVA analysis was performed with adjustments for baseline stature strata, midparental height, and investigative site ($p<0.001$). Additional analyses indicate final height gain ranging between 4.9 and 8.1 cm ($p<0.001$ for all analyses). This was considered to be close to final height since linear growth is 98% complete at the bone age of 14 years, a designated criterion for protocol completion in this study. Skeletal maturation was similar for the GH-treated and Control groups.

In the second of the randomized, controlled studies detailed in this report, study GDCI, the mean final height of the combined group of protocol completers receiving GH at a dose of 0.36 mg/kg/week or 0.27 mg/kg/week was 148.7 cm.

One aspect of GH treatment examined in this study was dose response. While the mean final height heights of the small number of protocol completers in this study were not statistically different, for the larger group of patients (the population with ≥ 6 months of efficacy data), the group receiving the higher GH dose had a significantly greater increase in height SDS [NCHS] from baseline to most recent visit ($p=0.012$). In addition, ANCOVA analysis of height at most recent visit in the population with ≥ 6 months of efficacy data, a statistical trend was seen in favor of the higher GH dosage group. Both of these findings suggest a possible dose-response effect for GH.

Five Lilly, European studies provide supportive evidence of the efficacy of GH in increasing final height in patients with Turner syndrome. The mean final height of 150.1 ± 5.0 cm for the pooled patients from these 5 European studies. The patients in these studies began therapy relatively late, at mean ages of 11.6 to 14.1. Hence the potential gain in final height is likely less than that reported in the North American studies, in which the mean ages at study entry were 9.7 to 11.7 years.

Improvements in Height with Respect to Reference Standards

One goal of these studies has been to bring the height of patients with Turner syndrome within, or as close as possible to, the normal female reference. The studies document a gain in height of approximately 1.0 to 1.5 SDS from baseline to most recent

visit. The mean heights of treated patients in both these studies improved to a position at the lower limit of the normal reference range, at approximately of -2.5 SDS below the mean of the NCHS standard, both for the final height of protocol completers in study GDCT, and for the height at most recent visit for the all patients with ≥ 6 months of efficacy data in study GDCI.

As compared with Turner syndrome reference standards such as those published by Lyon et al.(1985) and Ranke et al. (1988), GH-treated patients showed substantial height increases, both during treatment, and at final height. The mean final height of patients who completed the protocol in study GDCT was more than 1.0 SDS above the mean of the age-matched subjects of the Lyon reference standard. In study GDCI, the mean height at the most recent visit evaluated for the population with ≥ 6 months of efficacy data was approximately 1.5 SDS above the mean of the age-matched subjects of the Lyon standard. Similar findings were observed for the final height data for the combined European studies, compared with the Ranke reference standard.

Risks

Two deaths in patients receiving GH and one in a control patient all resulted from cardiovascular events and are not considered related to GH therapy. However, otitis media and other "ear disorders," occurred with a statistically significant increase in frequency in GH-treated patients compared to control patients in study GDCT. The explanation for this finding remains uncertain. Although an alteration in growth of membranous bones in response to growth hormone might be postulated, no evidence for overgrowth of the jaw was identified in a two year study from the Netherlands (Rongen-Westerlaken 1991). There was no statistically significant increase in occurrence of other adverse events potentially increased by GH therapy. Specifically, compared to Control patients in Study GDCT, GH-treated patients showed no statistically significant increase in headache, edema, hypothyroidism, or pigmented nevi, known to be increased at baseline in patients with Turner syndrome. In the GDCI dose-response study, no notable differences in adverse events occurred between patients treated with a weekly GH dose of 0.27 mg/kg or 0.36 mg/kg.

Despite concerns regarding GH-induced reduction of insulin sensitivity, glucose homeostasis was not disturbed in these studies. This is evidenced by absence of significant change in fasting glucose, 2 hour postprandial glucose, or in hemoglobinA_{1C}. However, approximately half of the patients had increased 2 hour postprandial insulin concentrations intermittently during study GDCI, including at baseline for a number of patients.

As expected for patients with Turner syndrome, who have a high rate of thyroid problems at baseline, up to 20% of the treated patients developed primary

hypothyroidism during these studies. However, in study GDCT, there was no significant difference between patients who received or did not receive GH treatment for rates of development of hypothyroidism. A number of additional patients had minor alterations in thyroid hormone concentrations without frank hypothyroidism, however, since the studies were not designed to evaluate pathogenesis of derangements of thyroid function tests in a systematic manner, the cause of these laboratory findings remains unknown, and is considered not clinically significant.

With respect to the antigenicity of exogenously administered growth hormone it is notable that development of clinically significant antibody response to GH did not occur.

The current, approved package label for Humatrope includes among various warnings the following precautions and adverse events which may also apply to GH therapy in patients with Turner syndrome:

"For patients with diabetes mellitus, the insulin dose may require adjustment when somatropin therapy is instituted. Because human growth hormone may induce a state of insulin resistance, patients should be observed for evidence of glucose intolerance. Patients with diabetes or glucose intolerance should be monitored closely during somatropin therapy.

Hypothyroidism may develop during treatment with somatropin, and inadequate treatment of hypothyroidism may prevent optimal response to somatropin. Therefore, patients should have periodic thyroid function tests and be treated with thyroid hormone when indicated.

Intracranial hypertension (IH) with papilledema, visual changes, headache, nausea and/or vomiting has been reported in a small number of patients treated with growth hormone products. Symptoms usually occurred within the first eight (8) weeks of the initiation of growth hormone therapy. In all reported cases, IH-associated signs and symptoms resolved after termination of therapy or a reduction of the growth hormone dose. Funduscopic examination of patients is recommended at the initiation and periodically during the course of growth hormone therapy.

Patients should be monitored carefully for any malignant transformation of skin lesions.

Growth hormone has not been shown to increase the incidence of scoliosis. Progression of scoliosis can occur in children who experience rapid growth. Because growth hormone increases growth rate, patients with a history of scoliosis who are treated with growth hormone should be monitored for progression of scoliosis.

Other adverse drug events that have been reported in growth hormone-treated patients include the following: 1) Metabolic: Infrequent, mild and transient peripheral or generalized edema. 2) Musculoskeletal: Rare carpal tunnel syndrome. 3)Skin: Rare

increased growth of pre-existing nevi. Patients should be monitored carefully for malignant transformation. 4) Endocrine: Rare gynecomastia. Rare pancreatitis."

Conclusion

GH treatment of short stature due to Turner syndrome significantly increases final height. GH-treated patients had final height 5.4 cm greater than control patients followed concurrently in the same study. The average benefit of approximately 5 cm or more in patients who begin therapy on average at around 11 years of age is consistent with most other reports with similar durations of therapy. There are no new risks associated with GH therapy in Turner syndrome, however otitis media or other ear disorders may occur more commonly in GH-treated patients. Physicians and patients will be advised of this risk through package labeling. Other adverse events, such as impairment of glucose tolerance, increased occurrence of thyroid disease, or increased occurrence of benign skin neoplasms such as pigmented nevi did not occur statistically more often in GH-treated patients than in non-GH treated controls. The benefit/risk ratio for GH use in these studies justifies approval for this indication of treatment of short stature in Turner syndrome.

APPENDIX A: GLOSSARY

Baseline Age Strata	In study GDCI patients were stratified by age at baseline into one of four groups. The Baseline age strata were: 5, 6, and 7 years; 8 and 9 years; 10 and 11 years; ≥12 years.
Baseline Stature Strata	In Study GDCT, patients were stratified by height into one of three groups, designated as Lower, Middle and Upper stature strata, based on the patient's height standard deviation score at baseline. These stature strata represent groups of patients with similar height for their age, based on historical data for Turner syndrome compiled by Lyon et al. and were designed so that each stratum could be expected to contain approximately one-third of enrolled patients.
Bone Age	Bone Age represents an estimate of skeletal maturation determined by comparison of a radiograph of the patient's left hand with known standards for skeletal maturation [in this study, the Atlas of Skeletal Maturation by Greulich & Pyle (Greulich and Pyle 1959)].
Chronological Age	In these studies, decimal age is used to represent chronological age. Decimal age is calculated as follows: [Visit Date - Birth Date]/365.25.
Final Height	Final height refers to the height attained at completion of linear growth. In study GDCT, the criteria used to define study completion (and attainment of final height) were bone age ≥ 14 years and growth velocity < 2.0 cm/yr. In study GDCI the criteria used to define achievement of final height were bone age ≥15 years and growth velocity <2.0 cm/year. In the controlled studies reported here, the term Final Height, as it appears in tables and statistical analyses, refers both to patients who met Final Height criteria and to those who came close to this in the opinion of the investigators. Thus the term Final Height as used in this report, refers more accurately to near final height.
Growth Velocity	Growth velocity is the rate of growth in cm/year as calculated from the difference between two height measurements divided by the time elapsed between those measurements.

Growth Velocity SDS

[Ranke]

Growth Velocity SDS [Ranke] is a standard deviation score for growth velocity using as a reference standard growth velocity data for Turner syndrome at various chronological ages reported by Ranke et al. (Ranke et al. 1988). Growth Velocity SDS (standard deviation scores) were calculated compared to reference data for Turner syndrome females [Ranke]. The SDS was defined as: [patient's growth velocity - mean growth velocity for the reference data at the patient's age]/ standard deviation for the reference data at the patient's age. The Ranke reference data contain mean growth velocity and standard deviation for each year of chronological age. The SDS for each patient was calculated for her exact chronological age using interpolation. The oldest available age for the Ranke data was 18 years, and the Ranke data had no standard deviation for ages 2, 3, 17, and 18. Growth Velocity SDS [Ranke] was therefore undefined for patients in this study who were older than 16 years.

Height SDS [Lyon]

Height SDS [Lyon] is a standard deviation score for height using as a reference standard the height of patients with Turner syndrome at various chronological ages reported by Lyon et al. (Lyon et al. 1985). The Lyon reference data contain mean height and smoothed standard deviation for each year of chronological age. The SDS for each patient was calculated for her exact chronological age using interpolation. The oldest available age for the Lyon data was 20 years, so Height SDS [Lyon] was undefined for patients in this study who were older than 20 years.

Height SDS [NCHS]

Height SDS [NCHS] is a standard deviation score for height using as a reference standard the height of normal females at various chronological ages (NCHS Growth Charts 1976). The NCHS reference data contain mean height and standard deviation for intervals of chronological age (generally six-month intervals). The SDS for each patient was calculated using data from the applicable age interval.

Population With ≥ 6

Months Efficacy Data — The population in these studies represents all randomized patients who had efficacy data at Visit 3 or beyond (scheduled 180 days after randomization).

Midparental Height — Midparental height is a gender adjusted average height of a patient's parents, that represents the mean genetic target height for the patient. For females this is calculated as: [(father's height minus 13 cm) plus mother's height]/2 (Tanner et al. 1975).

Protocol Completer — In these studies, a Protocol Completer was defined as a patient who in the opinion of the investigator, fulfilled or almost fulfilled the criteria for achievement of Final Height.

Safety Population — The safety population in these analyses was defined as comprising all randomized patients who took any study medication.

Standard Deviation Score — The Standard Deviation Score (SDS) for a given variable for a given patient is derived by subtracting the age-matched population mean value for that variable from the patient's value. The value obtained is then divided by the age-matched population standard deviation for that variable. For example, to calculate the height SDS in the Lyon Turner reference population for a patient who is 9.6 years old and 124.3 cm tall, the mean height at 9.6 years (117.2 cm) is subtracted from 124.3 cm. The residual 7.1 cm is divided by the reference-population standard deviation for height at 9.6 yrs (5.5 cm). The result is the standard deviation score (1.3 SDS Lyon). In summary the following is the method for calculation of calculation of height SDS: [patient's height - mean height for the reference data at the patient's age]/standard deviation for the reference data at the patient's age.

APPENDIX B: Data Analysis Methods

Study GDCT

The summary data presented for study GDCT represent an interim analysis of an ongoing, randomized, open-label study. Since this was an interim analysis, the significance level was set at p=0.005 to maintain the type 1 error rate for the final analysis at p=0.048 (O'Brien and Fleming, 1979). All tests for primary and secondary efficacy variables were evaluated using the p=0.005 significance level. Statistical tests of other efficacy variables were provided for descriptive purposes only. Data analyzed in this report include all clinical report forms received by the Lilly data management center as of 8 February 1996. The SAS® software (versions 6.09) (SAS Institute Inc., 1990) was used to perform all analyses. Except where otherwise noted, a p-value of 0.050 was considered statistically significant.

Pooling by Investigative Site

Because of sample size concerns for the primary efficacy variable, the 13 investigative sites were pooled into three geographical regions comprising: 1) British Columbia, Alberta, Manitoba; 2) Ontario; 3) Quebec, Nova Scotia. Details of investigative sites pooled to form these geographical groups can be found in the Clinical Study Report for study GDCT. Geographically pooled sites were consistently used in all efficacy and safety analyses which adjusted for site.

Definitions of Populations

The ***safety population*** is defined as those patients who were randomized, and either received any study medication or had post-baseline safety data. This is the population used in safety analyses The ***population with ≥ 6 months efficacy data*** is defined as those patients who were randomized and had height data at Visit 3 (180 days) or beyond. The ***protocol completers*** were those patients designated by the investigators as having fulfilled the criteria for attainment of final height.

Patient Disposition

Patient accountability and primary reasons for discontinuation were summarized for all patients and by treatment groups.

Patient Characteristics

Patient demographic and baseline characteristics measured at entry were summarized for both the patients with ≥ 6 months of efficacy data and patients who completed the protocol. Baseline comparability assessments between the treatment groups were performed only for the population with ≥ 6 months of efficacy data and not considered

for the protocol completers due to small sample sizes in each treatment group. The baseline comparability for continuous variables was performed using a two-way analysis of variance (ANOVA) (Neter et al. 1990) with effects for treatment and geographically pooled investigative site. For the categorical variables, baseline comparability was assessed using a Cochran-Mantel-Haenszel statistic (Mantel and Haenszel, 1959) stratifying by geographically pooled site.

Efficacy

Rationale for Selection of Efficacy Variables for Analysis

The primary aim of this study was to determine the efficacy of GH treatment in improving the final height of patients with Turner syndrome. Therefore, the variable designated as the ***primary efficacy variable*** in this analysis is final height for protocol completers. This variable compares the GH-Treated patients with the Control patients at a time at which only a small amount of residual growth remains. This analysis represents the most accurate evaluation of the therapeutic effect of supplemental human growth hormone in patients with Turner syndrome. Other efficacy variables analyzed in support of the primary efficacy variable were final height SDS according to standards of NCHS, and final height SDS according to standards of Lyon, in the subpopulation of protocol completers. These parameters represent height standard deviation scores for final height based on NCHS (normal female) and Lyon et al. (Turner syndrome) height data. (Mathematical definitions of some variables used in efficacy analyses are provided in the glossary, Appendix A.) After 9 years of age untreated patients with Turner syndrome generally grow along a curve approximately 3 SD below the height curve of normal females, and achieve mean final height that is approximately 20 cm below the mean for normal females. One of the aims of GH therapy in patients with Turner syndrome is to improve the height of affected individuals with respect to the normal female population with whom they interact. Therefore, to evaluate the ability of GH treatment to achieve this goal, the GH-treated versus Control groups were analyzed for height SDS based on the NCHS normal female data. Similar data analysis and comparisons were made between GH-Treated and Control groups, using the Turner syndrome growth data of Lyon et al. (1985). This latter analysis provides an assessment of the growth of GH-Treated versus Control patients in this study in comparison with the well-utilized standard curves for growth or patients with Turner syndrome published by Lyon et al. (Lyon et al. 1985). Also provided are change-from-baseline analyses of each of these variables (height, height SDS [NCHS], and height SDS [Lyon]).

Because approximately half of the patients enrolled are still actively participating in this study, it was considered important to analyze the efficacy of GH therapy in the full study population, not only those who have been designated as protocol completers.

Analyses were therefore performed on the data from the population with ≥ 6 months of efficacy data, at the most recent visit at which efficacy data were available. Variables analyzed were height, height SDS [NCHS], height SDS [Lyon], as well as each of the change in each of these from baseline. It should be noted that these analyses are not designed to evaluate the effect of GH therapy upon final height. Instead, the use of the height standard deviation score (SDS) allows comparison of treated versus control patients across a wide spectrum of ages, at the most recent evaluable visit prior to the data cutoff date, in an age-adjusted manner.

Some specific analyses among those described above were designated in advance of analysis as primary or secondary efficacy variables. As mentioned earlier, the primary efficacy variable is final height for protocol completers. Secondary variables were most recent height SDS [NCHS] and most recent height adjusted for bone age, in both cases for the population with ≥ 6 months of efficacy data.

Treatment Comparisons

For analysis of the primary and secondary efficacy variables the treatment groups (GH-treated and Control) were compared at protocol completion and at the most recent available visit prior to the February 1996 data cutoff date.

Between-group comparisons for all efficacy variables were performed using an ANCOVA model incorporating the effects for treatment, geographically pooled investigative site, baseline stature strata, and midparental height. Midparental height was included since a near-significant difference in midparental height was observed between the treatment groups. Some analyses in the submission called for adjustment for midparental height *a priori,* because of a known correlation between midparental height and adult height.

Analyses for the population with ≥ 6 months of efficacy data were performed using an ANCOVA model which incorporated an effect for bone age, in additional to those for treatment, geographically pooled investigative site, stature strata, and midparental height. The estimated effect of GH therapy from these analyses thus takes into account the effects of growth hormone on skeletal maturation.

Tests of Interactions

Tests of interaction between treatment and geographically pooled sites were performed for the primary and secondary efficacy variables using an ANOVA model which incorporated the effects for treatment, geographically pooled investigative site, stature strata, and the treatment-by-site interaction.

Tests of interaction between treatment and baseline stature strata were performed using an ANOVA model incorporating the effects for treatment, geographically pooled investigative site, stature strata, and the treatment-by-strata interaction.

Compliance

Compliance with GH therapy was analyzed for the GH-treated patients in the safety population. Patient compliance is defined as the total number of injections recorded divided by the total number of expected injections, based on the number of years the patient was in the study. In addition, total study compliance is presented as the percent of GH-treated patients who were 80%-120% compliant. No statistical testing was performed.

Exposure

Since only treated patients had actual drug exposure, the duration of study participation is analyzed for both GH-treated and Control patients and is presented as "Years in Study", both for patients in the safety population and for patients who completed the study. Years in study is defined as the number of years from the first visit to the most recent visit recorded. No statistical testing was performed.

Treatment-Emergent Events

The frequency and percentage of treatment-emergent events were summarized overall and by treatment group. A treatment-emergent event is defined as any event which: (a) had an onset date on or after start of treatment (start of study participation for control patients), or (b) worsened in severity on or after the start of treatment. For events with ≥5% incidence overall, the proportion of patients with treatment-emergent events was tested for homogeneity between the two treatment groups using Fisher's exact test (Armitage and Berry, 1987).

Because of concern that development or worsening of some events potentially associated with growth hormone therapy may also occur in this study, treatment-emergent events of special interest were identified for analysis in this report. These events included bone disorder (to include scoliosis), edemas, hyperglycemia, hypertension, hypothyroidism, increased nevi, and lymphedema. The frequency and percentage of treatment-emergent events of special interest were summarized overall and by treatment group.

Laboratory Data

For continuous laboratory variables, descriptive statistics (sample size, mean, standard deviation, median, minimum, and maximum) were analyzed by treatment group for baseline, most recent visit, and change from baseline at most recent visit results. For

categorical laboratory variables, frequencies and percentages of result values were analyzed for data obtained at baseline and most recent visit. A two-way ANOVA with effects for treatment group and geographically pooled investigative site was performed to assess treatment group differences for fasting glucose, fasting insulin, and hemoglobin A_{1C}. For selected laboratory tests, the frequency and percentage of patients in each treatment group who had laboratory results that were outside the designated clinically significant cut points were analyzed.

Study GDCI

This report represents an interim analysis of an ongoing, randomized, double-blind study. Data analyzed in this report include all clinical report forms received by the Lilly data management center as of 8 February 1996. The SAS® software system (version 6.09) (SAS Institute Inc. 1990) was used to perform all analyses. A p-value of 0.050 was considered statistically significant for all analyses.

Pooling by Treatment Group

The treatment groups into which patients were randomized was previously defined in the Efficacy Data Study B9R-MC-GDCI section. For most of the analyses, the data from the individual treatment groups were pooled to allow comparison of results on the basis of GH dose, or on the basis of treatment with low dose estrogen from an early age (Appendix D). Thus, groups were pooled by either GH dose, by low dose estrogen versus placebo estrogen treatment, or by receipt of GH versus placebo injections within the first 18 months (for safety analyses only). The treatment groups comprising the pooled groups are as follows:

<u>By GH Dose</u>	
GH0.36 group	- GH0.36/LDE, GH0.36/Placebo, Placebo/Placebo
GH0.27 group	- GH0.27/LDE, GH0.27/Placebo
<u>By GH versus Placebo injections within the first 18 months</u>	
GH	- GH0.36/LDE, GH0.36/Placebo, GH0.27/LDE, GH0.27/Placebo
Placebo Injections	- Placebo/Placebo
<u>By Low Dose Estrogen versus Placebo Estrogen</u>	
Low Dose Estrogen	- GH0.36/LDE, GH0.27/LDE
Placebo	- GH0.36/Placebo, GH0.27/Placebo, Placebo/Placebo

Pooling by Investigative Site

Due to sample size concerns, pooling by investigative site was performed for the efficacy analysis of protocol completers, and for other efficacy and safety analyses as

noted in the report. The 47 active investigative sites were pooled into five geographical regions: Central, Northeast, South, Southeast and West. Details of the investigative site pooling are available in the Clinical Study Report for study GDCI.

Definitions of Populations

The ***safety population*** is defined as comprising all randomized patients who took any study medication. The ***population with ≥ 6 months efficacy data*** is defined as all randomized patients who had efficacy data at Visit 3 or beyond (scheduled 180 days after randomization). In the Placebo/Placebo group, patients must also have had efficacy data at Visit 9 or beyond (scheduled 180 days after switch to GH treatment at Visit 7). The ***protocol completers*** in this study are those patients who were identified by the investigators as having fulfilled the criteria for achievement of final height (bone age ≥ 15 years, growth velocity <2 cm/year).

Patient Disposition

Patient accountability and primary reasons for discontinuation were summarized for all patients and by individual treatment group.

Patient Characteristics

Demographic and other baseline characteristics were summarized for the population with ≥ 6 months of efficacy data. Baseline comparability assessments between the five treatment groups were also performed for the population with ≥ 6 months of efficacy data. The baseline comparability for continuous variables was performed using a two-way analysis of variance (ANOVA) (Neter et al. 1990) with effects for treatment and geographically pooled investigative site. For the categorical variables, baseline comparability was assessed using a Cochran-Mantel-Haenszel statistic (Mantel and Haenszel 1959) stratifying by geographically pooled site.

Efficacy

Rationale for Selection of Efficacy Variables for Analysis

The primary aim of this study is to evaluate the efficacy of GH at two different doses in increasing linear growth of patients with Turner syndrome. Although final height was not part of the initial study design, an amendment was made to the protocol in 1993 to include this. The documents submitted to the FDA focus on the dose response aspects of this study, since this was the major objective of the study design. However, for the purpose of this present document, final height is key question to be addressed and has therefore been presented in as the first point of discussion of efficacy in this study.

Because many of the patients enrolled are still actively participating in the study, it was considered important to analyze the efficacy of GH therapy at two different doses, in the full study population, not only those who had completed the protocol. Analyses were therefore performed on the data from all patients who had at least 6 months efficacy data. The primary analysis was a comparison between groups of height SDS [NCHS] at most recent visit. This analysis is intended to provide an evaluation of the comparative effect of two different doses of GH upon the heights of patients with Turner syndrome with respect to the normal female reference standard published by the National Center for Health Statistics. Rather than evaluating raw height data, age-related differences in height are adjusted for by the use of height standard deviation scores (SDS). Analyses of height (cm) and height SDS [Lyon], as well the change in each of these from baseline, are provided in support. The dose-related effect of GH on linear growth rate was evaluated by assessing Growth Velocity SDS [Ranke]. This parameter compares growth rate of the different dosage groups in relation to the growth velocity standards for untreated patients with Turner syndrome, published by Ranke et al.

Mathematical definitions of terms used in efficacy analyses are provided in the glossary for chronological age, standard deviation scores (SDS) and midparental height.

Treatment Comparisons

The GH0.36 and GH0.27 groups were compared at protocol completion and at the most recent visit available prior to the February 1996 data cutoff point.

All tests for primary and secondary efficacy variables were evaluated using the $p \leq 0.050$ significance level. No adjustment to the significance level was made for this interim analysis since the study is scheduled to close December 1996 regardless of the preliminary results.

Between-dose comparisons for all efficacy variables (except height at most recent visit) were performed using an ANOVA model incorporating the effects for GH dose, low dose estrogen, geographically pooled investigative site, and baseline age strata. Analysis of height at most recent visit in all patients with ≥ 6 months efficacy data, were performed using an ANCOVA model which incorporated an additional effect for bone age.

A near-significant difference in midparental height was observed among the five treatment groups; therefore, to confirm results, GH0.27 versus GH0.36 group comparisons were performed using an analysis of covariance (ANCOVA) (Neter et al. 1990) incorporating effects for GH dose, low dose estrogen, geographically pooled investigative site, baseline age strata, and midparental height.

Tests of Interactions

Tests of interaction between GH dose group and geographically pooled site were performed using an ANOVA model which incorporated the effects for GH dose, low dose estrogen, geographically pooled investigative sites, baseline age strata, and the GH dose group-by-site interaction. Interaction testing for height at most recent visit was performed using an ANCOVA model with an additional effect for bone age.

The interactions between GH dose groups and baseline age strata, as well as between GH dose groups and low dose estrogen groups, were analyzed using similar ANOVA (or ANCOVA) models.

Compliance

Compliance with GH treatment was assessed for all patients in the safety population, overall, and for the pooled GH0.27 and GH0.36 groups. Patient compliance is defined as the total number of injections recorded divided by the total number of expected injections, based on the number of years the patient was in the study. In addition, total study compliance is presented as the percent of all safety patients who were 80%-120% compliant.

Exposure

Exposure to GH was assessed by analysis of the number of years the patient participated in the study. Years in study was analyzed for patients in the safety population and for the subgroup of patients who completed the study (protocol completers). Years in study is defined as the number of years from the first visit to the most recent visit recorded prior to the February, 1996 data cutoff date.

Treatment-Emergent Events

The frequency and percentage of treatment-emergent events were summarized overall and by pooled GH0.27 and GH0.36 treatment groups. A treatment-emergent event is defined as any event which: (a) had an onset date on or after start of treatment or (b) worsened in severity on or after the start of treatment.

A comparison of all GH-treated patients versus those receiving placebo injections was also provided by summarizing the frequency and percentage of treatment-emergent events occurring within the first eighteen months of treatment. This was the period before the change of the group with the lowest growth velocity (determined at the time of the present analysis to be the Placebo injection/Placebo estrogen group) was reassigned to join the group receiving 0.36 mg/kg/week GH and placebo estrogen. These statistics were provided overall, for all GH-treated patients combined, and for patients receiving Placebo injections.

Because patients with Turner syndrome are at increased risk for a variety of health problems in a number of body systems, treatment-emergent events of special interest were identified for analysis in this report to evaluate the effect of GH therapy on development or progression of such disorders. These events included bone disorder (to include scoliosis), edemas, hyperglycemia, hypertension, hypothyroidism, increased nevi, and lymphedema. The frequency and percentage of treatment-emergent events of special interest were summarized overall and for the GH0.27 and GH0.36 groups.

Laboratory Data

All descriptive statistics for the laboratory data are presented for the pooled GH0.27 and GH0.36 groups. For continuous laboratory variables, descriptive statistics (sample size, mean, standard deviation, median, minimum, and maximum) are presented for baseline, most recent visit, and change from baseline to most recent visit results. For categorical laboratory variables, frequencies and percentages of results were analyzed for data obtained at baseline and most recent visit. For selected key laboratory tests, the frequency and percentage of patients in each pooled GH dose group who had laboratory results that fell outside certain designated cut points were analyzed.

APPENDIX C: Supplementary Analyses of Efficacy

Since the preparation of the submission, further analyses of Turner Syndrome efficacy data from Studies GDCT and GDCI have been undertaken. In brief, these analyses address the following:

- In the submission, ANCOVA analyses that account for the midparental height of patients omitted those patients with incomplete parental height information at baseline. In the analysis of GDCT protocol completers, one patient had missing midparental height. Appendix C.1 documents the alternative approach of utilizing the efficacy data for that patient by an using approximated midparental height. The most conservative of these analyses estimates the effect of GH therapy upon height to be an increase of 4.9 cm.

- Several of the patients in GDCT who were declared to be protocol complete did not meet completion criteria, and there are several patients strictly meeting the criteria who have not been declared complete. An analysis of GDCT patients strictly meeting protocol completion criteria is presented in Appendix C.2, and is more supportive of efficacy than the corresponding analyses presented in the submission, with an estimated increase in height due to GH therapy of 6.4 cm.

- Since baseline height is known to be positively correlated with adult height in Turner patients, the GDCT design included stratification at baseline for height relative to age. This stratification is necessarily accounted for in analyses present in the submission, and is an important explanatory variable of height attained at completion. Further examination of the GDCT data reveals that, despite this accounting for baseline stature strata, the actual baseline height and age of the patients are still remarkably strong explanatory variables of height attained at completion. An important observation in these analyses is that midparental height and stature stratum are no longer informative variables, once baseline height and age are included in the analysis. Additionally, accounting in this way for baseline height and age largely resolves the discrepancy present in earlier analyses between the GH effect for height, and the GH effect for height change-from-baseline. Appendix C.3 presents analyses utilizing these variables, which lead to an estimated height increase due to GH therapy of 6.6 cm among declared protocol completers, and of 8.1 cm among patients meeting the protocol completion criteria.

- The criteria for protocol completion in study GDCI were more stringent than those for study GDCT, in particular requiring patients to have met or exceeded a bone age of 15 years. Since there were only 31 protocol completers in GDCI, in an effort to increase the number of analyzable patients, the GDCT protocol completion bone age criterion of 14 years was applied to the GDCI data. This led to 83 analyzable patients. Results are presented in Appendix C.4, and for efficacy are nearly identical to the results from the 31 patients completing by GDCI criteria, yielding an estimated final height of 148.3 cm.

Appendix C.1: Missing Midparental Height Data

In the submission, ANCOVA analyses that account for the midparental height of patients omitted those patients with incomplete parental height information at baseline. In particular, in the analysis of GDCT protocol completers, patient 106-2708 was omitted since her mother's height was unavailable.

A more robust analysis utilizes this patient's efficacy information at the expense of making a reasonable approximation of her mother's height. Using a population mean height for women of 164 cm, and repeating earlier analyses, leads to the estimates of height gain presented in Table C.1.1. Since patient 106-2708 was a tall patient in the Control group, estimates of height gain are reduced compared to Table 6. Several different values were used as estimates of the mother's height; the estimates in Table C.1.1 are not particularly sensitive to the value used.

Appendix C.3 presents alternative analyses that utilize each patient's baseline height and baseline age, obviating the need for midparental height adjustment.

Table C.1.1. Declared Protocol Completers (GDCT) GH (n=27) vs Control (n=19)

	Mean GH Effect (ANCOVA) [a] Mean ± SE	p-value (ANCOVA) [a]
Height (cm)	4.9 ± 1.3	< 0.001
Height SDS (NCHS)	0.7 ± 0.2	0.011
Height SDS (Lyon)	0.9 ± 0.2	< 0.001
Δ Height (cm)	7.4 ± 1.7	< 0.001
Δ Height SDS (NCHS)	0.7 ± 0.3	0.035
Δ Height SDS (Lyon)	1.0 ± 0.1	< 0.001

[a] ANCOVA accounts for stature strata, geographical site, and midparental height. Midparental height has been imputed for one patient with missing mother's height (see text of Appendix C.1).

Appendix C.2: Patients Strictly Meeting Protocol Completion Criteria

The protocol completion criteria of GDCT required that the patient have bone age ≥ 14 years and growth velocity < 2 cm/year. Among the 46 declared protocol completers, 18 GH-treated patients and 14 Control patients actually met these criteria. A further 4 GH-treated patients and 2 Control patients in GDCT met these criteria at their most recent visit, but had not yet been declared protocol complete by the investigator. Table C.2.1 presents parallel analyses, to those of Tables 6 and C.1.1, for the patients strictly meeting protocol-completion criteria. The mean gain in height due to GH in the patients strictly meeting protocol completion criteria is 6.4 cm. The GH-treated patients had a mean change in height from baseline of 8.8 cm more than the Control patients.

Table C.2.1. Patients Strictly Meeting Protocol Completion Criteria (GDCT) GH (n=22) vs Control (n=16)

	Mean GH Effect (ANCOVA) [a] Mean ± SE	p-value (ANCOVA) [a]
Height (cm)	6.4 ± 1.4	< 0.001
Height SDS (NCHS)	0.9 ± 0.2	0.001
Height SDS (Lyon)	1.0 ± 0.2	< 0.001
Δ Height (cm)	8.8 ± 2.0	< 0.001
Δ Height SDS (NCHS)	1.2 ± 0.3	< 0.001
Δ Height SDS (Lyon)	1.2 ± 0.2	< 0.001

[a] ANCOVA accounts for stature strata, geographical site, and midparental height. Midparental height has been imputed for one patient with missing mother's height (see text of Appendix C.1).

Appendix C.3: Explanatory Value of Baseline Height and Baseline Age

Since baseline height is known to be positively correlated with adult height in Turner patients, the GDCT design included stratification at baseline for height relative to age. This stratification is necessarily accounted for in analyses present in the submission, and is an important explanatory variable of height attained at completion. Further examination of the GDCT data reveals that, despite this accounting for baseline stature strata, the actual baseline height and age of the patients are still remarkably strong explanatory variables of height attained at completion.

Table C.3.1 presents analyses of the GDCT declared protocol completers that utilize baseline height and baseline age of patients, via ANCOVA. The Lyon height SDS was used as the baseline height, since it is most representative of the patient's height for her age. These baseline variables were first included in the ANCOVA, but when either was not a significant explanatory variable at the p=0.05 level, it was subsequently omitted from the ANCOVA. Table C.3.1 reports the final ANCOVA models that result; the third and fourth columns indicate whether the baseline height and baseline age variables were significant and hence included in the ANCOVA. An important

observation in these analyses is that midparental height and stature stratum are no longer informative variables, once baseline height and age are included in the analysis.

Table C.3.1. Declared Protocol Completers (GDCT): Adjusted for Baseline Height and Baseline Age GH (n=27) vs Control (n=19)

	Mean GH Effect (ANCOVA) [a] Mean ± SE	p-value (ANCOVA) [a]	Baseline Height SDS (Lyon) significant	Baseline Age significant
Height (cm)	6.6 ± 0.8	< 0.001	Yes	No
Height SDS (NCHS)	1.0 ± 0.2	< 0.001	Yes	No
Height SDS (Lyon)	1.0 ± 0.1	< 0.001	Yes	Yes
Δ Height (cm)	6.5 ± 0.8	< 0.001	No	Yes
Δ Height SDS (NCHS)	1.0 ± 0.2	< 0.001	Yes	Yes
Δ Height SDS (Lyon)	1.0 ± 0.1	< 0.001	Yes	Yes

[a] ANCOVA accounts for baseline height SDS (Lyon), baseline age, and geographical site. Baseline height SDS (Lyon) and/or baseline age are removed from the ANCOVA when not of significant explanatory value at $p<0.05$. In all cases, both stature stratum and midparental height are statistically insignificant explanatory variables, in the presence of the remaining variables, and are excluded from the ANCOVA.

In Table C.3.1, for declared protocol completers, the estimated height increase due to GH is 6.6 cm. The GH-treated patients had a mean change in height from baseline of 6.5 cm more than the Control patients. A feature of these analyses is that accounting in this way for baseline height and age largely resolves the discrepancy present in earlier analyses between the GH effect for height, and the GH effect for height change-from-baseline.

Table C.3.2. Patients Strictly Meeting Protocol Completion Criteria (GDCT): Adjusted for Baseline Height and Baseline Age GH (n=22) vs Control (n=16)

	Mean Effect (ANCOVA) [a] Mean ± SE	p-value (ANCOVA) [a]	Baseline Height SDS (Lyon) significant	Baseline Age significant
Height (cm)	8.1 ± 0.8	< 0.001	Yes	No
Height SDS (NCHS)	1.2 ± 0.2	< 0.001	Yes	No
Height SDS (Lyon)	1.2 ± 0.1	< 0.001	Yes	Yes
Δ Height (cm)	7.6 ± 0.8	< 0.001	No	Yes
Δ Height SDS (NCHS)	1.5 ± 0.2	< 0.001	Yes	Yes
Δ Height SDS (Lyon)	1.1 ± 0.1	< 0.001	No	Yes

[a] ANCOVA accounts for baseline height SDS (Lyon), baseline age, and geographical site. Baseline height SDS (Lyon) and/or baseline age are removed from the ANCOVA when not of significant explanatory value at $p<0.05$. In all cases, both stature stratum and midparental height are statistically insignificant explanatory variables, in the presence of the remaining variables, and are excluded from the ANCOVA.

In Table C.3.2, for patients strictly meeting protocol completion criteria, the estimated height increase due to GH is 8.1 cm. The GH-treated patients had a mean change in height from baseline of 7.6 cm more than the Control patients. As with other analyses of GDCT patients strictly meeting protocol completion criteria, these analyses are supportive of greater magnitude of efficacy than those with the declared protocol completers.

Appendix C.4: Analysis of GDCI patients with bone age 14 or greater

The criteria for protocol completion in study GDCI were more stringent than those for study GDCT, in particular requiring patients to have met or exceeded a bone age of 15 years. Since there were only 31 protocol completers in GDCI, in an effort to increase the number of analyzable patients, the GDCT protocol completion bone age criterion of 14 years was applied to the GDCI data. (This was done without regard to growth velocity, since no comparison between groups is being made.) This led to 83

analyzable patients. Results are presented in Table C.4.1, and for efficacy are nearly identical to the results from the 31 patients completing by GDCI criteria (Table 13), yielding an estimated final height of 148.3 cm.

Table C.4.1. Summary Data for Patients with Bone Age of 14 or Greater (GDCI)

Mean ± SD	At Baseline (n=83)	At Completion (n=83)
Age (y)	11.2 ± 1.9	16.3 ± 1.3
Bone Age (y)	9.0 ± 1.6	14.9 ± 0.9
Height (cm)	124.6 ± 8.7	148.3 ± 6.3
Height SDS [NCHS]	-3.1 ± 1.1	-2.3 ± 1.1
Height SDS [Lyon]	0.2 ± 0.8	1.5 ± 1.0
Duration of GH Therapy (y)	NA	4.9 ± 1.5
Δ Height (cm)	NA	23.8 ± 8.5
Δ Height SDS [NCHS]	NA	0.8 ± 1.0
Δ Height SDS [Lyon]	NA	1.2 ± 0.6

APPENDIX D: Low Dose Estradiol - Study GDCI

The main purpose of the present report is to address efficacy and safety of GH treatment in patients with Turner syndrome. However, in study GDCI an important objective was to evaluate the efficacy of low dose estradiol in combination with GH in enhancing linear growth in patients with Turner syndrome. In this study, patients were randomized at study entry to receive one of three GH doses (0.36 mg/kg/week; 0.27 mg/kg/week or placebo), in combination with one of two oral medications, either ethinyl estradiol, or placebo. The treatment groups at study entry were as follows:

1. GH 0.27 mg/kg/week (0.09 mg/kg/dose, three times per week), with low dose ethinyl estradiol; designated GH0.27/LDE
2. GH 0.27 mg/kg/week (0.09 mg/kg/dose, three times per week) with placebo tablets; designated GH0.27/Placebo
3. GH 0.36 mg/kg/week (0.12 mg/kg/dose, three times per week), with low dose ethinyl estradiol; designated GH0.36/LDE)
4. GH 0.36 mg/kg/week (0.12 mg/kg/dose, three times per week), with placebo tablets; designated GH0.36/Placebo
5. placebo injections with placebo tablets (designated Placebo/Placebo).

Study Design for Estradiol

Oral study drug material (ethinyl estradiol or placebo) is administered according to chronological age and body weight (Table D.1). The dose of oral study drug material was assigned according to the patient's chronological age at the admission visit. The dose is not increased as the patient's age increases, although the dosage is adjusted for weight changes at each visit, if necessary. The following exceptions were noted:

- No oral study drug material was administered to patients less than 8 years old, or weighing <20 kg.
- Patients 8 years old or older at Visit 1 but weighing less than 20 kg began oral study drug treatment at Visit 7 (18 months) according to the patient's age at Visit 1 and weight at Visit 7, if >20 kg. Dosage is adjusted for weight changes at each subsequent visit, if necessary (Table D.1).

- Patients less than 8 years old at Visit 1 began oral study drug at Visit 7 (18 months) or Visit 13 (36 months). Therapy began at the visit at which the patient was at least 8 years old and weighed at least 20 kg. The dosage of oral study drug was assigned according to the patient's age at the visit she began oral study drug therapy (Visit 7 or 13 only) and is subsequently adjusted for weight changes, as necessary.

Dosing of oral study drug is undertaken in the following manner:

- Active ethinyl estradiol tablets are one μg strength.
- Patients at least 8 years old but not yet 10 years old receive one tablet per 20 kg of body weight, with no oral study drug administered if the patient weighed less than 20 kg at the admission visit (Visit 1). This dose is equal to approximately 25 - 50 ng of ethinyl estradiol per kg; (0 ng/kg for patients receiving placebo).
- Patients at least 10 years old but not yet 12 years old receive one tablet per 10 kg of body weight. This dose is equal to approximately 67 - 100 ng of ethinyl estradiol per kg (0 ng/kg for patients receiving placebo). No oral study drug is administered if patient weighed less than 20 kg at the admission visit (Visit 1).
- Patients at least 12 years old receive one tablet per 5 kg of body weight. This dose is equal to approximately 160 - 200 ng of ethinyl estradiol per kg (0 ng/kg for patients receiving placebo).

The blinded oral study drug therapy is discontinued when patients are prescribed, by the investigator or their own physician, open-label estrogen to induce feminization, or estrogen/progesterone combination therapy to induce menstrual cycling. This was permitted in study patients after 13.5 years of age.

Table D.1. Dosing of Oral Study Drug - Study GDCI

Chronological Age (y in years)	Body Weight (z in kg)	Number of Tablets Per Dose	Ethinyl Estradiol Dose (ng/kg/day)
$5 \leq y < 8$	All Weights	0	0
$8 \leq y < 10$	$z < 20$	0	0
	$20 \leq z < 40$	1	50 - 25
	$40 \leq z < 60$	2	50 - 33
	$60 \leq z < 80$	3	50 - 37.5
$10 \leq y < 12$	$20 \leq z < 30$	2	100 - 67
	$30 \leq z < 40$	3	100 - 75
	$40 \leq z < 50$	4	100 - 80
	$50 \leq z < 60$	5	100 - 83
	$60 \leq z < 70$	6	100 - 86
$12 \leq y$	$20 \leq z < 25$	4	200 - 160
	$25 \leq z < 30$	5	200 - 167
	$30 \leq z < 35$	6	200 - 171
	$35 \leq z < 40$	7	200 - 175
	$40 \leq z < 45$	8	200 - 178
	$45 \leq z < 50$	9	200 - 180
	$50 \leq z < 55$	10	200 - 182
	$55 \leq z < 60$	11	200 - 183
	$60 \leq z$	12	200 -

Note: Patients receiving placebo tablets follow the same schedule for tablet number. The dosing of ethinyl estradiol is unknown to patients and investigators but is provided here to aid interpretation of the treatment regimen.

Efficacy Results

Because the number of patients in the protocol completing subgroup who received low dose estrogen was small (n=14), the results of the low dose estrogen treatment aspect of this study are provided only for the large study population with at least 6 months efficacy data (n=135 for low dose estrogen; n=89 for placebo). These data therefore represent only interim results and do not address final height.

Patients in two of the treatment groups in this study received low dose estrogen at an early age, from 8 years of age until 13.5 years of age (if weight >20 kg). Patients in the other three treatment groups received placebo tablets between 8 and 13.5 years of age and started standard estrogen replacement therapy after 13.5 years of age. To evaluate the potential effects of low dose estrogen at a young age and its interaction with GH on height, the following groups were pooled for the purpose of making

statistical comparisons: Placebo (comprising all three subgroups of patients who received oral placebo, pooled with respect to GH dose) and low dose estrogen (LDE; the two subgroups that received low dose ethinyl estradiol from 8 yrs of age, pooled with respect to GH dose).

Height and height SDS data for the pooled groups from the population with at least 6 months efficacy data are presented in Table D.2. Analysis of baseline patient characteristics revealed differences in baseline height SDS between the oral placebo and low dose estrogen groups. At baseline, the mean height of the low dose estrogen group was 0.4 SDS by NCHS standards below that of the group receiving oral placebo (p=0.011); it was 0.3 SDS lower than that of the placebo group when analyzed according to Lyon standards (p=0.031). There was also a trend or the low dose estrogen group to have lower pretreatment growth velocity (p=0.090).

Table D.2. Baseline and Most Recent Visit - Placebo vs Low Dose Estrogen

Mean ± SE	Placebo n=135	Low Dose Estradiol n=89	p-value
At Baseline			
Height (cm)	118.5 ± 13.1	117.1 ± 12.2	0.389 [a]
Height SDS [NCHS]	-2.8 ± 0.9	-3.2 ± 1.1	0.011 [a]
Height SDS [Lyon]	0.2 ± 0.9	-0.1 ± 0.9	0.031 [a]
Midparental Height (cm)	163.3 ± 5.8 (n=130)	162.7 ± 5.9 (n=84)	0.460 [a]
At Most Recent Visit			
Bone Age (y)	12.6 ± 2.5 (n=134)	12.9 ± 2.4 (n=89)	0.288 [a]
Height (cm)	142.4 ± 0.6	139.4 ± 0.7	<0.001 [b]
Δ Height	24.2 ± 0.6	21.7 ± 0.7	0.004 [b]
Height SDS [NCHS]	-2.4 ± 0.1	-2.5 ± 0.1	0.226 [b]
Δ Height SDS [NCHS]	0.6 ± 0.1	0.5 ± 0.1	0.640 [b]
Height SDS [Lyon]	1.3 ± 0.1	1.3 ± 0.1	0.684 [b]
Δ Height SDS [Lyon]	1.2 ± 0.1	1.2 ± 0.1	0.900 [b]

[a] By ANOVA analysis including effects for pooled geographical site and GH dose.

[b] By ANCOVA analysis with adjustments for age strata, pooled geographical site, baseline age, baseline height SDS [Lyon], midparental height, GH dose, bone age.

The analyses shown in Table D2 provide quite disparate results, making it difficult to evaluate the effect of low dose estrogen on the height of these patients. While the most recent height and the change in height over the duration of study are greater for the placebo group than the low dose estrogen group, the standard deviation scores for the height are very similar between groups, even when bone age is accounted for in the analysis.

There are other features of the study design that have not yet been analyzed which may contribute to the outcome of patients in the low dose estrogen versus placebo groups. These include the actual dose of estradiol received by the patient, the dose per kg (as shown in Table D1, the dose per kg varies within and between age strata) and the age at which low dose estrogen and subsequent standard estrogen replacement were instituted. Since the primary purpose of this document is to address the efficacy of GH treatment of short stature in Turner syndrome, these analyses have not yet been performed.

In summary, there is some evidence from this study that patients who receive low dose estrogen have a smaller gain in height than those who not. However, given the importance of estrogen for other aspects of health such as bone mineral accretion and cardiovascular status, there is insufficient evidence to draw conclusions regarding the relative benefits and risks of early low dose estrogen therapy in this population.

APPENDIX E: Efficacy Analysis Lilly-Sponsored European Studies

This section summarizes the efficacy data for five European Lilly-sponsored studies addressing GH treatment of short stature of patients with Turner syndrome. These studies, which were conducted in an open-label fashion, were undertaken between 1987 and 1991 in France, Germany, the United Kingdom, the Netherlands and Norway. They were designed as short term studies (6 - 12 months) to evaluate GH effect on growth rate at dosages ranging from 0.18 to 0.33 mg/kg/week. In general they were single-dose studies, however the German study evaluated efficacy at two different GH doses. There were no upper limits for age of enrollment in these studies, however the study conducted in the UK included only those patients with bone age less than 12 yrs; the Dutch study had 2 treatment groups, starting GH treatment either before or after 12 years of age.

Table E.1. Baseline Age of Patients Enrolled in European Studies

Country	n	Baseline Age Mean ± SD
France	59	12.9 ± 1.7
Germany	91	11.6 ± 1.3
Netherlands	54	14.1 ± 2.2
Norway	29	12.1 ± 1.4
UK	20	13.7 ± 1.9

All of these studies were conducted over a limited period. They were not designed to address final height. The indication of treatment of short stature in Turner syndrome was approved on the basis of the significant increases in growth velocity seen in these studies. After approval for this indication, follow-up of the original study patients was undertaken in the Netherlands and Germany. To obtain follow-up data on the patients treated in France, UK and Norway, Dr J. Van den Broeck personally collected all data from the patients' clinical records. Thus, the available final height data were obtained retrospectively, without comparison to a control group.

Two hundred and fifty three patients were enrolled in these studies. Of these, final or near final height data (defined by growth velocity less than 9 mm/year) are available for

78 patients across all five studies. The average height of these 78 patients was 150.1 ± 5.0 cm. Only the final height data from France and the Netherlands are available separately. These data are shown in Table E.2.

Table E.2. Final Height Data - France and Netherlands

	France (n=28) Mean ± SD	Netherlands (n=33) Mean ± SD
Baseline Age (y)	12.9 ± 1.7	14.1 ± 2.2
Baseline Height SDS (Ranke)*	-0.4 ± 0.7	0.4 ± 0.9
Duration of Therapy (mths)	49 ± 14	51 ± 15
Final Age (y)	19.0 ± 1.5	20.0 ± 1.9
Final Height (cm)	147.8 ± 3.9	151.3 ± 5.4
Final Height SDS (Ranke)*	0.2 ± 0.6	0.8 ± 0.9

*Height SDS are according to the standards of Ranke et al. These standards differ from the Lyon standards used in the North American studies in that the same height represents a greater SDS when analyzed by Ranke standards than when analyzed by Lyon standards.

The data obtained from the European studies are supportive of the efficacy of GH in treatment of short stature in Turner syndrome. This is evidenced by the fact that patients in the Dutch and French studies improved their height relative to the Ranke reference standard by approximately 0.4 and 0.6 SD scores respectively. It is important to recognize that treatment was initiated late in both of these studies and available growth potential in these patients was therefore limited. Earlier initiation of GH treatment would likely have provided greater increases in final height, as seen in the North American studies detailed in this submission.

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APPENDIX G: Label

HUMATROPE®
SOMATROPIN (rDNA ORIGIN) FOR INJECTION

DESCRIPTION

Humatrope® (Somatropin, rDNA Origin, for Injection) is a polypeptide hormone of recombinant DNA origin. Humatrope has 191 amino acid residues and a molecular weight of about 22,125 daltons. The amino acid sequence of the product is identical to that of human growth hormone of pituitary origin. Humatrope is synthesized in a strain of *Escherichia coli* that has been modified by the addition of the gene for human growth hormone.

Humatrope is a sterile, white, lyophilized powder intended for subcutaneous or intramuscular administration after reconstitution. Each vial of Humatrope contains 5 mg somatropin (15 IU* or 225 nanomoles); 25 mg mannitol; 5 mg glycine; and 1.13 mg dibasic sodium phosphate. Phosphoric acid and/or sodium hydroxide may have been added at the time of manufacture to adjust the pH. This product is oxygen sensitive. Each vial is supplied in a combination package with an accompanying 5-mL vial of diluting solution. The diluent contains water for injection with 0.3% *m*-cresol as a preservative and 1.7% glycerin added at the time of manufacture.

Humatrope is a highly purified preparation. The 1.7% glycerin content makes the reconstituted product nearly isotonic at a concentration of 2 mg of Humatrope/mL diluent. Reconstituted solutions have a pH of approximately 7.5.

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* The units per vial of Humatrope have changed from approximately 13 IU to 15 IU. This does not represent a change in product purity or the quantity (mg) of somatropin per vial. The change in units is a result of harmonizing the defined specific activity of the current reference standard with the international WHO (World Health Organization) reference standard. The specific activity of the International Standard for somatropin is defined as 3 International Units per mg of protein (previously designated as approximately 2.67 IU/mg). Humatrope is now labeled based on a specific activity of 3 IU/mg. This change in reference standard activity does not affect the recommended weekly dosage of 0.18 mg of somatropin per kg of body weight for pediatric patients. However, due to the reference standard change the weekly units administered will be measured as 0.54 IU/kg of body weight (previously approximately 0.48 IU/kg of body weight).

CLINICAL PHARMACOLOGY

General:

Linear Growth--Humatrope stimulates linear growth in pediatric patients who lack adequate normal endogenous growth hormone. In vitro, preclinical, and clinical testing have demonstrated that Humatrope is therapeutically equivalent to human growth hormone of pituitary origin and achieves equivalent pharmacokinetic profiles in normal adults. Treatment of growth hormone-deficient pediatric patients and patients with Turner syndrome with Humatrope produces increased growth rate and IGF-I (Insulin-like Growth Factor-I/Somatomedin-C) concentrations similar to those seen after therapy with human growth hormone of pituitary origin.

In addition, the following actions have been demonstrated for Humatrope and/or human growth hormone of pituitary origin.

A. *Tissue Growth*--1. Skeletal Growth: Humatrope stimulates skeletal growth in pediatric patients with growth hormone deficiency. The measurable increase in body length after administration of either Humatrope or human growth hormone of pituitary origin results from an effect on the growth plates of long bones. Concentrations of IGF-I, which may play a role in skeletal growth, are low in the serum of growth hormone-deficient pediatric patients but increase during treatment with Humatrope. Elevations in mean serum alkaline phosphatase concentrations are also seen. 2. Cell Growth: It has been shown that there are fewer skeletal muscle cells in short-statured pediatric patients who lack endogenous growth hormone as compared with normal pediatric populations. Treatment with human growth hormone of pituitary origin results in an increase in both the number and size of muscle cells.

B. *Protein Metabolism*--Linear growth is facilitated in part by increased cellular protein synthesis. Nitrogen retention, as demonstrated by decreased urinary nitrogen excretion and serum urea nitrogen, follows the initiation of therapy with human growth hormone of pituitary origin. Treatment with Humatrope results in a similar decrease in serum urea nitrogen.

C. *Carbohydrate Metabolism*--Pediatric patients with hypopituitarism sometimes experience fasting hypoglycemia that is improved by treatment with Humatrope. Large doses of human growth hormone may impair glucose tolerance. There is an increased incidence of glucose intolerance in untreated patients with Turner

syndrome. Administration of human growth hormone to normal adults and patients with Turner syndrome resulted in increases in mean serum fasting and postprandial insulin levels. However, mean glucose and hemoglobin A_{1C} levels remained in the normal range.

D. *Lipid Metabolism*--In growth hormone-deficient patients, administration of human growth hormone of pituitary origin has resulted in lipid mobilization, reduction in body fat stores, and increased plasma fatty acids.

E. *Mineral Metabolism*--Retention of sodium, potassium, and phosphorus is induced by human growth hormone of pituitary origin. Serum concentrations of inorganic phosphate increased in patients with growth hormone deficiency after therapy with Humatrope or human growth hormone of pituitary origin. Serum calcium is not significantly altered in patients treated with either human growth hormone of pituitary origin or Humatrope.

Pharmacokinetics:

Absorption—Humatrope has been studied following intramuscular, subcutaneous, and intravenous administration in adult volunteers. The absolute bioavailability of somatropin is 75% and 63% after subcutaneous and intramuscular administration, respectively.

Distribution—The volume of distribution of somatropin after intravenous injection is about 0.07 L/kg.

Metabolism—Extensive metabolism studies have not been conducted. The metabolic fate of somatropin involves classical protein catabolism in both the liver and kidneys. In renal cells, at least a portion of the breakdown products of growth hormone is returned to the systemic circulation. In normal volunteers, mean clearance is 0.14 L/hr/kg. The mean half-life of intravenous somatropin is 0.36 hours, whereas subcutaneously and intramuscularly administered somatropin have mean half-lives of 3.8 and 4.9 hours, respectively. The longer half-life observed after subcutaneous or intramuscular administration is due to slow absorption from the injection site.

Excretion–Urinary excretion of intact Humatrope has not been measured. Small amounts of somatropin have been detected in the urine of pediatric patients following replacement therapy.

Special Populations

Geriatric–The pharmacokinetics of Humatrope has not been studied in patients greater than 60 years of age.

Pediatric–The pharmacokinetics of Humatrope in pediatric patients is similar to adults.

Gender–No studies have been performed with Humatrope. The available literature indicates that the pharmacokinetics of growth hormone is similar in both men and women.

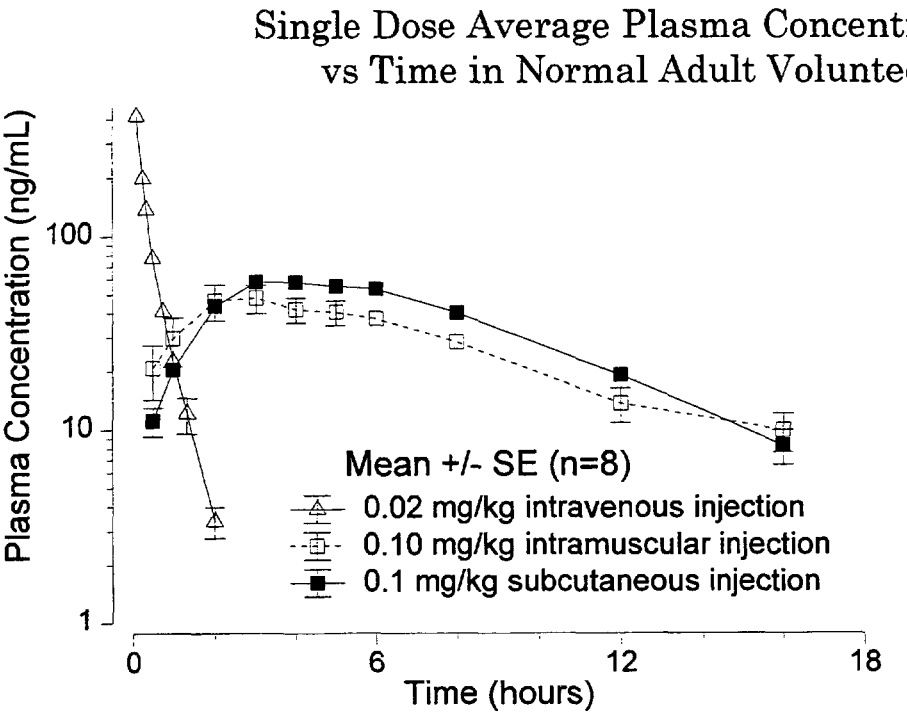
Race–No data are available.

Renal, Hepatic insufficiency–No studies have been performed with Humatrope.

Table 1
Summary of Somatropin Parameters in the Normal Population

	C_{max} (ng/mL)	$t_{1/2}$ (hr)	$AUC_{0-\infty}$ (ng·hr/mL)	Cls (L/kg·hr)	Vß (L/kg)
0.02 mg (0.05 IU*)/kg iv					
MEAN	415	0.363	156	0.135	0.0703
SD	75	0.053	33	0.029	0.0173
0.1 mg (0.27 IU*)/kg im					
MEAN	53.2	4.93	495	0.215	1.55
SD	25.9	2.66	106	0.047	0.91
0.1 mg (0.27 IU*)/kg sc					
MEAN	63.3	3.81	585	0.179	0.957
SD	18.2	1.40	90	0.028	0.301

Abbreviations: C_{max} = maximum concentration; $t_{1/2}$ = half-life;
$AUC_{0-\infty}$ = area under the curve; Cls = systemic clearance;
Vß = volume distribution; iv = intravenous; SD = standard deviation;
im = intramuscular; sc = subcutaneous.
* Based on previous International Standard of 2.7 IU = 1 mg



Effects of Humatrope treatment in adults with somatotropin deficiency

Two multicenter trials in adult onset somatotropin deficiency (n=98) and two studies in childhood onset somatotropin deficiency (n=67) were designed to assess the effects of replacement therapy with Humatrope. The primary efficacy measures were body composition (lean body mass and fat mass), lipid parameters, and the Nottingham Health Profile. The Nottingham Health Profile is a general health-related quality of life questionnaire. These four studies each included a 6-month randomized, blinded, placebo-controlled phase followed by 12 months of open-label therapy for all patients. The Humatrope dosages for all studies were identical: one month of therapy at 0.00625 mg/kg/day followed by the proposed maintenance dose of 0.0125 mg/kg/day. Adult onset patients and childhood onset patients differed by diagnosis (organic versus idiopathic pituitary disease), body size (normal versus small for mean height and weight), and age (mean = 44 versus 29 years). Lean body mass was determined by bioelectrical impedance analysis (BIA), validated with potassium 40. Body fat was assessed by BIA and sum of skinfold thickness. Lipid subfractions were analyzed by standard assay methods in a central laboratory.

Humatrope-treated adult onset patients, as compared to placebo, experienced an increase in lean body mass (2.59 versus -0.22 kg, $p<0.001$) and a decrease in body fat (-3.27 versus 0.56 kg, $p<0.001$). Similar changes were seen in childhood onset somatotropin deficient patients. These significant changes in lean body mass persisted throughout the 18 month period as compared to baseline for both groups, and for fat mass in the childhood onset group. Total cholesterol decreased short term (first 3 months) although the changes did not persist. However, the low HDL cholesterol levels observed at baseline (mean = 30.1 mg/mL and 33.9 mg/mL in adult onset and childhood onset patients) normalized by the end of 18 months of therapy (a change of 13.7 and 11.1 mg/dL for the adult onset and childhood onset groups, $p<0.001$). Adult onset patients reported significant improvements as compared to placebo in the following 2 of 6 possible health related domains: physical mobility and social isolation (Table 2). Patients with childhood onset disease failed to demonstrate improvements in Nottingham Health Profile outcomes.

Two additional studies on the effect of Humatrope on exercise capacity were also conducted. Improved physical function was documented by increased exercise capacity (VO_2 max, $p<0.005$) and work performance (Watts, $p<0.01$) (J Clin Endocrinol Metab 1995; 80:552-557).

Table 2

Changes[a] in Nottingham Health Profile Scores[b] in Adult Onset Somatotropin Deficient Patients

Outcome Measure	Placebo (6 Months)	Humatrope Therapy (6 Months)	Significance
Energy Level	-11.4	-15.5	NS
Physical Mobility	-3.1	-10.5	p <0.01
Social Isolation	0.5	-4.7	p <0.01
Emotional Reactions	-4.5	-5.4	NS
Sleep	-6.4	-3.7	NS
Pain	-2.8	-2.9	NS

[a]An improvement in score is indicated by a more negative change in the score.

[b]= To account for multiple analyses, appropriate statistical methods were applied and the required level of significance is 0.01.
NS = not significant

Efficacy Studies:

Turner Syndrome- One long-term, randomized, open-label multicenter concurrently controlled study and two long-term, open-label multicenter, historically controlled studies were conducted to evaluate the efficacy of growth hormone for the treatment of patients with short stature due to Turner syndrome.

In the randomized study comparing growth hormone-treated patients to a concurrent control group who received no growth hormone, the growth hormone-treated patients who received a dose of 0.3 mg/kg/week from a mean age of 11.7 years attained a mean and near final height of 146.0 ± 6.2 cm (n=27, mean ± SD) as compared to the control group who

attained a near final height of 142.1 ± 4.8 cm (n=19). By analysis of covariance†, the effect of growth hormone therapy was a height increase of 5.4 cm (p = 0.001).

The effect of long-term growth hormone treatment (0.375 mg/kg/week given either 3 times per week (tiw) or daily) on adult height was determined by comparing adult heights in the treated patients with those of age-matched patients with Turner syndrome who never received any growth-promoting therapy. The greatest improvement in adult height was observed in patients who received early growth hormone treatment and estrogen after age 14. In one study, this resulted in an adult height gain of 7.4 cm vs. matched historical controls by analysis of covariance.

In a second study, patients were randomized to receive estrogen replacements therapy (conjugated estrogens, 0.3 mg escalating to 0.625 mg daily) at either age 12 or 15 years. Compared with matched historical controls, early GH therapy combined with estrogen replacement at age 12 years resulted in an adult height gain of 5.9 cm (n=26), compared to 8.3 cm (n=29) in patients initiating estrogen at age 15 years.

Table 3
Summary Table of Efficacy Results

Study/Group	N at Adult Height	GH Age (yr)	Estrogen Age (yr)	GH Duration (yr)	Adult Height Gain (cm)*
GDCT	27	11.7	13	4.7	5.4
85-023	17	9.1	15.2	7.6	7.4
85-044: A	29	9.4	15	6.1	8.3
B	26	9.6	12.3	5.6	5.9
C	51	12.7	13.7	3.8	5

*Analysis of covariance vs controls

These studies confirm that when patients with short stature associated with Turner syndrome are treated appropriately with growth hormone, there is a significant gain in final height.

† Analysis of covariance includes adjustments for baseline height relative to age and for mid-parental height.

INDICATIONS AND USAGE

Pediatric Patients--Humatrope is indicated for the long-term treatment of pediatric patients who have growth failure due to an inadequate secretion of normal endogenous growth hormone.

Humatrope is indicated for the treatment of short stature associated with Turner Syndrome in patients whose epiphyses are not closed.

Adult Patients--Humatrope is indicated for replacement of endogenous somatotropin in adults with somatotropin deficiency syndrome who meet both of the following two criteria:

1. Adult Onset: Patients who have somatotropin deficiency syndrome, either alone or with multiple hormone deficiencies (hypopituitarism), as a result of pituitary disease, hypothalamic disease, surgery, radiation therapy, or trauma;

or

Childhood Onset: Patients who were growth hormone-deficient during childhood who have somatotropin deficiency syndrome confirmed as an adult before replacement therapy with Humatrope is started.

and

2. Biochemical diagnosis of somatotropin deficiency syndrome, by means of a negative response to a standard growth hormone stimulation test [maximum peak < 5 ng/mL when measured by RIA (polyclonal antibody) or < 2.5 ng/mL when measured by IRMA (monoclonal antibody)].

CONTRAINDICATIONS

Humatrope should not be used for growth promotion in pediatric patients with closed epiphyses.

Humatrope should not be used when there is any evidence of activity of a tumor. Intracranial lesions must be inactive and antitumor therapy complete prior to the institution of therapy. Humatrope should be discontinued if there is evidence of tumor growth.

Humatrope should not be reconstituted with the supplied Diluent for Humatrope by patients with a known sensitivity to either *m*-cresol or glycerin.

WARNING

If sensitivity to the diluent should occur, the vials may be reconstituted with Sterile Water for Injection, USP. When Humatrope is reconstituted in this manner, (1) use only one reconstituted dose per vial, (2) refrigerate the solution (36° to 46°F [2° to 8°C]) if it is not used immediately after reconstitution, (3) use the reconstituted dose within 24 hours, and (4) discard the unused portion.

PRECAUTIONS

Therapy with Humatrope should be directed by physicians who are experienced in the diagnosis and management of patients with growth hormone deficiency, Turner syndrome and adult patients with either childhood-onset or adult-onset somatotropin deficiency.

Patients with growth hormone deficiency secondary to an intracranial lesion should be examined frequently for progression or recurrence of the underlying

disease process. In pediatric patients, clinical literature has demonstrated no relationship between somatropin replacement therapy and CNS tumor recurrence. In adults, it is unknown whether there is any relationship between somatropin replacement therapy and CNS tumor recurrence.

Patients should be monitored carefully for any malignant transformation of skin lesions.

For patients with diabetes mellitus, the insulin dose may require adjustment when somatropin therapy is instituted. Because human growth hormone may induce a state of insulin resistance, patients should be observed for evidence of glucose intolerance. Patients with diabetes or glucose intolerance should be monitored closely during somatropin therapy.

In patients with hypopituitarism (multiple hormonal deficiencies) standard hormonal replacement therapy should be monitored closely when somatropin therapy is administered. Hypothyroidism may develop during treatment with somatropin, and inadequate treatment of hypothyroidism may prevent optimal response to somatropin. Patients with Turner syndrome have an inherently increased risk of developing autoimmune thryoid disease. Therefore, patients should have periodic thyroid function tests and be treated as indicated.

Excessive glucocorticoid therapy will inhibit the growth promoting effect of human growth hormone. Patients with coexisting ACTH deficiency should have their glucocorticoid replacement dose carefully adjusted to avoid an inhibitory effect on growth.

Pediatric patients with endocrine disorders, including growth hormone deficiency, may develop slipped capital epiphyses more frequently. Skeletal abnormalities including scoliosis are commonly seen in untreated Turner syndrome patients. Any pediatric patient with the onset of a limp during growth hormone therapy should be evaluated.

Patients with epiphyseal closure who were treated with growth hormone replacement therapy in childhood should be re-evaluated according to the criteria in *INDICATIONS AND USAGE* before continuation of somatropin therapy at the reduced dose level recommended for somatropin-deficient adults.

Intracranial hypertension (IH) with papilledema, visual changes, headache, nausea and/or vomiting has been reported in a small number of patients treated with growth hormone products. Symptoms usually occurred within the first eight (8) weeks of the initiation of growth hormone therapy. In all reported cases, IH-associated signs and symptoms resolved after termination of therapy or a reduction of the growth hormone dose. Funduscopic examination of patients is recommended at the initiation and periodically during the course of growth hormone therapy. Patients with Turner syndrome may be at increased risk for development of IH.

Experience in patients above 60 years is lacking.

Experience with prolonged treatment in adults is limited.

Growth hormone has not been shown to increase the incidence of scoliosis. Progression of scoliosis can occur in children who experience rapid growth. Because growth hormone increases growth rate, patients with a history of scoliosis who are treated with growth hormone should be monitored for progression of scoliosis.

Patients with Turner Syndrome should be evaluated carefully for otitis media and other ear disorders since these patients have an increased risk of ear or hearing disorders. In a randomized, concurrent controlled trial, there was a statistically significant increase, as compared to untreated controls, in otitis media (43% vs 26%) and ear disorders (18% vs 5%) in patients receiving Humatrope. A similar increase in otitis media was observed in an 18 month placebo-controlled trial. In addition, patients with Turner syndrome are at risk for cardiovascular disorders (e.g. stroke, aortic aneurysm, hypertension) and these conditions should be monitored closely.

Carcinogenesis, Mutagenesis, Impairment of Fertility--Long-term animal studies for carcinogenicity and impairment of fertility with this human growth hormone (Humatrope) have not been performed. There has been no evidence to date of Humatrope-induced mutagenicity.

Pregnancy--Pregnancy Category C--Animal reproduction studies have not been conducted with Humatrope. It is not known whether Humatrope can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. Humatrope should be given to a pregnant woman only if clearly needed.

Nursing Mothers--There have been no studies conducted with Humatrope in nursing mothers. It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when Humatrope is administered to a nursing woman.

Information for Patients--Patients being treated with growth hormone and/or their parents should be informed of the potential benefits and risks associated with treatment. If home use is determined to be desirable by the physician, instructions on appropriate use should be given, including a review of the contents of the patient information insert. This information is intended to aid in the safe and effective administration of the medication. It is not a disclosure of all possible adverse or intended effects.

If home use is prescribed, a puncture resistant container for the disposal of used syringes and needles should be recommended to the patient. Patients and/or parents should be thoroughly instructed in the importance of proper needle disposal and cautioned against any reuse of needles and syringes (*see* Information for the Patient insert).

ADVERSE REACTIONS

Pediatric Patients--Approximately 2% of 481 naive and previously treated clinical trial patients treated with Humatrope have developed antibodies to growth hormone, as demonstrated by a binding capacity determination threshold ≥0.02 μg/mL. Nevertheless, even these patients experienced increases in linear growth and other salutary effects of Humatrope and did not experience any unusual adverse events. Although growth-limiting antibodies have been observed with other growth hormone preparations (including products of pituitary origin), antibodies in patients treated with Humatrope have not limited growth. The long-term implications of antibody development are uncertain at this time.

Of the 232 naive and previously treated clinical trial patients receiving Humatrope for 6 months or more, 4.7% had serum binding of radiolabeled growth hormone in excess of twice the binding observed in control sera when the

serum samples were assayed at a tenfold dilution. Among these patients were 160 naive patients, of whom 6.9% had positive serum binding. In comparison, 74.5% of 106 naive patients treated for 6 months or more with somatrem (produced by Lilly) in a similar clinical trial had serum binding of radiolabeled growth hormone of at least twice the binding observed in control sera.

In addition to an evaluation of compliance with the treatment program and of thyroid status, testing for antibodies to human growth hormone should be carried out in any patient who fails to respond to therapy.

In studies with growth hormone-deficient pediatric patients, injection site pain was reported infrequently. A mild and transient edema, which appeared in 2.5% of patients, was observed early during the course of treatment.

Leukemia has been reported in a small number of pediatric patients who have been treated with growth hormone, including growth hormone of pituitary origin as well as of recombinant DNA origin (somatrem and somatropin). The relationship, if any, between leukemia and growth hormone therapy is uncertain.

Adult Patients--In clinical studies in which high doses of Humatrope were administered to healthy adult volunteers, the following events occurred infrequently: headache, localized muscle pain, weakness, mild hyperglycemia, and glucosuria.

In the first 6 months of controlled blinded trials, adult onset somatotropin-deficient adults experienced a statistically significant increase in edema (Humatrope 17.3% vs. placebo 4.4%, p=0.043) and peripheral edema (11.5% vs. 0% respectively, p=0.017). In patients with adult onset somatotropin deficiency syndrome, edema, muscle pain, joint pain, and joint disorder were reported early in therapy and tended to be transient or responsive to dosage titration.

Two out of 113 adult onset patients developed carpal tunnel syndrome after beginning maintenance therapy without a low dose (0.00625 mg/kg/day) lead-in phase. Symptoms abated in these patients after dosage reduction.

All treatment-emergent adverse events with ≥5% overall incidence during 12 or 18 months of replacement therapy with Humatrope are shown in Table ~~3~~ 4 (adult onset patients) and in Table ~~4~~ 5 (childhood onset patients).

Adult patients treated with Humatrope who had been diagnosed with growth hormone deficiency in childhood reported side effects less frequently than those with adult onset somatotropin deficiency.

Table ~~3~~ 4

Treatment-Emergent Adverse Events with ≥5% Overall Incidence in Adult Onset Patients Treated with Humatrope for Either 12 or 18 Months

	12 Months hGH Exposure (N=44)		18 Months hGH Exposure (N=52)	
Adverse Event	n	%	n	%
Edema	5	11.4	11	21.2
Arthralgia	6	13.6	9	17.3
Paresthesia	6	13.6	9	17.3
Myalgia	4	9.1	7	13.5
Pain	6	13.6	7	13.5
Rhinitis	5	11.4	7	13.5
Peripheral Edema	8	18.2	6	11.5
Back Pain	4	9.1	5	9.6
Headache	3	6.8	4	7.7
Hypertension	2	4.6	4	7.7
Acne	0	0	3	5.8
Joint Disorder	1	2.3	3	5.8
Surgical Procedure	1	2.3	3	5.8
Flu Syndrome	3	6.8	2	3.9

Abbreviations: hGH = Humatrope; N = number of patients receiving treatment in the period stated; n = number of patients reporting each treatment-emergent adverse event.

Table ~~4~~ 5

Treatment-Emergent Adverse Events with ≥5% Overall Incidence in Childhood Onset Patients Treated with Humatrope for Either 12 or 18 Months

	12 Months hGH Exposure (N=30)		18 Months hGH Exposure (N=32)	
Adverse Event	n	%	n	%
Flu Syndrome	3	10.0	5	15.63
SGOT Increased	2	6.67	4	12.50
Headache	2	6.07	3	9.38
Asthenia	1	3.33	2	6.25
Cough Increased	0	0	2	6.25
Edema	3	10.00	2	6.25
Hypesthesia	0	0	2	6.25
Myalgia	2	6.67	2	6.25
Pain	3	10.00	2	6.25
Rhinitis	2	6.67	2	6.25
SGPT Increased	2	6.67	2	6.25
Respiratory Disorder	2	6.67	1	3.13
Gastritis	2	6.67	0	0
Pharyngitis	2	6.67	1	3.13

Abbreviations: hGH = Humatrope; N = number of patients receiving treatment in the period stated; n = number of patients reporting each treatment-emergent adverse event; SGOT =

serum glutamic oxaloacetic transaminase, or AST; SGPT = serum glutamic pyruvic transaminase, or ALT.

Other adverse drug events that have been reported in growth hormone-treated patients include the following:

1) Metabolic: Infrequent, mild and transient peripheral or generalized edema. 2) Musculoskeletal: Rare carpal tunnel syndrome. 3) Skin: Rare increased growth of pre-existing nevi. Patients should be monitored carefully for malignant transformation. 4) Endocrine: Rare gynecomastia. Rare pancreatitis.

OVERDOSAGE

Acute overdosage could lead initially to hypoglycemia and subsequently to hyperglycemia. Long-term overdosage could result in signs and symptoms of gigantism/acromegaly consistent with the known effects of excess human growth hormone. (See recommended and maximal dosage instructions given below.)

DOSAGE AND ADMINISTRATION

The Humatrope dosage and administration schedule should be individualized for each patient. Therapy should not be continued if epiphyseal fusion has occurred. Patients who fail to respond adequately while on Humatrope therapy should be evaluated to determine the cause of unresponsiveness.

Pediatric Patients--

Growth hormone-deficient pediatric patients--The recommended weekly dosage is 0.18 mg/kg (0.54 IU/kg) of body weight. It should be divided into equal doses given either on 3 alternate days or 6 times per week. The maximal replacement weekly dosage is 0.3 mg/kg (0.90 IU/kg) of body weight divided into equal doses given on 3 alternate days. The route of administration should be by subcutaneous or intramuscular injection. The dosage and administration schedule for Humatrope should be individualized for each patient.

Turner Syndrome--A weekly dosage of up to 0.375 mg/kg (1.125 IU/kg) of body weight administered by subcutaneous injection is recommended. It should be

divided into equal doses given either daily or on 3 alternate days.

Adult Patients--

Somatotropin-deficient adult patients--The recommended dosage at the start of therapy is not more than 0.006 mg/kg/day (0.018 IU/kg/day) given as a daily subcutaneous injection. The dose may be increased according to individual patient requirements to a maximum of 0.0125 mg/kg/day (0.0375 IU/kg/day).

During therapy, dosage should be titrated if required by the occurrence of side effects. To minimize the occurrence of adverse events in patients with increasing age or excessive body weight, dose reductions may be necessary.

Each 5-mg vial of Humatrope should be reconstituted with 1.5 to 5 mL of Diluent for Humatrope. The diluent should be injected into the vial of Humatrope by aiming the stream of liquid against the glass wall. Following reconstitution, the vial should be swirled with a GENTLE rotary motion until the contents are completely dissolved. DO NOT SHAKE. The resulting solution should be inspected for clarity. It should be clear. If the solution is cloudy or contains particulate matter, the contents MUST NOT be injected.

Before and after injection, the septum of the vial should be wiped with rubbing alcohol or an alcoholic antiseptic solution to prevent contamination of the contents by repeated needle insertions. Sterile disposable syringes and/or needles should be used for administration of Humatrope. The volume of the syringe should be small enough so that the prescribed dose can be withdrawn from the vial with reasonable accuracy.

STABILITY AND STORAGE

Before Reconstitution--Vials of Humatrope as well as the Diluent for Humatrope are stable when refrigerated (36° to 46°F [2° to 8°C]). Avoid freezing Diluent for Humatrope. Expiration dates are stated on the labels.

After Reconstitution--Vials of Humatrope are stable for up to 14 days when reconstituted with Diluent for Humatrope and stored in a refrigerator at 36° to 46°F (2° to 8°C). Avoid freezing the reconstituted vial of Humatrope.

HOW SUPPLIED

Vials:

5 mg (No. 7335)--(6s) NDC 0002-7335-16, and 5-mL vials of Diluent for Humatrope (No. 7336)

CAUTION--Federal (USA) law prohibits dispensing without prescription.

Literature revised October 8, 1996

ELI LILLY AND COMPANY, Indianapolis, IN 46285, USA

Printed in USA

PA 1643-A AMP

Endocrinologic and Metabolic Drugs Advisory Committee #65

Food and Drug Administration
Center for Drug Evaluation and Research

Bethesda Holiday Inn, 8120 Wisconsin Avenue, Bethesda, MD

December 11, 1996

NDA 20-720; **Rezulin™**, (trogildizone) Parke Davis Research,
a Division of Warner-Lambert
NDA 20-719; **Prelay™**, (trogildizone) Sankyo U.S.A.

DRAFT AGENDA

Endocrinologic and Metabolic Drugs Advisory Committee #65

Food and Drug Administration
Center for Drug Evaluation and Research
Bethesda Holiday Inn, 8120 Wisconsin Avenue, Bethesda, MD

December 11, 1996

8:00 Call to Order, Introductions, Opening Comments
Henry G. Bone III, MD, Chair
Endocrinologic and Metabolic Drugs Advisory Committee
Meeting Statement: Kathleen Reedy, Executive Secretary
Endocrinologic and Metabolic Drugs Advisory Committee

NDA 20-720; **Rezulin™**, (trogildizone) Parke Davis Research, a Division of Warner-Lambert
NDA 20-719; **Prelay™**, (trogildizone) Sankyo U.S.A.

8:15 Open Public Hearing

8:45 Introduction to the Proceedings :
G. Alexander Fleming, MD, Group Leader
Division of Metabolic and Endocrine Drug Products, FDA

8:50 Overview of Data Submitted in Support of Approval
Introduction:
Efficacy:
Safety:
Benefit/Risks:

10:30 Break

10:45 FDA Presentation of Issues:
Division of Metabolic and Endocrine Drug Products

11:30 Lunch

Interactive Discussion of Issues

12:30 Issue #1: a: Rationale for defining the pivotal studies' patient populations.
b: Assumption that patients in these studies would not have responded to re-instituted sulfonylurea therapy

1:00 Issue #2: Estimation of the clinical significance of troglitazone's treatment effects

1:30 Issue #3: Recommended dosing and how it was selected

2:00 Issue #4: Estimation of specific risks
a: Cardiovascular
b: Body compartment fluid distribution
c: Carcinogenicity

2:30 Questions

3:30 Adjourn

Endocrinologic and Metabolic Drugs Advisory Committee #65

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DRAFT

Questions

1. What is the clinical significance of the troglitazone treatment effects, i.e. reduced HbA_{1c} levels and total insulin dosage, observed in the two pivotal studies?

2. Are the study designs and efficacy endpoints adequate to assess the efficacy and safety of this drug for the proposed patient population?

3. Based on the efficacy and safety data presented, and your assessment of the overall benefits compared to the risks of troglitazone therapy, do you recommend that this drug be approved for use in the proposed patient population?

4. If approval is recommended, what measures should be taken after approval to refine understanding of this therapy's efficacy and resolve its remaining safety issues.

DRAFT

DRAFT

STATISTICAL REVIEW AND EVALUATION

NDA #: 20-719 and 20-720/ Drug Class 1P

APPLICANT: Parke-Davis Pharmaceutical Research/Sankyo Co. Ltd.

NAME OF DRUG: Prelay™ /Rezulin™ (troglitazone tablets)

INDICATION: Type II Diabetes

DOCUMENTS REVIEWED: Volumes 2.1, 2.2, 2.216 thru 2.229

MEDICAL REVIEWER: John Gueriguian, MD (HFD-510).

This review is arranged in four sections. Section I gives a brief introduction of the two studies under this submission. Sponsor's efficacy results and conclusions are described in Section II. This reviewer's evaluation of these studies is contained in Section III. Section IV contains reviewer's conclusions that may be conveyed to the sponsor.

I. INTRODUCTION

The sponsor submitted two double-blind, placebo-controlled, multicenter 6-month clinical studies in NIDDM patients requiring insulin: Studies 991-040 and 991-068.

Study 991-040 was designed to evaluate the effect of troglitazone on measures of glycemic control in these insulin-requiring patients by focusing on reductions in hemoglobin A_{1c} (HbA_{1c}) and fasting serum glucose (FSG) levels. Reducing insulin dose was not a principle efficacy measure in this study. In this study, 351 patients were randomly assigned to receive either placebo, troglitazone 200 mg (QAM), or troglitazone 600 mg (QAM).

The objective of study 991-068 was to evaluate the effectiveness of troglitazone therapy in reducing daily insulin dose while improving glucose control as measured by capillary blood glucose. In this study, 222 patients were randomly assigned to receive either placebo, troglitazone 200 mg (QAM), or troglitazone 400 mg (QAM).

Key Words: Multicenter Studies, Parallel Studies, Fixed Dose, Insulin.

II. SPONSOR'S EFFICACY RESULTS

This section reviews the sponsor's results and conclusions of the primary and secondary efficacy variables (per protocol) from the two double-blind, placebo-controlled, multicenter 6-month clinical studies in NIDDM patients requiring insulin: Studies 991-040 and 991-068.

Patient Disposition/Characteristics

The disposition of patients for each placebo-controlled study and the number of patients in the primary efficacy analyses (intent-to-treat population) are summarized in the Table below. The study completion rates were high, ranging from 87% to 91% of patients completing across treatment groups. The percent of patients included in the primary efficacy analysis (intent-to-treat) was 97% to 100% across both the studies.

	Study 991-040			Study 991-068		
	Placebo	Troglitazone		Placebo	Troglitazone	
		200 mg/day	600 mg/day		200 mg/day	400 mg/day
Pts. Randomized to Treatment, N	118	116	116	71	75	76
Pts. Completing Study, N (%)	105 (89)	107 (91)	103 (89)	62 (87)	65 (87)	67 (88)
Withdrawals, N (%)						
Adverse Events	5 (4.2)	0 (0.0)	5 (4.3)	2 (2.8)	4 (5.3)	3 (3.9)
Lack of Efficacy	Not Tracked	Not Tracked	Not Tracked	0 (0.0)	0 (0.0)	1 (1.3)
Lost to Follow-Up	2 (1.7)	2 (1.7)	1 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)
Voluntary Withdrawal	4 (3.4)	5 (4.3)	4 (3.4)	0 (0.0)	0 (0.0)	0 (0.0)
Other/Administrative	2 (1.7)	2 (1.7)	3 (2.6)	6 (8.0)	6 (8.0)	5 (6.5)
Intent-to-Treat, N(%)	118 (100)	116 (99)	116 (100)	69 (97)	73 (97)	74 (97)

According to the sponsor, patients selected for the controlled studies were ≥ 18 years of age with NIDDM, who required ≥ 30 units of insulin/day, and had prior sulfonylurea therapy that did not result in adequate glycemic control. Patients were to have FSG levels of > 140 mg/dL and fasting C-peptide levels of ≥ 0.8 ng/mL (for Study 991-040) or ≥ 1.5 ng/mL (for Study 991-068). Hemoglobin A_{1c} (HbA_{1c}) levels were to be > 7% (8%-12% for Study 991-040), which indicates that these patients had not achieved good glycemic control on insulin therapy. Of the 573 patients who were randomized to treatment in these 2 placebo-controlled studies, approximately half were women (see Table below); the mean age was 56 to 57 years across studies. Characteristics were also similar across studies with respect to race, baseline FSG and HbA_{1c}. On

average, patients had been diagnosed with NIDDM for 10 to 11 years and had been taking insulin for 4 to 5 years. Exogenous insulin usage averaged from 70 to 75 units/day across both studies.

	Study 991-040			Study 991-068		
	Placebo	Troglitazone		Placebo	Troglitazone	
		200 mg/day	600 mg/day		200 mg/day	400 mg/day
	N=118	N=116	N=116	N=71	N=75	N=76
Sex, N (%)						
Men	60 (50.8)	54 (46.6)	53 (45.7)	35 (49.3)	44 (58.7)	38 (50.0)
Women	58 (49.2)	62 (53.4)	63 (54.3)	36 (50.7)	31 (41.3)	38 (50.0)
Race, N(%)						
Caucasian	82 (69.5)	81 (69.8)	78 (67.2)	55 (77.5)	52 (69.3)	61 (80.3)
Black	18 (15.3)	18 (15.5)	24 (20.7)	7 (9.9)	13 (17.3)	6 (7.9)
Hispanic	16 (13.6)	14 (12.1)	12 (10.3)	7 (9.9)	8 (10.7)	7 (9.2)
Other	2 (1.6)	3 (2.6)	2 (1.8)	2 (2.8)	2 (2.7)	2 (2.6)
Age (years)						
Mean	55.8	55.6	56.0	57.1	57.7	57.7
Range	26 - 72	26 - 73	33 - 72	36 - 81	37 - 78	36 - 81
Gly. Parameters Mean (SD)						
FSG (mg/dL)	219 (46)	214 (45)	215 (49)	230 (60)	225 (64)	222 (52)
HbA1c (%)	9.4 (1.1)	9.5 (1.1)	9.3 (1.1)	9.0 (1.4)	9.5 (1.7)	9.0 (1.3)
C-Peptide (ng/mL)	1.7 (0.6)	1.6 (0.6)	1.7 (0.6)	2.8 (1.5)	2.2 (1.1)	2.5 (1.2)
Years Diagnosed With NIDDM						
Mean	9.8	9.7	10.4	9.9	10.5	11.5
Duration of Insulin Use(years)						
Mean	4.7	5.3	5.1	4.5	4.5	4.4
Total Daily Insulin Dose(Units)						
Mean	75	72	70	75	72	71
Range	24 - 276	32 - 290	22 - 193	27 - 144	28 - 145	27 - 139

Results of Major Efficacy Variables

Study 991-040

The following table summarizes the mean change from baseline in HbA_{1c} (%) and FSG (mg/dL) following 6 months of therapy for the ITT population. The primary efficacy variable for this study was the change in HbA_{1c} (%) from baseline to the end of the study. Changes from baseline were analyzed using ANCOVA with treatment and

study center as factors and baseline as covariate. Troglitazone treatment groups were compared to placebo using step-down tests for linear trends. Baseline was defined as the mean of a patient's values recorded at weeks -8, -6, -4, -2, and 0. Study completion was defined as having a week 24 or week 26 visit. If both of these values were available, an average of the two values was used for analysis. If only one value was available, the single value was used for analysis.

	Study 991-040					
	Placebo		Trog. 200 mg		Trog. 600 mg	
	N=118		N=116		N=116	
	Mean	SE	Mean	SE	Mean	SE
HbA1c (%)						
Baseline Mean	9.43	1.07	9.51	1.08	9.32	1.14
Mean Change from Baseline*	-0.12	0.10	-0.84	0.10	-1.41	0.10
Adjusted Mean Difference from Placebo			-0.72	0.14	-1.29	0.14
			p<0.0001		p<0.0001	
Fasting Serum Glucose (mg/dL)						
Baseline Mean	219.18	45.66	213.80	44.83	214.69	49.35
Mean Change from Baseline*	0.76	4.64	-34.92	4.72	-48.79	4.43
Adjusted Mean Difference from Placebo			-35.68	6.61	-49.56	6.62
			p<0.0001		p<0.0001	

*: Least Squares Mean adjusted for center and baseline

Study 991-068

The primary efficacy variable was the proportion of responders. Responders were patients having at least a 50% decrease from baseline in mean total daily insulin dose (TDID) **and** improved glycemic control as evidenced by ***either*** (a) $\geq$ 15% decrease from baseline in mean blood glucose (MBG) ***or*** (b) a MBG $\leq$ 140 mg/dL at Week 26. Baseline was defined as the measurement (or measurements in the case of MBG and mean TDID) taken just prior to randomization (Week 0).

For the ITT population, step-down comparisons of proportions in the troglitazone treatment groups (200 and 400 mg/day) versus placebo were performed by sequential application of the Cochran-Mantel-Haenszel (CMH) test for linear trend with centers as strata. To cover the case where monotonicity of dose response might not be supported by the data, pairwise comparison of each troglitazone treatment group with placebo were conducted using the CMH test for general association with a Bonferroni adjustment for the 2 comparisons. Due to small number of patients per center, homogeneity of the association between treatment and response across centers could not be adequately evaluated by the Breslow-Day test. The following table gives the number and percentage of responders at Week 26 for ITT population.

	Placebo N=69	Trog. 200 mg N=73	Trog. 400 mg N=74
Responders	5 (7.3%)	16 (21.9%)	20 (27.0%)
P-Values (CMH test):			
Linear Trend		0.008	0.006
General Association		0.008	0.006

Changes from baseline in HbA_{1c}, MBG and FSG were secondary variables to evaluate the effect of troglitazone on glycemic control. The following table summarizes the mean change from baseline in HbA_{1c} (%), MBG (mg/dL) and FSG (mg/dL) following 26 weeks of therapy for ITT population. Clearly, none of these secondary variables were found to be statistically significant.

	Study 991-068					
	Placebo		Trog. 200 mg		Trog. 400 mg	
	Mean	SE	Mean	SE	Mean	SE
HbA1c (%)						
N	69		73		72	
Baseline Mean	8.97	1.39	9.52	1.65	9.00	1.29
Adjusted Mean Change at Week 26	-0.09	0.14	-0.13	0.14	-0.41	0.14
Adjusted Mean Difference from Placebo			-0.04	0.19	-0.33	0.18
95% CI*			(-0.46, 0.37)		(-0.74, 0.08)	
MBG (mg/dL)						
N	67		67		74	
Baseline Mean	186.8	44.0	190.3	46.7	177.5	38.7
Adjusted Mean Change at Week 26	-11.1	4.10	-14.4	4.10	-20.2	3.90
Adjusted Mean Difference from Placebo			-3.4	5.4	-9.1	5.3
95% CI*			(-15.4, 8.7)		(-20.9, 2.7)	
Fasting Serum Glucose (mg/dL)						
N	70		75		75	
Baseline Mean	229.7	59.6	225.2	64.2	221.5	52.2
Adjusted Mean Change at Week 26	-4.5	7.20	0.4	6.80	-22.0	6.80
Adjusted Mean Difference from Placebo			4.8	9.4	-17.5	9.4
95% CI*			(-16.0, 25.7)		(-38.4, 3.3)	

*: 95% CI (Mean Trog. minus Mean Placebo) based on Dunnett's Procedure

Sponsor's Conclusion

Sponsor stated that troglitazone therapy, at doses of 200 to 600 mg/day, is effective in significantly reducing total daily insulin dose and improving glycemic control in NIDDM patients requiring insulin.

III. STATISTICAL REVIEWER'S EVALUATION

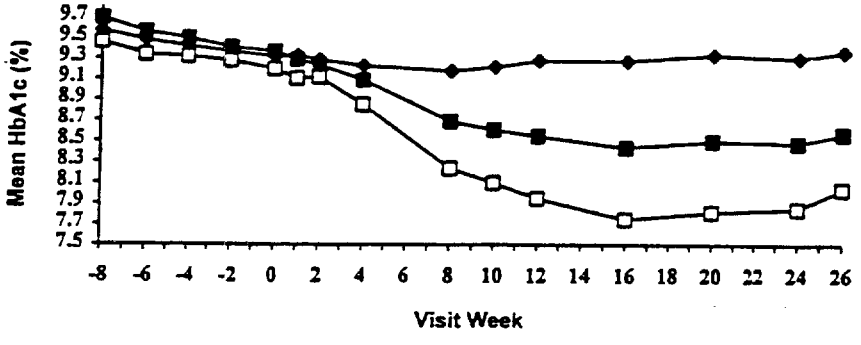
This reviewer's evaluation of the two studies for ITT population is contained in this section.

Study 991-040

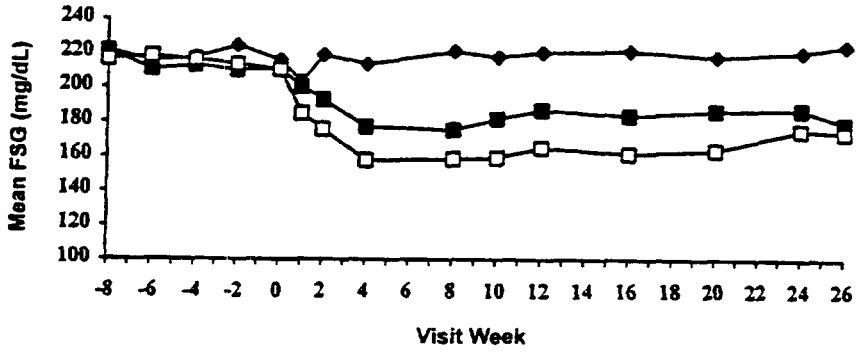
According to the protocol, the primary objective of this study was to determine the efficacy of troglitazone on the glycemic control assessed by reduction in HbA_{1c}, fasting serum glucose (FSG), C-peptide and fructosamine in patients with NIDDM treated with insulin. Further, change from baseline was considered to be the primary endpoint. The sponsor stated that the analysis of the primary parameters would be performed on the intent-to-treat and evaluable patient samples.

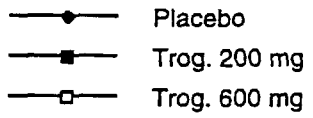
This reviewer replicated sponsor's results for the data provided by the sponsor. The time course of HbA_{1c}, FSG and C-peptide for placebo, troglitazone 200 mg and troglitazone 600 mg groups is provided below. These graphs show observed means at each visit. There were statistically significant reductions for HbA_{1c}, FSG and C-peptide when troglitazone 200 mg and 600 mg groups were compared with placebo at 26 weeks.

Mean HbA_{1c} at Each Study Visit (ITT Population)

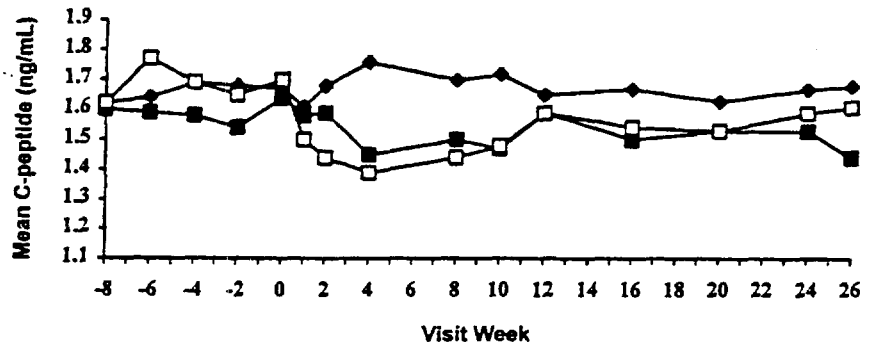


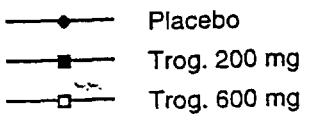
Mean FSG at Each Study Visit (ITT Population)





Mean C-peptide at Each Study Visit (ITT Population)





Study 991-068

According to the protocol, the objective of this study was to determine if troglitazone could decrease the insulin requirement in NIDDM patients taking insulin and provide evidence of improved glycemic control.

Further, according to Amendment 3 (dated September 15, 1995), the primary efficacy parameter was the proportion of patients having at least a 50% decrease from baseline in mean total daily insulin dose (TDID) **and *either*** (a) $\geq$ 15% decrease from baseline in mean blood glucose (MBG) ***or*** (b) a MBG $\leq$ 140 mg/dL at Week 26. Patients would be classified as "responder" or "nonresponder" based on their last available mean TDID and MBG; patients with at least 50% decrease from baseline in mean total daily insulin dose (TDID) **and *either*** (a) $\geq$ 15% decrease from baseline in mean blood glucose (MBG) ***or*** (b) a MBG $\leq$ 140 mg/dL would be considered responders. Comparison of responders in the troglitazone groups versus placebo would be performed by sequential application of the CMH test for linear trend with study centers as strata.

This reviewer replicated sponsor's results for the data provided by the sponsor. There were 20 responders (27%) in the troglitazone 400 mg group, 16 (22%) in the troglitazone 200 mg group and 5 (7%) in the placebo group for the ITT population at Week 26. The differences in responders were statistically significant for both comparisons: troglitazone 400 mg versus placebo and troglitazone 200 mg versus placebo. There were no statistically significant differences between the troglitazone 400 mg and 200 mg groups. These results are summarized in the following table.

Number (%) of Responders at Week 26 for ITT Population

	Placebo N=69	Trog. 200 mg N=73	Trog. 400 mg N=74
Responders	5 (7.3%)	16 (21.9%)	20 (27.0%)
P-Values (CMH test):			
Linear Trend		0.008	0.006
General Association		0.008	0.006

Based on sequential application of the CMH test for linear trend with study centers as strata, results are considered statistically significant as p-value is < 0.049.

Based on CMH test for general association with study centers as strata and Bonferroni's adjustment for multiple comparisons, results are considered statistically significant as p-value is < 0.0245.

IV. STATISTICAL REVIEWER'S CONCLUSIONS THAT MAY BE CONVEYED TO THE SPONSOR

The sponsor has submitted the results of two studies: 991-040 and 991-068 as proof of the effectiveness of troglitazone therapy, at doses of 200 to 600 mg, in significantly reducing total daily insulin dose and improving glycemic control in NIDDM patients requiring insulin.

Study 991-040

Analyses conducted by the sponsor as well as by this reviewer detected statistically significant differences in favor of troglitazone 200 mg/day and 600 mg/day over placebo patients for the primary efficacy variables spelled out in the protocol.

Study 991-068

Analyses conducted by the sponsor as well as by this reviewer detected statistically significant differences in favor of troglitazone 200 mg/day and 400 mg/day over placebo patients for the primary efficacy variables spelled out in the protocol.

DRAFT

Baldeo K. Taneja, Ph.D.
Mathematical Statistician (Biomed)

Concur: Mr. Marticello

Dr. Nevius

cc:
Archival NDA 20-720
HFD-510
HFD-510/Dr. Sobel/Dr. Fleming/Dr. Gueriguian/Ms. Galliers/Mr. Johnson
HFD-715/Division File/Dr. Nevius/Mr. Marticello/Dr. Taneja

This review contains 9 pages of text.

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PARKE-DAVIS
People Who Care

Irwin G. Martin, Ph.D.
Vice President, FDA Liaison
Worldwide Regulatory Affairs

November 25, 1996

NDA 20-720
Rezulin™ (troglitazone) Tablets

Re: Briefing Document

Ms. Kathleen R. Reedy
Advisors and Consultants Staff
HHS/FDA
CDER/ACS/HFD-21
Chatman Building Room 200
1901 Chatman Avenue
Rockville, Maryland 20852

Dear Ms. Reedy:

In preparation for December 11, 1996, meeting of the Endocrinologic and Metabolic Drugs Advisory Committee the Parke-Davis Research Division of Warner-Lambert and Company has prepared for you a briefing document on Rezulin™ (troglitazone) tablets for the treatment of Type II diabetes patients inadequately controlled on insulin.

This document summarizes information on the following topics:

- Troglitazone enhances insulin action in almost all models of insulin resistance by increasing insulin-stimulated glucose uptake in skeletal muscle and adipose tissue, and decreasing hepatic glucose output.

- One hundred and thirty-nine preclinical toxicity studies characterized the toxicologic profile troglitazone and support its clinical use.

- Absorption, distribution, metabolism and excretion of troglitazone and metabolites were studied in mice, rats, rabbits, dogs, monkeys and humans.

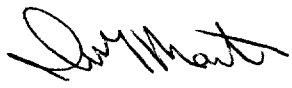
- Clinical data demonstrates that troglitazone significantly improves indicators of glycemic control and has the added benefit of reducing exogenous insulin requirements

This information is derived from our pending NDA 20-720. An additional NDA for troglitazone, NDA 20-719, was submitted to the FDA by Sankyo U.S.A. Corporation. The NDA is identical to the Parke-Davis NDA with the exception of the tradename Prelay™. As noted in the attached letter from Sankyo U.S.A., Parke-Davis will be speaking on behalf of both companies.

We hope that the material provided facilitates a highly productive meeting. We have also provided for your convenience, a copy of the draft package insert for Rezulin.

The Parke-Davis staff will be pleased to answer your questions during our meeting on December 11, 1996. We are looking forward to an informative discussion.

Sincerely,



Irwin G. Martin

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Attachment

PHONE: (212) 753-4432 FAX: (212) 308-2491

SANKYO U.S.A. CORPORATION

780 THIRD AVENUE, 47TH FLOOR
NEW YORK, NY 10017

November 25, 1996

NDA 20-719
Prelay™ (troglitzaone) Tablets

Ms. Kathleen R. Reedy
Advisors and Consultants Staff
Food and Drug Administration
HHS/FDA
CDER/ACS/HFD-21
Chatman Building Room 200
1901 Chatman Avenue
Rockville, Maryland 20852

Dear Dear Ms. Reedy:

The attached document from Parke-Davis / Warner-Lambert should be considered supportive of both our NDA 20-719 for Prelay™ as well as Parke-Davis' NDA 20-720 for Rezulin™ (brands of troglitazone).

The NDAs are identical but for the trade names. Parke-Davis is, therefore, authorized to speak on behalf of Sankyo U.S.A. Corporation for all matters pertaining to the Prelay™ NDA data.

Representatives from Sankyo U.S.A. Corporation will be available to discuss any other questions you might have.

Sincerely,

David Woodward, Ph.D.
Vice President
Development

Troglitazone
Tablets

ADVISORY COMMITTEE BRIEFING DOCUMENT

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1. OVERVIEW

1.1. Introduction

Troglitazone, (also known as Rezulin™, CI-991, and CS-045), is a novel drug for treating insulin resistance. It is not related chemically or functionally to the sulfonylurea or biguanide classes of oral antidiabetic agents. Troglitazone does not stimulate insulin release or mimic its action. By enhancing insulin-mediated glucose disposal and reducing hepatic glucose output, troglitazone reverses the insulin-resistant state characteristic of noninsulin- dependent diabetes mellitus (NIDDM). Troglitazone is under development for the treatment of Type II diabetes and potentially other insulin-resistant disease states. The advantages of troglitazone therapy include significant improvements in both hyperglycemia, with concomitant hyperinsulinemia, as well as dyslipidemia and blood pressure.

The clinical development of troglitazone is directed at two populations of patients with Type II diabetes. The current New Drug Application (NDA) focuses on patients inadequately controlled on insulin.

The American Diabetes Association estimates that over 43% of patients with Type II diabetes are insulin requiring. Troglitazone offers patients who require insulin a unique advantage over currently available interventions. When added to insulin therapy, it helps patients achieve glycemic control while potentially reducing the dose of required exogenous insulin.

1.2. Background

Troglitazone was discovered by Sankyo Co, Ltd, Tokyo, Japan, which filed an Investigational New Drug (IND) application in January 1989.

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In August 1995, Parke-Davis met with the FDA to propose development of an NDA based on results of a 12-week pilot study (991-063) in which patients with Type II diabetes who required insulin were able to improve glycemic control and reduce or eliminate their insulin use. This was an unexpected, but medically important discovery. The clinical development plan was redirected based on remarkable data in insulin-requiring Type II patients. It was agreed that the NDA, planned for July 1996, would include two 6-month pivotal studies in patients with Type II diabetes requiring insulin. One study would be conducted by Parke-Davis (991-068) and the other one (991-040) would be the study being conducted by Sankyo, U.S.A., under the Parke-Davis IND. A pre-NDA meeting was held in January 1996 to review the content and format of the NDA.

1.3. Clinical Program

Troglitazone has been evaluated in 74 clinical pharmacology and/or clinical studies conducted by the three codevelopers. More than 3,800 healthy subjects or patients have been exposed to troglitazone (Figure 1). The clinical efficacy section of the current NDA focuses primarily on the safety and efficacy of Parke-Davis studies but all safety data and supportive efficacy data from the Sankyo and Glaxo studies are included. The clinical pharmacology studies of the three codevelopers are reported collectively.

Troglitazone studies for this NDA submission include 16 clinical pharmacology and 12 clinical studies covering 359 healthy subjects and 1,273 patients exposed to troglitazone. The majority of the studies included patients with Type II diabetes although three studies were conducted in patients with impaired glucose tolerance (IGT) and one in patients with polycystic ovary syndrome (PCOS).

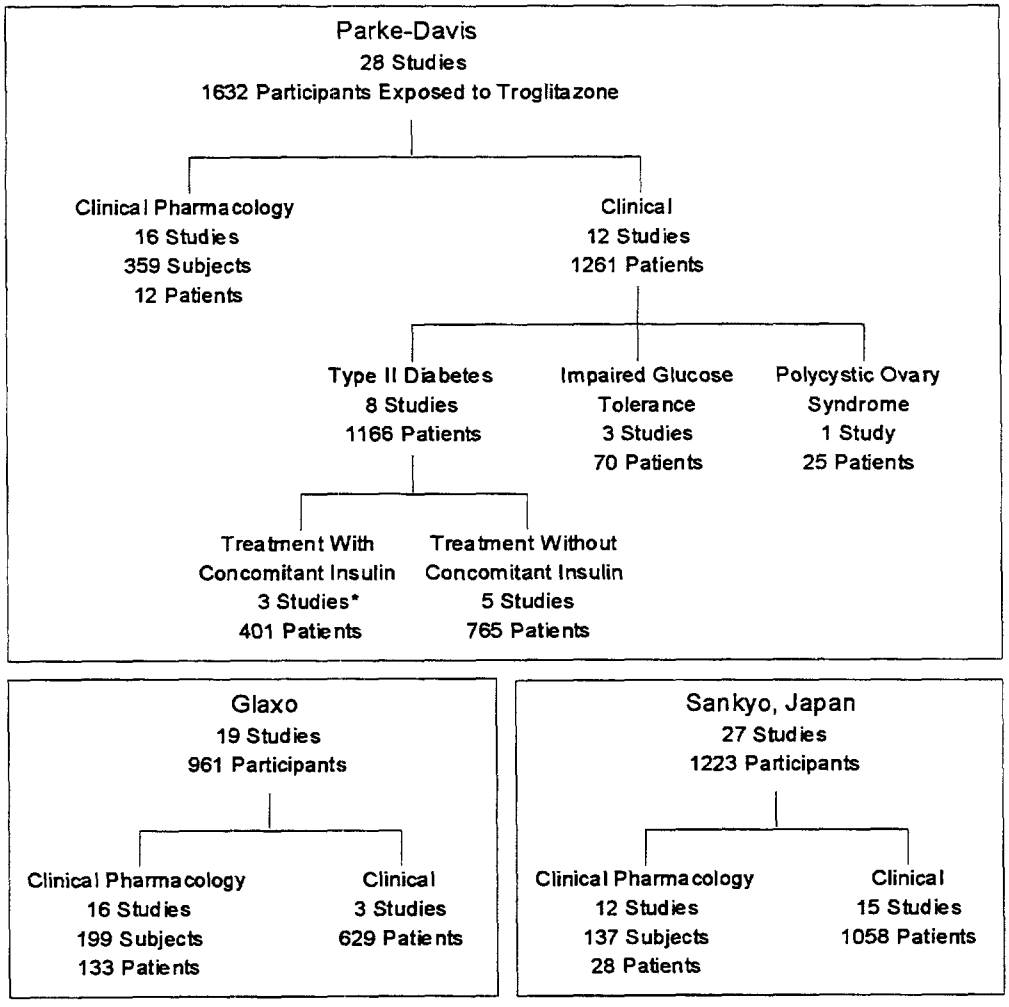

FIGURE 1. Overview of Clinical Pharmacology and Clinical Studies of Patient Exposure to Troglitazone

* Includes 991-40 conducted by Sankyo, USA

1.4. Future Direction

The development of troglitazone will continue in all potential disease states that could benefit from reversing insulin resistance including Type II diabetes and PCOS.

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1.5. Summary

This is a briefing document that summarizes all preclinical and clinical information within the NDA. This document also summarizes the development of troglitazone for patients with Type II diabetes who are inadequately controlled on insulin. Generally, Type II patients requiring exogenous insulin have advanced disease and are the most difficult patients to achieve glycemic control. The results of this comprehensive clinical evaluation provide data to support the use of Rezulin in this Type II diabetes population.

2. PRECLINICAL PHARMACOLOGY

2.1. Introduction

Type II diabetes results from a combination of environmental and complex genetic factors. Although the primary lesion in Type II diabetes is unknown, both insulin resistance and reduced insulin secretion contribute to the disease. A number of studies suggest that insulin resistance usually precedes the appearance of glucose intolerance. The pancreatic β-cells respond to peripheral insulin resistance by increasing basal and postprandial insulin secretion. As the disease progresses, the further aggravation of insulin resistance produces an increased secretory load on the pancreas, leading to a

deterioration in glucose-induced insulin secretion, and the development of frank diabetes.

Hyperglycemia, hyperinsulinemia, and insulin resistance all contribute to the many complications of diabetes. Insulin resistance and/or hyperinsulinemia, even in the absence of hyperglycemia and overt diabetes, is thought to be a major risk factor for atherosclerosis, peripheral vascular disease, and hypertension. Indeed, the majority of diabetes-related deaths are attributed to complications arising from cardiovascular disease and stroke.

The presumed central role of insulin resistance in the pathophysiology of Type II diabetes suggests that the enhancement of insulin action might be an effective pharmacological approach. The discovery of troglitazone represents the first breakthrough in this effort. This drug enhances insulin action in almost all models of insulin resistance by increasing insulin-stimulated glucose uptake in skeletal muscle and adipose tissue, and decreasing hepatic glucose output. Additionally, troglitazone reduces hypertriglyceridemia, both by decreasing lipid synthesis in the liver and increasing clearance from the circulation.

The precise molecular events involved in the mechanism of action of troglitazone are only partly understood. Exposure of cells in tissue culture to thiazolidinediones can modulate insulin action, but these compounds have little direct effect on early signaling pathways for insulin. Recent efforts to understand the effects of thiazolidinediones on adipocyte differentiation have revealed the key role played by peroxisome proliferator activated receptor gamma (PPARγ). Although the natural activators of this nuclear receptor have not been identified, the thiazolidinediones can serve as ligands for this transcription factor, producing transcriptional activation upon binding. Consequently, the activated PPARγ regulates the expression of genes that encode for proteins central in controlling carbohydrate and lipid metabolism, thus producing the insulin sensitizing effects of these compounds.

2.2. The Antidiabetic Effects of Troglitazone Result from the Improvement of Insulin Resistance

To assess the pharmacological activities of troglitazone, studies were conducted on several genetic and acquired animal models of insulin resistance which possess symptoms most closely associated with human Type II diabetes, including obesity, hyperglycemia, hyperinsulinemia, and hypertriglyceridemia.

Treatment of KK and ob/ob mice with troglitazone produced a 40% to 45% decrease in plasma glucose and insulin levels. Plasma lactate, triglycerides, free fatty acids, and ketone bodies also declined, possibly contributing to the antihyperglycemic effects of troglitazone. A reduction in lactate, a gluconeogenic substrate, reduces glucose production by the liver, and a reduction in free fatty acids and ketone bodies alleviates the repressive effects that these metabolites exert on peripheral glucose uptake. There was no change in food intake or body weight with troglitazone treatment.

Nearly identical results were achieved in two other genetically obese diabetic rodent models, the db/db mouse and the Zucker fatty rat, and in a number of nongenetic models of insulin resistance. Marked reductions in fasting plasma glucose, insulin, and lipids were observed in these animals. Moreover, glucose tolerance was essentially normalized in Zucker fatty rats treated with troglitazone, decreasing both postprandial glucose and insulin levels following an oral glucose challenge. Troglitazone also prevented the induction of diabetes by dexamethasone in the female Zucker rat.

In contrast to the antihyperglycemic actions of troglitazone observed in insulin-resistant rodent models of diabetes, the drug did not significantly lower plasma glucose levels in streptozotocin-treated rats, a model of insulin-dependent diabetes. However, insulin tolerance tests performed in these rats indicated that treatment with troglitazone significantly improved insulin sensitivity, increasing the rate of plasma glucose lowering by an insulin challenge. Moreover, combined treatment with troglitazone potentiated the antihyperglycemic actions of exogenously administered insulin, and lowered the insulin dose required to achieve a set plasma glucose level.

Impaired islet cell function is a characteristic trait of Type II diabetes. Whether improvement of insulin resistance by troglitazone can restore pancreatic function, islet morphology, insulin content, and glucagon content was examined in db/db and KK diabetic mice at varying stages of diabetes. In both models, 3- to 4-week treatment with troglitazone (7 to 200 mg/kg) produced increased regranulation and insulin content of the pancreas, without effecting pancreatic weight, islet number, or glucagon content. This effect was accompanied by a decrease in exocrine cell number and normalization of the pattern of exocrine cell distribution within the islet.

Because troglitazone both decreases fasting plasma insulin levels and increases pancreatic regranulation, the effects of troglitazone on insulin secretion were assessed by a pancreatic perfusion. Treatment with troglitazone alone did not affect insulin secretion, but did enhance insulin secretion when administered in combination with a sulfonylurea. This increase in plasma insulin was associated with decreased plasma glucose levels in both fed and fasted nondiabetic animals. However, even in fasted animals, combination therapy did not produce hypoglycemia.

2.3. Troglitazone Reduces Hepatic Glucose Production

Inappropriate elevations in hepatic glucose production due to increased gluconeogenesis is a major cause of fasting hyperglycemia in Type II diabetes. To determine if troglitazone affects gluconeogenesis, the rate of glucose production was assessed in diabetic KK mice treated with troglitazone. In KK mice gluconeogenesis is markedly elevated compared to nondiabetic control animals. Troglitazone reduced the rates of gluconeogenesis in diabetic mice but was without effect in normal mice.

To determine the enzymatic step at which troglitazone influences the glycolytic/gluconeogenic pathway, the levels of the glycolytic intermediates were measured from livers of control and troglitazone-treated db/db and KK mice and subject to crossover analysis. A crossover point was observed between fructose-6-phosphate and fructose-1,6-bisphosphate, suggesting that troglitazone affects the interconversion of the two intermediates. Drug treatment led to a significant decrease in fructose-1,6-bisphosphatase activity, with no effect on phosphofructokinase activity. Concentrations of fructose-2,6-bisphosphate, an allosteric modulator of both enzymes,

remained unchanged with treatment, suggesting that the reduction in gluconeogenesis results from a decrease in fructose-1,6-bisphosphatase protein.

Gluconeogenesis was also evaluated in HepG2 cells treated with or without troglitazone. Forty-eight hour treatment with troglitazone caused a dose-dependent reduction in the production of glucose from lactate over a two-hour period. This effect was seen in the absence of insulin, and was not enhanced further by insulin addition. These data are consistent with liver perfusion experiments, supporting the premise that troglitazone decreases glucose output from the liver via the inhibition of gluconeogenesis.

2.4. Troglitazone Increases Insulin-Dependent Glucose Utilization

Peripheral insulin resistance in Type II diabetes is characterized by an attenuation of insulin-stimulated glucose utilization. The acute effect of troglitazone on glucose disposal in normal Sprague-Dawley rats was evaluated. In a hyperinsulinemic, euglycemic clamp assay, troglitazone infusion increased the overall rate of glucose disposal in vivo. Since hepatic glucose output is completely suppressed by insulin infusion, the increased glucose infusion rate reflected a direct effect of troglitazone on peripheral glucose uptake.

This effect of troglitazone was also observed in a hindlimb perfusion model. This isolated system maintains muscle integrity and insulin responsiveness, allowing for the evaluation of glucose metabolism without the influence of changes in endogenous hormones and metabolic feedback loops. Insulin alone increased glucose uptake 2.8-fold over basal levels. Perfusion with troglitazone further increased glucose uptake (1.79 ± 0.17 µmol/min for troglitazone plus insulin vs 1.24 ± 0.13 µmol/min for insulin alone). The production of lactate and pyruvate as well as oxygen consumption were also increased by insulin treatment, and further enhanced with troglitazone infusion.

Adipose tissue is highly responsive to insulin and plays a pivotal role in metabolic homeostasis. To determine if troglitazone affects adipose cell function, [^{14}C]2-deoxyglucose uptake by insulin was evaluated in adipocytes isolated from treated and untreated animals. Insulin produced a dose-dependent increase in

[^{14}C]2-deoxyglucose uptake in adipocytes from both groups. Troglitazone treatment significantly enhanced basal glucose uptake, and shifted the insulin dose response curve to the left.

To explore the mechanism by which glucose and lipid metabolism is regulated by troglitazone, direct evaluations were performed in cultured muscle and adipose cells. Exposure of L6 myocytes to troglitazone for one hour had no effect on glucose uptake. However, 72-hour treatment with troglitazone increased basal glucose uptake 2-fold without further affecting insulin-stimulated uptake. Analysis of glucose transporter content in treated cells indicated a 2.9 ± 1.3-fold increase in the Glut1 transporter, and a small but significant increase (1.9 ± 0.3-fold) in Glut4 transporter levels.

Treatment of fully differentiated 3T3-L1 adipocytes with 0.5 to 5 μM troglitazone for 48 hours caused a 2-fold increase in basal glucose uptake. No additional enhancement of insulin-stimulated glucose uptake was observed. This increase in basal uptake was caused by a 2-fold elevation in the Glut1 glucose transporter, due to an increase in Glut1 gene expression. This increase correlates with effects on glucose uptake in adipocytes isolated from troglitazone-treated Zucker rats, suggesting that increases in peripheral glucose uptake in rats may be due to increased glucose transporter synthesis.

Insulin promotes the formation of glycogen from glucose. The effect of troglitazone on the activity of glycogen synthase was studied in both HepG2 (human hepatoma) and BC3H-1 (mouse muscle) cells. Treatment of both cell types with troglitazone produced a dose-dependent increase in glycogen synthase activity. Addition of insulin and troglitazone together did not produced any further increase in activity. The increase in activity was not due to an elevation in glycogen synthase protein, but to the conversion of glycogen synthase from the glucose-6-phosphate dependent (D) to the glucose-6-phosphate independent (I) form.

2.5. Troglitazone Improves the Insulin Resistance Syndrome

In humans, insulin resistance is thought to be closely associated with a collection of metabolic abnormalities known as insulin-resistance syndrome, or Syndrome X, whose manifestations include glucose intolerance, dyslipidemia, vascular disease, obesity, and

hypertension. Although it is not known whether insulin resistance is a causative factor for any or all of these symptoms, they represent a major set of risk factors for cardiovascular disease. Moreover, some evidence suggests that lipid peroxides, also known to be elevated in Type II diabetes, may play an important role in atherogenesis. Troglitazone was synthesized with an α-tocopherol substitution, producing a bifunctional drug that combines the insulin sensitizing activity of a thiazolidinedione with a potent inhibitor of lipid peroxidation. Therefore, studies were designed to assess the effects of troglitazone on plasma triglycerides and lipid peroxides in laboratory models.

Troglitazone treatment dramatically decreased plasma triglyceride and free fatty acids in Zucker rats. VLDL triglycerides were significantly lower (78%) in treated animals. LDL and chylomicron levels were also decreased, but these values failed to show statistical significance, whereas cholesterol levels were unchanged with treatment. Overall the HDL/VLDC plus LDL reaction was increased by almost 50% in troglitazone-treated female Zucker rats. The decrease in triglyceride levels resulted from both an increase in triglyceride clearance and a decrease in hepatic triglyceride output.

The oxidation of LDL particles enhances their uptake by macrophages and monocytes, increasing the formation of plaque-forming foam cells. Antioxidants reduce lipid peroxidation, perhaps contributing to the decreased formation of foam cells. Twenty micromolar troglitazone inhibited LDL peroxidation by 90%. This antioxidant activity was significantly greater and of longer duration than that seen with α-tocopherol (35%). Additionally, troglitazone inhibited $CuSO_4$-induced oxidation of LDL more effectively than did the antioxidant probucol and other thiazolidinediones. This inhibition of LDL oxidation resulted in the formation of lipoprotein particles which were considerably less atherogenic, based on their ability to induce cholesterol ester formation in macrophages.

2.6. Troglitazone Regulates Transcription Via Activation of the Peroxisome Proliferator Activated Receptor (PPAR)γ

A number of studies have suggested that thiazolidinediones exert their primary insulin-sensitizing effects through the regulation of transcription. This has been examined in

cultured adipocyte cells. Confluent 3T3-L1 preadipocytes can be differentiated into adipocytes. Addition of troglitazone at the initiation of differentiation increases both the rate at which the cells differentiate, as well as the percent of preadipocytes which become adipocytes. However, troglitazone does not significantly influence the stimulation of mitogenesis by insulin or serum in 3T3-L1 fibroblasts. The transcription factor C/EBPα, which is both necessary and sufficient for the conversion of preadipocytes to adipocytes, is induced two to three days after the initiation of differentiation and is maintained at high levels in the adipocyte. Troglitazone treatment increased the rate of accumulation of C/EBPα, but did not affect the levels of C/EBPα message after differentiation was complete. This suggests that troglitazone influences factors which regulate the onset of C/EBPα production, but not those involved in modulating the total amount of C/EBPα expressed.

Recent studies have demonstrated that thiazolidinediones induce adipocyte differentiation by interacting with members of the peroxisome proliferator activated receptor (PPAR) family. In combination with other C/EBP family members, these nuclear receptors are believed to induce synthesis of C/EBPα and thus promote adipocyte differentiation. Thiazolidinediones can serve as ligands for PPARγ, a PPAR family member highly expressed in adipose tissue, but also found in other tissues. This interaction occurs over a concentration range similar to that required for transactivation of a heterologous promoter. PPAR activation thus initiates the cascade of transcriptional events which culminate in the expression of C/EBPα and adipocyte differentiation.

Data now suggest that the modulation of PPARγ by troglitazone plays a critical role in regulating intermediary metabolism, although the full spectrum of genes that respond to this drug, either directly or indirectly, await further characterization. One important target may be glucokinase, which catalyzes the conversion of glucose to glucose-6-phosphate, the rate-limiting step in glucose utilization in hepatocytes. Insulin increases glucokinase activity by stimulating the expression of the glucokinase gene. Both insulin and troglitazone induced transcription from the glucokinase promoter within 2 hours in a dose-dependent manner. Additionally, troglitazone decreased the EC_{50} required for insulin to initiate transcription 100-fold. No effect was seen on the maximal activation of glucokinase transcription. This study suggests that the hepatic insulin sensitization produced by troglitazone may result from both a direct insulin-

mimetic and insulin-sensitizing effect of the compound on glucokinase gene transcription.

2.7. Pharmacological Actions Related to Possible Adverse Reactions

The general pharmacological effects of troglitazone were evaluated in at variety of species including normal rats, mice, beagles, rabbits, and guinea pigs. At doses up to 1000 mg/kg, troglitazone had no significant effects on gross behavior or motor activities, the central nervous system, respiratory system, digestive system, cardiovascular system, or urinary system. Additionally troglitazone treatment did not affect body temperature, platelet aggregation, blood coagulation, spontaneous sleep, or gastric secretion. Cardiac ventricular weight was increased in Wistar female rats treated with 400 mg/kg of drug for 14 days, although similar increases in cardiac weight were not observed in two diabetic rat models treated with high doses (800 mg/kg) of the drug. This cardiac hypertrophy did not affect ventricular contractile performance over a range of aortic perfusion pressures, but was associated with increased blood volume and water balance. No other significant side effects were observed in the animal models tested.

3. TOXICOLOGY

3.1. Introduction

The toxicologic profile of troglitazone was evaluated in mice, rats, dogs, and monkeys. Toxicity and toxicokinetic data support selection of monkey as the most appropriate nonrodent species for assessment of preclinical safety. Carcinogenic potential was assessed in mice and rats. Additional studies characterized effects observed in multidose studies and toxicity of several related compounds. Reproductive toxicity studies were conducted in rats and rabbits, and genotoxic potential was evaluated in vitro and in vivo. Initial studies evaluated toxicity of crystalline troglitazone; additional studies evaluated amorphous troglitazone which is more bioavailable and will be the marketed formulation. All definitive studies were conducted in compliance with US FDA Good Laboratory Practice regulations.

Pharmacokinetic data indicated that human troglitazone metabolites (M-1, sulfate conjugate; M-2, glucuronide conjugate; and M-3, a quinone) were also found in mice, rats, and monkeys. Troglitazone was rapidly absorbed in these species; time to peak plasma concentration (tmax) was one to two hours in mice and rats and three hours in monkeys. Troglitazone was widely distributed to most tissues at concentrations lower than in plasma and was present at high concentrations in liver, gastrointestinal tract, and adipose tissue. Troglitazone exposure in toxicology studies increased with increasing dose but was less than dose proportional. Exposures were greater in female mice and rats than in males given the same dose; no gender differences were seen in monkeys. M-1 metabolite exposures were greater in female than in male mice and were higher in male than in female rats. M-3 metabolite exposures were similar in both genders in all species, and were lower than troglitazone or M-1 metabolite values.

The initial recommended human dose expected to be effective in most patients is 200 or 400 mg once daily, with associated exposures up to 13.4 μg·hr/mL, upon which comparisons to exposures in toxicology studies are based. The maximum recommended human dose which may be required is 600 mg once daily, with an associated exposure of 22.1 μg·hr/mL.

3.2. Acute Toxicity

No drug-related clinical signs, deaths, body weight changes, or gross pathologic findings were observed in mice or rats given single oral or intraperitoneal doses of crystalline troglitazone up to 5000 mg/kg. There were no drug-related clinical signs, deaths, or changes in body weight or food consumption in monkeys given single oral doses of 2000 mg/kg. No deaths or significant effects were observed on Day 1 of multidose studies in mice, rats, or monkeys given amorphous troglitazone at 1200 mg/kg, the maximum achievable dose based on dose volume and solubility limitations.

3.3. Multidose Toxicity

The toxicity of crystalline and amorphous troglitazone following repeated oral [illegible] monkeys. Two week intravenous

studies in rats and monkeys were conducted to support a clinical absolute bioavailability study; minimal effects were observed in both species.

Mouse. Increased body weight, food consumption, and liver weight, and hepatocellular hypertrophy were observed in mice given crystalline troglitazone by gavage for up to 52 weeks. Amorphous troglitazone was clinically well-tolerated in mice given up to 1200 mg/kg for 2 or 13 weeks. Reversible increases in body weight gain, heart weight, liver weight, and hepatocellular hypertrophy were observed in 2-week studies. Increased hepatic enzymes (cytochrome P450 (CYP), carnitine acetyltransferase, and peroxisomal cyanide-insensitive fatty acid β-oxidation) were observed. In the 13-week dose range-finding study for the carcinogenicity study, decreased hemoglobin (Hb) and hematocrit (Hct) and increased liver and heart weights were observed at 400, 800, and 1200 mg/kg but not at 50 mg/kg. Centrilobular hepatocellular hypertrophy was observed in both sexes at all doses. Fatty changes and hypocellularity were noted in bone marrow at ≥400 mg/kg. Splenic extramedullary hematopoiesis was more pronounced at 1200 mg/kg. All changes were reversible within four weeks. Troglitazone plasma AUC at 400 mg/kg, the lowest dose associated with increased heart weight, was 195 μg·hr/mL in males and 304 μg·hr/mL in females.

Rat. Increased body weight, food consumption, and liver weight; decreased erythrocyte count, Hb, and Hct; and hepatocellular hypertrophy were observed in rats given crystalline troglitazone by gavage for up to 52 weeks. Heart weight increased in females given 1200 mg/kg for 52 weeks. Similar findings were observed with amorphous troglitazone in studies up to 52 weeks duration, although the effects were more pronounced due to higher exposure. Erythrocyte count, Hb, and Hct were decreased significantly when exposure exceeded 162 μg·hr/mL. Increased liver weight was observed at the highest dose (800 mg/kg in males and 200 mg/kg in females) and hepatocellular hypertrophy occurred in males at all doses (100, 400, and 800 mg/kg) and in females at the mid (50 mg/kg) and high (200 mg/kg) doses in the 52-week study. Troglitazone exposure at these doses ranged from 21.1 to 1060 μg·hr/mL. Increased hepatic enzymes (CYP, carnitine acetyltransferase, and peroxisomal cyanide-insensitive fatty acid β-oxidation) were noted in two-week studies. Hypertrophy and hyperplasia of brown adipose tissue were observed in males at ≥100 mg/kg and in females at ≥25 mg/kg after 13, 26, and 52 weeks. Heart weight increased after two weeks in females at ≥200 mg/kg, and after 13, 26, or 52 weeks in

≥400 mg/kg and in females at ≥50 mg/kg; troglitazone exposures at these doses were ≥192 μg·hr/mL. No significant histopathologic changes accompanied heart weight increases in rats treated for up to 26 weeks. At 52 weeks, microscopic changes in heart were subtle and not considered toxicologically significant based on low incidence and minimal severity.

A series of investigational studies was conducted to assess drug-induced changes in erythrocyte parameters and heart weight increases. Changes in erythrocyte parameters and increased heart weight in rats occurred within three to five days after treatment initiation. Circulating blood volume was increased, and circulating erythrocyte number and bone marrow erythropoiesis were unchanged. The diuretic furosemide did not affect troglitazone-induced increases in heart weight. Increases in both wet and dry heart weight and protein per gram of heart were ameliorated when troglitazone was coadministered with the angiotensin-converting enzyme inhibitor, temocapril, or the angiotensin II antagonist, CS-866, without affecting the drug-induced increase in plasma volume. Administration of insulin alone increased urine and plasma volume and heart weight, as well as decreased erythrocyte count and hematocrit. Functional studies revealed no changes in left ventricular performance in hearts from female rats given 50 or 400 mg/kg for two weeks or in blood pressure or electrocardiograms in rats given 800 mg/kg for two weeks.

Dog. Although monkey is the most appropriate nonrodent species, subacute studies in dogs explored toxicity of crystalline troglitazone, which was clinically well-tolerated when given at up to 400 mg/kg for up to four weeks or at 100 mg/kg for 13 weeks. Nondose-related reversible 2- to 18-fold increases in alanine aminotransferase (ALT) were observed at ≥3 mg/kg. Liver weight increased at ≥12.5 mg/kg, and hepatocellular hypertrophy was noted at 400 mg/kg. No changes were observed in the heart.

Monkey. Crystalline troglitazone was clinically well-tolerated in monkeys given up to 800 mg/kg for up to 52 weeks. Decreased cholesterol and increased liver weight without microscopic changes were the only notable findings. With amorphous troglitazone at maximum achievable doses, decreased cholesterol, increased liver weight, hepatocellular hypertrophy, and increased hepatic enzymes (CYP, peroxisomal cyanide-insensitive fatty acid β-oxidation) were noted. In the ongoing 52-week study, decreased erythrocyte count, Hb, and Hct were observed in males at 300, 600, and

1200 mg/kg. Blood pressure and electrocardiograms were not affected. Serial echocardiographic evaluations did not reveal changes in heart volumes. Troglitazone exposure at Week 52 ranged from 47.3 to 76.3 μg·hr/mL.

3.4. Carcinogenicity

In studies conducted by the Sankyo Company with crystalline troglitazone, no evidence of carcinogenicity was observed in B6C3F1 mice given up to 100 mg/kg for 78 weeks or in Fischer 344 rats given up to 450 mg/kg for 104 weeks. Additional studies conducted by Parke-Davis evaluated the carcinogenic potential of amorphous troglitazone. Relevance of tumor data was based on the entire battery of statistical results and consideration of tumor incidence, background rates, and patterns and trends of tumor histologic types for tumors with positive dose trends.

Mouse. Based on a 13-week dose range-finding study in which dose-related increased heart and liver weights and hepatocellular hypertrophy were observed at 400, 800, and 1200 mg/kg, B6C3F1 mice were given amorphous troglitazone at 50, 400, or 800 mg/kg by gavage for 104 weeks. Doses selected were associated with exposures approximately 2 to 23 times the human therapeutic level (13.4 μg·hr/mL). Plasma troglitazone concentrations during the carcinogenicity study approximated values in the 13-week dose range-finding study, indicating that toxicokinetics did not change appreciably with time.

There were no drug-related clinical signs, effects on body weight, food consumption, and ophthalmic parameters, or clinically significant changes in clinical laboratory parameters. After 104 weeks, liver and heart weight increased in both sexes at 400 and 800 mg/kg. Nonneoplastic microscopic changes included increased size and coalescence of fat droplets within adipocytes of interscapular brown adipose tissue, increased incidence of bone marrow fatty changes and hypocellularity in both sexes at all doses, and hepatocellular vacuolar changes in males at 400 mg/kg and in both sexes at 800 mg/kg.

Statistically-significant, dose-related trends toward decreased survival were identified in both sexes using the product-limit method. Pairwise comparisons to vehicle controls indicated mortality rates were statistically-significantly increased in males at 400 and 800 mg/kg and in females at 800 mg/kg; most deaths occurred during the

final 6 months of the study. Survival was sufficient for a valid carcinogenicity study based on published criteria. Overall survival at 104 weeks was 83%, 90%, 82%, 63%, and 58% in males and 77%, 73%, 70%, 62%, and 48% in females in vehicle controls, placebo controls, and at 50, 400, and 800 mg/kg, respectively. No predominant cause of death was apparent.

Compared to vehicle controls, Peto analysis identified statistically significant positive-dose trends for one specific tumor type in males (hemangiosarcoma) and four specific tumor types in females (hemangiosarcoma, hepatocellular carcinoma, alveolar-bronchiolar carcinoma, and uterine adenocarcinoma). Of the specific tumor types identified by Peto analysis, alveolar-bronchiolar carcinomas and uterine adenocarcinomas in females were not considered drug-related given the lack of significance in additional tests in the statistical battery and the low incidences, which were comparable to the historical control ranges.

The increased incidence of hemangiosarcoma in females at 400 mg/kg and in both sexes at 800 mg/kg was statistically significant in additional trend and pairwise statistical tests. Onset of hemangiosarcoma was shortened in females at 400 and 800 mg/kg and latency was decreased in females at 400 mg/kg and in both sexes at 800 mg/kg. Hemangiosarcomas were distributed similarly between intercurrent deaths and mice surviving to termination, suggesting that these tumors were not the sole cause of death in these animals. There were no primary hemangiosarcomas in the lung, and no pulmonary metastases of any tumor were found. There appears to be a predisposition for development of hemangiosarcoma in mice, and a trend towards increased spontaneous occurrence of these tumors is apparent. In addition, based on background incidence and metastatic potential, troglitazone-induced hemangiosarcomas in mice appear biologically different from those in humans.

Additional tests confirmed statistical significance of increased hepatocellular carcinomas in females at 800 mg/kg. Microsomal enzyme induction may be relevant to the increased incidence of hepatocellular carcinoma in that an association between enzyme induction and promotion of hepatocarcinogenesis in rodents, particularly mice, has been postulated. The incidence of hepatocellular carcinomas in males was not increased at any dose, and the relevance of experimentally-induced mouse liver tumors to human drug therapy remains controversial.

Troglitazone exposure at doses associated with increased tumor incidence in mice were at least 16 times the human therapeutic level. There were no increased tumor incidences at 50 mg/kg, with exposures 2 to 4 times human therapeutic level.

Rats. Wistar rats were given amorphous troglitazone by gavage for 104 weeks; males received 100, 400, or 800 mg/kg and females received 25, 50, or 200 mg/kg. Doses selected were based on a 13-week study in which dose-related increased liver weight and hepatocellular hypertrophy in males at 400 to 1200 mg/kg and in females at 50 to 400 mg/kg, and increased heart weight in males at 1200 mg/kg and in females at 400 mg/kg were observed. Doses selected were associated with exposures in males and females approximately 2 to 12 times and 5 to 47 times the human therapeutic level (13.4 μg·hr/mL), respectively. Plasma troglitazone concentrations during the carcinogenicity study approximated values in the 13-week study, indicating that toxicokinetics did not change appreciably with time.

Body weight of drug-treated males and low- and mid-dose females was similar to controls throughout the study. In high-dose females, body weight increased during the first six months and decreased during the last 12 months of the study. Food consumption increased in males at all doses and in mid- and high-dose females. No drug-related ophthalmic changes were observed. Erythrocyte count was decreased in high-dose females at termination. Heart weight increased significantly in both sexes at the mid and high doses. Liver weight increased in mid- and high-dose males.

Nonneoplastic microscopic changes were observed in heart, brown adipose tissue, liver, and bone marrow. The incidence and severity of ventricular dilatation, myocardial fibrosis, and karyomegaly of atrial cardiomyocytes in males at the mid and high doses and in females at all doses were greater than in controls, and correlated with heart weight increases and macroscopic findings. Increased incidence of atrial thrombosis was seen in males at the mid-dose and in both sexes at the high doses. These lesions were morphologically similar to spontaneous lesions also occurring in these rats. Changes considered secondary to myocardial lesions included diffuse centrilobular hepatocellular necrosis in both sexes at the high doses, an increased incidence of alveolar macrophages in low-dose females and both sexes at the mid and high doses, and subcutaneous edema in mid-dose males and both sexes at the high doses.

Increased size and coalescence of fat droplets within adipocytes, and increased fibrosis and fibroplasia in intra- and interlobular septa were observed microscopically in brown adipose tissue in both sexes at all doses, and correlated with macroscopic findings of enlargement and firmness of interscapular brown adipose tissue. Similar change in brown adipose tissue in other locations, including paravertebral, thoracic and lumbar, mediastinal, and perirenal sites, were observed in high-dose males and in females at all doses. Centrilobular hepatocellular hypertrophy correlated with increased liver weight in mid-dose males and in both sexes at the high doses. Bone marrow fatty change and hypocellularity were observed in mid- and high-dose females.

Survival was considered adequate for a valid evaluation of tumorigenic potential based on published criteria. Group survival was ≥63% at Week 80, with at least 38 surviving animals per group. Survival at the high doses was reduced in both sexes during the last 6 months of the study. At termination, survival was 47%, 45%, 45%, 45%, and 25% in males and 53%, 65%, 62%, 68%, and 17% in females in the vehicle and placebo controls, and at the low, mid, and high doses, respectively.

Compared to vehicle control, Peto analysis identified statistically significant positive-dose trends for angiolipoma of skin and liposarcoma of skin in males, and adenocarcinoma of large intestine, fibrosarcoma of skin, liposarcoma of skin, and schwannoma of uterus (malignant) in females. Based on low incidences and lack of statistical corroboration in the overall statistical battery, the occurrence of these tumors could not be attributed to drug treatment.

3.5. Special Studies

Troglitazone is a racemate with two chiral centers, one of which undergoes epimerization. The toxicity of the two stable optical isomers (2S5RS, 2R5RS) were similar to those of troglitazone in mice and rats given single or repeated oral doses. The antigenic potential of crystalline troglitazone in mice, rats, guinea pigs, and rabbits was low. Crystalline troglitazone did not cause ocular irritation in rabbits or contact skin sensitization in guinea pigs. Effects in mice and rats given troglitazone in the diet or by gavage were similar.

Acute toxicity, antigenicity, and mutagenicity of troglitazone metabolites and related substances also were assessed. There were no compound-related effects in mice or rats given single oral or intravenous doses of M-1, M-2, or M-3 metabolites, related substances ROY-1302 (troglitazone analogue) and ROY-1788 (troglitazone dimer), or thermally decomposed troglitazone.

Death occurred in mice given single oral doses of ROY-1993 (impurity) at 2000 mg/kg. M-3 metabolite and thermally decomposed troglitazone did not induce antibody formation, and M-3 metabolite, ROY-1302, ROY-1788, ROY-1993, and thermally decomposed troglitazone were not mutagenic in bacteria.

3.6. Reproductive Toxicity

Fertility and general reproductive function in rats were not affected by crystalline troglitazone at up to 1000 mg/kg given prior to and throughout mating and gestation. Toxicokinetics were evaluated retrospectively to estimate exposure over the dose range evaluated in reproductive toxicology studies. Pregnant rats were given crystalline troglitazone on Gestation Days 7 or 17, or on Lactation Day 10, and exposures ranged from 32.2 to 38.6 μg·hr/mL at 200 mg/kg and from 62.2 to 107 μg·hr/mL at 2000 mg/kg, or 2 to 8 times the human therapeutic level. Toxicology findings at high doses in these studies and pharmacokinetic data indicate exposure to crystalline troglitazone in rat reproduction studies was adequate to assess reproductive toxicity potential.

In rats given crystalline troglitazone during gestation, fetal body weight and offspring body weight gain were decreased at 2000 mg/kg; no effects were observed at 20 or 200 mg/kg, and there was no evidence of teratogenicity at any dose. A tissue distribution study with crystalline troglitazone in pregnant rats indicated troglitazone concentrations in fetal tissue were two to three times higher than in maternal plasma.

There were no effects on maternal and fetal parameters or teratogenicity in rabbits given crystalline troglitazone during the critical period of organogenesis at 40 to 1000 mg/kg, the highest dose achievable due to dose volume limitations. Based on a toxicokinetic study in pregnant rabbits, teratology studies were repeated with amorphous troglitazone. Hemorrhagic maternal mammary tissue, decreased fetal

weights, and delayed ossification were observed at 40 and 100 mg/kg; no effects were observed at 15 mg/kg, and there was no evidence of teratogenicity at any dose. In pregnant rabbits given a single oral dose of 40 mg/kg, the lowest-effect dose in the definitive study, estimated exposure was 82.2 μg·hr/mL, six times the human therapeutic level.

Delayed postnatal development attributed to reduced body weight gain during lactation was the only effect in offspring of rats given crystalline troglitazone at 40, 200, or 1000 mg/kg in a perinatal/postnatal study; no effects were observed at 10 or 20 mg/kg. Exposures at 200 mg/kg were ≥32.2 μg·hr/mL. Troglitazone also was present in milk of lactating rats at concentrations similar to those in plasma. Thus, in addition to in utero exposure, offspring in the perinatal/postnatal study were exposed during the lactation period.

3.7. Genetic Toxicity

Troglitazone was not mutagenic in bacteria at doses up to 10,000 μg/plate, with or without metabolic activation. No structural chromosome aberrations (SCA) were observed in Chinese hamster V79 cells exposed continuously to 0.7 to 2.9 μg/mL for 24 or 48 hours without metabolic activation or to 16 to 64 μg/mL for 6 hours with metabolic activation. Aneuploid cells and giant cell forms were observed after exposure to 2.9 μg/mL without activation for 48 hours. With activation, the number of cells with endoreduplicated chromosomes was increased at 58 and 64 μg/mL. In another assay in Chinese hamster lung cells, SCA frequency was not increased with continuous exposure of up to 64 μg/mL without metabolic activation for 24 or 48 hours. Pronounced cytotoxicity and increased SCA frequency were observed following six hours exposure to 178 μg/mL without activation and at 163 μg/mL with activation.

Results of the in vitro mouse lymphoma mutation assay at cytotoxicity-limited concentrations up to 30 μg/mL were equivocal. Minimal but statistically significant increases in mutant frequency were observed in two of five trials without metabolic activation and in two of six trials with activation. To clarify if this variability was related to microtiter plate methodology used in these trials, studies are currently in progress with agar plate methodology.

Micronucleus frequency in bone marrow was not increased in mice given single oral doses of crystalline troglitazone at 500 to 5000 mg/kg. An additional mouse micronucleus study with amorphous troglitazone is currently in progress. No unscheduled DNA synthesis was observed in hepatocytes isolated 2 or 24 hours postdose from rats given single oral doses of amorphous troglitazone at 1000, 1500, or 2000 mg/kg.

3.8. Overall Discussion and Assessment

Adequate preclinical studies have characterized the toxicologic profile of troglitazone. In pharmacology safety studies in mice, rats, and dogs, there were no significant central nervous, respiratory, cardiovascular, digestive, urinary, or smooth muscle effects. Acute toxicity in mice, rats, and monkeys is low. Decreased erythrocyte parameters were the only significant effects observed in the definitive chronic study in monkeys given amorphous troglitazone at three to five times the human therapeutic level (13.4 μg·hr/mL). In multidose studies in rodents given amorphous troglitazone, brown adipose tissue changes, increased liver weight, hepatocellular hypertrophy, decreased erythrocyte parameters, and increased heart weight were consistent findings. Changes in brown adipose tissue may be a response to protracted pharmacologic effects of troglitazone on adipocytes. Increased liver weight was attributed to hepatocellular hypertrophy associated with induction of CYP, carnitine acetyltransferase, cyanide-insensitive fatty acid β-oxidation, and minimal peroxisome proliferation.

Reversible decreases in erythrocyte parameters were attributed to hemodilution due to fluid accumulation and increased plasma volume. Splenic extramedullary hematopoiesis in mice was considered a physiologic response to decreased erythrocyte parameters. Bone marrow fatty change and hypocellularity were observed in mice and rats after prolonged exposure. Reversible heart enlargement without significant microscopic changes were observed in mice and rats in long-term studies with troglitazone exposures exceeding 14 times the human therapeutic level. There were no significant changes in cardiac function in troglitazone-treated rats, and cardiovascular function appears to improve in diabetic or insulin-resistant rats given troglitazone. In addition, no effects on blood pressure, ECG, or heart weight were observed in monkeys. Heart size was not changed based on serial echocardiographic evaluations.

In humans, no adverse cardiac events or changes in left ventricular mass were observed in long-term clinical trials at doses up to 800 mg/day, and stroke volume and other measures of cardiac output improved (see Section 5.2.5).

Troglitazone did not affect fertility and was not teratogenic in rats or rabbits. Compared to the human therapeutic level, estimated exposures in these studies was at least two to eight times higher in rats and six times higher in rabbits. No significant effects on perinatal/postnatal development in rats were elicited at high exposures.

While additional in vitro and in vivo studies are in progress, genetic toxicity assays conducted to date suggest that troglitazone does not possess significant genotoxic potential.

Incidences of hepatocellular carcinoma in female mice and hemangiosarcoma in male and female mice were increased in the 104-week carcinogenicity study. The biologic characteristics of hemangiosarcomas in mice differ from those in humans, and the increased incidence was observed at 16 times the human therapeutic level. Liver tumors in mice were found at 23 times the human therapeutic exposure. No tumors of any type were increased in mice at two to four times the human therapeutic level. No increased incidence of any tumor type was observed in rats given troglitazone for 104 weeks at 2 to 12 times the human therapeutic level in males and 5 to 47 times in females. The collective evaluation of these data suggest minimal carcinogenic risk.

One hundred thirty-nine studies evaluated the toxicity of crystalline and amorphous troglitazone in mice, rats, dogs, monkeys, and rabbits. These preclinical studies adequately characterized the toxicologic profile of troglitazone and support its clinical use.

4. PRECLINICAL AND CLINICAL PHARMACOKINETICS AND PHARMACODYNAMICS

4.1. Introduction

Absorption, distribution, metabolism, and excretion (ADME) of troglitazone and metabolites were studied in mice, rats, rabbits, dogs, monkeys and humans. Initial studies in preclinical species were conducted using the crystalline formulation of troglitazone, but due to increased plasma concentrations obtained with the amorphous formulation, additional studies were conducted using amorphous troglitazone. Human pharmacokinetic studies were conducted using the amorphous formulation of troglitazone. Toxicokinetic studies conducted with crystalline or amorphous material to support the Parke-Davis toxicology evaluation are reported in the toxicology summary.

4.2. Pharmacokinetics

Following oral administration of troglitazone, maximum plasma concentrations are achieved within one to three hours in all species including humans. The extent of absorption was increased by 30% to 80% when troglitazone was administered with food in man. Troglitazone elimination t½ values generally ranged from 1 to 10 hours in preclinical species and 16 to 34 hours in humans. Multiple-dose administration in man results in attainment of steady state within one week. There was no change in steady state systemic exposure to troglitazone and its major metabolites over time (Weeks 12 to 48 of troglitazone treatment).

Three metabolites of troglitazone have been identified in plasma for all species studied including humans: a sulfate (M-1), glucuronide (M-2) conjugate, and a quinone metabolite (M-3). The relative proportions of troglitazone and M-1, M-2, and M-3 in mice, rats, dogs, monkeys, and humans calculated as a percentage of the sum of troglitazone and metabolite AUC values at doses producing similar troglitazone AUC values is summarized in Table 1. Based on in vivo exposure, the relative proportions of troglitazone, M-1, M-2, and M-3 in monkey plasma following oral administration most closely resembles humans, thus, making monkey the most appropriate nonrodent species for the toxicology studies.

TABLE 1. Approximate Relative Proportions Calculated as a Percentage of the Sum of AUC(0-24) Values for Troglitazone and Metabolites in Mice, Rats, Dogs, Monkeys, and Humans Following Oral Administration of Amorphous Troglitazone

Species	Troglitazone	M-1	M-2	M-3
Mouse[a]	<60	20	20	<5
Rat (Male)	10	90	<5	<5
Rat (Female)	90	10	<5	<5
Dog	20	80	<1	<1
Monkey	10	70	5	20
Human	10	80	5	10

[a] All strains except KK mice

Significant gender differences in troglitazone disposition were observed in rats. M-1 concentrations in male rats were greater than troglitazone concentrations, whereas, troglitazone concentrations in female rats were higher than M-1 concentrations. No significant gender differences were observed in mice, dogs, monkeys, and humans.

4.3. Distribution

Troglitazone was highly bound to serum proteins in all species. In vitro binding in human, dog, male rat, and female rat serum and mouse plasma was greater than 98%. In vivo binding was comparable. Albumin appeared to be the dominant binding protein. Binding was not affected in vitro by the presence of other highly protein-bound drugs.

Troglitazone distributed rapidly and widely throughout the body, with a volume of distribution greater than 0.73 L/kg in mice and rats and ranging from 0.28 to 0.87 L/kg in dogs. In humans, mean steady-state apparent volume of distribution ($V_d\beta/F$) of troglitazone ranged from 10.5 to 26.5 L/kg.

Following multiple dosing of [^{14}C]troglitazone to rats, the steady-state tissue/blood concentration ratio was three or less in all tissues except white fat, brown fat, bone marrow, and large intestine, where ratios ranged from three to six. Liver ratio was highest at approximately 30. Troglitazone is transferred to the fetus in rats and rabbits and into the milk from nursing rats.

4.4. Metabolism

The proposed metabolite pathway for troglitazone is shown in Figure 2. Results of a

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FIGURE 2. Proposed Metabolic Pathway of Troglitazone

See above

See previous page

4.5. Excretion

In preclinical species, following intravenous and/or oral administration of [^{14}C]troglitazone, radioactivity is excreted primarily in feces (>87%) with a minor amount in urine (<7%). Biliary secretion has been shown to be the major elimination pathway in rats, dogs, and monkeys.

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FIGURE 3. Structure of Troglitazone

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4.7. Additional Human Pharmacokinetics and Pharmacodynamics

In humans, mean steady-state Cmax and AUC(0-24) values for all analytes increased proportionally over the clinical dose range of 200 to 600 mg/day (Table 2).

TABLE 2. Mean Pharmacokinetic Parameter Values for Troglitazone, M-1, and M-3 Following Administration of 200, 400, and 600 mg QD for 7 Days to Human Volunteers

Dose (mg/Day)	Cmax (μg/mL)	tmax (hr)	AUC(0-24) (μg·hr/mL)	CL/F (mL/min)
Troglitazone				
200	0.90	2.9	7.4	500
400	1.61	2.7	13.4	601
600	2.82	2.7	22.1	496
M-1				
200	5.94	5.1	77.8	ND
400	10.2	5.1	138	ND
600	17.8	4.7	216	ND
M-3				
200	0.79	3.8	9.0	ND
400	1.36	3.5	16.7	ND
600	2.39	3.9	26.6	ND

ND = Not determined.

4.8. Special Populations

4.8.1. Formal Studies

4.8.1.1. Renal Impairment

Pharmacokinetics of troglitazone, M-1, and M-3 were determined in 20 human subjects with various degrees of renal function following administration of a single 400-mg dose of troglitazone. There was no significant correlation between troglitazone, M-1, and M-3 pharmacokinetic parameter values and creatinine clearance values. Unbound troglitazone AUC values were comparable across all subjects. Based on pharmacokinetics, troglitazone dose adjustment in patients with impaired renal function is not required.

4.8.1.2. Hepatic Impairment

A study of troglitazone, M-1, and M-3 pharmacokinetics was performed in healthy subjects and patients with liver disease (Childs-Pugh Grade B and C). Subjects received a single 400-mg dose of troglitazone. Systemic exposure to troglitazone was similar in healthy subjects and in patients with moderate to severe impaired hepatic function. Mean AUC values of M-1 and M-3 were 5- and 2-fold higher, respectively, in patients with hepatic impairment than in healthy subjects. The clinical significance of these changes is unknown. Thus, cautious use of troglitazone is recommended in patients with liver disease.

4.8.1.3. Elderly

Pharmacokinetics of troglitazone, M-1, and M-3 were evaluated in healthy young (aged 24-39 years) and elderly (aged 70-81 years) subjects. Subjects received a single oral dose of 400 mg troglitazone or placebo, followed one week later by 400 mg troglitazone or placebo BID for 13 days, with a single 400-mg troglitazone dose or placebo on the morning of Day 14. Twelve young and 11 elderly subjects received troglitazone. Age-related differences in AUC(0-∞) of troglitazone, M-1, and M-3 were not observed following single-dose administration. Mean Day 14 AUC(0-12) value was similar to mean Day 1 AUC(0-∞) value in the elderly group, but mean Day 14 AUC(0-12) value was lower than the mean Day 1 AUC(0-∞) value in young subjects. Thus, mean AUC(0-12) values for all analytes were unexpectedly lower (by approximately 20%-36%) in young compared with elderly subjects following multiple-dose administration. Mean tmax and elimination t½ of troglitazone, M-1, and M-3 were similar in the two groups.

While small differences in steady-state AUC values were observed in this study involving a small number of subjects, no significant age-dependent effect on steady-state troglitazone, M-1, and M-3 pharmacokinetics was observed in a larger population analysis. Thus it is not likely that age-dependent dose adjustments will be necessary.

4.8.1.4. Patients With Type II Diabetes

The pharmacokinetics of troglitazone, M-1, and M-3 in patients with Type II diabetes was compared to age- (within 3 years), weight- (within 5 kg), and gender-matched healthy subjects. Subjects received 400 mg troglitazone QD with food for 15 days. Steady state plasma concentrations of troglitazone, M-1, and M-3 were achieved by the fifth day of drug dosing, and their accumulation was modest (1.2- to 2.2-fold). Mean troglitazone t_{max}, CL/F, and λz values were similar, indicating that the absorption and elimination of troglitazone are not significantly different in these populations. Mean troglitazone elimination t½ value at steady state was approximately 24 hours in both groups. Thus, pharmacokinetics of troglitazone, M-1, and M-3 are essentially the same in patients with Type II diabetes and in age-, weight-, and gender-matched healthy subjects. This finding was confirmed in a larger population analysis.

4.8.2. Population Pharmacokinetic Analysis

Population analysis was used to examine the effects of age, gender, presence of Type II diabetes, race, smoking, and body weight on steady-state pharmacokinetics of troglitazone, M-1, and M-3. Pharmacokinetic parameter estimates were obtained from 255 subjects in 8 studies. Age, gender, presence of Type II diabetes, race and smoking did not affect steady state pharmacokinetics of troglitazone and its metabolites. Although body weight was a statistically significant covariate, the predictive ability of these demographic factors, in combination, on steady-state pharmacokinetics of troglitazone, M-1, and M-3 was low (R^2<0.2). Based on pharmacokinetics, dose adjustment in relation to these factors is not required.

4.8.3. Drug-Drug Interactions

4.8.3.1. Pharmacokinetic

- **Acetaminophen**: Coadministration of single doses of troglitazone (400 mg) and acetaminophen (1 gm) did not affect the plasma concentrations or metabolism of either drug compared to each agent administered alone.

- **Glyburide**: Following coadministration of troglitazone (200 mg QD × 12 days) and glyburide (3.5 mg QD × 12 days) to patients with Type II diabetes, minimal changes in steady-state pharmacokinetic parameters of either drug were observed compared to each drug regimen administered alone. Plasma protein binding of both drugs was unaltered.

- **Cholestyramine**: Coadministration of single doses of troglitazone (400 mg) and cholestyramine (12 gm) reduced troglitazone bioavailability by 70%. Thus, coadministration of these drugs is not recommended.

4.8.3.2. Pharmacodynamic

- **Warfarin**: Multiple-dose administration of troglitazone (800 mg QD × 15 days) had no effect on prothrombin time in patients receiving chronic warfarin therapy.

- **Glyburide**: Coadministration of glyburide with troglitazone (600 mg QD) for 6 weeks had additive effects on lowering serum glucose levels in patients with Type II diabetes.

- **Ethanol**: Moderate consumption of alcohol with meals did not increase the risk of hypoglycemia in patients with Type II diabetes treated with troglitazone (200 mg QD × 45 days).

4.8.4. Pharmacodynamics

4.8.4.1. Glycemic and Lipid Effects

4.8.4.1.1. Healthy Subjects

Single and multiple doses of troglitazone (200 to 800 mg) administered for two weeks did not have significant effects on fasting plasma glucose, insulin, glucagon, C-peptide, triglyceride, HDL, or VLDL levels, or on blood pressure. These results are consistent with findings in preclinical models (no hypoglycemic response was observed in euglycemic paradigms). Total and LDL-cholesterol levels in plasma were lower in subjects following administration of multiple doses of troglitazone compared with

placebo. These reductions increased with increasing troglitazone dose from 200 through 400 mg BID and then plateaued.

Similarly following administration of 200 mg troglitazone TID for 14 days, there was no clinically significant effect on fasting serum fructosamine and triglycerides; mean and peak glucose, insulin, and nonesterified fatty acids; basal stimulation of insulin secretion, and/or ratio of insulin secretion to glucose AUC.

4.8.4.1.2. Patients With Type II Diabetes

Following administration of 800 mg troglitazone QD or 400 mg troglitazone BID for six weeks to patients with Type II diabetes, fasting, and postprandial glucose were reduced to a similar degree (21% to 23%), with placebo as baseline. Troglitazone was also effective in reducing fasting (800 mg QD, 36%; 400 mg BID, 18%) and postprandial (800 mg QD, 39%; 400 mg BID, 18%) insulin, nonesterified fatty acids (42% to 45%), and triglycerides (20% to 23%) compared with placebo. Additionally, insulin resistance, as defined by (insulin × [fasting plasma glucose])/22.5 based on a homeostasis model, was lower following administration of 800 mg troglitazone QD (54%) and 400 mg troglitazone BID (43%) compared with placebo. No differences were seen in C-peptide and serum fructosamine levels or β-cell function following troglitazone or placebo administration.

In another study , patients with fasting glucose values ≥150 mg/dL, maintained by diet therapy or 5 mg glyburide, received troglitazone 200 mg BID for 6 weeks. At Week 6, the effect of troglitazone on change from baseline in fasting plasma glucose and HbA_{1c}, diurnal variation in plasma glucose levels and insulin resistance index (IRI) were evaluated. By Week 6, mean fasting plasma glucose decreased from 180 to 133 mg/dL and HbA_{1c} decreased from 11% to 9%. Additionally, the sum of plasma glucose levels, a measure of diurnal variation in plasma glucose, decreased from 1416 to 1133 mg/dL. The sum of IRI levels measured concurrently decreased from 110 to 80 μU/mL. These results indicate that troglitazone improves fasting plasma glucose and postprandial plasma glucose.

Similar results were observed in a multicenter study, dose-ranging study. A weak, but statistically significant correlation between changes in fasting serum glucose and troglitazone dose was evident.

4.8.5. Pharmacodynamics/Pharmacokinetics

The relationship between troglitazone systemic exposure (determined by AUC or mean trough plasma concentrations) and changes in fasting serum glucose was explored.as a supplemental analysis to a multicenter Phase 2 clinical study. Following a 4-week baseline period, patients with Type II diabetes received placebo, 200, 400, 600, or 800 mg troglitazone QD for 12 weeks. Change from baseline in fasting serum glucose following 12 weeks of therapy was determined and related to troglitazone dose, mean through plasma concentrations, and AUC values.

There was a weak but statistically significant correlation between change from baseline in fasting serum glucose and troglitazone dose. Although fasting serum glucose values tended to be lower with increasing troglitazone trough concentrations, the relationship between change in fasting serum glucose and mean trough plasma concentrations was of little predictive value. Separation by dose revealed a similar relationship. Changes in fasting serum glucose did not correlate with troglitazone AUC(0-24). A similar relationship was observed within a given dose level. In an additional clinical trial no relationship was seen between trough plasma troglitazone concentrations and HbA_{1c} and fasting serum glucose following administration of 200 to 800 mg troglitazone QD or 200 and 400 mg troglitazone BID (Study C93-009). Thus, pharmacokinetic estimators of systemic troglitazone exposure do not improve the prediction of glycemic response beyond that obtained based upon knowledge of the administered dose.

5. CLINICAL STUDIES

5.1. Efficacy in Pivotal Clinical Studies

5.1.1. Introduction

This section summarizes the efficacy of troglitazone (CI-991) in the treatment of patients with noninsulin-dependent diabetes mellitus (Type II diabetes) who are inadequately controlled on insulin. Data in this summary demonstrates that troglitazone significantly improves indicators of glycemic control and has the added benefit of reducing exogenous insulin requirements in this patient population.

5.1.2. Clinical Studies of the Mechanism of Action

Although the precise molecular events involved in the mechanism of action of troglitazone are only partly understood, the thiazolidinediones have been shown to act as ligands for receptors that regulate the transcription of a number of genes critical to the control of glucose and lipid metabolism. Troglitazone induces the glucokinase gene, for example. Troglitazone also increases the expression of the Glut 1 and Glut 4 glucose transporters in the presence of insulin in adipocyte and myocyte cell lines. These results support the improvement in glucose disposal rate (GDR) observed in several pilot studies in patients with impaired glucose tolerance (IGT) and Type II diabetes.

Glucose Disposal Rate

Study 991-001 was a 6-week, open-label study to assess the effects of troglitazone 200 mg BID on GDR and hepatic glucose production in 15 patients with Type II diabetes using the hyperinsulinemic-euglycemic clamp method. The euglycemic clamp procedure was conducted at baseline and after 4 to 6 weeks of troglitazone therapy; fasting serum glucose (FSG) was also assessed.

Mean GDR increased by 47% (from 3.6-5.3 mg/kg/min, $p < 0.001$) following 4 to 6 weeks of troglitazone therapy (Figure 3). Hepatic glucose production (HGP) was above normal at baseline, indicative of the Type II diabetes population; HGP

decreased by 15% following 6 weeks of troglitazone therapy. The mean reduction from baseline in FSG at the end of treatment was 48.3 mg/dL (mean baseline FSG, 232.3 mg/dL); 50% of the patients had either an FSG normalized to 124 mg/dL or a ≥20% decrease in FSG.

Improvement in insulin sensitivity was also demonstrated by the hyperinsulinemic-euglycemic clamp method in Study 991-003 in which patients with IGT showed a 30% increase (from 4.7-6.1 mg/kg/min p = 0.004) in mean GDR following 12 weeks of troglitazone 200 mg BID therapy (Figure 4). Patients treated with placebo had a 14% decrease (from 5.1-4.4 mg/kg/min) in mean GDR.

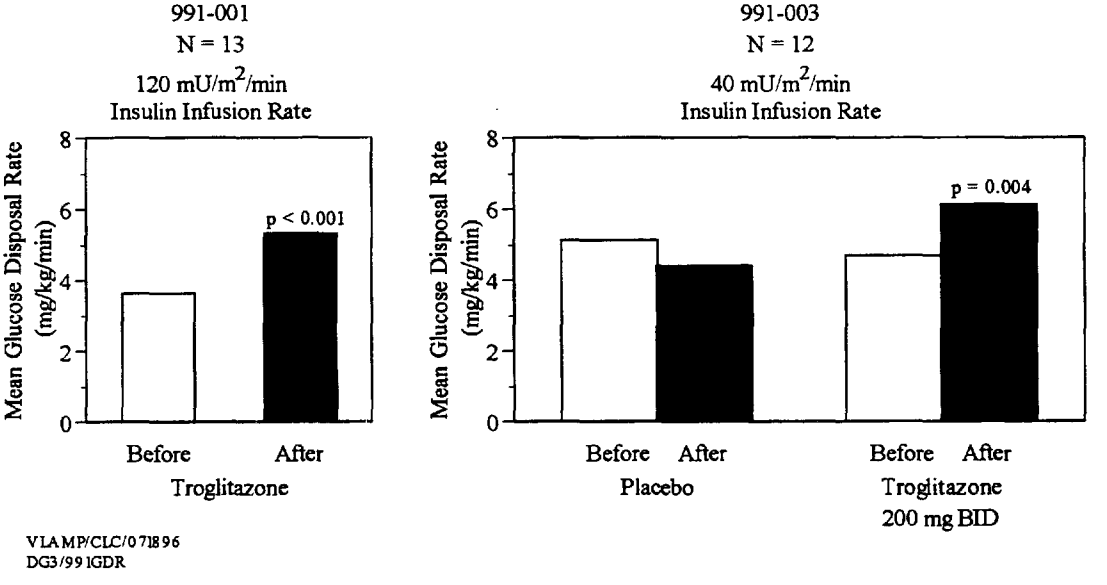

FIGURE 4. Glucose Disposal Rate (Euglycemic Clamp)

Insulin Sensitivity Index

The insulin sensitivity index (S_I), the surrogate measure of insulin sensitivity obtained during an intravenous glucose tolerance test (IVGTT) (minimal-model analysis), also demonstrated the positive effect of troglitazone therapy. Patients with IGT (Study 991-003) showed significant (p = 0.004) improvement in S_I following 12 weeks

of troglitazone therapy (Figure 5). Improvement in S_I was also observed in troglitazone-treated patients in Study 991-056 in which 37 Hispanic women with IGT and a history of gestational diabetes were treated with either placebo or troglitazone 200 or 400 mg QD for 12 weeks (Figure 5). Women treated with 200 mg troglitazone showed a 39% mean increase in S_I, while women treated with 400 mg troglitazone had an 88% mean increase in S_I following 12 weeks of treatment.

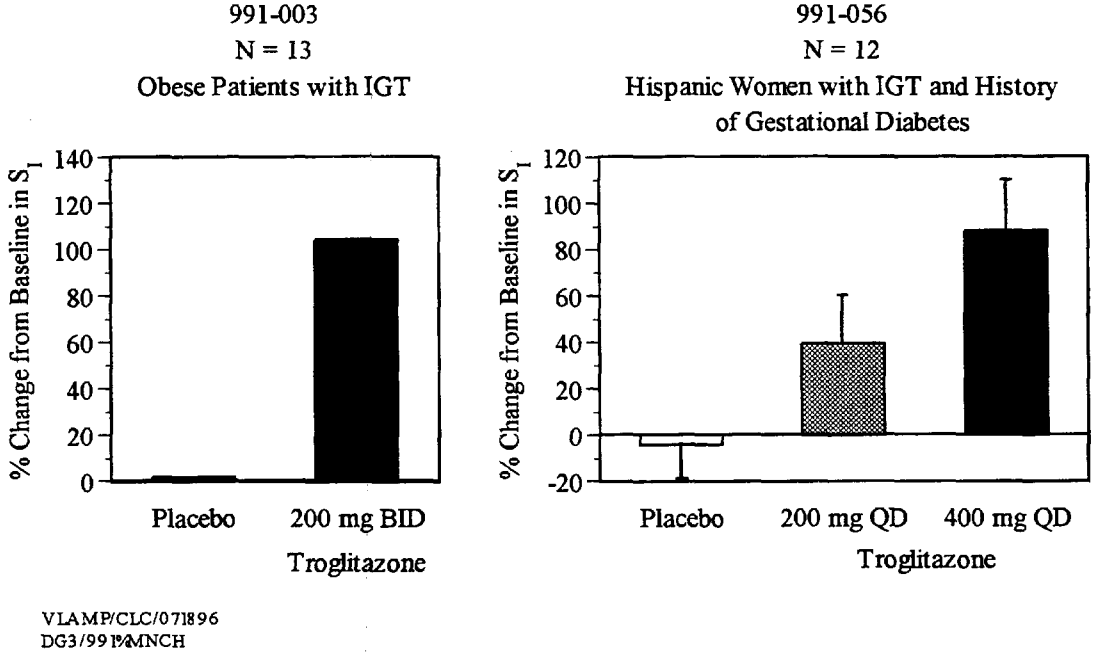

FIGURE 5. Percent Mean Change From Baseline in S_I at Week 12

Taken together, these observations are consistent with an improvement in insulin sensitivity, demonstrating that troglitazone treats the insulin resistance that is the hallmark of Type II diabetes. This is reflected by the improvement in the peripheral GDR and S_I.

5.1.3. Placebo-Controlled Pivotal Studies: 991-040, 991-068

5.1.3.1. Study Designs

The 2 pivotal clinical studies, 991-040 and 991-068, were complementary to one another in both study design and objective. Study **991-040** was a 6-month, double-blind, placebo-controlled, multicenter study in which 351 patients were randomly assigned to receive either placebo, 200 mg troglitazone (QAM), or 600 mg troglitazone (QAM). The main objective of this study was to determine the effect of troglitazone therapy on parameters of glycemic control (HbA_{1c}, FSG).

Study **991-068** was a 26-week, double-blind, placebo-controlled, multicenter study in which 222 patients were randomly assigned to receive either placebo, 200 mg troglitazone (QAM), or 400 mg troglitazone (QAM). The objective of this study was to determine if troglitazone therapy would reduce the insulin requirements in insulin-requiring Type II diabetes patients while providing evidence of improved glycemic control as measured by capillary blood glucose.

The algorithm for insulin dosage adjustments is the following: at randomization, the baseline total daily insulin dose (TDID) and capillary blood glucose (CBG) were calculated and recorded on worksheets for use in comparisons at subsequent visits. At each subsequent clinic visit, the current CBG and TDID were calculated. The CBG was compared to the baseline CBG. If the current CBG was 95% of baseline, the insulin dose could be decreased by 25% of the baseline TDID. If the criterion was not met no adjustment was necessary. This same decision tree was applied at each visit with comparisons to baseline. The decrease insulin dose in this algorithm was always at 25% of the baseline. However, insulin dose could be adjusted at any time for reasons of safety.

Patients in both studies were insulin-requiring Type II diabetes patients. Figure 6 illustrates the design for each placebo-controlled study.

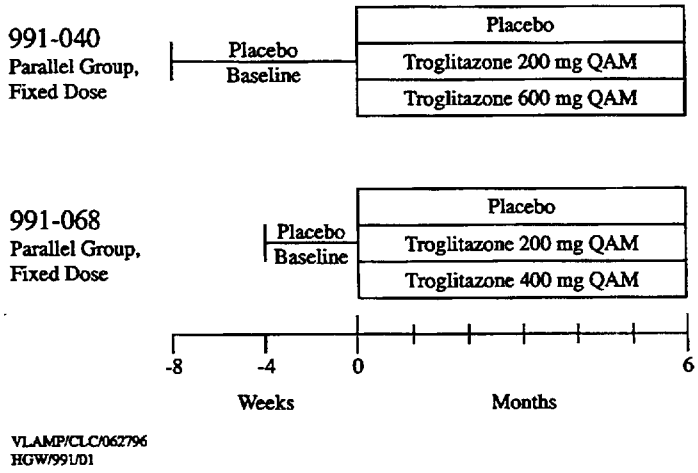

FIGURE 6. Placebo-Controlled Study Designs

5.1.3.2. Patient Characteristics/Disposition

Patients selected for the controlled studies were adults with Type II diabetes, who required ≥30 units of insulin/day and had prior antidiabetic therapy (sulfonylurea or metformin failure) that did not result in adequate glycemic control. Patients were to have FSG levels of >140 mg/dL and fasting C-peptide levels of ≥0.8 ng/mL (991-040) or ≥1.5 ng/mL (991-068). Hemoglobin A_{1c} (HbA_{1c}) levels were to be >7% (8%-12% for 991-040), indicating that these patients had not achieved good glycemic control on insulin therapy.

On average, patients in these studies had been diagnosed with Type II diabetes for 10 to 11 years and had been taking insulin for 4 to 5 years (Table 3). Exogenous insulin usage averaged from 70 to 75 units/day across both studies.

The study completion rate was high, ranging from 87% to 91% of patients completing across treatment groups for both studies.

In 991-068, an inclusion criteria was prior failure on either sulfonylurea or metformin. Fifty-four percent of the patients in this study were treated with maximal labeled dose

of the medication and 24% were on a half-maximal dose. While this exact information is not available on the patients in 991-040, these patients had a mean C-peptide of 1.64 ng/mL, lower than the mean value of 2.5 ng/mL from 991-068, indicating less beta cell function than the 991-068 population.

TABLE 3. Baseline Patient Characteristics in Placebo-Controlled Studies: All Patients

Study/ Treatment	991-040			991-068		
	Placebo N = 118	Troglitazone 200 mg/day N = 116	Troglitazone 600 mg/day N = 116	Placebo N = 71	Troglitazone 200 mg/day N =75	Troglitazone 400 mg/day N=76
Sex, N (%)						
Men	60 (50.8)	54 (46.6)	53 (45.7)	35 (49.3)	44 (58.7)	38 (50.0)
Women	58 (49.2)	62 (53.4)	63 (54.3)	36 (50.7)	31 (41.3)	38 (50.0)
Race, N (%)						
Caucasian	82 (69.5)	81 (69.8)	78 (67.2)	55 (77.5)	52 (69.3)	61 (80.3)
Black	18 (15.3)	18 (15.5)	24 (20.7)	7 (9.9)	13 (17.3)	6 (7.9)
Hispanic	16 (13.6)	14 (12.1)	12 (10.3)	7 (9.9)	8 (10.7)	7 (9.2)
Other	2 (1.6)	3 (2.6)	2 (1.8)	2 (2.8)	2 (2.7)	2 (2.6)
Age, Mean (yr)	55.8	55.6	56.0	57.1	57.7	57.7
Range	26-72	26-73	33-72	36-81	37-78	36-81
Glycemic Parameters, Mean (SD)						
FSG, mg/dL	219 (46)	214 (45)	215 (49)	230 (60)	225 (64)	222 (52)
HbA_{1c}, %	9.4 (1.1)	9.5 (1.1)	9.3 (1.1)	9.0 (1.4)	9.5 (1.7)	9.0 (1.3)
C-Peptide, ng/mL	1.7 (0.6)	1.6 (0.6)	1.7 (0.6)	2.8 (1.5)	2.2 (1.1)	2.5 (1.2)
Years Diagnosed With Type II diabetes, Mean	9.8	9.7	10.4	9.9	10.5	11.5
Duration of Insulin Use, Years	4.7	5.3	5.1	4.5	4.5	4.4
Total Daily Insulin Dose, Units						
Mean	75	72	70	75	72	71
(Range)	(24-276)	(32-290)	(22-193)	(27-144)	(28-145)	(27-139)
Body Mass Index, kg/m^2						
Mean	35	35	35	35	34	35

5.1.3.3. Results of Major Efficacy Parameters

5.1.3.3.1. Glycemic Effect at Month 6: 991-040

Study 991-040 was specially designed to evaluate the effect of troglitazone on measures of glycemic control in insulin-requiring Type II diabetes patients. Table 4 summarizes the mean change from baseline in HbA_{1c} and FSG following 6 months of therapy (last observation carried forward, LOCF) for patients in Study 991-040. Figure 7 illustrates change from baseline in HbA_{1c} and FSG according to treatment. Patients treated with 200 and 600 mg troglitazone showed a significant ($p < 0.0001$) reduction in HbA_{1c} and FSG compared with patients who received placebo. HbA_{1c} decreased by a mean of 1.29% in patients treated with 600 mg troglitazone and 0.72% in patients treated with 200 mg troglitazone compared with placebo.

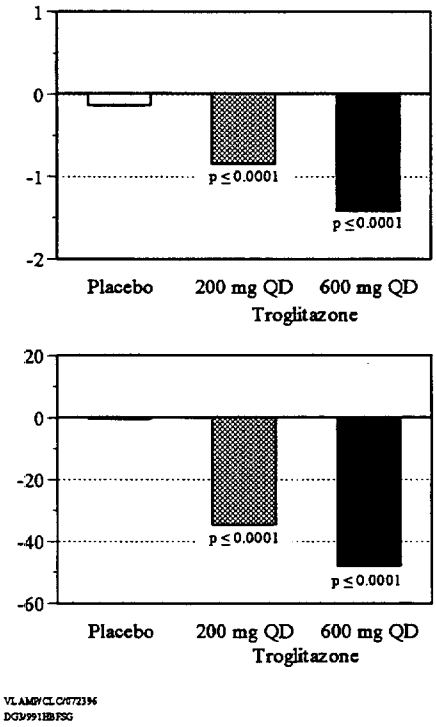

FIGURE 7. Adjusted Mean Change From Baseline in HbA_{1c} and FSG at Month 6 (ITT): 991-040

TABLE 4. Mean Change From Baseline in Glycemic Parameters at Month 6 (ITT): 991-040

Parameter	991-040		
	Placebo	Troglitazone 200 mg	Troglitazone 600 mg
HbA_{1c}, %			
N	118	116	116
Mean Baseline (SD)	9.43 (1.07)	9.51 (1.08)	9.32 (1.14)
Adjusted Mean Change (SE)	-0.12 (0.10)	-0.84 (0.10)	-1.41 (0.10)
Adjusted Mean Difference From Placebo (SE)	--	-0.72 (0.14)**	-1.29 (0.14)**
FSG, mg/dL			
N	118	116	116
Mean Baseline (SD)	219.2 (45.7)	213.8 (44.8)	214.7 (49.4)
Adjusted Mean Change (SE)	0.76 (4.6)	-34.9 (4.7)	-48.8 (4.7)
Adjusted Mean Difference From Placebo (SE)	--	-35.7 (6.6)**	-49.6 (6.6)**
C-Peptide, ng/mL			
N	118	116	116
Mean Baseline (SD)	1.66 (0.63)	1.59 (0.64)	1.68 (0.58)
Adjusted Mean Change (SE)	0.02 (0.04)	-0.12 (0.04)	-0.13 (0.04)
Adjusted Mean Difference From Placebo (SE)	--	-0.13 (0.06)*	-0.14 (0.06)*

* Significantly different from placebo ($p < 0.05$) based on step-down test for linear trend within analysis of covariance.
** $p \leq 0.0001$

Figure 8 further illustrates the level of improvement in HbA_{1c} following 6 months of troglitazone therapy. Thirty percent of patients treated with 200 mg troglitazone and 57% of patients treated with 600 mg troglitazone had an HbA_{1c} value below 8% at the end of the study, compared with 11% of placebo-treated patients.

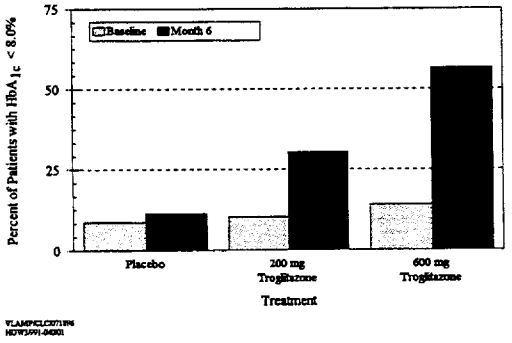

FIGURE 8. Distribution of Patients (%) With HbA_{1c} Level of <8.0% (ITT): 991-040

5.1.3.3.2. Change in Insulin Requirements: 991-040

Although **Study 991-40** was specifically designed to assess changes in parameters of glycemic control, insulin dose was also monitored throughout the study to monitor changes in insulin requirements. For safety reasons, insulin dose was to be reduced when FSG level reached ≤100 mg/dL; the level of dose reduction was at the investigator's discretion. However, a number of investigators began decreasing insulin levels before patients reached the ≤100 mg/dL FSG threshold due to the dramatic decreases in FSG observed during the study.

Patients treated with 200 and 600 mg troglitazone had a mean reduction in total daily insulin dose of 15% and 42%, respectively (compared to a 1.4% increase for patients treated with placebo). Despite this unexpected reduction in insulin dose, patients treated with 600 mg troglitazone showed a 1.4% mean decrease in HbA_{1c} following six months of therapy.

5.1.3.3.3. Responder Analysis: 991-068

Study **991-068** was specifically designed to assess the number of patients that were able to significantly reduce insulin requirements and improve glycemic control as measured by capillary blood glucose. Patients were instructed to measure capillary blood glucose on a daily basis. The average preprandial home blood glucose value for the seven days prior to a clinic visit was calculated as the **mean blood glucose (MBG)**. By agreement with the FDA, patients who showed a ≥50% reduction from baseline in total daily insulin dose **and either** a ≥15% reduction in MBG **or** an MBG ≤140 mg/dL, were classified as **responders** as a special definition for the analysis of this study.

Troglitazone-treated patients had a significantly greater ($p < 0.01$) **responder** rate compared with patients who received placebo (Table 5). Twenty-two percent of patients treated with 200 mg troglitazone and 27% of patients treated with 400 mg troglitazone were classified as **responders** compared with 7% of placebo-treated patients.

TABLE 5. Number (%) of Responders to Treatment and Related Parameters at Week 26 (ITT): 991-068

	Placebo N = 69	Troglitazone 200 mg N = 73	Troglitazone 400 mg N = 74
Responders, N (%)	5 (7.3)	16 (21.9)*	20 (27.0)*
Patients Discontinuing Insulin Therapy, N (%)[a]	1 (1.4)	5 (6.8)	11* (14.7)
Reduction in Total Daily Insulin Dose (TDID)			
Mean Change, units	-13	-30**	-41**
Percent Reduction	14%	41%	58%
Patients With MBG ≤160 mg/dL and 40% Reduction in TDID,[a] %	16%	23%	44%**

Inferences on proportions based on Cochran-Mantel-Haenszel; changes in daily insulin dose based on ANCOVA. Both procedures used step-down tests of linear trend, to determine which doses significantly differed from placebo.
* $p \leq 0.01$
** $p \leq 0.001$

Overall, patients treated with 400 mg troglitazone were able to reduce their total daily insulin dose by 58%, and 15% of patients in this treatment group were able to discontinue insulin therapy by the end of the study.

In addition to the number of responders who met the relatively stringent "responder" criteria for insulin reduction and glycemic control, a large number of patients were able to achieve an MBG of ≤160 mg/dL and a 40% reduction in insulin dose. For this criteria, 23% of patients with 200 mg troglitazone and 44% of patients treated with 400 mg troglitazone met this criteria, compared with 16% of placebo-treated patients. Many practicing clinicians see an MBG ≤160 mg/dL as an important target to reach.

Glycemic Control: 991-068

Since the main objective of Study 991-068 was to determine if troglitazone therapy could reduce exogenous insulin requirements while providing evidence of improved glycemic control, decreases in HbA_{1c} of the magnitude seen in Study 991-40 were not anticipated. The mean changes from baseline in HbA_{1c} were -0.1% for placebo, -0.1% for 200 mg troglitazone, and -0.4% for 400 mg troglitazone at Month 6. These changes were not significantly different from placebo, however, the difference between placebo and 400 mg troglitazone approached significance ($p = 0.077$). Decreases in

HbA_{1c} were greater at Month 3 for patients treated with 200 and 400 mg troglitazone; mean changes from baseline at Month 3 were -0.2% for placebo, -0.5% for 200 mg, and -0.8% for 400 mg troglitazone.

The subsequent increase in HbA_{1c} and FSG at Month 6, after declines in these parameters earlier in the study, is illustrated in Figure 9. It appears that the decrease in total daily insulin dose, which was particularly evident in patients treated with 400 mg troglitazone, resulted in the subsequent increase in FSG followed by an increase in HbA_{1c}. This trend, although less pronounced, was also observed in patients treated with 200 mg troglitazone (data not shown). This trend may have resulted in a number of investigators deviating from the algorithm used to determine the level of insulin adjustment based on MBG from home monitoring. Physicians may have aggressively decreased insulin doses further while MBG plateaued or in some cases, increased. This may have been the reason that overall glycemic control was reduced in the latter half of the study.

However, of the patients who were classified as **responders**, those treated with troglitazone had a mean reduction in HbA_{1c} of 1% (200 and 400 mg troglitazone) at Month 6 compared to a 0.4% decrease for the placebo-treated responders.

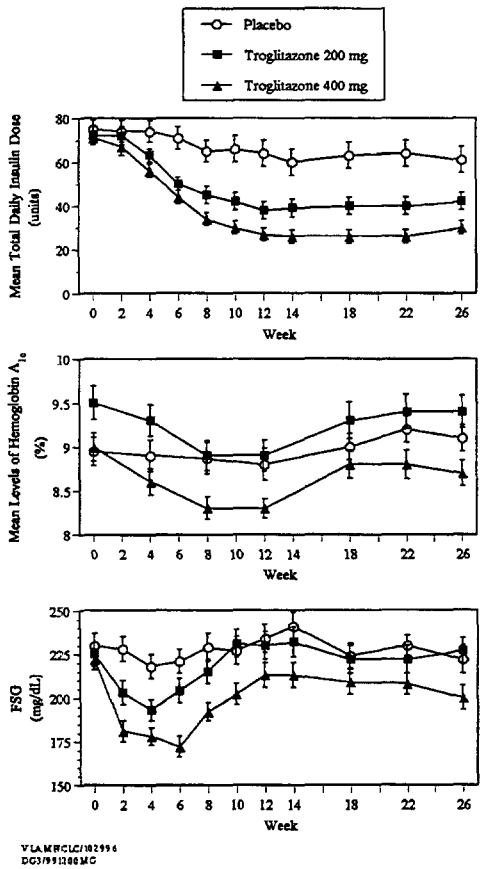

FIGURE 9. Comparison of Insulin Dose With HbA_{1c} and FSG Values Over Time (All Available Patients at Each Time Point, N = 61-75): 991-068

5.1.3.4. Lipids

5.1.3.4.1. Clinical Experience With Troglitazone

Troglitazone has reduced triglyceride levels and increased HDL levels in the general Type II diabetes population. Increase in total cholesterol and LDL cholesterol have also been consistent. However, there is evidence that the atherogenic risk was not increased as a result of these increases. In general, the increase in total cholesterol and HDL resulted in no change in the total cholesterol/HDL ratio. These effects of

troglitazone on the lipid profile of Type II diabetes patients have been consistent in the four Phase 2/3 studies conducted by Parke-Davis and further described below. These results are also confirmed by the Phase 2 studies conducted in Europe by Glaxo, Inc.

5.1.3.4.2. Lipids: Studies 991-040 and 991-068

Mean baseline levels of total cholesterol and HDL were generally within the borderline risk category at baseline in both studies; mean triglyceride levels were also within a borderline risk category, indicative of a Type II diabetes population. Table 6 summarizes the change from baseline in lipid parameters for patients in Studies 991-040 and 991-068. Each lipid parameter is addressed separately in the following sections.

TABLE 6. Mean Change From Baseline in Lipid Parameters at Month 6 in Placebo-Controlled Studies (ITT): 991-040 and 991-068

Parameter	991-040			991-068		
	Placebo N = 118	Troglitazone 200 mg N = 116	Troglitazone 600 mg N = 116	Placebo N = 69	Troglitazone 200 mg N = 73	Troglitazone 400 mg N = 72
Total Cholesterol, mg/dL						
Baseline (SD)	211 (56)	218 (38)	208 (40)	207 (42)	216 (71)	211 (41)
Adjusted Change From Baseline (SE)	-2 (3)	8 (3)	11 (3)	1 (5)	9 (5)	21 (5)
Adjusted Difference From Placebo (SE)	--	10 (5)*	13 (5)*	--	8 (6)	20 (6)
LDL Cholesterol, mg/dL						
Baseline (SD)	122 (48)	135 (34)	125 (34)	121 (37)	120 (33)	127 (31)
Adjusted Change From Baseline (SE)	2 (2)	9 (2)	13 (3)	-1 (3)	6 (3)	10 (3)
Adjusted Difference From Placebo (SE)	--	7 (3)*	11 (4)**	--	6 (4)	11 (4)*
HDL Cholesterol, mg/dL						
Baseline (SD)	37 (9)	39 (9)	38 (9)	41 (11)	42 (11)	43 (10)
Adjusted Change From Baseline (SE)	1 (1)	2 (1)	3 (1)	-1 (1)	1 (1)	3 (1)
Adjusted Difference From Placebo (SE)	--	1 (1)*	2 (1)**	--	2 (1)*	4 (1)**
Triglycerides, mg/dL						
Baseline (SD)	263 (364)	222 (112)	239 (175)	267 (215)	292 (414)	223 (154)
Adjusted Change From Baseline (SE)	-13.0 (11)	-25 (11)	-38 (11)	4 (19)	-4 (18)	1 (18)
Adjusted Difference From Placebo (SE)	--	-13 (15)	-25 (15)	--	-8 (24)	-3 (24)

* $p \leq 0.05$ based on step-down test for linear trend within analysis of covariance.

** $p \leq 0.001$ based on step-down test for linear trend within analysis of covariance.

5.1.3.4.2.1. Triglycerides

Triglyceride levels decreased by a maximum of 36 mg/dL for troglitazone-treated patients in Study 991-040 and by 4 mg/dL in Study 991-068 (Table 6). However, decreases were statistically significant ($p < 0.05$) at Week 12 in Study 991-040 and mean triglyceride levels decreased into the acceptable range (<200 mg/dL) at Weeks 4 and 8 for patients treated with 400 mg troglitazone in Study 991-068.

This shift in triglyceride level over the course of the studies may be explained by the effects of exogenous insulin on triglyceride metabolism. It is well known that exogenous insulin use results in decreased triglyceride levels due to its effect on hepatic VLDL formation. Therefore, a decrease in exogenous insulin would be expected to result in an increase in triglyceride levels over time. The expected decrease of 15% to 20% in triglyceride levels for troglitazone-treated patients during the first 4 weeks precedes the dramatic reduction of daily insulin doses by the investigators. As the insulin doses were further decreased, the triglyceride levels increased.

5.1.3.4.2.2. LDL

LDL increased by a mean of 6 to 12 mg/dL compared with placebo in Studies 991-040 and 991-068. Although these increases were statistically significant, the clinical significance (atherogenic risk) can only be established through a more detailed analysis of LDL composition. A summary of preliminary findings regarding LDL characterization follows:

Mean levels of Apo B remained unchanged (Apo B averaged from 115-124 mg/dL across treatments at baseline and 118-124 mg/dL at Month 6) at all troglitazone doses, indicating that the increase in LDL was not due to an increase in the levels of Apo B. LDL particle size increased consistently in all troglitazone-treated patients (0.3-0.4 nm); LDL particle size decreased in the placebo-treated patients. Susceptibility of LDL to oxidation was consistently decreased in troglitazone-treated patients (lag time increased 5.7-34.8 minutes), while placebo-treated patients were more susceptible to LDL oxidation (lag time decreased 8.4 minutes).

LDL subfractions, including Apo B, were measured at all 24 sites as part of Study 991-032. In addition, susceptibility of LDL to oxidation (copper oxide method) and LDL particle size (NMR spectroscopy) were measured from 10 patients (8 troglitazone, 2 placebo) studied at one site (Drs Jerrold Olefsky and Peter Reaven).

Small, dense LDL has been shown to be more readily oxidized than larger, more buoyant LDL. Once oxidized, LDL takes on a number of properties that increase its atherogenicity and may explain why small, dense LDL is associated with an increased risk of atherosclerosis. Large, buoyant LDL resists oxidation compared with small, dense, more atherogenic LDL. Large LDL particles are less likely to undergo oxidation and uptake by macrophages and monocytes, therefore, decreasing the formation of plaque-forming foam cells. Other small, dense LDL markers of atherogenic risk include apolipoprotein B (Apo B).

Therefore, analysis of the subfractions of total LDL indicate that troglitazone does not increase atherogenic risk, as might be inferred by looking only at the increase in total LDL.

5.1.3.4.2.3. HDL and Total Cholesterol

Mean levels of HDL increased significantly ($p < 0.001$) in troglitazone-treated patients, pushing levels well above 40 mg/dL and further from the high-risk category for HDL. Increases were similar in both studies.

Mean total cholesterol levels increased significantly ($p < 0.05$) in troglitazone-treated patients in both studies. However, mean levels remained within the borderline risk range, similar to baseline levels.

In general, the mean total cholesterol/HDL ratio was ≥ 5 at baseline for patients in Studies 991-040 and 991-068; this indicates that these patients were at an increased atherogenic risk at baseline. The increase in total cholesterol was offset by the increase in HDL and the mean cholesterol/HDL ratios remained virtually unchanged in both studies.

5.1.3.4.2.4. Atherogenic Risk

Atherogenic risk factors include decreased levels of HDL-cholesterol, elevated levels of triglycerides, free fatty acids, and components of the total LDL-cholesterol; small, dense LDL particles, and Apo B. Patients with Type II diabetes characteristically exhibit an atherogenic lipid profile with high levels of triglycerides and small, dense LDL particles that are more easily oxidized than large LDL particles; oxidation of LDL enhances their uptake by macrophages and contributes to foam cell and plaque formation. To understand the effects of troglitazone on lipids, all lipid parameters must be taken into consideration.

Clinical studies with troglitazone have demonstrated consistent changes in lipid parameters indicative of a reduction in atherogenic risk profiles in troglitazone-treated patients compared with placebo. Consistent changes in lipid parameters have shown increased HDL, reductions in circulating free fatty acids and triglycerides, with modest reductions or no change in Apo B levels, increased LDL particle size, and decreased susceptibility of LDL to oxidation. Taken collectively, these changes indicate troglitazone may be increasing hepatic clearance of small, dense LDL, the more atherogenic subfractions of LDL, and producing larger, more buoyant, less atherogenic LDL. These consistent lipid changes in troglitazone-treated patients demonstrate that troglitazone does not adversely effect the atherogenic risk profile of patients with Type II diabetes.

5.1.4. Uncontrolled Pilot Study in Insulin-Requiring Type II diabetes Patients: 991-063 (12-Week Phase and 6-Month Extension)

5.1.4.1. Study Design

Study **991-063** was a 12-week, open-label, single-center pilot study in 17 insulin-requiring Type II diabetes patients. The study was designed to determine if troglitazone therapy (400 mg QAM) could be substituted for insulin in these patients while still maintaining the same level of glycemic control. The study was continued for an additional 6 months to monitor the progress of these patients. Patients were to have been inadequately controlled on maximal doses of sulfonylureas to qualify for the study and have been receiving ≤80 units of insulin per day.

All patients received 400 mg troglitazone QAM while maintaining baseline levels of insulin therapy. In general, if home-monitored blood glucose was within ±10% of baseline fasting serum glucose following 2 weeks of troglitazone therapy, the daily insulin dose was halved. Insulin dosage adjustments could occur every 2 weeks, halving the daily insulin dose if home-monitored blood glucose remained within ±10% of baseline FSG. Mean change from baseline in FSG at Week 12 was the primary efficacy parameter.

These patients were clearly uncontrolled on insulin therapy, with a mean baseline FSG of 239 mg/dL and mean HbA_1 of 12%. All 17 patients completed the 12-week phase and the 6-month extension.

5.1.4.2. Results of Major Efficacy Parameters

Six patients (35%) were able to discontinue insulin therapy by Week 12 of the study (Table 7). The mean total daily insulin dose decreased from baseline by 37 units (63%) at Week 12 and glycemic control also improved on average with a mean reduction in FSG of 27 mg/dL and a 0.3% decline in HbA_1 at Week 12.

By the end of 9 months of troglitazone therapy, another patient (41% total) was able to discontinue insulin therapy. Mean total daily insulin dose continued to decline by 42 units (72%) and HbA_1 declined by nearly a full percentage point compared to baseline.

TABLE 7. Mean Change From Baseline in Major Efficacy Parameters at Week 12 and Month 9 (ITT): 991-063

Parameter	Week 12 400 mg Troglitazone N = 17	Month 9 400 mg Troglitazone N = 17
Capillary Blood Glucose[a], mg/dL		
Baseline (SD)	204.5 (61.5)	Not done at
Change From Baseline (SD)	-23.1 (52.3)	Month 9
FSG, mg/dL		
Baseline (SD)	238.9 (55.9)	235.8 (56.3)[b]
Change From Baseline (SD)	-26.7 (76.7)	-18.5 (70.5)
HbA_1, %		
Baseline (SD)	11.8 (2.0)	11.8 (2.0)[b]
Change From Baseline (SD)	-0.3 (1.3)	-0.8 (2.0)
C-Peptide, ng/mL		
Baseline (SD)	2.80 (0.93)	2.83 (0.95)[b]
Change From Baseline (SD)	0.06 (1.10)	0.33 (1.17)
Total Daily Insulin Dose, Units		
Baseline (SD)	58 (25.0)	58 (25.0)
Change From Baseline (SD)	-37 (23.0)	-42 (25.1)
Patients Discontinuing Insulin Therapy, N (%)	6 (35)	7 (41)

[a] From home glucose monitoring
[b] N = 16.

Lipids

Mean values of total cholesterol were approximately 200 mg/dL at baseline. Mean total cholesterol increased from baseline at Week 12, however, stabilized by Month 9 (Table 8). HDL increased at Week 12 and Month 9, shifting the mean level further from the high-risk range (≤35 mg/dL). Mean triglyceride levels were elevated at baseline, characteristic of the diabetic population; mean levels increased by 9 mg/dL at Week 12 in response to decreased insulin dose. However, with the stabilization of insulin dose, triglyceride levels decreased by 72 mg/dL (22%) by Month 9.

Although LDL levels increased by 18% by Month 9, the levels remained within the baseline risk category (borderline). This increase in the LDL level is likely due to the increased triglyceride and small LDL-particle clearance and increased hepatic production of large LDL particles.

TABLE 8. Mean Change From Baseline in Lipid Parameters at Week 12 and Month 9 (ITT): 991-063

Parameter	Week 12 400 mg Troglitazone N = 17		Month 9 (LOCF) 400 mg Troglitazone N = 16	
Total Cholesterol, mg/dL				
Baseline (SD)	197.8	(35.1)	199.1	(35.8)
Change From Baseline (SD)	28.5	(33.7)	29.8	(27.8)
LDL Cholesterol, mg/dL				
Baseline (SD)	124.4	(40.0)	124.8	(41.2)
Change From Baseline (SD)	19.9	(25.4)	22.0	(28.5)
HDL Cholesterol, mg/dL				
Baseline (SD)	37.1	(8.2)	37.1	(8.4)
Change From Baseline (SD)	2.9	(4.6)	4.8	(4.5)
Triglycerides, mg/dL				
Baseline (SD)	330.8	(366.1)	334.2	(377.9)
Change From Baseline (SD)	9.1	(123.2)	-71.6	(199.4)

LOCF = Last observation carried forward.

5.1.5. Supportive Efficacy Data in Type II diabetes Patients Not Requiring Insulin

A number of Parke-Davis placebo-controlled clinical studies provide evidence of the effectiveness of troglitazone in improving glycemic control in patients with Type II diabetes that do not require insulin therapy. In addition, a Parke-Davis study and a Sankyo Co, LTD (Japan) study provide evidence of long-term (1-2 years) effectiveness of troglitazone.

5.1.5.1. Parke-Davis Sponsored Studies

5.1.5.1.1. Short-Term, Placebo-Controlled Studies: 991-006 and 991-031

Study **991-006** was a 6-week, placebo-controlled study that compared the efficacy of QAM, QHS, and BID dosing regimens for a 400-mg daily dose of troglitazone. The 54 Type II diabetes patients chosen for this study (35 men, 19 women) were obese

(BMI 28-35 kg/m^2), hyperinsulinemic (C-peptide of ≥2 ng/mL), and had an FSG of 160 to 240 mg/dL during the placebo-baseline phase.

The QAM dosing regimen was found to be the most effective in reducing FSG; the mean change from baseline was -59.6 mg/dL, which was significantly different ($p < 0.001$) from placebo and the QHS dosing regimen ($p < 0.05$). There were no significant changes from baseline in HbA_{1c} due to the short duration of the study.

Study **991-031** was a double-blind, placebo-controlled study in which 792 patients with Type II diabetes (472 men, 320 women; mean age 58 years) were randomized to receive placebo, or 200, 400, 600, or 800 mg troglitazone (QAM) for 12 weeks. Mean baseline FSG was 247 mg/dL, mean HbA_{1c} was 9.2%, and mean C-peptide was 2.5 ng/mL.

The mean change from baseline in HbA_{1c} and FSG were significantly ($p < 0.001$) different from placebo at the 200- through 800-mg dose of troglitazone at Week 12 (Table 9).

TABLE 9. Major Efficacy Parameters at Week 12 (Evaluable Patients): 991-031

Parameter	Placebo	Troglitazone[a]			
		200 mg	400 mg	600 mg	800 mg
FSG, mg/dL					
N	105	120	119	121	110
Mean Baseline	240.6	229.9	237.0	229.2	239.1
Adjusted Mean Change From Baseline	13.8	-26.1	-33.6	-34.7	-45.6
Adjusted Mean Difference From Placebo (95% CI)	NA	-39.9* (-52, -28)	-47.4* (-60, -35)	-48.6* (-61, -36)	-59.5* (-72, -47)
HbA_{1c}, %					
N	103	119	118	120	109
Mean Baseline	9.23	9.03	9.06	8.99	9.15
Adjusted Mean Change From Baseline	1.24	0.37	0.40	0.11	-0.06
Adjusted Mean Difference From Placebo (95% CI)	NA	-0.87* (-1.2, -0.5)	-0.84* (-1.2, -0.5)	-1.13* (-1.5, -0.8)	-1.30* (-1.7, -0.9)

* $p < 0.001$, based on step-down tests for trend and Dunnett's Test, 2-sided.
NA = Not applicable.
CI = Confidence interval.
[a] Based on bioavailability studies, the 200, 400, 600, and 800 mg doses of troglitazone were approximately equivalent to 150, 300, 400, and 600 mg doses of troglitazone tablets used in Studies 991-040 and 991-068.

5.1.5.1.2. Long-Term, Positive-Controlled Study: 991-042 (1 and 2 Years)

Study **991-042** was a 2-year, open-label, positive-controlled study designed to assess the effects of troglitazone on cardiac mass and function in 154 patients with Type II diabetes. As in all clinical studies, patients with evidence of heart disease were excluded from the study. After a 2-week washout period, patients were randomized to receive either 800 mg troglitazone QAM or glyburide ≤20 mg QD or BID (titrated during the first 6 weeks of treatment to achieve FSG ≤200 mg/dL) for 1 to 2 years.

The mean age of the 154 patients was 54 years and mean duration of diabetes was 6.4 years. Mean baseline FSG and HbA_{1c} were 255 mg/dL and 9.1%, respectively.

Patients treated with troglitazone showed clinically significant decreases from baseline in HbA_{1c}, FSG, and plasma insulin levels after 1 year of troglitazone treatment (Table 10). Patients who continued troglitazone therapy for 2 years continued to show improvement in glycemic parameters; HbA_{1c} decreased from baseline by 1.0%, mean decrease in FSG was 60 mg/dL.

TABLE 10. Mean Change From Baseline in Glycemic Parameters for Patients Completing 1 or 2 Years of Treatment: 991-042

Parameter	Troglitazone 800 mg[a]		Glyburide ≤20 mg	
	1 Year[b]	2 Years	1 Year[a]	2 Years
HbA_{1c}, %				
N	45	27	60	44
Baseline (SD)	8.8 (1.6)	8.9 (1.6)	9.0 (1.4)	9.1 (1.5)
Change From Baseline (SD)	-0.5 (1.8)	-1.0 (1.8)	-0.2 (1.4)	-0.3 (1.6)
FSG, mg/dL				
N	45	27	60	44
Baseline (SD)	229.2 (72.7)	236.4 (73.6)	251.2 (69.3)	256.8 (70.4)
Change From Baseline (SD)	-39.7 (61.0)	-59.7 (71.6)	-48.7 (73.4)	-42.6 (71.9)
Plasma Insulin, µIU/mL				
N	46	27	60	44
Baseline (SD)	18.7 (16.5)	19.6 (18.6)	15.5 (10.2)	15.5 (12.7)
Change From Baseline (SD)	-4.9 (13.0)	-6.8 (15.9)	3.4 (12.7)	2.2 (9.8)
C-Peptide, ng/mL				
N	46	27	61	44
Baseline (SD)	2.8 (1.3)	2.9 (1.4)	2.8 (1.2)	2.8 (1.1)
Change From Baseline (SD)	-0.6 (1.1)	-0.7 (1.2)	0.1 (1.0)	-0.1 (0.9)

[a] Based on bioavailability studies, the 800-mg dose of troglitazone was approximately equivalent to a 600-mg dose of troglitazone used in Studies 991-040 and 991-068.
[b] Based on observed cases

5.1.5.2. Sankyo Co, Ltd (Japan) 2 Year, Uncontrolled Clinical Study: 136-035

Following a 16-week, uncontrolled, open-label clinical study (136-034), Type II diabetes patients showing good glycemic control and tolerance to troglitazone were enrolled in a 1-year, open-label study (inclusive of 16-week phase). Patients continued to receive the dose of troglitazone last received during the 16-week phase

(200-800 mg/day BID) based on the discretion of the investigator. Patients who were receiving concomitant sulfonylurea therapy were to maintain the same dose throughout the long-term phase of the study. Type II diabetes patients chosen for the study were to be uncontrolled by diet or sulfonylurea therapy. Of the 194 patients evaluated for efficacy (204 enrolled), 110 were women, 84 were men, and 82% were 50 years or older.

Mean fasting plasma glucose (FPG) was 141 mg/dL (181 mg/dL at baseline) at the end of 16 weeks for the 57 patients on troglitazone monotherapy; mean FPG continued to be maintained between 137 to 150 mg/dL during the long-term portion of the study. HbA_{1c} was maintained between 7.0% to 7.3% (reduced from 8.7% at baseline) for these same patients over the 12-month period.

For patients on concomitant sulfonylurea therapy, mean FPG was 150 mg/dL (191 mg/dL at baseline) at the end of 16 weeks. Mean FPG was maintained between 152 to 164 mg/dL during the 12-month study. HbA_{1c} was maintained at 7.7% to 8.2% (9.19% at baseline) over the 12-month period for troglitazone-treated patients on concomitant sulfonylurea therapy. These results indicate that troglitazone maintains its effectiveness over 12 months of therapy.

5.1.6. Dosage Information

5.1.6.1. Recommended Doses and Insulin Adjustment

Troglitazone is recommended for the treatment of patients with Type II diabetes who are inadequately controlled on insulin. The usual dose of troglitazone is 400 mg once daily. The recommended starting dose for this patient population is 200 mg/day.

The recommended dosing range for troglitazone is 200 to 600 mg/day for this patient population. The maximal response at any dose is seen at four weeks or later. For patients not achieving adequate glycemic control, the troglitazone dose should be increased by 200 mg at 2- to 4-week intervals to a maximum of 600 mg/day. It is recommended that the insulin dose be decreased by 10% to 25% when FSG levels decrease to <120 mg/dL in patients receiving concomitant insulin and troglitazone. Further reductions should be individualized based on glucose-lowering response.

5.1.6.2. Dosing Interval

Study **991-006** compared a 400-mg/day dose of troglitazone administered in 3 different dosing regimens to noninsulin-requiring Type II diabetes patients; every morning (QAM), every night (QHS), and BID (200 mg in AM and in PM). Although all dosing regimens resulted in decreases in FSG, the QAM dosing regimen was superior in reducing FSG following 6 weeks of treatment. Therefore, it is being recommended that troglitazone be administered as a single dose (QD) in the morning and taken with food.

5.1.7. Discussion

Of the approximately 16 million diabetics (known and estimated undiagnosed) in the United States, 90% have Type II diabetes. Despite the fact that almost half of the diagnosed Type II diabetes patients are treated with insulin, they often have inadequate glycemic control (HbA_{1c} 9% to 10%). Given the phenotype of the majority of Type II diabetics, (ie, obese and highly insulin-resistant) intensive insulin treatment such as was used in the Diabetes Control and Complications Trial (DCCT) in Type I diabetes may not be optimal therapy. The insulin therapy that is required to improve or maintain glycemic control in these patients often requires in excess of 100 units per day. These high doses of insulin result in weight gain and the potentiation of other comorbid conditions, such as cardiovascular disease. Given that these patients are very insulin-resistant, as evidenced by the high doses of exogenous insulin required to improve metabolic control, a therapy that directly treats insulin resistance would be optimal.

Troglitazone, as demonstrated by the studies cited in this document, effectively treats insulin resistance. Its efficacy is demonstrated by the ability to improve glycemic control while significantly decreasing insulin requirements in this refractory population of obese, insulin-requiring patients. The data from two complementary studies have been presented to substantiate these claims. Study 991-040 specifically addressed the ability of troglitazone used in combination with insulin to optimize glycemic control. This study enrolled patients who were receiving at least 30 units of insulin per day and were poorly controlled as evidenced by an HbA_{1c} over 9%. During the course of this study, HbA_{1c} decreased significantly ($p < 0.0001$) in patients treated with both 200 and

600 mg of troglitazone. Importantly, 30% of patients treated with 200 mg of troglitazone and 57% of those receiving 600 mg of troglitazone achieved an HbA_{1c} level less than 8% by the end of the study as compared with 11% of placebo-treated patients. This level of glycemic control was present in less than 10% of these patients prior to beginning troglitazone therapy. Accompanying this significant improvement in glycemic control, was a reduction in insulin requirements of 15% and 42% in the two treatment groups, respectively. These data clearly demonstrate the efficacy of troglitazone in this obese, insulin-resistant population.

Study 991-068 addressed a different hypothesis. Based upon the results obtained in pilot Study 991-063, target criteria integrating both glycemic control and exogenous insulin dose reduction were created. During this study, investigators were to decrease the patient's insulin dose in response to a decrease in capillary blood glucose. A stringent target combination of glycemic control as measured by at least a 15% reduction in capillary blood glucose or a glucose less than 140 mg/dL and at least a 50% reduction in total daily insulin dose was used to define responders. A significantly greater number of patients receiving either 200 or 400 mg of troglitazone met these criteria compared to the placebo group. Further evidence of the improved glycemic control in the group of patients who met these response criteria is documented by the 1% fall in mean HbA_{1c} in both the 200- and 400-mg dose group. Further analysis of a group of patients who met the clinically meaningful criteria of a blood glucose level ≤160 mg/dL and at least a 40% decrease in daily insulin dose further demonstrates the combined efficacy in improving glycemic control and decreasing insulin requirements. Forty-four percent of the patients in the 400-mg dose group met these criteria compared with 16% in the placebo group. In addition, 15% of patients in the 400-mg treatment group were able to completely discontinue insulin compared with 1.5% in the placebo group.

Further examination of the 991-068 data points out an important observation regarding the use of troglitazone in combination with insulin. Both blood glucose and HbA_{1c} reached a nadir in the first half of the study. As would be expected, the decrease in blood glucose preceded the decrease in HbA_{1c}. In fact, the HbA_{1c} value reached statistical significance in both the 200- and 400-mg treatment groups at Week 12. While the statistical significance was not present at the end of the study, the HbA_{1c} values were lower by a mean of 0.4% at the end of the study in the 400 mg group.

The subsequent increase in both glucose and HbA_{1c} following Week 12 requires further discussion. There appear to be three possible explanations for the increase in glucose and $HbA_{1c,}$ which occurred during the last half of the study. The first possibility is that drug effectiveness decreases with time. There are two important pieces of information that provide evidence against this hypothesis. The first being in the 1- and 2-year analyses of 991-042, a monotherapy study using troglitazone, secondary failure rates are extremely low and mean HbA_{1c} values decrease and stay suppressed throughout both 1 and 2 years of therapy. Further evidence is provided by the analysis of the 9-month data from the 991-063 pilot study in insulin-requiring patients. In this study, the decreases in HbA_{1c} were maintained throughout the 9 months of the study. No evidence of secondary failure is present.

The second possible explanation is that Weeks 10 to 14 of this study coincided with the end of calendar year holiday season. Due to rapid enrollment into this study, nearly all patients were on the same date enter schedule. Glucose control for diabetes is predictably less well-controlled during this period of the year. It is possible that the increases in blood glucose, which translated into the increases in $HbA_{1c,}$ are due to this so called "Holiday Effect" that was also observed in the placebo group.

The third and most likely explanation is found in examining the exogenous insulin dose curve during the course of this study. In spite of the instructions to the investigators to decrease insulin doses only in response to a falling capillary glucose, it is clear that physicians aggressively decreased insulin doses further in the face of capillary blood glucoses that were either staying the same or in some cases increasing. This trend is also evidenced in examining the blood glucose data from the 991-040 study in which a slight increase in blood glucose was evident at the end of the study.

There appears to be a critical balance between the dose of exogenous insulin and troglitazone that leads to optimal glucose control. Clearly, some patients are able to completely discontinue insulin with a significant improvement in glycemic control. However, in the majority of patients, further lowering of insulin dose beyond a critical point causes an increase in glucose and a lessening of the improved metabolic control. Physicians will need to decide where that balance between glycemic control and insulin dose falls for their individual patients.

The improvement in insulin resistance in patients receiving troglitazone is further born out by its impact on lipid profiles which has been demonstrated in multiple studies. As insulin resistance is improved in patients, triglyceride levels decrease. It is important to note that these prior studies have not been conducted in insulin-requiring Type II diabetes patients. It is very important to take into account the impact of exogenous insulin on triglyceride levels when examining triglyceride data in insulin-requiring Type II diabetes patients. It is a well-known clinical phenomenon that starting Type II diabetes patients on insulin results in a decrease of plasma triglyceride levels due to suppression of hepatic VLDL formation. Therefore, decreasing insulin doses in patients may actually lead to an increase in triglyceride levels. The triglyceride data from Studies 991-040 and 991-068, in which exogenous insulin doses were effectively reduced, may at first glance appear to be contradictory. During the first 4 to 6 weeks of both studies, dramatic falls of triglycerides occurred. However, as insulin doses are decreased, triglyceride levels that had been suppressed actually begin to increase again. This is presumably due to the decrease in exogenous insulin dose and the increase in hepatic VLDL production that accompanies this effect. Further examination of the triglyceride data from the 991-063 pilot study confirms that this increase in triglycerides following the initial decrease is transient and with longer term therapy, in this case up to 9 months, triglyceride levels once again decrease and the triglyceride-lowering effect of troglitazone is evident. It is important for the clinician to understand that the initial decrease of triglycerides seen when patients are started on troglitazone, may be temporarily mitigated as insulin doses are decreased; however, with ongoing treatment, further triglyceride-lowering should be evident.

Following treatment with troglitazone, an increase in LDL and total cholesterol occurs while the ratios of total cholesterol to HDL and LDL to HDL do not change. The increase in total cholesterol is due to this increase in both HDL and LDL cholesterol. However, it is important to examine the Apo B subfraction in order to understand the impact of troglitazone on total LDL. The Apo B subfraction of LDL is believed to be one of the small-dense particles that increases the atherogenic risk of elevated LDL concentrations. Examination of data from the 991-032 study indicates that Apo B fractions are not increased in patients receiving troglitazone in spite of the total LDL cholesterol increasing. Further evidence is found in studying the LDL particle size following troglitazone therapy. LDL particle size increases following administration of troglitazone, as cholesterol is shifted from VLDL into LDL. However, the LDL

particles that the patients are producing are the so called "large fluffy" less atherogenic LDL particles, rather than the "small dense" LDL particles which are associated with increased atherogenic risk. In addition, susceptibility of LDL to oxidation is decreased following treatment with troglitazone, providing further evidence that the increase in LDL is not increasing atherogenic risk. Taken together, the lack of change in total cholesterol/HDL ratio, the lack of change in the LDL Apo B fraction, and the increase in LDL particle size provide evidence that there is no attendant increase in atherogenic risk for troglitazone-treated patients.

There have been no clinically significant changes in patient weight in any study where patients received troglitazone monotherapy for up to 2 years. Patients treated with concomitant insulin therapy in the pilot Study 991-063 had a mean weight reduction of 5 pounds following 9 months of troglitazone therapy. However, in the 991-040 study, there was a statistically significant increase in patient weight of 3.0% and 3.8% for patients treated with 200 and 600 mg troglitazone , respectively, compared with a 0.8% mean increase in weight for placebo-treated patients; this may or may not be of clinical significance. The most likely explanation for this effect may be found in examining the diet instructions given to these patients. Unlike all other studies, the patients in Study 991-040 were instructed to follow a weight-maintenance diet; ie, they were told to maintain their baseline weight throughout the study by altering their eating habits if necessary. This was done to remove any effect that weight fluctuation might have had on parameters of glycemic control. However, these instructions may have encouraged patients to continue with poor eating habits, resulting in significant weight gain. Despite the weight gain however, glycemic control did improve significantly in troglitazone-treated patients in Study 991-040. In addition, patients receiving concomitant insulin and troglitazone in Study 991-068 did not exhibit significant changes in weight.

In summary, both pivotal studies accomplished their respective protocol-defined primary endpoints and demonstrated the clear efficacy of troglitazone in both improving glycemic control (991-040) and in demonstrating the combined benefit of lowering insulin dose and improving glycemic control as measured by home glucose monitoring (991-068).

5.1.8. Conclusions Regarding Efficacy

- Troglitazone therapy, at doses of 200 to 600 mg, is effective in significantly reducing total daily insulin dose and improving glycemic control in Type II diabetes patients requiring insulin.

5.2. Safety in Clinical and Clinical Pharmacology Studies

5.2.1. Number of Participants and Duration of Exposure to Troglitazone

During studies included in this submission, troglitazone was administered to 3816 healthy subjects and patients worldwide (Table 11), with approximately 228 patients exposed for ≥1 year.

TABLE 11. Overview of Source and Number of Participants

Source	Healthy Subjects		Patients		
	Placebo	Troglitazone	Placebo	Troglitazone	Sulfonylurea
Parke-Davis Studies (US)					
Clinical Pharmacology	8	359	0	12	0
Clinical	0	0	412	1261	77
Sankyo Studies (Japan)					
Clinical Pharmacology	28	137	0	28	0
Clinical	0	0	294	1058	6
TOTAL	50	695	916	3121	83

The safety of troglitazone was evaluated in 28 studies sponsored by Parke-Davis in the US, one of which was conducted by Sankyo US. Sixteen of these were clinical pharmacology studies, during which troglitazone was administered to 359 healthy subjects and 12 patients at daily doses of 200 to 1600 mg for up to 3 weeks. In the

12 clinical studies, 412 patients received placebo, 1261 received troglitazone, and 77 received glyburide (Table 12).

TABLE 12. Parke-Davis Clinical Studies: Source and Number of Patients

Study	Duration	Placebo	Troglitazone	Glyburide
Patients With NIDDM Treated With Concomitant Insulin				
Controlled Studies				
991-040	26 Weeks	118	233	0
991-068	26 Weeks	71	151	0
Uncontrolled Study				
991-063	9 Months	0	17	0
Subtotal		189	401	0
Patients With NIDDM Not Treated With Concomitant Insulin				
Controlled Studies				
991-002	12 Weeks	2	1	0
991-006	6 Weeks	15	39	0
991-031	12 Weeks	158	634	0
991-042	96 Weeks	0	77	77
Uncontrolled Study				
991-001	12 Weeks	0	14[a]	0
Subtotal		175	765	77
Patients With Impaired Glucose Tolerance				
Controlled Studies				
991-003	12 Weeks	8	14	0
991-041	12 Weeks	26	29[b]	0
991-056	12 Weeks	14	27[c]	0
Subtotal		48	70	0
Patients With Polycystic Ovary Syndrome				
Uncontrolled Study				
991-050	12 Weeks	0	25	0
TOTAL		412	1261	77

[a] Patient 6 was enrolled but never received troglitazone; data for this patient are not included in the database. One patient was enrolled twice (as Patients 9 and 99) and is treated as 2 patients.

[b] Four of these patients met criteria for Type II diabetes.

[c] Patient 28 was randomized but never received troglitazone; data for this patient are not included in the database.

The cumulative duration of treatment with troglitazone is summarized for clinical studies in Table 13. Seventy-three percent of the 1261 patients were treated for ≥12 weeks. Specifically, 281 patients were treated for ≥26 weeks and 46 for ≥48 weeks. Thirty-four patients received troglitazone for >1 year. Patients were exposed to troglitazone for a total of 22,321 patient-weeks, or 428 patient-years. An additional 616 patients were treated with troglitazone in studies not included in the submission (991-032 and 991-057), for a total of approximately 273 patient-years of additional exposure.

TABLE 13. Parke-Davis Clinical Studies: Cumulative Exposure to Troglitazone by Daily Dose

(Number of Patients)

Duration	Troglitazone Dose (mg/day)				Any Troglitazone
	200	400	600	800	
<1 Day to 1 Day	376	371	278	236	1261
>1 Day to <12 Weeks	375	370	276	236	1257
≥12 Weeks to <26 Weeks	299	244	218	156	917
≥26 Weeks to <48 Weeks	113	43	78	47	281
≥48 Weeks	0	0	0	46	46
Total Patient-Weeks	6686	5270	4540	5825	22321

In clinical pharmacology studies conducted by Sankyo Co, Ltd in Japan (Table 11), subjects and patients were treated with troglitazone at dosages of 100 to 800 mg/day for up to 12 weeks. Of the 1058 patients with Type II diabetes exposed to troglitazone in clinical studies, 24 were treated in a 7-day study; 204 in a 1-year, open-label extension; and the remainder in studies with treatment periods of 8 to 16 weeks.

5.2.2. Overview of Adverse Events

5.2.2.1. Parke-Davis Studies

In clinical pharmacology studies, the overall incidence of adverse events for subjects treated with troglitazone was 61%, with 40% of subjects experiencing events attributed to study medication. The most frequent events (occurring in >5% of subjects) were headache, dizziness, nausea, asthenia, and rhinitis. With the exception of rhinitis, these were also the most frequent events attributed to study treatment. Although a dose relationship was not evident for adverse events, only 8 subjects were treated with placebo and most of the troglitazone exposure was at the 400-mg dose, making dose-response difficult to evaluate. Most adverse events were mild to moderate. Among troglitazone-treated subjects, 7 events were reported as severe: asthenia, headache (3 reports), pain, cerebral ischemia (transient ischemic attack), and unintended pregnancy. Subjects treated with placebo reported 4 severe events: chest pain, headache, insomnia, and pharyngitis.

In clinical studies conducted by Parke-Davis, the overall incidence of adverse events among patients treated with troglitazone was 79%, similar to that for placebo-treated patients (81%, Table 14). The incidence of cardiovascular and metabolic/nutritional events was similar for troglitazone and placebo, while adverse events in the digestive system occurred less often among troglitazone-treated patients (24%) compared with those who received placebo (32%). Adverse events occurring in >5% of troglitazone-treated patients were infection (upper respiratory or skin infections), headache, pain (pain in extremities), accidental injury, asthenia, back pain, nausea, dizziness, flu syndrome, peripheral edema, diarrhea, rhinitis, pharyngitis, and urinary tract infection. These events occurred at a similar incidence among placebo-treated patients.

Few adverse events (25%) were associated with troglitazone treatment. Adverse events tended to occur during the first 4 weeks of treatment with troglitazone and did not appear to be dose-related. Adverse events were similar for patients <65 and ≥65 years of age, for patients of various races, and for men and women. Most patients with adverse events had events that were mild to moderate.

TABLE 14. Parke-Davis Clinical Studies: Adverse Events Occurring in ≥1% of Troglitazone-Treated Patients, by Body System[a]
[Number (%) of Patients]
(Page 1 of 4)

BODY SYSTEM/ Adverse Event	Placebo N = 412		Troglitazone N = 1261		Glyburide N = 77	
BODY AS A WHOLE	225	(55)	649	(51)	55	(71)
Infection	100	(24)	241	(19)	42	(55)
Headache	50	(12)	157	(12)	11	(14)
Pain	61	(15)	136	(11)	6	(8)
Accidental Injury	23	(6)	111	(9)	11	(14)
Asthenia	26	(6)	85	(7)	3	(4)
Back Pain	19	(5)	84	(7)	5	(6)
Flu Syndrome	23	(6)	64	(5)	8	(10)
Abdominal Pain	26	(6)	46	(4)	4	(5)
Chest Pain	17	(4)	33	(3)	4	(5)
Fever	11	(3)	19	(2)	1	(1)
Cellulitis	1	(<1)	16	(1)	0	(0)
Neck Pain	3	(1)	15	(1)	2	(3)
Allergic Reaction	5	(1)	14	(1)	4	(5)
Abscess	3	(1)	12	(1)	1	(1)
Chills	3	(1)	10	(1)	1	(1)
Moniliasis	4	(1)	10	(1)	1	(1)
Face Edema	3	(1)	9	(1)	0	(0)
Malaise	3	(1)	8	(1)	0	(0)
CARDIOVASCULAR SYSTEM	50	(12)	139	(11)	5	(6)
Hypertension	18	(4)	33	(3)	2	(3)
Retinal Vascular Disorder	7	(2)	11	(1)	0	(0)
Cardiovascular Disorder	0	(0)	10	(1)	0	(0)
Peripheral Vascular Disorder	4	(1)	10	(1)	0	(0)
Angina Pectoris	6	(1)	9	(1)	0	(0)
Vasodilatation	1	(<1)	9	(1)	1	(1)
Coronary Artery Disorder	3	(1)	8	(1)	0	(0)
Migraine	2	(<1)	8	(1)	0	(0)
Congestive Heart Failure	3	(1)	7	(1)	0	(0)
Postural Hypotension	1	(<1)	7	(1)	1	(1)
DIGESTIVE SYSTEM	132	(32)	297	(24)	31	(40)
Nausea	18	(4)	80	(6)	2	(3)
Diarrhea	26	(6)	70	(6)	3	(4)
Dyspepsia	29	(7)	51	(4)	3	(4)

[a] The totals for each body system may be less than the number of patients with adverse events if a patient had >1 event in that system. Body system totals include events occurring in <1% of patients.

TABLE 14. Parke-Davis Clinical Studies: Adverse Events Occurring in ≥1% of Troglitazone-Treated Patients, by Body System[a]
[Number (%) of Patients]
(Page 2 of 4)

BODY SYSTEM/ Adverse Event	Placebo N = 412		Troglitazone N = 1261		Glyburide N = 77	
DIGESTIVE SYSTEM (continued)						
Vomiting	13	(3)	29	(2)	2	(3)
Constipation	11	(3)	23	(2)	2	(3)
Flatulence	11	(3)	19	(2)	1	(1)
Increased Appetite	3	(1)	13	(1)	0	(0)
Tooth Disorder	8	(2)	13	(1)	3	(4)
Gastroenteritis	6	(1)	10	(1)	3	(4)
Anorexia	3	(1)	9	(1)	1	(1)
Gastrointestinal Disorder	8	(2)	9	(1)	0	(0)
Abnormal Stools	3	(1)	8	(1)	0	(0)
Liver Function Tests Abnormal	2	(<1)	8	(1)	2	(3)
Periodontal Abscess	4	(1)	7	(1)	2	(3)
Thirst	1	(<1)	7	(1)	0	(0)
HEMIC AND LYMPHATIC	10	(2)	45	(4)	3	(4)
Ecchymosis	5	(1)	16	(1)	2	(3)
Anemia	2	(<1)	14	(1)	0	(0)
METABOLIC AND NUTRITIONAL DISORDERS	52	(13)	153	(12)	20	(26)
Peripheral Edema	26	(6)	66	(5)	7	(9)
Hypoglycemia	6	(1)	28	(2)	7	(9)
Hyperlipemia	5	(1)	13	(1)	1	(1)
SGPT Increased	1	(<1)	10	(1)	1	(1)
Hypercholesteremia	2	(<1)	9	(1)	0	(0)
SGOT Increased	1	(<1)	7	(1)	0	(0)
MUSCULOSKELETAL SYSTEM	71	(17)	170	(13)	8	(10)
Arthralgia	16	(4)	45	(4)	2	(3)
Leg Cramps	19	(5)	44	(3)	1	(1)
Arthritis	10	(2)	30	(2)	1	(1)
Myalgia	10	(2)	29	(2)	1	(1)
Tendon Disorder	2	(<1)	10	(1)	1	(1)
Bursitis	2	(<1)	8	(1)	0	(0)
Myasthenia	3	(1)	8	(1)	0	(0)

[a] The totals for each body system may be less than the number of patients with adverse events if a patient had >1 event in that system. Body system totals include events occurring in <1% of patients.

TABLE 14. Parke-Davis Clinical Studies: Adverse Events Occurring in ≥1% of Troglitazone-Treated Patients, by Body System[a]
[Number (%) of Patients]
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BODY SYSTEM/ Adverse Event	Placebo N = 412		Troglitazone N = 1261		Glyburide N = 77	
NERVOUS SYSTEM	86	(21)	260	(21)	17	(22)
Dizziness	20	(5)	76	(6)	4	(5)
Insomnia	9	(2)	30	(2)	1	(1)
Depression	9	(2)	29	(2)	3	(4)
Anxiety	14	(3)	24	(2)	0	(0)
Neuropathy	11	(3)	23	(2)	3	(4)
Hypesthesia	5	(1)	21	(2)	1	(1)
Paresthesia	10	(2)	19	(2)	1	(1)
Somnolence	4	(1)	13	(1)	1	(1)
Vertigo	1	(<1)	12	(1)	0	(0)
Amnesia	0	(0)	10	(1)	0	(0)
Hypertonia	5	(1)	10	(1)	3	(4)
Nervousness	3	(1)	10	(1)	0	(0)
Reflexes Decreased	6	(1)	10	(1)	0	(0)
Incoordination	0	(0)	8	(1)	0	(0)
Ataxia	0	(0)	7	(1)	0	(0)
RESPIRATORY SYSTEM	98	(24)	231	(18)	24	(31)
Rhinitis	29	(7)	66	(5)	8	(10)
Pharyngitis	20	(5)	61	(5)	2	(3)
Sinusitis	26	(6)	55	(4)	8	(10)
Cough Increased	17	(4)	48	(4)	4	(5)
Bronchitis	10	(2)	24	(2)	3	(4)
Dyspnea	8	(2)	19	(2)	0	(0)
Lung Disorder	7	(2)	13	(1)	0	(0)
Asthma	8	(2)	11	(1)	1	(1)
Pneumonia	3	(1)	7	(1)	0	(0)

[a] The totals for each body system may be less than the number of patients with adverse events if a patient had >1 event in that system. Body system totals include events occurring in <1% of patients.

TABLE 14. Parke-Davis Clinical Studies: Adverse Events Occurring in ≥1% of Troglitazone-Treated Patients, by Body System[a]
[Number (%) of Patients]
(Page 4 of 4)

BODY SYSTEM/ Adverse Event	Placebo N = 412		Troglitazone N = 1261		Glyburide N = 77	
SKIN AND APPENDAGES	57	(14)	181	(14)	23	(30)
Rash	18	(4)	50	(4)	7	(9)
Dry Skin	5	(1)	24	(2)	2	(3)
Pruritus	8	(2)	20	(2)	2	(3)
Skin Disorder	4	(1)	15	(1)	5	(6)
Nail Disorder	2	(<1)	14	(1)	1	(1)
Sweating	5	(1)	13	(1)	0	(0)
Fungal Dermatitis	5	(1)	11	(1)	2	(3)
Acne	0	(0)	9	(1)	1	(1)
Herpes Simplex	5	(1)	9	(1)	1	(1)
Skin Ulcer	5	(1)	9	(1)	3	(4)
Alopecia	0	(0)	8	(1)	1	(1)
Vesiculobullous Rash	3	(1)	8	(1)	0	(0)
SPECIAL SENSES	64	(16)	166	(13)	20	(26)
Retinal Disorder	20	(5)	38	(3)	2	(3)
Amblyopia	5	(1)	32	(3)	1	(1)
Cataract Specified	6	(1)	22	(2)	4	(5)
Otitis Media	8	(2)	14	(1)	7	(9)
Ear Pain	3	(1)	12	(1)	2	(3)
Abnormal Vision	0	(0)	11	(1)	0	(0)
Retinal Edema	7	(2)	10	(1)	0	(0)
Conjunctivitis	3	(1)	9	(1)	1	(1)
Eye Disorder	9	(2)	8	(1)	2	(3)
Eye Pain	2	(<1)	8	(1)	1	(1)
Glaucoma	3	(1)	8	(1)	1	(1)
UROGENITAL SYSTEM	64	(16)	177	(14)	24	(31)
Urinary Tract Infection	26	(6)	66	(5)	8	(10)
Vaginal Moniliasis	7	(2)	34	(3)	2	(3)
Vaginitis	4	(1)	26	(2)	1	(1)
Polyuria	4	(1)	12	(1)	0	(0)
Hematuria	5	(1)	9	(1)	1	(1)
Prostatic Disorder	2	(<1)	8	(1)	0	(0)
ANY BODY SYSTEM	332	(81)	996	(79)	72	(94)

[a] The totals for each body system may be less than the number of patients with adverse events if a patient had >1 event in that system. Body system totals include events occurring in <1% of patients.

Overall, the incidence of adverse events was similar for patients with Type II diabetes who were treated with troglitazone and concomitant insulin compared with those receiving placebo and concomitant insulin in the 2 pivotal, placebo-controlled studies. With the exception of dizziness, all of the adverse events that occurred in ≥5% of the total population of troglitazone-treated patients also occurred frequently among patients receiving insulin (Table 15). In general, the incidence of adverse events was similar for patients treated with troglitazone or placebo, suggesting that these events are related to the progression of diabetes. Hypoglycemia was reported more frequently among troglitazone-treated patients receiving insulin (6%) compared with patients receiving placebo and concomitant insulin (3%).

Similar types of adverse events occurred among patients with Type II diabetes receiving concomitant insulin and patients in the other populations, most of whom (N = 765) were patients with Type II diabetes who were not treated with insulin. These events tended to occur at a higher incidence among patients treated with insulin compared with the other populations.

TABLE 15. Parke-Davis Clinical Studies: Adverse Events Reported at a Frequency ≥5% of Troglitazone-Treated Patients, by Indication [Number (%) of Patients]

BODY SYSTEM/ Adverse Event	Placebo		Troglitazone		Glyburide[c] N = 77
	Insulin[a] N = 189	Other[b] N = 223	Insulin[a] N = 401	Other[b] N = 860	
ANY BODY SYSTEM	172 (91)	160 (72)	360 (90)	636 (74)	72 (94)
Infection	68 (36)	32 (14)	124 (31)	117 (14)	42 (55)
Pain	45 (24)	16 (7)	73 (18)	63 (7)	6 (8)
Headache	24 (13)	26 (12)	61 (15)	96 (11)	11 (14)
Accidental Injury	12 (6)	11 (5)	48 (12)	63 (7)	11 (14)
Back Pain	15 (8)	4 (2)	43 (11)	41 (5)	5 (6)
Peripheral Edema	22 (12)	4 (2)	35 (9)	31 (4)	7 (9)
Sinusitis	20 (11)	6 (3)	36 (9)	19 (2)	8 (10)
Retinal Disorder	20 (11)	0 (0)	38 (9)	0 (0)	2 (3)
Flu Syndrome	19 (10)	4 (2)	33 (8)	31 (4)	8 (10)
Nausea	11 (6)	7 (3)	33 (8)	47 (5)	2 (3)
Rhinitis	20 (11)	9 (4)	33 (8)	33 (4)	8 (10)
Diarrhea	18 (10)	8 (4)	28 (7)	42 (5)	3 (4)
Pharyngitis	13 (7)	7 (3)	29 (7)	32 (4)	2 (3)
Hypertension	17 (9)	1 (<1)	27 (7)	6 (1)	2 (3)
Cough Increased	13 (7)	4 (2)	30 (7)	18 (2)	4 (5)
Asthenia	13 (7)	13 (6)	25 (6)	60 (7)	3 (4)
Urinary Tract Infection	17 (9)	9 (4)	26 (6)	40 (5)	8 (10)
Hypoglycemia	6 (3)	0 (0)	25 (6)	3 (<1)	7 (9)
Arthralgia	6 (3)	10 (4)	21 (5)	24 (3)	2 (3)
Dyspepsia	19 (10)	10 (4)	21 (5)	30 (3)	3 (4)
Abdominal Pain	17 (9)	9 (4)	20 (5)	26 (3)	4 (5)
Chest Pain	10 (5)	7 (3)	19 (5)	14 (2)	4 (5)
Arthritis	7 (4)	3 (1)	19 (5)	11 (1)	1 (1)
Depression	5 (3)	4 (2)	19 (5)	10 (1)	3 (4)
Dry Skin	4 (2)	1 (<1)	19 (5)	5 (1)	2 (3)

[a] Includes patients with Type II diabetes treated with concomitant insulin

[b] Includes patients with Type II diabetes not treated with concomitant insulin, patients with impaired glucose tolerance, and patients with polycystic ovary disease

[c] All patients who received glyburide had Type II diabetes and were not treated with concomitant insulin therapy

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5.2.2.3. Sankyo Studies (Japan)

Limited safety data were available from studies conducted by Sankyo. Adverse events that were reported in clinical pharmacology studies included headache and gastrointestinal symptoms. Few adverse events were reported in clinical studies. The types of events reported most often were gastrointestinal events, edema, dizziness, rash, and headache.

5.2.3. Deaths, Serious Adverse Events, and Withdrawals Because of Adverse Events

Overall, the incidence of deaths, serious adverse events, and withdrawals because of adverse events among troglitazone-treated patients was low and similar to that observed for placebo-treated patients (Table 16).

TABLE 16. Summary of Deaths, Serious Nonfatal Adverse Events, and Withdrawals Because of Adverse Events
[Number (%) of Participants]

	Placebo	Troglitazone	Glyburide
Parke-Davis Studies			
Clinical Pharmacology Studies			--
N	8	371	0
Deaths	0	0	NA
Serious, Nonfatal Adverse Events	0	1 (<1)	NA
Withdrawals Because of Adverse Events	0	2 (<1)	NA
Clinical Studies			
Studies Included in the Submission			
N	412	1261	77
Deaths	0	2[a] (<1)	1 (1)
Serious, Nonfatal Adverse Events[b]	28 (7)	85 (7)	8 (10)
Withdrawals Because of Adverse Events[b]	17[c] (4)	73[d] (6)	9 (12)
Studies Not Reported as of December 31, 1995[e]			
N	80	616	0
Deaths	0	0	NA
Serious, Nonfatal Adverse Events	4 (5)	40 (6)	NA
Glaxo Studies			
Clinical Pharmacology Studies			
N	63	316	0
Deaths	0	1 (<1)	0
Serious Adverse Events	0	1 (<1)	0
Withdrawals Because of Adverse Events	1 (2)	4 (1)	0
Clinical Studies			
N	153	629	0
Deaths	0	1 (<1)	0
Serious Adverse Events	7 (5)	24 (4)	0
Withdrawals Because of Adverse Events	20 (13)	68 (11)	0
Sankyo Studies			
Clinical Pharmacology Studies			
N	28	165	0
Deaths	0	0	0
Serious Adverse Events	0	0	0
Withdrawals Because of Adverse Events	0	0	0
Clinical Studies			
N	294	1058	6
Deaths	1	0	0
Serious Adverse Events	0	11 (1)	0
Withdrawals Because of Adverse Events	6 (2)	42 (4)	0

NA = Not applicable.
[a] Both patients died after termination from the study.
[b] Includes TESS and non-TESS events
[c] Patient 449 (Study 991-040, Center 20) was erroneously reported as withdrawn because of adverse events.
[d] Patient 10 (Study 991-001) withdrew because of an adverse event after completing the minimum treatment period.
[e] Complete research report not available by December 31, 1995.

5.2.3.1. Deaths

Three deaths occurred among patients participating in Parke-Davis clinical studies. Two of these patients were treated with troglitazone, one of whom died because of a cerebrovascular accident 48 days after discontinuing treatment and one who died of myelodysplastic syndrome 242 days after treatment. The third patient died of a myocardial infarction on Day 417 of treatment with glyburide.

Two participants in studies died after study termination. One subject with moderate hepatic impairment had two serious adverse events, injured foot prior to dosing and peritonitis, which resulted in death 77 days following administration of a single dose in a clinical pharmacology study. One patient who had participated in a clinical study died of a myocardial infarction during followup, 26 days after study participation.

One placebo-treated patient died of a myocardial infarction during a clinical study conducted by Sankyo.

5.2.3.2. Serious Adverse Events

One subject in a Parke-Davis clinical pharmacology study experienced a serious adverse event (ovarian cancer) approximately 6 months after treatment with single doses of troglitazone once per week for 4 weeks. She was treated with surgery and radiotherapy, and has not yet recovered. The investigator considered this event definitely not related to treatment with troglitazone. In Parke-Davis clinical studies, serious adverse events that were treatment emergent (not present at baseline or increased in intensity or frequency from baseline) occurred in 6% of patients treated with troglitazone in Parke-Davis studies, compared with 6% of patients treated with placebo and 8% of those treated with glyburide. No single event occurred in >1% of patients, and few events were considered associated with study treatment. Serious adverse events that occurred in at least 5 troglitazone-treated patients (≥0.4%) were cellulitis (6 patients) and angina pectoris (5 patients). A variety of carcinomas were reported by 5 troglitazone-treated patients (<1%), 2 placebo-treated patients (<1%), and 1 glyburide-treated patient (1%). None of the cancers were associated with study treatment.

In and Sankyo studies, the incidence of serious adverse events was similar for participants treated with troglitazone or placebo, representing a variety of events similar to those observed in the Parke-Davis studies (Table 16).

5.2.3.3. Withdrawals Because of Adverse Events

The incidence of withdrawals from Parke-Davis studies because of adverse events was similar for subjects and patients treated with placebo or troglitazone (Table 16). Among troglitazone-treated patients, withdrawals because of treatment-emergent adverse events occurred in 3% to 5% of patients treated at 200 to 600 mg/day, compared with 11% of patients treated at 800 mg/day. No event led to withdrawal of >1% of patients and few events were associated with study treatment. Eight patients were withdrawn because of abnormal liver function tests.

In and Sankyo studies, withdrawals because of adverse events occurred at a similar incidence for troglitazone- and placebo-treated participants (Table 16). Although the incidence was higher in clinical studies compared with Parke-Davis studies, approximately half of the withdrawals occurred in one study conducted in elderly patients. The types of adverse events that led to withdrawal from and Sankyo studies were similar to those observed in Parke-Davis studies.

5.2.3.4. Studies Not Included in the Submission

Two clinical studies conducted by Parke-Davis (991-032 and 991-057) were clinically complete but not summarized at the time of this submission. No patients died during these studies. Twenty-two (7%) of the 322 troglitazone-treated patients in Study 991-032 had serious adverse events, as did 4 (5%) of the 80 patients who received placebo. Eighteen (6%) of 294 patients who received troglitazone in Study 991-057 had serious adverse events. The types of serious adverse events that occurred during these studies were similar to those reported in other clinical studies.

Study 991-055, a study of troglitazone in combination with glyburide, was ongoing at the time of data cutoff for this submission. Of the 539 patients enrolled as of April 24, 1996, 66 (12%) had serious adverse events. Events that occurred in 3 or

more patients (>5%) were myocardial infarction (7 patients), chest pain (4 patients), and angina, coronary artery disease, third degree heart block, and cellulitis (3 patients each). Two deaths occurred, one caused by a myocardial infarction and one by acute pulmonary edema. Neither death was considered related to study treatment. --

and Sankyo are continuing to report deaths and serious adverse events to Parke-Davis from their ongoing studies in Europe. Data available to Parke-Davis through April 24, 1996 were reviewed. The types and incidence of deaths and serious adverse events were consistent with the profile of other studies.

5.2.4. Clinical Laboratory Data

5.2.4.1. Parke-Davis Studies (US)

5.2.4.1.1. Hypoglycemia

Hypoglycemia was defined as a glucose value <50 mg/dL as determined by the central laboratory or recorded in patient diaries (self-monitored). No occurrences of hypoglycemia were reported among patients treated without concomitant insulin. Among patients treated with insulin, the occurrence of hypoglycemia appeared to be related to the management of insulin dosage as glycemic control improved.

Overall, the incidence of hypoglycemia, whether documented by glucose values or reported as an adverse event, was higher for patients in Study 991-040, where insulin dosage was not specifically adjusted unless glucose values were ≤100 mg/dL or two readings (Table 17). In Study 991-068, the protocol-specified adjustment of insulin dosage appeared to minimize the occurrence of glucose values <50 mg/dL. A higher percentage of troglitazone-treated patients experienced hypoglycemic adverse events, which may have been related to decreases in glucose values that were clinically significant for individual patients but did not meet the 50 mg/dL criteria for documented hypoglycemia. All symptoms of hypoglycemia were reversed with snacks or glucose tablets, with the exception of one troglitazone-treated patient who lost consciousness and required emergency treatment with intravenous dextrose.

TABLE 17. Incidence of Hypoglycemia in Insulin-Treated Patients

	Study 991-040			Study 991-068		
	Placebo N = 118	TRG 200 N = 117	TRG 600 N = 116	Placebo N = 71	TRG 200 N = 75	TRG 400 N = 76
Hypoglycemia						
As an Adverse Event	40 (34)	48 (41)	71(61)	6 (8)	14 (19)	11(14)
Adverse Event With Self-Monitored Glucose <50 mg/dL	9 (8)	16 (14)	27(23)	3 (4)	6 (8)	4 (5)
FSG <50 mg/dL (central lab)	0	1 (<1)	0	0	0	1 (1)

TRG = Troglitazone; FSG = Fasting serum glucose.

5.2.4.1.2. Liver Function Tests

The incidence of elevations in bilirubin, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) above the upper limit of normal was lower for troglitazone-treated patients compared with placebo (Table 18). Elevations meeting threshold criteria for clinically important changes occurred at a similar incidence in both groups.

A total of 20 troglitazone-treated patients had elevations meeting criteria for clinically important changes in bilirubin (>1.25 × ULN), AST, and/or ALT (>3 × ULN). Eight of the 20 patients had baseline values above the upper limit of normal and 3 had a history of hepatic disease. Ten of the 20 patients had values that returned to the normal range at the last observation, 7 with continued treatment and 3 after discontinuation of troglitazone therapy.

A total of 8 patients were withdrawn from troglitazone therapy because of abnormal liver function tests, 1 of whom did not have elevations above threshold values. Two of the 8 patients had elevations in liver function tests reported as serious adverse events; 1 patient had a history of hepatitis and exposure to toxic solvents, and one had liver biopsy results consistent with an idiosyncratic drug reaction.

TABLE 18. Incidence of Elevations in Liver Function Tests

Test	Threshold	Placebo N = 395	Troglitazone N = 1201	Glyburide N = 76
Bilirubin	>ULN	25 (6)	24 (2)	6 (8)
	>1.25 × ULN	3 (1)	5 (<1)	3 (4)
AST	>ULN	52 (13)	127 (11)	22 (29)
	> 2 × ULN	8 (2)	19 (2)	2 (3)
	> 3 × ULN	3 (1)	13 (1)	0 (0)
ALT	>ULN	78 (20)	115 (10)	29 (38)
	>2 × ULN	6 (2)	25 (2)	5 (7)
	>3 × ULN	2 (1)	14 (1)	0 (0)

ULN = Upper limit of normal; AST = Aspartate aminotransferase; ALT = Alanine aminotransferase.

5.2.4.1.3. Hematology

Mean and median hemoglobin, hematocrit, and erythrocyte count, while remaining within the normal range, tended to decrease in all groups, including placebo and glyburide. The magnitude of these minor changes was 3% to 4% among patients treated with troglitazone and 1% to 2% for those receiving placebo. The percentage of individual patients with decreases in hemoglobin below the normal range was similar for patients treated with troglitazone (5%) compared with placebo (4%). Decreases of ≥2 g/dL occurred more often among troglitazone-treated patients (9%) than placebo (4%); however, most of these patients had values that remained within the normal range. A similar pattern was observed for hematocrit. There was no evidence of drug-induced anemia or suppression of erythropoiesis, as indicated by minor changes in mean and median red blood cell indices and reticulocyte count that were similar across treatment groups.

To determine whether decreases in hemoglobin were associated with perturbations in the red cell mass, plasma volume, other parameter of erythropoiesis, or hemolysis, a study was undertaken in 8 healthy subjects per group, who were treated with either placebo, 200 mg, or 600 mg troglitazone QD for 6 weeks.

Following 6 weeks of administration of 200 and 600 mg QD of troglitazone, plasma volume was increased 5.7% and 7.8%, respectively, compared to placebo. These changes increased with increasing dose but were not statistically significant. The red cell mass however, was not reduced but was slightly increased in both the 200- (7.5%) and 600-mg (2%) dose groups compared to placebo. These changes were not statistically significant and did not show any dose relationship. Adjusted mean reticulocyte counts for troglitazone-treated subjects were similar to those for subjects receiving placebo at 6 weeks.

Mean hemoglobin levels were slightly decreased (0.3 g/dL in the 200 mg troglitazone group and 0.4 g/dL in the 600 mg troglitazone group) compared to placebo. These changes were not statistically significant, and there were no changes in the erythrocyte count, size, or corpuscular hemoglobin concentration, or in hematocrit. In addition, the mean erythropoietin levels did not change during the dosing period in either the 200- or 600-mg dose groups, and the soluble transferrin levels were unchanged. This lack of effect of troglitazone on red cell mass, reticulocyte count, erythropoietin levels, and transferrin receptor levels suggest that 6 weeks of troglitazone administration does not suppress or perturb erythropoiesis. The observed dose-dependent increases in plasma volume suggest that the observed decrease in hemoglobin may be dilutional in nature as a result of plasma volume expansion.

There was no evidence that troglitazone was associated with increased RBC destruction or hemolysis. Troglitazone treatment was not associated with increased reticulocyte count or changes in mean corpuscular volume, serum haptoglobin levels were unchanged and normal, unconjugated bilirubin and LDH levels did not change, and Coombs tests were negative.

Transient decreases in neutrophil counts occurred infrequently and resolved with continued treatment. Nine troglitazone-treated patients (<1%) and one glyburide-treated patient (<1%) had at least one absolute neutrophil count <1,000/mm^3 during treatment. Eight of these nine patients had WBC and neutrophil counts within the normal range at the last visit. The ninth patient had a history of thrombocytopenia and autoimmune hemolytic anemia. With the exception of two patients with a history of hematologic disease, there were no decreases in erythrocyte or platelet counts below normal range. None of these changes were reported as adverse events.

5.2.4.1.4. Other Parameters

Mean and median changes from baseline to final value for all other clinical laboratory parameters were minimal and tended to be similar between patients treated with troglitazone and placebo. The percentage of patients with values changing to outside the normal range or to outside threshold criteria was low and also tended to be similar between groups. Changes in individual patients were not generally associated with study treatment.

5.2.4.1.5. Study 991-040

In Study 991-040, changes in clinical laboratory parameters that were considered clinically significant by the investigator or were outside the alert ranges defined by Sankyo were reported as adverse events and were summarized in a separate database. Differences in the incidence of laboratory adverse events were noted for decreased hemoglobin and hematocrit, and increased creatine kinase and LDH. However, mean hemoglobin and hematocrit and median creatine kinase did not change significantly from baseline and values remained within the normal range. Mean LDH increased significantly over the course of the study, but also remained within the normal range. With the exception of LDH, the profile of laboratory adverse events was similar to the results of clinical laboratory analyses in studies conducted by Parke-Davis. In the overall Integrated Summary of Safety (ISS) database, the mean percent change from baseline for LDH was 7% for troglitazone-treated patients compared with -2% for placebo-treated patients. Less than 1% of patients in either group has LDH values >3 times the upper limit of normal. The percentage of patients with values changing from normal at baseline to high at the end of the study was 5% for the troglitazone group and 3% for the placebo group.

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5.2.4.3. Sankyo Studies (Japan)

The most common laboratory abnormalities reported in clinical studies conducted by Sankyo were slight decreases in erythrocytes, hemoglobin, and hematocrit, and slight increases in LDH. These were generally not considered serious abnormalities.

5.2.5. Echocardiographic Data

Echocardiograms were performed in Study 991-042, an open-label, randomized study assessing the effects of troglitazone 800 mg/day compared with glyburide <20 mg/day on cardiac mass and function over a 96-week treatment period. Formulations used in this study delivered approximately 600 and 700 mg/day of troglitazone during Years 1 and 2, respectively, compared with tablets used in pivotal Phase 3 studies. Mean left ventricular mass index values for troglitazone-treated patients were not clinically or statistically different from baseline or from patients treated with glyburide. The mean change from baseline to Week 96 was -1.76 g/m^2 for troglitazone-treated patients and -6.2 g/m^2 for patients receiving glyburide. Mean cardiac index was increased at Week 96, with a change from baseline of 0.31 $L/min/m^2$, compared with a decrease in the glyburide group (-0.1 $L/min/m^2$). Mean stroke volume was also increased for patients who received troglitazone, with a change from baseline of 4.6 mL/m^2 compared with a change of -1.6 mL/m^2 for glyburide-treated patients. Mean change from baseline was -6.2 torr for peripheral resistance and -2.9 mm Hg for arterial pressure among patients who received troglitazone.

Echocardiograms were also performed in a subset of patients treated in Study THZ-P01 conducted by Results for the 53 patients included in the analysis showed no clinically significant increase in end diastolic or systolic left ventricular diameter following any of the troglitazone dosages compared with placebo.

5.2.6. Conclusions Regarding Safety

Troglitazone is safe and well-tolerated when administered at dosages of 200 to 800 mg/day to patients with Type II diabetes who are currently uncontrolled on insulin. Results of safety evaluations in this population indicate that:

- The incidence of adverse events is similar for troglitazone and placebo.

- The most frequent adverse events (≥5% of troglitazone-treated patients) are infection, headache, pain, accidental injury, asthenia, back pain, nausea, peripheral edema, dizziness, diarrhea, rhinitis, pharyngitis, flu syndrome, and urinary tract infection. These events occur at a similar incidence among placebo-treated patients.

- The incidence of adverse events is similar for patients with Type II diabetes treated with troglitazone and concomitant insulin or placebo and concomitant insulin.

- The incidence of adverse events is similar for patients <65 and ≥65 years of age, for patients of various races, and for men and women.

- Serious adverse events and withdrawals because of adverse events are infrequent and occur at a similar incidence in patients treated with troglitazone and placebo.

- When initiating treatment with troglitazone, hypoglycemia may occur among patients treated with concomitant insulin. Insulin dosage should be adjusted in response to improvements in blood glucose values.

- Small decreases in hemoglobin and hematocrit within the normal range are more common in patients treated with troglitazone than placebo and are related to a modest increase in plasma volume without change in red cell mass.

- Troglitazone does not increase cardiac mass or impair cardiac function.

6. DISCUSSION OF RISK/BENEFIT RELATIONSHIP

6.1. Introduction

Approximately 16 million people in the US suffer from diabetes and only half are aware of their disease. Less than 10% of all diabetics have Type I diabetes or insulin-dependent diabetes mellitus (NIDDM) and 90% have Type II diabetes. About 200,000 people die each year due to diabetes or diabetes-related complications. The estimated annual total cost of diabetes in the US is over $90 billion. Over $37 billion are hospital- related care costs accounting for 26 million days of hospital care.

Type II diabetes is characterized by a sequence of events that start long before the classical symptoms of diabetes appear. Insulin resistance, defined as the state in which greater amounts of insulin are required to elicit a quantitatively normal response marks the early stages in the pathophysiology of Type II diabetes. Hyperglycemia is manifested when the pancreatic secretory capacity is no longer able to meet the increasing demands for insulin.

When diet and exercise fail to maintain glycemic control, sulfonylureas have been prescribed to stimulate the pancreatic beta cells to secrete more insulin. Metformin, a member of the biguanide family, recently has been approved either as monotherapy or in combination with sulfonylureas. Eventually, a great percentage of patients fail oral treatment and require exogenous insulin therapy. A recent survey indicated that 43% of adults with Type II diabetes use exogenous insulin. According to the American Diabetes Association (ADA) clinical practice guidelines, tight control of hyperglycemia remains the main goal to minimize long-term complications, including vision loss, kidney and nerve damage, and heart disease. Near normal glycemic level can only be accomplished in this population by intensive insulin therapy. However, large doses of insulin may be required to reach euglycemic targets, especially in obese patients and this is usually not achievable in the usual practice setting.

According to the ADA guidelines, the risk-to-benefit ratio of intensive insulin treatment may not be as favorable in Type II diabetes as it is in Type I patients. The enthusiasm over the benefits of intensive therapy is hindered by the associated risks, practicality, and cost of implementation. The fact that mean HbA_{1c} levels >9% are

common in Type II diabetes patients requiring insulin further emphasizes the reluctance of physicians to push the insulin dose to the levels often required to achieve glycemic control. The results of the Diabetes Control and Complication Trial (DCCT) in Type I patients and similar studies in Type II diabetes patients have only emphasized the dire need of this patient population for a practical and safe therapeutic intervention that would accomplish improved glycemic control without the associated risks.

Troglitazone is a novel drug that works primarily by reversing insulin resistance. It improves glycemic control in Type II diabetes patients receiving exogenous insulin.

6.2. Benefits of Troglitazone Therapy

Troglitazone is an oral once-a-day antihyperglycemic agent that works by reversing insulin resistance. Troglitazone does not stimulate insulin secretion nor does it mimic its action. It is an effective therapy for Type II diabetes as reflected by improvement in parameters of glycemic control, including FSG and HbA_{1c}. In addition, troglitazone treatment results in a significant improvement in the postprandial profile of blood glucose, insulin, and C-peptide during a meal challenge.

For patients with Type II diabetes, the addition of troglitazone to insulin provides a practical and easy-to-implement intervention to help achieve and maintain near normoglycemia without intensive insulin therapy. In fact, the glycemic control benefits attained with troglitazone therapy are associated with a significant reduction in the amount of exogenous insulin required. A complete elimination of exogenous insulin was achieved while improving glycemic control for some patients. The reduction and/or elimination of exogenous insulin is an important factor in reducing or eliminating the risk of hypoglycemia and associated risks of intensive therapy including increased appetite and weight gain. The benefits of tight glycemic control can be translated into a reduction in the risk of retinopathy, nephropathy, and neuropathy, as demonstrated in the DCCT. The reduced exogenous use of insulin is of particular benefit to the elderly and other patient populations where insulin therapy poses a significant and limiting impact on the patient's activity.

In addition to the reduction of exogenous insulin use, a decrease in C-peptide levels (indicative of reduced endogenous insulin secretion) has also been observed with troglitazone treatment. The reversal of insulin resistance leads to the amelioration of the hyperinsulinemic state and potentially reduced manifestations of Syndrome X, including hypertension and accelerated atherosclerosis.

Troglitazone treatment is associated with an improvement in the dyslipidemia observed in diabetic patients. A reduction in triglycerides along with increases in HDL cholesterol levels have been observed with troglitazone treatment. While the magnitude of these changes varies according to study design and patient population, the direction of these changes is consistent. Troglitazone treatment was associated with a slight increase in total cholesterol and LDL cholesterol. However, further studies have shown that LDL oxidation is significantly delayed with troglitazone treatment. Moreover, the size of the LDL particles has been shown to increase denoting a favorable change in density and atherogenicity of LDL. No clinically significant change was observed in low-density lipoprotein Apolipoprotein B (LDL-Apo B) with troglitazone treatment. The impact of troglitazone treatment on lipid metabolism is potentially indicative of a reduced atherogenic risk and potentially reduced cardiovascular morbidity.

Diabetic patients treated with troglitazone exhibit a reduction in mean arterial pressure. Measures of cardiac function have indicated an increase in stroke volume and reduced peripheral resistance with troglitazone treatment. Since the patient population studied thus far have included mainly normotensive diabetic patients, the benefit would potentially be greater in a hypertensive diabetic population.

Troglitazone is metabolized by the liver and is excreted with hepatic bile secretions. Compromised renal function, observed in greater proportions in the diabetic population, does not present a significant limitation. Troglitazone would be the ideal choice of treatment for patients with renal function impairment or complications without increasing the risk of lactic acidosis.

6.3. Risks of Troglitazone Treatment

Troglitazone alone is not associated with any risk of hypoglycemia because it does not stimulate insulin release nor does it mimic its action. The clinical experience with troglitazone monotherapy is thus far devoid of any hypoglycemic events. The addition of troglitazone to insulin, on the other hand, could theoretically increase the risk of hypoglycemia during the initiation of troglitazone therapy. The clinical experience of troglitazone and insulin combination has demonstrated that true hypoglycemic events (defined as glucose <50 mg/dL) were infrequent. Only one reported case required urgent care or hospitalization. Upon initiation of troglitazone therapy in an insulin using Type II diabetes patient, the reversal of insulin-resistance could potentially render the exogenous insulin dose excessive. Once the full therapeutic effect of troglitazone is achieved and a new reduced and stable insulin dose is established, the risk of hypoglycemia is reduced. Over the long run, the lower daily dose of insulin carries a lower risk of hypoglycemia. Nevertheless, the same clinical guidelines and recommendations for insulin monotherapy should be followed for insulin/troglitazone combination therapy. If standard care practices and specific package insert guidelines are followed during initiation of therapy, the risk of hypoglycemia is virtually eliminated.

Preclinical studies have demonstrated that rodents treated with troglitazone at blood levels exceeding 14 times the therapeutic blood levels in humans, had reversible increases in heart weight. The suspension of the clinical development of other thiazolidinediones has generated a level of concern in the scientific community implicating cardiac safety as a class effect. Although treatment of primates with troglitazone showed no cardiac mass changes, a rigorous cardiac study in humans, at doses above those used clinically in trials, was performed to definitively resolve this issue. The results of that study have demonstrated conclusively that any adverse effects of troglitazone treatment has no adverse effect on cardiac mass or function. In fact, patients treated with troglitazone experienced an improvement in cardiac function. Troglitazone treatment poses no known risk on cardiac mass or function.

Although the percentage of patients experiencing a reduction in hemoglobin, hematocrit, or white blood cell (WBC) below normal limits were similar across troglitazone and placebo groups, a slight decrease in erythrocyte parameters and WBC

count were noted in human trials. The magnitude of these changes in the troglitazone group (1200 patients) is confined to a mean change from baseline of -4% in hemoglobin, -4% in hematocrit, and -5% for WBC count. By comparison, patients in the placebo group (390 patients) experienced changes of -1% in hemoglobin, -2% in hematocrit, and <-1 % for WBC. The effect of troglitazone treatment on important aspects of RBC homeostasis have been carefully studied. Formal red cell mass and plasma volume determinations have been performed in both humans and preclinical models. Red cell mass remained unchanged after troglitazone treatment. Examination of RBC indices, erythropoietin, reticulocytes count, iron levels, bilirubin, and soluble transferrin receptors, indicates no evidence of bone marrow suppression, hemorrhage or hemolysis. Plasma volume showed a slight increase (4%), along with comparable decreases in albumin and total protein after drug administration. Collectively, the data suggest that a hemodilution could account for the changes observed in erythrocyte and WBC counts. Small decreases in hemoglobin, hematocrit, and WBC count which occur with troglitazone treatment are of no clinical significance. However, patients experiencing changes outside the normal range should be evaluated for a possible concurrent illness. Pre-existing conditions, such as anemia, should be treated prior to or concomitant with troglitazone therapy according to standards of clinical care.

The process of evaluating the toxicological profile of troglitazone included a total of 139 studies in mice, rats, dogs, monkeys, and rabbits. These studies conclusively support the clinical use of troglitazone. An increased incidence of hemangiosarcoma was reported in B6C3F1 female mice receiving 400 and 800 mg/kg troglitazone and male mice receiving 800 mg/kg for two years. These tumors appear spontaneously in B6C3F1 mice and the increased incidence was observed at blood levels 16 times that of the human therapeutic level. No tumors in mice were observed at two to four times the human therapeutic level. No increase in the incidence of these tumors was observed in rats with exposure up to 47 times that of man. Collectively, these studies support the conclusion that the increased incidence of hemangiosarcoma are species specific and present minimal carcinogenic risk to humans.

The data from all clinical studies indicate that troglitazone has an excellent margin of safety. Treatment with troglitazone with doses up to 800 mg/day is well-tolerated. Serious adverse events are mainly reflective of the natural progression of Type II diabetes and occur at incidences that are comparable between troglitazone and placebo

groups. Withdrawals due to an adverse event are infrequent and occur at comparable rates for patients treated with troglitazone and placebo. No deaths have been attributed to treatment with troglitazone. The incidence of the most frequently reported adverse events is comparable for troglitazone and placebo. These include (troglitazone vs placebo) infection (19% vs 24%), headache (12% vs 12%), pain (11% vs 15%), accidental injury (9% vs 6%), asthenia (7% vs 6%), back pain (7% vs 5%), nausea (6% vs 4%), peripheral edema (5% vs 6%), dizziness (6% vs 5%), diarrhea (6% vs 6%), rhinitis (5% vs 7%), pharyngitis (5% vs 5%), flu syndrome (5% vs 6%), and urinary tract infection (5% vs 6%). Accordingly, troglitazone treatment does not appear to increase the known risk of adverse events.

7. SUMMARY AND CONCLUSIONS

Troglitazone is a novel, effective, and safe therapy for reversing insulin resistance and improving glycemic control in Type II diabetes patients using insulin. What separates troglitazone from other available therapies for this population is its unique ability to achieve improved glycemic control without placing the patient at undue risk of hypoglycemia and hazards associated with intensive insulin therapy. The benefits of reduced end-organ complications (retinopathy, nephropathy, neuropathy, and cardiovascular disease) gained by improved glycemic parameters cannot be overemphasized. The metabolic advantages of troglitazone therapy extend beyond the glycemic benefits. Reduced exogenous insulin, potential improvement in the lipid profile, improved blood pressure, and suitability for treatment in patients with impaired renal function provide clinicians with a therapeutic tool that has, to a large extent, been lacking for this population. The risks of troglitazone treatment are minimal. The adverse event profile does not appear to be different from placebo. Patient education and instruction on insulin dose adjustment upon initiation of troglitazone therapy should reduce the risk of hypoglycemia. The observed changes to erythrocyte and WBC parameters appear to be of no clinical significance. In conclusion, the benefits of troglitazone therapy outweigh the potential risk in this population of Type II diabetes patients requiring insulin.