

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*  
**21-884**

**PHARMACOLOGY REVIEW(S)**

**MEMORANDUM**

Dec. 12, 2005

TO: File

FROM: Kenneth L. Hastings, Dr.P.H., D.A.B.T.

SUBJECT: NDA 21-884

I concur with Dr. Herman Rhee and Dr. Jeri El-Hage that the marketing application for rhIGF-1/rhIGFBP-3 (mecasermin rinfabate) is approvable based on submitted pharmacology/toxicology data.

---

Kenneth L. Hastings, Dr.P.H., D.A.B.T.  
Associate Director for Pharmacology and Toxicology  
Office of Drug Evaluations II & III

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/s/

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Kenneth Hastings  
12/12/2005 04:15:16 PM  
PHARMACOLOGIST

Comments on N21-884 Iplex (mecasermin fabate)  
From A. Jacobs 9/20/05

I have reviewed the nonclinical material, and I concur with the recommendations of the memo of Sept 7, 2005, by the Supervisor, Jeri El Hage:

There are no pharm/tox approval issues for the proposed indication.  
If approval is sought for other indications, additional toxicology studies, including carcinogenicity evaluations, may be needed.  
Dr. El Hage's labeling recommendations are appropriate.

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/s/

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Leah Ripper

9/20/2005 01:44:05 PM

CSO

I am DFSing this review for Abigail Jacobs, Associate  
Director for Pharmacology/Toxicology



DEPARTMENT OF HEALTH AND HUMAN SERVICES  
PUBLIC HEALTH SERVICE  
FOOD AND DRUG ADMINISTRATION  
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 21-884  
SERIAL NUMBER: 000  
DATE RECEIVED BY CENTER: 1/05/2005  
PRODUCT: rhIGF-1/rhIGFBP-3 (mecasermin rinfabate),  
Iplex®  
INTENDED CLINICAL POPULATION: Growth failure due to IGF-1 deficiency  
SPONSOR: Insmmed Inc.  
DOCUMENTS REVIEWED: June 27, 2005  
REVIEW DIVISION: Division of Metabolic Endocrine Drug  
Products (HFD-510)  
PHARM/TOX REVIEWER: Herman Rhee, Ph.D.  
PHARM/TOX SUPERVISOR: Jeri El-Hage, Ph.D.  
DIVISION DIRECTOR: David Orloff, M.D.  
PROJECT MANAGER: Enid Galliers

Date of submission to Division File System (DFS): September 12, 2005

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## EXECUTIVE SUMMARY

### I. Recommendations

#### A. Recommendation on approvability: Approval

Preclinical pharmacology and toxicology recommends approval of NDA 21-884, based on the preclinical IGF-1 alone and/or IGF-1/IGFBP-3 studies that were submitted.

#### B. Recommendation for nonclinical studies:

As previously discussed, the sponsor is advised that carcinogenicity testing with IGF-1/IGFBP-3 is recommended to support approval of future non-orphan indications.

#### C. Recommendations on labeling:

##### Carcinogenesis, Mutagenesis, Impairment of Fertility:

Long term animal studies for the evaluation of carcinogenicity have not been performed with iPlex (rhIGF-1/rhIGFBP-3). The genotoxic potential of iPlex has not been assessed. rhIGF-1 tested negative for genotoxic potential in the Ames test and in chromosomal aberration assays conducted with human lymphocytes or rat peripheral lymphocytes. Animal fertility studies have not been performed with iPlex. Effects of rhIGF-1 on fertility and reproductive performance were assessed in male and female rats administered 0.4, 2 and 10 mg/kg/day, subcutaneously (times clinical exposures with the MRHD based on body surface area). rhIGF-1 had no effects on mating, fertility, or reproductive performance in rats

##### Pregnancy: Teratogenic Effects: Pregnancy Category C

Animal reproduction studies have not been conducted with iPlex<sup>TM</sup> [mecasermin rinfabate (rDNA origin) injection]. Effects of rhIGF-1 on embryofetal development were assessed in rats and rabbits.

Subcutaneous administration of 2, or 1.5, or 0.5 mg/kg/day rhIGF-1 to pregnant rats during organogenesis had no effects on embryofetal development (1.5, and 0.5 times therapeutic exposures with MRHD based on body surface area). Subcutaneous administration of 0.2, 0.5 or 1.25 mg/kg/day rhIGF-1 to rabbits during organogenesis resulted in an increased incidence of fetal loss but no fetal anomalies. Increased early resorptions were observed in rabbits treated with 1.25 mg/kg and increased preimplantation loss was observed (exposures equivalent to 1.25 mg/kg based on body surface area).

A second rabbit embryofetal development study was conducted to determine the role of hypoglycemia in rhIGF-1 mediated fetal loss. Rabbits were administered subcutaneous doses of 0, 0.5, and 1.25 mg/kg/day rhIGF-1, 1.25 or 2.5 mg/kg rhIGF-1 plus glucose supplementation, or

2.5 IU/kg/day insulin. A comparable degree of hypoglycemia was observed in rabbits treated with 1.25 mg/kg rhIGF-1 alone or 2.5 IU/kg insulin. Animals treated with 0.5 mg/kg rhIGF-1 or rhIGF-1 plus glucose maintained normal glucose levels. Similar to the initial rabbit study, an increase in early fetal resorptions was observed in rabbits treated with 1.25 mg/kg/day rhIGF-1 (2 times MRHD based on body surface area). This finding was not observed in insulin-treated rabbits despite a comparable degree of drug-induced hypoglycemia. A dose-related increase in postimplantation loss was observed in all rhIGF-1 treated groups ( $\geq 0.5$  times MRHD based on body surface area). While the incidence of fetal loss was somewhat reduced in glucose supplemented rabbits, it was not clearly attributable to drug-induced hypoglycemia since significant fetal loss was still observed in normoglycemic rhIGF-1 treated rabbits.

Nursing Mothers:

It is not known whether iPlex<sup>TM</sup> [mecasermin rinfabate (rDNA origin) injection] is excreted in human milk. Because many drugs are excreted in human milk.

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## II. Summary of nonclinical findings

### A. Brief overview of nonclinical findings

The rhIGF-1 (Recombinant human insulin-like growth factor 1) and rhIGFBP-3 (Recombinant human insulin-like growth factor 1 binding protein) components are produced by recombinant DNA expression technology in *E. coli*. The nature of association of rhIGF-I and rhIGFBP-3 is non-covalent and the dissociation constant is approximately 50 pM. The rhIGF-I component of the rhIGF-I/rhIGFBP-3 complex is a single chain polypeptide consisting of 70 amino acid residues with a molecular weight is 7649 Daltons. The primary structure of rhIGF-I is identical to that of natural IGF-I isolated from human serum. The rhIGFBP-3 is a single chain polypeptide consisting of 264 amino acid residues with a molecular weight of 28732 Daltons. It appears that the primary structure of rhIGFBP-3 is also identical to that of natural IGFBP-3. Natural IGFBP-3 isolated from human plasma is glycosylated. While the rhIGFBP-3 that is produced in *E. coli* is nonglycosylated, the glycosylated and nonglycosylated IGFBP-3 bind IGF-I with similar affinities.

In the normal human circulation less than 1-2% of the total serum IGF-I exists in free form. Most circulating IGF-I is found predominantly in association with the growth hormone-dependent IGF binding protein-3 (IGFBP-3). It appears that IGFBP-3 occupied with IGF-I further combines with a third serum protein, the acid-labile subunit (ALS). This final ternary complex represents the natural physiologic reservoir of IGF-I. The free IGF-I can readily cross the vascular endothelium to form a ternary complex which restricts the IGF-I to the circulation. The complex provides prolonged action of IGF-I (half-life < 15 min. in the free form to > 12 h in the ternary complex).

The *in vitro* growth-promoting effects of rhIGF-1 have been demonstrated using the chick femur assay and 3T3 mouse fibroblasts. It is well documented that IGF-I stimulates matrix synthesis in chondrocytes, and was shown to exert effects on chick pelvic cartilages and femurs *in vitro*. In chick femur studies, rhIGF-I induced a dose-dependent increase in the weight of the femora up to a dose of approximately 100 ng/mL over 3 days, with plasma derived human IGF-I showing equivalent effects to rhIGF-I. In a similar study of rhIGF-I, IGF-II and insulin, rhIGF-I and IGF-II were equipotent in terms of the growth-promoting effects, whereas insulin had a 10-fold lower potency. In mouse 3T3 fibroblasts, rhIGF-I was shown to increase mitogenic activity in a dose-dependent manner.

*In vivo* studies with rhIGF-I/rhIGFBP-3 demonstrate anabolic activities that are similar to those observed with rhIGF-I. Systemic administration of either rhIGF-I/rhIGFBP-3 or rhIGF-I to ovariectomized rats for 16 weeks resulted in significant and dose-dependent increases in cortical bone and lean body mass compared to control rats. In calorie-restricted rats, rhIGF-I/rhIGFBP-3 administration significantly stimulated muscle protein biosynthesis, whereas an equimolar dose of rhIGF-I failed to increase protein synthesis. In this study, plasma concentrations of IGF-I were 20% higher in rhIGF-I/rhIGFBP-3 treated rats as compared to control animals or animals treated with rhIGF-I. This observation was attributed to a prolonged half-life and a more physiologic concentration/kinetic behavior of IGF-I following rhIGF-I/rhIGFBP-3 administration compared to rhIGF-I administration in its free form.

In an experimental model of cancer cachexia, tumor-bearing mice treated with rhIGF-I/rhIGFBP-3 exhibited a significant attenuation in weight loss compared to control mice. An important observation in this study was that administration of rhIGF-I/rhIGFBP-3 did not affect net tumor growth. Moreover, administration of rhIGF-I/rhIGFBP-3 improved nutritional state, not only by attenuating weight loss, but by increasing food intake and improving glucose metabolism. It is safe to conclude that the primary and secondary pharmacological actions of rhIGF-I/rhIGFBP-3 and rhIGF-I are qualitatively similar. However, some pharmacokinetic and pharmacodynamic data demonstrate that administration of rhIGF-I in the form of rhIGF-I/rhIGFBP-3 significantly attenuates the acute hypoglycemic activity of rhIGF-I. In addition, in 13-week toxicology study in rats the mild hyperplasia in both male and female rats was observed in some tissues such as cardiac, renal, lung and gland tissues, which appear to be in IGF-1/IGFBP dose related.

The sponsor and its associates performed single and repeated toxicology studies of IGF-1 in mice, rats, chicks, rabbits, dogs and monkeys. In addition, 13-Week toxicology studies of IGF-1/IGFBP were carried out in rats, and monkeys at the top doses of 10 to 30 mg/kg/day (dose resulting in an exposure 1 to 4 times the average human dose, based on body surface area comparison). A 26-Week toxicology study was also performed in the rat with IGF-1 at a dose of 10 mg/kg/day. The 26-Week toxicology study in monkey utilized only IGF-1 alone at a dose of 1 mg/kg/day (0.2 times the average human dose based on body surface area comparison). Pharmacokinetic studies were also performed primarily in rats as reviewed in this NDA. The sponsor has not performed a long-term animal study for carcinogenicity with rhIGF-1/rhIGFBP-3. The genotoxicity and reproductive toxicity studies were conducted with IGF-1 alone.

B. Pharmacologic activity

The pharmacological activity of IGF-1 has been well documented both in animals and humans. The sponsor summarized the main pharmacological properties of IGF-1 in the NDA, based primarily on reports in the scientific literature. There were some preclinical data on IGF-1/IGFBP, which were performed by various contract laboratories as presented in this review.

B. Nonclinical safety issues relevant to clinical use

None.

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## 2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

## 2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-884

Review number: 1

Sequence number/date/type of submission: 000/Jan. 5, 2005/Commercial

Information to sponsor: Yes (x) No ( )

Sponsor and/or agent: Inmed Incomp., Glen Allen, VA 23060 (804)-565-3000

Manufacturer for drug substance: Avecia Biotechnology, Billingham Cleveland, UK

Reviewer name: Hee M (Herman) Rhee, Ph.D.

Division name: Division of Metabolic Endocrine Drug Products

HFD #: 510

Review completion date: August 25, 2005

## Drug:

Trade name: Iplex®

Generic name: rhIGF-1(Recombinant human insulin-like growth factor 1)/rhIGFBP-3(Recombinant human insulin-like growth factor 1 binding protein) (mecasermin rinfabate)

Code name: NA

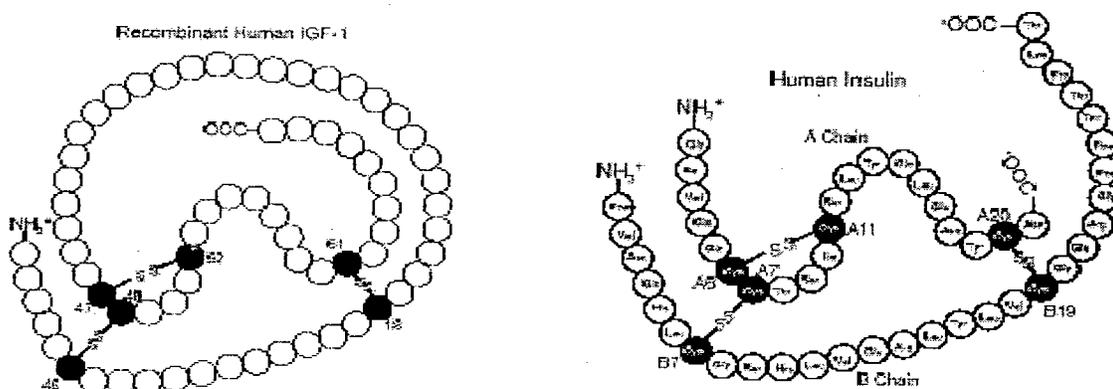
Chemical name: Protein consisting of 70 amino acid residues

CAS registry number: 67763-96-6

Molecular formula: IGF-1 is a protein of a polypeptide chain consisting of 70 amino acid residues while IGFBP-3 is a single polypeptide chain consisting of 264 amino acids

Molecular weight: IGF-1(7649 daltons), IGFBP-3(28,732 daltons)

Chemical structure of IGF-1 is shown below in reference to human insulin. Two dimensional structure of IGF-1 binding protein 3 is not available except its amino acid sequence as shown below.



**IGFBP-3:** GASSAGGLGP VVRCEPCDAR ALAQCAPPPA VCAELVREPG CGCC LTCAL SEGQPCGIYT ERCSGLRCQ  
 PSPDEARPLQ ALLDGRGLCV NASAVSRLRA YLLPAPPAPG NASESEEDRS AGSVESPSVS STHRVSDFPKF  
 HPLHSKIIII KKGHAKDSQR YKVDYESQST DTQNFSSSEK RETEYGPCRR EMEDTLNHLK FLNVLSPRGV  
 HIPNCDKKGK YKKKQCRPSK GRKRGFCWCV DKYGQPLPGY TKGKEDVHCY SMQS

IGFBP-3 Amino Acid Sequences

Relevant INDs/NDAs/DMFs: IND#50,140 (rhIGF and rhIGFBP-3, Celtrix/Insmed)

Drug class: Insulin-like Growth Factor

Intended clinical population: — treatment of children : — with growth failure due to severe growth hormone insensitivity syndrome(GHIS).

Clinical formulation: Clear, sterile, aqueous solution containing the rhIGF-1 and rhIGF-1BP (60 mg/ml), 50 mM sodium acetate, 105 mM sodium chloride, pH 5.5. A clinical dose of equimolar combination product of IGF-1 and IGF-1BP is 1- 2 mg/kg/day for GHIS subjects.

Route of administration: Subcutaneous

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission: The following studies have been reviewed below briefly, depending upon the nature and purpose of individual studies.

1. Insulin-Like Growth Factor 1 Bioassay using 3T3 BALB C Fibroblasts
2. IGF-1 and IGF-II Stimulate Net Growth of Chick Embryonic Bone in vitro, by inducing cell proliferation and proteoglycan synthesis, primarily in chondral epiphysis
3. Chick embryonic femur Bioassay for the Quantification of IGF-1.
4. Toxicokinetics study of rhIGF-1/IGFBP-3 in cynomolgus monkeys
5. Recombinant Human IGF-1 PK/PD study in Cynomolgus monkeys: Hypoglycemia
6. Study on glucose and IGF-1 blood levels after subcutaneous administration of rhIGF-1 to pigs
7. Recombinant Human IGF-1 PK/PD study in Cynomolgus monkeys: Metabolic effects
8. Effects on cardiovascular parameters recorded with telemetry in the conscious dog
9. Pharmacokinetics of rhIGF-1/IGFBP-3 following subcutaneous injection in male rats
10. Pharmacokinetics of rhIGF-1/IGFBP-3 following intravenous injection in male rats
11. Pharmacokinetics of rhIGF-1/IGFBP-3 following a single subcutaneous injection to SD rats
12. rhIGF-1/IGFBP-3: Single dose subcutaneous injection PK study in cynomolgus monkeys
13. Toxicokinetics study of rhIGF-1/IGFBP-3 in cynomolgus monkeys
14. PK profiles of IGF-1 and IGF-1/IGFBP-3 following their administration in pigs.
15. Pharmacokinetics of rhIGF-1 in the rats
16. Pharmacokinetics of rhIGF-1 in the dog following intravenous injection of 0.5 mg/kg and subcutaneous administration of 0.5 and 2.5 mg/kg

17. Recombinant Human IGF-1 PK/PD study in Cynomolgus monkeys: Phase I
18. Toxicity study of recombinant Human IGF-1 in Cynomolgus monkeys. PK evaluation of serum concentration
19. Autoradiographic distribution of IGF-1 in male mouse
20. 13-Week repeat dose subcutaneous toxicity study of rhIGF-1/IGFBP-3 in rats
21. 90-Day repeat dose subcutaneous toxicity study of rhIGF-1/IGFBP-3 in monkeys
22. 13-Week repeat dose subcutaneous toxicity study of rhIGF-1 in mice
23. Determination of antibodies against rh-IGF-1 in rat serum after repeated iv administration
24. 26-Week repeat dose subcutaneous toxicity study of rhIGF-1 in rats with 8-week recovery period
25. Analysis of rhIGF-1 and anti-IGF-1 antibodies in serum(26 Week subcutaneous toxicity study in rats with 8-week recovery period)
26. Determination of antibodies against rhIGF-1 in cynomolgus monkey serum during repeated iv injection
27. rhIGF-1 26 Week (subcutaneous bolus) toxicity study in cynomolgus monkeys
28. Study to evaluate the chromosome damaging potential of recombinant Human IGF-1 by its effects on cultured human lymphocytes using an in vitro cytogenetic assay
29. Study to evaluate the chromosome damaging potential of recombinant Human IGF-1 by its effects on rat peripheral blood lymphocytes using an in vitro cytogenetic assay
30. Study to evaluate the chromosome damaging potential of recombinant Human IGF-1 by its effects on rat peripheral blood lymphocytes treated in vivo and cultured in vitro
31. Gene Mutation test in bacteria: rh-IGF-1
32. Study of the effect of rhGH and IGF-1 on the growth of primary tumor and lung metastases from Lewis Lung carcinoma
33. Study of the effect of rhGH and IGF-1 on the growth of HT-29 human colon carcinoma implanted in nude mice
34. rhIGF-1: Study on fertility and pre- and post-natal development in the rat
35. rhIGF-1: An investigation of the effects on neonatal development in the rat
36. rhIGF-1: Teratology study in the rat
37. rhIGF-1: Teratology study in the rabbit
38. A study of the effects of rhIGF-1 with and without glucose administration on pregnancy in the rabbit
39. 14-day subcutaneous irritation study of rhIGF-1 in SD rats
40. A study on the local subcutaneous toxicity of rhIGF-1 in pigs.

Studies not reviewed within this submission: Some of the safety studies on IGF-1 alone which were published in many medical journals are not documented in this review.

## 2.6.2 PHARMACOLOGY

### 2.6.2.1 Brief summary

rhIGF-I/rhIGFBP-3 is produced by two separate *E. coli* strains: one containing the human gene for insulin-like growth factor I (IGF-I), the other containing the human gene for insulin-like growth factor-binding protein-3 (IGFBP-3). The two proteins are combined in a 1: 1 molar ratio of the rhIGF-I/rhIGFBP-3 complexes. IGFBP-3 from human plasma is glycosylated, whereas rhIGFBP-3 produced in *E. coli* is nonglycosylated. Glycosylated and non-glycosylated IGFBP-3 bind IGF-I with similar affinities.

The promotion of linear growth is the primary pharmacological effect of both rhIGF-I/rhIGFBP-3 and rhIGF-I. There are data that have been derived from experiments conducted in a variety of assay systems and species, including *in vitro* cellular proliferation and bone growth assays. The similar effects were documented in osteoporotic rat models, hypophysectomized rats, calorie-restricted rats and normal healthy monkeys. Of particular relevance to the treatment of growth hormone insensitivity syndrome is the observation that rhIGF-I promotes growth in models of growth hormone deficiency (i.e. the hypophysectomized rat).

The anabolic effects following repeated dosing with rhIGF-I/rhIGFBP-3 or rhIGF-I were qualitatively similar and considered to result from the known pharmacological effects of the test materials. However, severe hypoglycemia was noted on numerous occasions following dosing with rhIGF-I which resulted in many deaths, particularly in mice and rats in the 10 mg/kg/day dose groups. These effects were not apparent in rats treated with rhIGF-I/rhIGFBP-3 at doses up to 30 mg/kg/day (this dose is approximately equivalent to only 6 mg/kg/day rhIGF-I). With one exception, hypoglycemic shock was noted in all monkeys treated with 1 mg/kg/day rhIGF-I when food was withheld for serial blood sampling. Again, these effects were not observed in monkeys treated with 10 mg/kg/day rhIGF-I/rhIGFBP-3 (a dose approximately equivalent to 2 mg/kg/day rhIGF-I).

### 2.6.2.2 Primary pharmacodynamics

rhIGF-I is the bioactive component of the rhIGF-I/rhIGFBP-3 complex. The primary pharmacologic effect of IGF-I in children is the promotion of linear growth. Secondary pharmacologic actions of IGF-I include the induction of insulin sensitization and insulin-like effects. In normal human circulation, less than 2% of total IGF-I exist in the free form. Most circulating IGF-I is found in association with the growth hormone (GH)-dependent IGFBP-3 and this binary complex further associates with a third serum protein. GH-dependent acid-labile subunit forms a ternary complex of 150 kD, which represents the natural physiologic reservoir of IGF-I. The ternary complex, consisting of one mole each of IGF-I, IGFBP-3 and acid labile subunit is non-covalent in nature. Specific mechanisms exist for the release of IGF-I from the ternary complex in order for it to act on target cells inside and outside the vascular compartment. A small amount of free, bioavailable IGF-I is present in serum in equilibrium with the bound forms. In addition, proteolytic cleavage of IGFBP-3 and interaction of the ternary complex with proteoglycans have been shown to release IGF-I from the ternary complex.

Mean height standard deviation score(SDS) improved significantly during treatment with SomatoKine from  $-6.4 \pm 2.1$  at baseline to  $-6.1 \pm 2.1$  at Month 6 and  $-6.0 \pm 2.2$  at Month 12 ( $p < 0.002$  for both time points versus baseline). In another group, mean height SDS improved from  $-7.9 \pm 1.1$  at baseline to  $-7.5 \pm 1.1$  at Month 6 ( $p < 0.001$  versus baseline) as shown below(Table 2).

Table 2. Mean ( $\pm$ SD) Efficacy Results for Patients with GHIS Treated with [BRANDNAME].

Endpoint	Low Dose (Up to 1.0 mg/kg daily)			High Dose (Up to 2.0 mg/kg daily)	
	Pre-Tx/ Baseline (n=16)	Month 6 (n=16)	Month 12 (n=16)	Pre-Tx/ Baseline (n=9)	Month 6 (n=9)
Annualized Height Velocity (cm/yr)	$3.4 \pm 1.9$	$7.4 \pm 2.0$ ***	$6.4 \pm 1.6$ ***	$2.2 \pm 1.5$	$8.8 \pm 2.0$ ***
Height SDS	$-6.4 \pm 2.1$	$-6.1 \pm 2.1$ ***	$-6.0 \pm 2.2$ *	$-7.9 \pm 1.1$	$-7.5 \pm 1.1$ **

\*\*\* p < 0.0001 vs Pre-Tx / Baseline

\*\* p < 0.001 vs Pre-Tx / Baseline

\* p < 0.002 vs Pre-Tx / Baseline

#### Title: Insulin-Like Growth Factor 1 Bioassay using 3T3 BALB C Fibroblasts (Section 4.2.1.1.b.1)

##### 1. PURPOSE:

To access the potency of rhIGF-1 in stimulating nucleic acid incorporation to DNA because IGF-1 is known to be a potent mitogen. This study was performed at Kabi Pharmacia, Stockholm, Sweden.

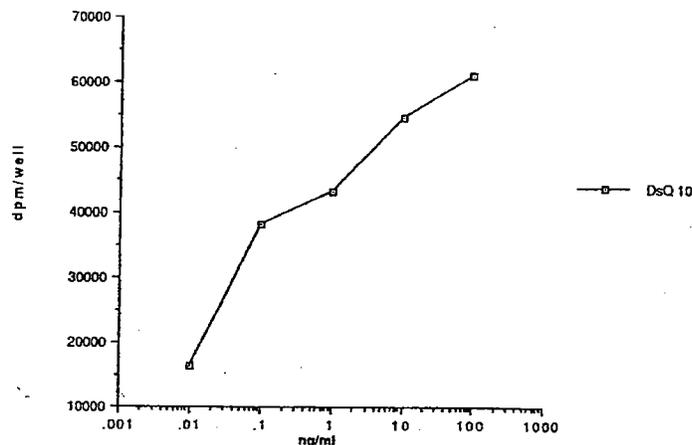
##### 2. METHODS:

A commercial cell line, 3T3 Balb C fibroblasts was seeded in 6 well multi-dishes and grown to confluency. rhIGF-I<sub>1</sub> was added at concentrations of 0.01-100 ng/ml, and a further incubation of 40 hours was performed. During this time, <sup>3</sup>H-thymidine was present in the incubation medium. After the incubation, the cells were washed and extraction performed by the addition of TCA. The TCA precipitate was dissolved and the radioactivity in the precipitate was counted.

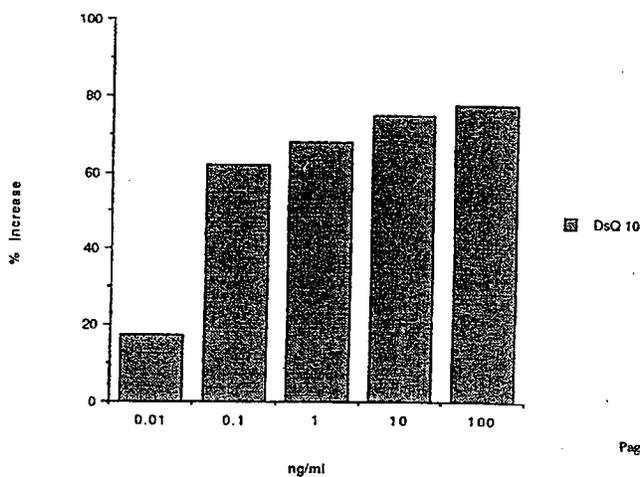
##### 3. RESULTS:

rhIGF-I stimulated the uptake of <sup>3</sup>H-thymidine into DNA in a dose dependent way in confluent cells as shown below. A smaller response was observed in non-confluent cells, probably due to a lower number of cells. A difference in sensitivity of the cells depending on the manufacturer of plastic dishes were observed, showing that when FALCON dishes were used, the dose response curve was moved to the left, and maximum response was obtained already at a concentration of 0.1 ng/ml. Similar results were obtained with several production batches of rhIGF-I.

4. CONCLUSIONS: RhIGF-I was shown to stimulate DNA-synthesis, measured as <sup>3</sup>H-thymidine uptake, in a dose-dependent way in 3T3 Balb C fibroblasts. This is in agreement with results reported for the plasma derived hIGF-I.



g 3 b % increase of 3-H thy uptake on Falcon dishes



**Title: IGF-1 and IGF-II Stimulate Net Growth of Chick Embryonic Bone in vitro, by Inducing Cell Proliferation and Proteoglycan Synthesis, Primarily in Chondral Epiphysis(S. 4.2.1.1.b.2)**

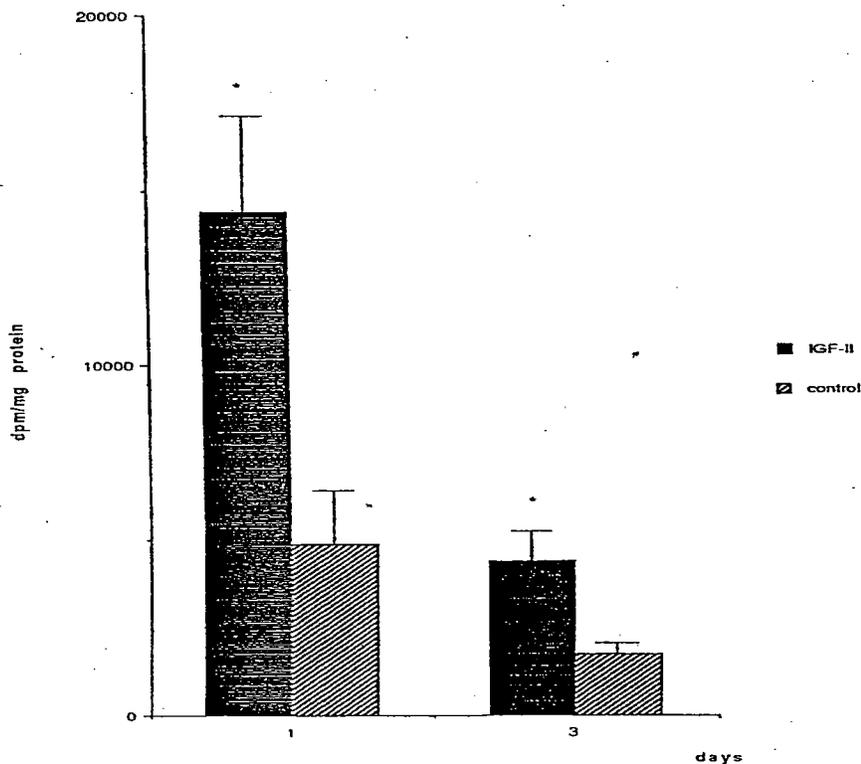
1. PURPOSE: IGFs have been reported to have potent effects on the proliferation of chondrocytes from various species including chicken and they are known to stimulate proteoglycan synthesis in several types of cartilage. Thus, the sponsor investigated the effects of IGF-1 and/or IGF-II on thymidine uptake in bone shafts and epiphyses. This study was performed at Kabi Pharmacia, Stockholm, Sweden.

2. METHODS: Chicken femora were pulsed for one hour with 1 µc/femur of <sup>3</sup>H-thymidine. The medium was removed and the femora washed with Phosphate Buffer Solution(PBS) and left in acetone for 5 minutes. The dried femora were weighed and hydrolyzed in 0.5 ml 6M HCl at 100 °c over night. The incorporated radioactivity was measured on a liquid scintillation counter. The direct counts obtained were finally normalized on a dpm/bone dry weight basis after a separation of bone shafts from epiphyses.

3. RESULTS:

Thymidine uptake in epiphyses of femora was examined after an incubation of 1 to 3 days with 100 ng/ml IGF-II pulse in eight-day old chicken femora. On day one radioactivity was increased significantly in IGF-II treated group, compared to the control. The effects were reduced significantly after the treatment for 3 days. Thymidine uptake in bone shafts was also increased after IGF-II treatment as in Fig. 3. The effect of IGF-II was visible even after its treatment for three days. In addition, the increases in thymidine uptake and bone net growth were compared. As presented in Fig 9, the bone weight of femora was significantly increased after IGF treated group compared to the control, which indicate the two events are well correlated, although the IGFs-mediated causal relationship is not clear at this time.

Section 4.2.1.1.b.2  
 Thymidine uptake in boneshafts. Figure 3



Femora were cultured ± IGF-II for 3 days and pulsed with tritiated thymidine the last hour of culture. Boneshafts were separated after the end of the culture period. n = 5-6 mean ± s.e.m.

\* P < 0.05 vs. respective control

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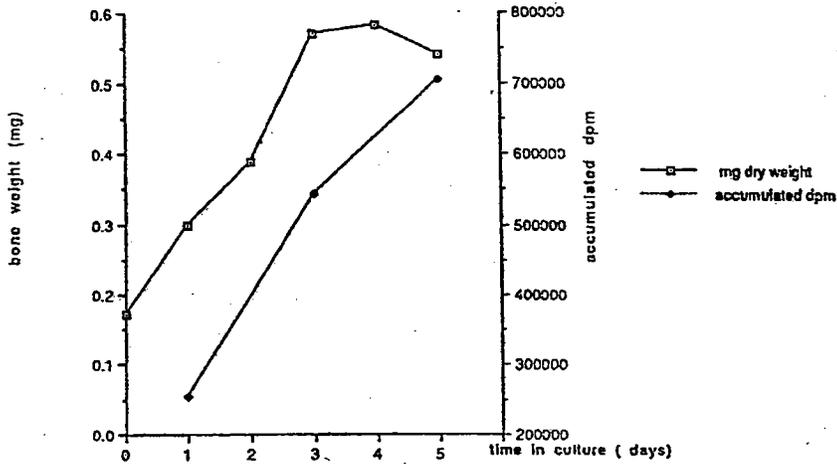
Section 4.2.1.1.b.2

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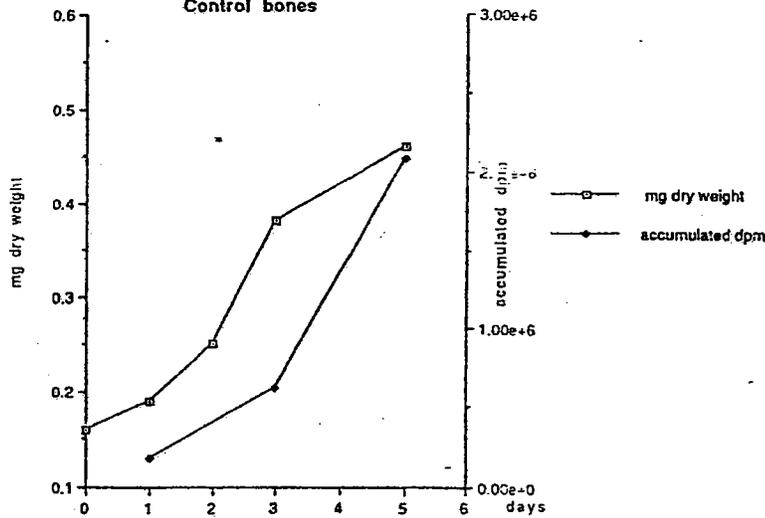
Correlation between accumulated bone weight and accumulated thymidine incorporated

Figure 9

IGF treated bones



Control bones



Femora were cultured with  $\pm 100$  ng/ml IGF-II and pulsed with tritiated thymidine for one hour at the end of culture.

**Title: Chick Embryonic Femur Bioassay for the Quantification of IGF-1(Section 4.2.1.1.b.3).**

1. Purpose: The sponsor wished to define and to quantify IGF-1 by bioassay. One of early methods was to determine the ability of IGF-1 in stimulating sulfate incorporation into proteoglycans of cartilage, from which the term of "Sulfation factors" originated. Cartilage is therefore a classical target tissue for IGF actions. This study was performed at Kabi Pharmacia, Stockholm, Sweden.

2. Methods: The two femora were carefully dissected out from chick embryos which were incubated in an egg Hatcher for 8 days. The isolated femora were then individually transferred to wells containing 0.5 ml of culture medium in a 24 well tissue culture plate. The plates were incubated over night at 37 °C in an incubator under humidified atmosphere of 5 % CO<sub>2</sub> in air. Following the over night incubation, the femora were transferred to 6 well tissue culture plates containing sterile-filtered fresh culture medium +/- additions of the IGF-I (0.1 mg/ml) to be tested.

One femur of the embryo was incubated in the medium supplemented with the peptide. The other served as a pair-matched control and was incubated in the medium alone. Control and test bones were incubated for 3 days at 37°C under a humidified atmosphere of 5% CO<sub>2</sub> in air. At the end of the incubation period the medium was removed and replaced with 1 ml acetone for 5 minutes. The femora were then left to dry in air. The dry weight of each femur was measured on a precision balance and the growth promoting effect of the peptide estimated as the ratio of the treated (T) femur to that of its pair-matched (C) control (=T/C ratio).

The T/C ratio response was recorded for dilutions of the in house IGF-I standard (DSQ10) to produce test doses in the range 1-1000 ng/ml. The initial observations from single and repeated assays were that the response to doses below 10 ng/ml was weak, highly variable, and frequently of low statistical significance. The response tended to reach saturating values on increasing the dose. Concentrations above 100 ng/ml did not produce a significant further T/C increase. On increasing doses up to 1000 ng/ml a decrease in T/C response was occasionally observed. On accepting data for control bones with weights between 0.2-0.4 mg for 6 assays performed during the time period. A final standard curve was constructed after numerous tests (See fig 6a and 6b). Log transformed T/C Ratios vs. log dose now produced an acceptable linear fit (R<sup>2</sup> = 0.95) although a slight curvature still was present. Indeed, a perfect linear fit was obtained if the dose-range was limited to 25-100 ng/ml. This linear regression curve extrapolated to an apparent minimal effective dose of 13 ng/ml.

3. Results: It appears that the chick embryo femora model provides a suitable bioassay for the determination of IGF-I biopotency. Standard curve linearity was observed for the dose range ~ ng/ml, which defines the practical limit of quantification if a 2-dose parallel line assay approach were to be adopted. The limit of detection was approximately ~ ng/ml (n= ~ The inter- and intra-assay variation was ~ , respectively, which is acceptable for this type of assay. On the negative side, the assay is laborious, time-consuming and of moderate "ruggedness". A major finding in this study was a correlation between the growth stimulatory response to added rhIGF-I and initial bone weights, which has led us to apply weight exclusion criteria in the performance of the assay.

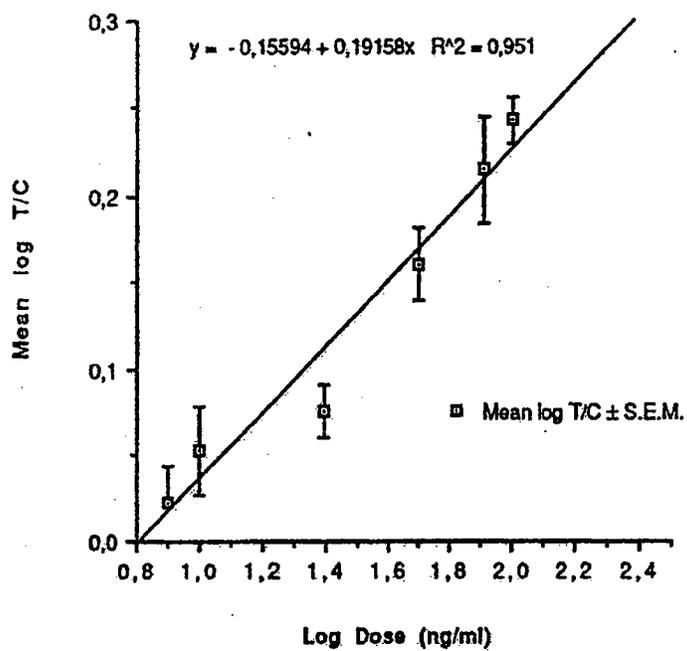


FIG.6a. DSQ10 STANDARD CURVE.

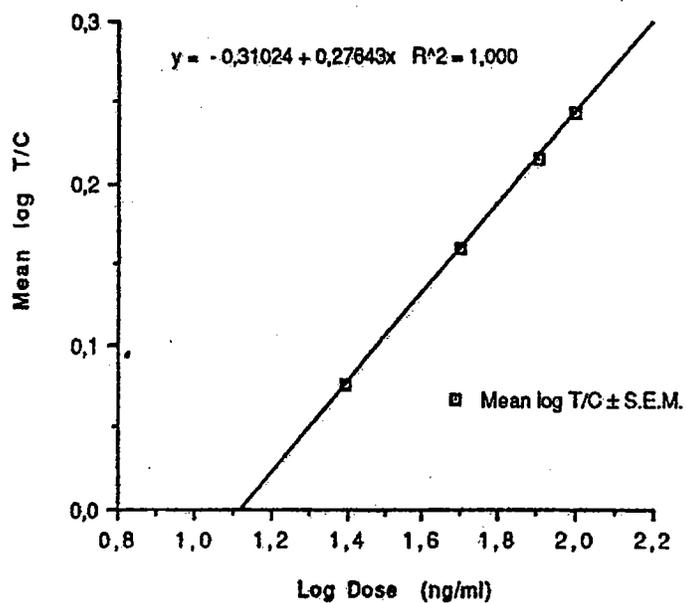


FIG.6b. STANDARD CURVE LINEARITY OVER DOSE RANGE 25-100 ng/ml.

### 2.6.2.3 Secondary pharmacodynamics

Some of studies listed below may be classified under this section as the secondary pharmacodynamic actions or safety pharmacology. Please see the individual studies for details. The major secondary pharmacological action of rhIGF-I/rhIGFBP-3 is the induction of glucose lowering effects due to the structural and functional homology of IGF-I with insulin. Under conditions of elevated circulating levels, IGF-I induces insulin-like effects by activating the insulin receptor in addition to its own receptor. A major consequence of this activation is the occurrence of hypoglycemia, the severity of which is primarily dependent on dose, species, and nutritional status. The administration of rhIGF-I/rhIGFBP-3 significantly attenuates the acute hypoglycemic effects of rhIGF-I. In the rat and the monkey, clinical signs of severe hypoglycemia were not apparent until doses of rhIGF-I/rhIGFBP-3, representing a 40- and 80-fold increase in rhIGF-I were administered, respectively.

### 2.6.2.4 Safety pharmacology

The sponsor has not submitted any data under the heading of "Safety Pharmacology", but some of their studies may be relevant to safety issues of the drug products. Although no safety pharmacology studies have been conducted with rhIGF-I/rhIGFBP-3 complex, the cardiovascular and respiratory effects of rhIGF-I have been studied in the anesthetized cat and conscious dog. The overall conclusion from the studies is that the effects of rhIGF-I were not remarkably different from those of the complex and are similar to those observed with insulin.

#### **Title: Effects on cardiovascular parameters recorded with telemetry in the conscious dog**

1. Purpose: To evaluate the cardiovascular effects of IGF-1 and insulin in telemetered conscious dog after single intravenous injection and repeated subcutaneous injections. The study was performed at Pharmacia and Upjohn Company.
2. Methods: The telemetric transmitter was implanted in a subcutaneous pocket on the flank. The pressure catheter was guided under the skin and inserted, via the saphanous artery, into the femoral artery and electrodes were positioned subcutaneously for registration of ECG. After administration of a bolus injection of 1.1 IU Insulin or 0.5 mg/kg rhIGF-I, ECG, blood pressure, heart rate and body temperature were registered every second minute for three to four hours.
3. Results: Treatment with these doses of rhIGF-I and insulin induced a similar decrease of blood glucose. The heart rate increased continuously during the first hour after treatment remained high for about one hour and returned to normal at 3 to 4 hours after treatment both with rhIGF-I as shown in Fig. 2. Concomitant with heart rate changes both systolic and diastolic pressure decreased continuously reaching a minimum about 90 minutes after treatment and returned to normal 3 to 4 hours after treatment. The rate of rise of dp/dt (first derivatives of intraventricular pressure) was not significantly altered after the IGF-1. Similar cardiovascular effects were obtained after a bolus injection of 1.1 IU insulin. One dog experienced hypoglycemic convulsions and was treated with glucose intravenously which normalized the cardiovascular changes. The sponsor concluded that cardiovascular changes induced with rhIGF-I were similar to changes induced with insulin, which is true in qualitative sense.

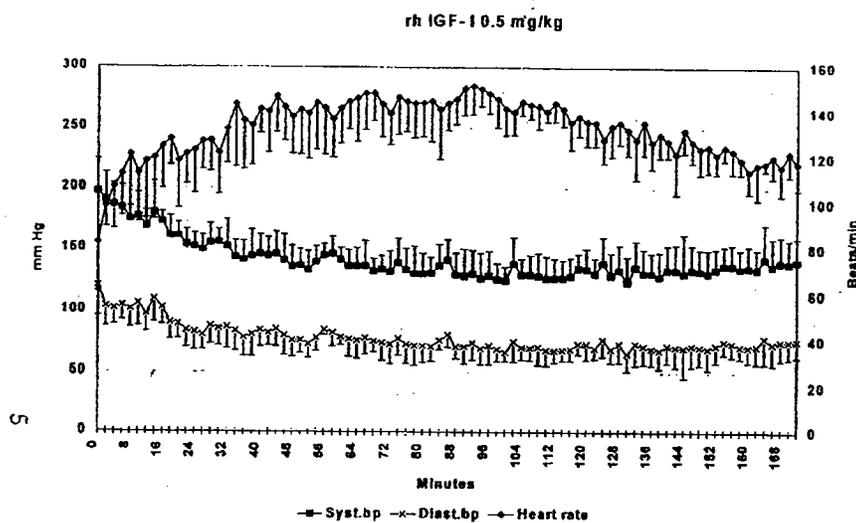


Fig. 2. The effect of an intravenous bolus injection of rh IGF-I 0.5 mg/kg on systolic and diastolic blood pressure and heart rate at different times after administration. Values are mean  $\pm$  SD of 6 animals.

#### 2.6.2.5 Pharmacodynamic drug interactions.

Non-clinical studies to evaluate the interaction of rhIGF-I/rhIGFBP-3 with other compounds have not been conducted. However, an enhanced glucose lowering effect is likely upon concomitant administration of insulin or other compounds that induce a glucose lowering effect.

#### 2.6.4. Pharmacokinetics/Toxicokinetics

The pharmacokinetic parameters of IGF-I following administration of free rhIGF-I have been investigated in rats, dogs and monkeys by both the intravenous and subcutaneous routes. In the rat and the monkey, administration of rhIGF-I resulted in a multi-phasic decline in serum IGF-I levels. Following 30 or 90 consecutive days of dosing with rhIGF-I or rhIGF-I/rhIGFBP-3 to monkeys, respectively, the pre-dose IGF-I serum concentrations were higher on the final day of dosing compared to day 1, indicating some degree of IGF-I accumulation. Intravenous administration of  $^{125}\text{I}$ -labeled rhIGF-I resulted in a wide distribution of radioactivity throughout the body, particularly in tissues with rich vascularization.

No metabolism studies have been conducted with rhIGF-I/rhIGFBP-3 or rhIGF-I. One excretion study has been conducted with rhIGF-I/rhIGFBP-3 in monkeys. Results obtained in this study showed that monkeys dosed with 25 or 100 mg/kg rhIGF-I/rhIGFBP-3 generally showed increased immunoreactive IGF-I or fragments of IGF-I in the urine through 72 hours after administration. It appears that an increased systemic exposure to rhIGF-I following administration of rhIGF-I/rhIGFBP-3 was observed, compared to administration of free rhIGF-I. A wide distribution of rhIGF-I throughout the body was noted, although this pattern of distribution may not be indicative of rhIGF-I/rhIGFBP-3 due to its ability

to bind ALS to form the large 150kD ternary complex. The excretion study with rhIGF-I/rhIGFBP-3 suggests that at relatively high doses, some immunoreactive IGF-I appears in the urine up to 72 hours after administration. However, it should be noted that this immunoreactive IGF-I may represent either full length IGF-I or immunoreactive IGF-I fragments or both.

In pediatric patients with severe GHIS, 1.0 mg/kg was administered by subcutaneous injection to 4 subjects in a pharmacokinetic sub-study of the phase 3 pivotal clinical trial. A summary of the pharmacokinetic parameters for IGF- I and IGFBP-3, uncorrected for baseline values, is presented in Table 1 and Figure 1. The assay employed does not distinguish between exogenous and endogenous IGF-I or IGFBP-3, respectively.

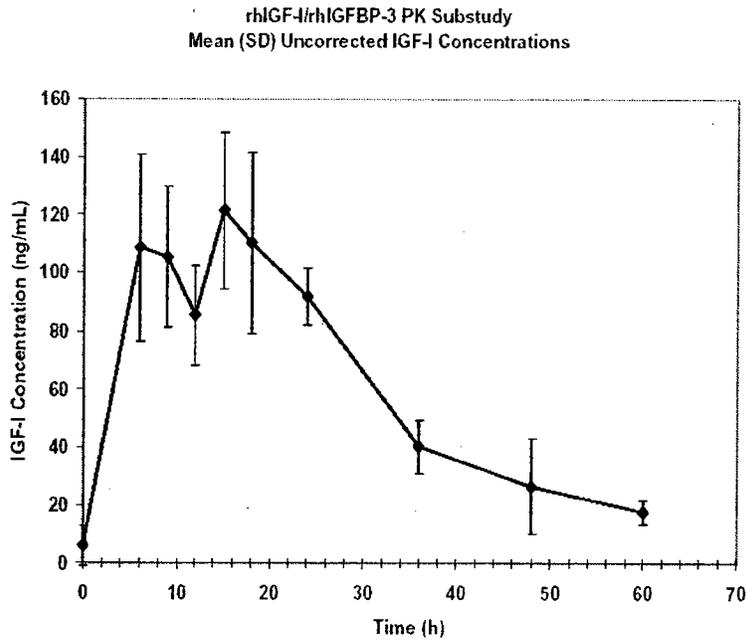
**Table 1. Pharmacokinetic Parameters in Patients with Severe GHIS Treated with [BRANDNAME] 1.0 mg/kg (n= 4); Mean (SD)**

	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (hr)	AUC <sub>0-60</sub> (ng hr/mL)	Half-life (hr)	Cl/F (mL/hr/kg)	V <sub>z</sub> /F (mL/kg)
IGF-I	133 (19)	11.3 (6.2)	3654 (237)	13.4 (2.7)	52.76 (2.94)	1018 (188)
IGFBP-3	1574 (401)	19.5 (9.0)	62525 (8352)	54.1 (31.6)	7.47 (3.97)	492 (32)

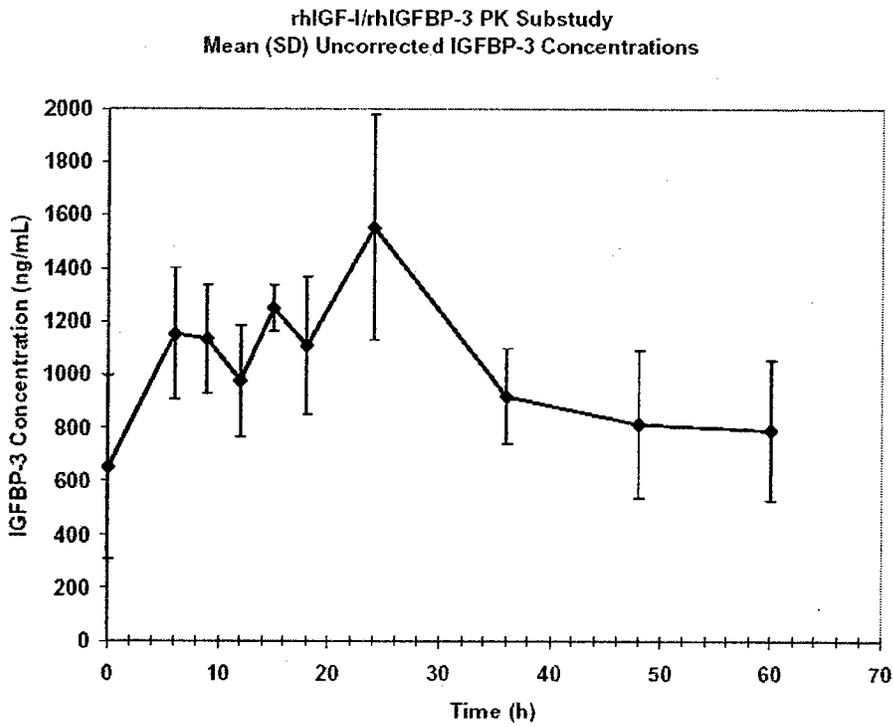
**Figure 1. Mean (SD) Uncorrected IGF-I (Panel A) and IGFBP-3 (Panel B) (ng/mL) Concentrations in Patients with Severe GHIS Treated with [BRANDNAME] 1.0 mg/kg (n=4)**

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Panel A.



Panel B.



**Title: Toxicokinetics Study of rhIGF-1/IGFBP-3 in Cynomolgus Monkeys (Section 4.2.1.2.a.1)**

**1. Purpose:**

To describe the plasma pharmacokinetics and urinary excretion of rhIGF-I/IGFBP-3 administered via intravenous (iv) injection to cynomolgus monkeys. The study was performed at

**2. Methods:**

Eighteen young adult male cynomolgus monkeys (2.3 to 3.3 kg) were randomly allocated into six groups of three monkeys each. Monkeys received a single intravenous administration of either rhIGF-I/IGFBP-3 (at 1, 10, 25, and 100 mg/kg); rhIGF-I (at 2.0 mg/kg); or rhIGFBP-3 (at 7.6 mg/kg -hereafter rounded to 8 mg/kg for clarity). Serial blood specimens were collected prior to dosing, at 5, 10, 15, 30, 45, 60 minutes after dosing, and at 2, 4, 8, 16, 24, 48, 72, and 96 hours post-dosing. The total volume of urine excreted was collected for urine drug concentration analyses at 0-24, 24-48, 48-72, and 72-96 hours after dosing. Serum concentration versus time curves were generated, the area under the curve (AUC) was determined by the trapezoid method, and serum clearance relative to body weight (CL/W) was determined by the ratio of dose to AUC.

**3. Results:**

Pharmacokinetic analysis demonstrated that the administration of rhIGF-I in a complex with rhIGFBP-3 results in slower clearance than the administration of equimolar doses of free rhIGF-I as shown in Table 2 below. The CL/W following administration of 10, 25, or 100 mg/kg rhIGF-I/IGFBP-3 was very similar for both rhIGF-I and rhIGFBP-3. The CL/W for 1 mg/kg rhIGF-I/IGFBP-3 was only slightly lower than that calculated for higher doses. One explanation for this profile is that ALS was saturated at approximately 1 mg/kg rhIGF-I/IGFBP-3. rhIGF-I and rhIGFBP-3 were also shown to be cleared from the circulation together following intravenous administration of rhIGF-I/IGFBP-3 even at a dose of 100 mg/kg. The fact that rhIGF-I and rhIGFBP-3 remain in approximately equimolar concentrations in the circulation suggests that rhIGF-I and rhIGFBP-3 remain associated in the circulation even at concentrations that probably exceed the binding capacity of ALS.

**Table 2:** Pharmacokinetic Parameters of rhIGF-I and rhIGFBP-3 (Mean  $\pm$  SD) Following IV Injection of rhIGF-I/IGFBP-3 in Monkeys.

Dose	N	AUC <sub>0-t</sub> (ng-hr/ml)		CL/W (ml/hr/kg)		C <sub>max</sub> (ng/ml)	
		IGF-I	IGFBP-3	IGF-I	IGFBP-3	IGF-I	IGFBP-3
1 mg/kg rhIGF-I/IGFBP-3	3	4611 $\pm$ 543	17615 $\pm$ 3695	44 $\pm$ 6	47 $\pm$ 9	4214 $\pm$ 239	13733 $\pm$ 1826
10 mg/kg rhIGF-I/IGFBP-3	3	29161 $\pm$ 1676	105953 $\pm$ 21042	69 $\pm$ 4	77 $\pm$ 14	42922 $\pm$ 6536	140433 $\pm$ 43343
25 mg/kg rhIGF-I/IGFBP-3	3	62236 $\pm$ 19773	293341 $\pm$ 557749	86 $\pm$ 28	70 $\pm$ 14	89538 $\pm$ 21477	464233 $\pm$ 83906
100 mg/kg rhIGF-I/IGFBP-3	3	329676 $\pm$ 73630	1703237 $\pm$ 732609	63 $\pm$ 12	54 $\pm$ 26	398672 $\pm$ 106105	1716133 $\pm$ 364160
2 mg/kg rhIGF-I	2	337 $\pm$ 181	-	6935 $\pm$ 3725	-	4428 $\pm$ 1447	-
6 mg/kg rhIGFBP-3	3	-	17815 $\pm$ 6653	-	509 $\pm$ 240	-	25967 $\pm$ 17406

AUC<sub>0-t</sub> = Area under the serum concentration versus time curve to last measurable time point.  
 CL/W = Serum clearance, dose (per kg) divided by AUC  
 C<sub>max</sub> = Maximum observed serum concentration

**Title: Recombinant Human Insulin-like Growth Factor-1 (rhIGF-1)  
 Pharmacokinetic/Pharmacodynamic Study in Cynomolgus Monkeys (Section 4.2.1.2.b.2)**

1. Purpose: The objective of this study was to measure metabolic parameters following intravenous injection, intravenous infusion and subcutaneous injection of Recombinant Human Insulin-like Growth Factor-1 (rhIGF-1) in Cynomolgus monkeys. This study was conducted at

2. Methods:

Eight Cynomolgus monkeys (Macaca fascicularis: 4 males and 4 females) were obtained from Two male and 2 female fasted Cynomolgus monkeys were dosed with intravenous bolus injections of 0 (Vehicle), 0.1, and 0.5 mg rhIGF-1/kg. Blood samples were taken at predose and periodically up to 24 h after dosing to measure the levels of glucose, insulin, free fatty acids, glucagon, cortisol and blood urea nitrogen. The blood sampling regimen and the metabolic parameters measured on each occasion of the treatment.

3. Results: There were no adverse reactions in the animals during the treatments. There was a dose related transient reduction in non esterified free fatty acids up to 60 minutes after the treatment and a reduction in insulin level up to 180 min post dose (last sampling point). A reduction in glucose level was also noted up to 1 hour post dose following treatment at 0.5 mg rhIGF-1/kg as shown below. The levels of glucagon were not affected significantly, though the mean values of cortisol were also increased initially, which was leveled off 60 minutes after the treatment at 0.5 mg rhIGF/kg dose. It appears that no other metabolic parameters were affected.

TABLE 1 (continued)

Metabolic Parameters: Individual and Mean Values  
Day 38: 0.5 mg rhIGF-1/kg<sup>1</sup>

Metabolic Parameters	Animal/ Sex	Time Relative to Dosing				
		0	+30 min	+60 min	+180 min	+24 h
Glucose	3σ	4.66	1.97	2.37	4.25	-
	4σ	5.60	1.44	1.36	2.77	-
	7σ	3.83	2.10	2.15	3.21	-
	8σ	4.22	1.19	1.05	2.11	-
	Mean		4.58	1.68	1.73	3.08
Insulin	3σ	86.1	19.3	10.2	8.3	-
	4σ	21.5	14.8	8.0	5.7	-
	7σ	56.8	10.1	5.9	5.0	-
	8σ	26.4	15.3	9.4	5.0	-
	Mean		47.7	14.9	8.4	6.0
Glucagon	3σ	394	775	775	281	-
	4σ	256	313	269	185	-
	7σ	141	550	388	116	-
	8σ	203	241	225	187	-
	Mean		249	470	414	192
Cortisol	3σ	1140	753	614	365	-
	4σ	855	1366	1103	1081	-
	7σ	796	1360	719	546	-
	8σ	789	1378	867	713	-
	Mean		895	1214	826	676
Non Esterified Fatty Acid	3σ	0.23	0.16	0.34	0.60	-
	4σ	0.40	0.12	0.28	0.55	-
	7σ	0.19	0.09	0.22	0.34	-
	8σ	0.67	0.09	0.24	0.72	-
	Mean		0.37	0.12	0.27	0.55
Blood Urea Nitrogen	3σ	7.8	7.7	7.6	7.4	7.2
	4σ	6.7	7.0	7.1	7.3	7.0
	7σ	8.5	8.4	8.4	7.5	6.9
	8σ	7.4	7.7	7.8	7.2	6.2
	Mean		7.6	7.7	7.7	7.4

- = Not applicable

**Title: Pharmacokinetics of rhIGF-1/IGFBP-3 following subcutaneous injection in male rats**

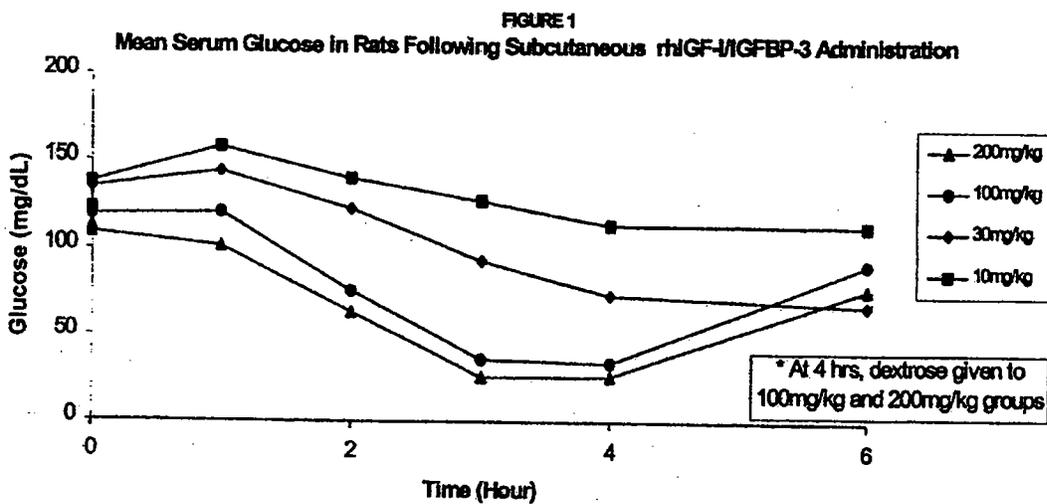
1. Purpose: To evaluate the extent of systemic exposure in adult male rats following subcutaneous (SC) administration of recombinant human insulin-like growth factor-1 and insulin-like growth factor binding protein-3 (rhIGF-I/IGFBP-3). In addition to measurements of serum IGF-I concentrations, serum glucose concentrations were determined. This study was performed by Celtrix Pharmaceuticals, Inc.

2. Methods: Adult male rats (250 to 300 grams) were administered 10, 30, 100, or 200 mg/kg recombinant human insulin-like growth/ insulin-like growth factor binding protein-3 (rhIGF-I/IGFBP-3) by bolus subcutaneous injection (SC). Blood samples were collected for determination of IGF-I and glucose concentrations. Single bolus injections of 10 and 30 mg/kg were well tolerated, but doses of 100 and 200 mg/kg produced severe hypoglycemia. The areas under the serum IGF-I concentration versus time curves (AUC) were calculated using the trapezoid method.

### 3. Results:

No remarkable abnormal clinical observations were noted in the 10 and 30 mg/kg dose groups. Animals in the higher dose groups had a decreased activity, tremors and, in some cases, convulsions. Animals displaying these signs were treated with dextrose. Three of eight animals in the 100 mg/kg dose group and all eight of the animals in the 200 mg/kg dose group displayed extreme lethargy, tremors, and/or convulsions. Despite the administration of dextrose, the effects were not completely resolved in the high dose group and all animals in Group 4 were euthanized at 8 or 16 hours following dosing.

Mean serum glucose is plotted versus time through the first six hours in Figure 1. It is clear that doses of 100 mg/kg and greater induce marked hypoglycemia. The sharp increase in serum glucose concentrations in the higher dose groups at four hours after injection corresponds to dextrose administration. All animals were given food after six hours and there is a corresponding increase in glucose concentrations. Pharmacokinetic data are summarized in a table below.



Dose mg/kg	AUC ng-hr/mL	Minimum Serum Glucose mg/dL	Abnormal Clinical Observations
10	22,958	106	None
30	32,574	61	None
100	42,856	28	tremors, convulsions 3/8
200	50,251	17	tremors, convulsions 8/8

Serum clearance was calculated and found to increase with dose. This phenomenon has been observed previously (PT-95-1121R).

### Title: Pharmacokinetics of rhIGF-1/IGFBP-3 following intravenous injection in male rats

1. Purpose: To characterize the pharmacokinetic profiles of rhIGF-I and IGFBP-3 following a single intravenous administration of rhIGF-1, rhIGFBP-3, or rhIGF-I/IGFBP-3. In addition, the sponsor wished to compare the pharmacokinetic data of IGF-1/IGFBPs after subcutaneous and iv administration in rats.

#### 2. Methods:

Adult male Sprague-Dawley rats (N=3/group) weighing approximately 400 grams were anesthetized and cannulas were placed into their jugular veins. Following a recovery period of two days, rats received an intravenous bolus injection of vehicle, rhIGF-I (at 0.02, 0.2, 2.0, or 5.0 mg/kg), rhIGF-I/IGFBP-3 (at 1, 10, 100, or 200 mg/kg), or rhIGFBP-3 (at 0.8, 8, 80, and 160 mg/kg). Blood specimens were collected just prior to dosing and at 5, 20, and 40 minutes and 1, 2, 3, 4, 6, 8, 24, 32, 48, 56 and 72 hours after administration. Serum clearance relative to body weight (CL/W) was determined by the following equation:  $CL/W = Dose/AUC$ .

#### 3. Results:

Effects of IGF-1 (0.02, 0.2, 2 and 5 mg/kg) and IGF-1/IGF-BP-3 on serum glucose kinetics after iv administration are summarized in Fig. 1. IGF-1 reduced blood glucose rapidly after 2 and 5 mg/kg. In the presence of IGFBP-3, however, the rate of reduction was somewhat blunted. It appears that IGFBP-3 itself had no effect on serum glucose level (Fig. 2). Pharmacokinetic data in this study are summarized in Table 2 below.

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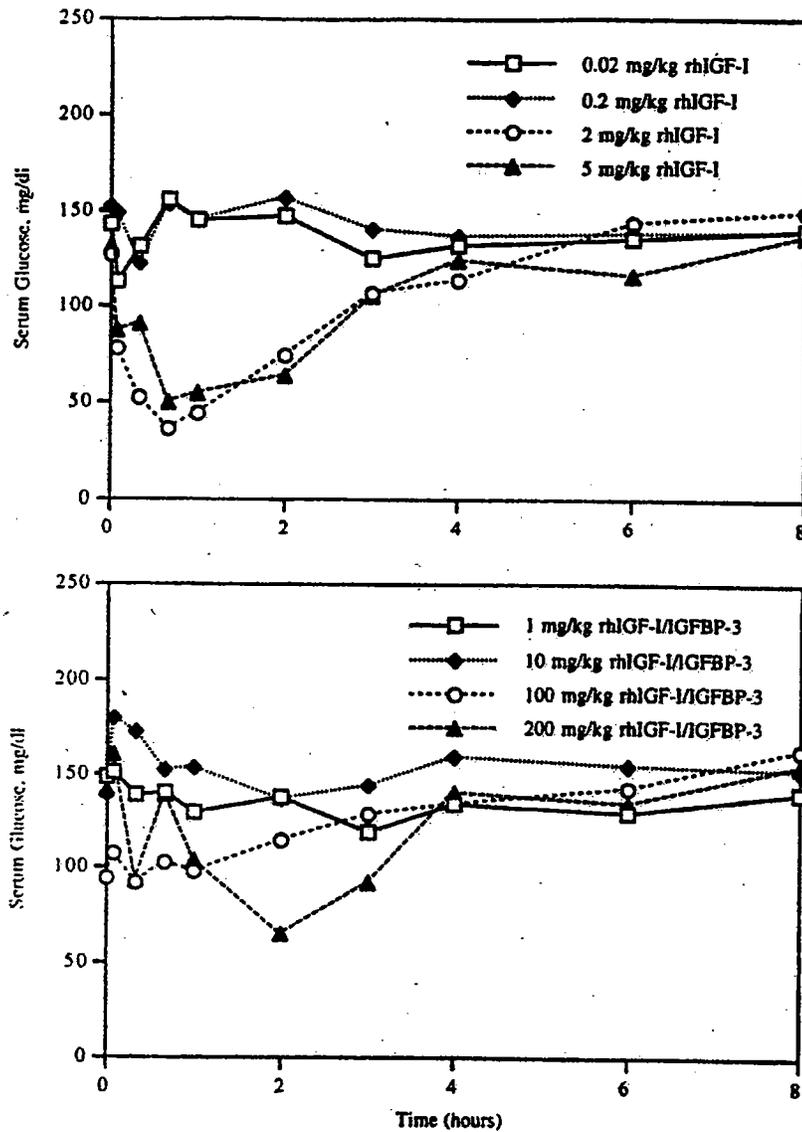


Figure 1: Serum glucose kinetics following I.V. administration of 0.02, 0.2, 2.0, and 5.0 mg/kg rhIGF-I (above) and 1, 10, 100, and 200 mg/kg rhIGF-I/IGFBP-3 (below) to rats. Each timepoint represents the mean of three animals.

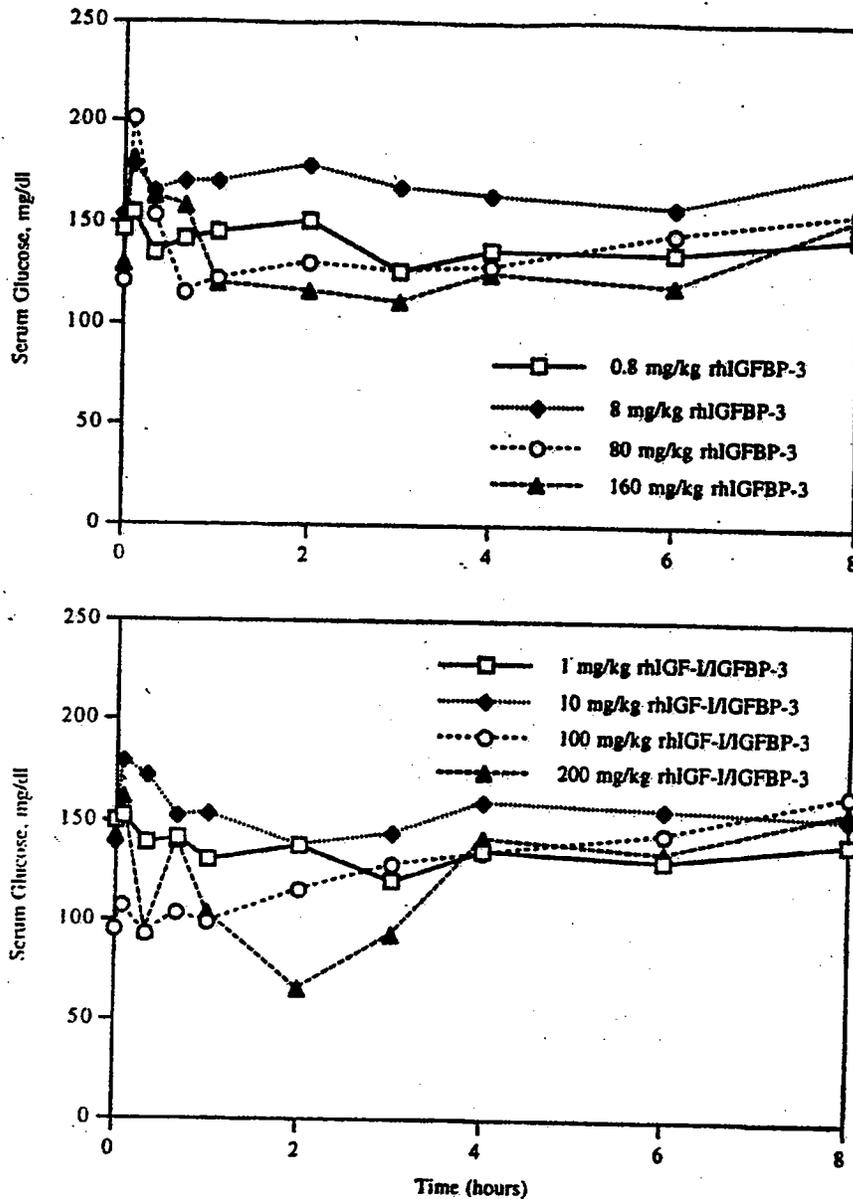


Figure 2: Serum glucose kinetics following I.V. administration of 0.8, 8, 80, and 160 mg/kg rhIGFBP-3 and 1, 10, 100, and 200 mg/kg rhIGF-I/IGFBP-3 to rats. Each timepoint represents the mean of three animals.

**Table 2** Pharmacokinetic Parameters (Mean  $\pm$  SD) of rhIGF-I Following IV Injection of rhIGF-I or rhIGF-I/IGFBP-3 in Rats.

Test Article & Treatment Dose (mg/kg)	AUClast (ng-hr/ml)	CL/W (ml/hr/kg)	Cmax (ng/ml)
0.02 mg/kg rhIGF-I	588 $\pm$ 12	34 $\pm$ 1	338 $\pm$ 29
0.2 mg/kg rhIGF-I	971 $\pm$ 554	249 $\pm$ 116	1081 $\pm$ 1062
2.0 mg/kg rhIGF-I	2734 $\pm$ 586	762 $\pm$ 144	7571 $\pm$ 2520
5.0 mg/kg rhIGF-I	5710 $\pm$ 836	889 $\pm$ 134	19911 $\pm$ 6644
1 mg/kg rhIGF-I/IGFBP-3	2875 $\pm$ 588	71 $\pm$ 14	637 $\pm$ 25
10 mg/kg rhIGF-I/IGFBP-3	12769 $\pm$ 4328	167 $\pm$ 48	4644 $\pm$ 1186
100 mg/kg rhIGF-I/IGFBP-3	38065 $\pm$ 6710	536 $\pm$ 89	46434 $\pm$ 1977
200 mg/kg rhIGF-I/IGFBP-3	79236 $\pm$ 10218	511 $\pm$ 69	116553 $\pm$ 19115

**Title: Pharmacokinetics of rhIGF-1/IGFBP-3 following a single subcutaneous injection to SD rats**

1. Purpose: The objectives of this study were to characterize rhIGF-1/rhIGFBP-3 and to assess the relative exposure between the products from different sites after a single subcutaneous injection in SD rats. The study was performed under OECD GLP guidelines in February, 2004 at the lot numbers of original and new materials were DP0204 and B2114/Complex/002, respectively.

2. Methods:

Sprague-Dawley CD/Hsd rats whose body weight ranged 211 to 261 g in males and 157 to 193 g in females were used in this study. The estimated age at the time of dosing was 6-11 weeks. The animals were divided into 6 groups and each group had 12 rats/sex/group. They received a single subcutaneous injection of either original or new rhIGF-1/IGFBP-3 at doses of 1, 10, and 30 mg/kg as shown below. Serial blood samples (0.5 ml) were taken from four sub-groups of 3 males and 3 females per group at the following time points:

- Sub-group 1: Predose, 0.5, 4, and 24 hours
- Sub-group 2: 5 and 40 minutes, 6 and 32 hours;
- Sub-group 3: 15 minutes, 1 and 8 hours;
- Sub-group 4: 30 minutes, 2 and 12 hours

Group	Test compound	Dose (mg/kg)	Number of Animals	Animal numbers	
				Male	Female
1	Original	1	12M + 12F	1 - 12	73 - 84
2	Original	10	12M + 12F	13 - 24	85 - 96
3	Original	30	12M + 12F	25 - 36	97 - 108
4	New	1	12M + 12F	37 - 48	109 - 120
5	New	10	12M + 12F	49 - 60	121 - 132
6	New	30	12M + 12F	61 - 72	133 - 144

Results: Pharmacokinetic data of IGF-1 such as achieved dose level, C<sub>max</sub>, AUC<sub>0-32hr</sub> in the original and new materials are summarized below. The rate of systemic exposure, which is characterized by C<sub>max</sub>, AUC increased as doses increased, though the increases were not dose-proportional. Pharmacokinetic parameters of IGF-1 are summarized below.

#### Original material

Nominal dose level (mg/kg)	Achieved dose level ratio		C <sub>max</sub> ratio		AUC <sub>32</sub> ratio	
	Males	Females	Males	Females	Males	Females
	1	1	9	1	1	1
10	9.5	9.6	11.9	13.9	12.3	13.2
30	27.8	27.8	13.3	17.0	16.6	21.1

#### New material

Nominal dose level (mg/kg)	Achieved dose level ratio		C <sub>max</sub> ratio		AUC <sub>32</sub> ratio	
	Males	Females	Males	Females	Males	Females
	1	1	1	1	1	1
10	9.8	9.8	9.0	8.0	10.5	8.2
30	30.5	30.5	10.2	10.2	15.2	13.7

Pharmacokinetic parameters of human IGF-1 are presented in Table 7 and summarized below:

Group	Nominal dose level (mg/kg)	C <sub>max</sub> (ng/mL)		T <sub>max</sub> (hours)		AUC <sub>32</sub> (ng.h/mL)		t <sub>1/2</sub> (hours)	
		Males	Females	Males	Females	Males	Females	Males	Females
		1	527	408	6	4	5430	4420	5.4
Original material	10	6270	5690	4	4	67000	58200	4.0	4.2
Original material	30	7020	6930	4	2	90400	93100	4.3	4.3
Original material	1	675	717	4	4	5920	5880	8.2 <sup>a</sup>	8.5 <sup>a</sup>
New material	10	6080	5730	4	4	61900	48400	3.8	3.7
New material	30	6870	7280	4	4	89800	80500	3.7	4.7
New material									

<sup>a</sup> Value should be regarded as an estimate since terminal rate constant could not be adequately determined (regression coefficient was <0.95 and fraction of variance accounted for <0.90)

The maximum mean measured serum concentrations (C<sub>max</sub>) of human IGFBP-3 generally occurred at 2 or 4 hours post-dose, although for the original material, C<sub>max</sub> occurred at 1 hour in

males at the 30 mg/kg dose level. The serum concentrations declined fairly rapidly, and were below the limit of quantification (< —

ng/ml) by 24 hours at the low dose (1 mg/kg) and by 32 hours at the 10 mg/kg dose level. At 32 hours post-dose, after administration of 30 mg/kg, serum concentrations were below the limit of quantification following administration of the original material, but were still quantifiable following administration of the new material. The relationships between maximum mean serum concentrations (C<sub>max</sub>) of human IGFBP-3, areas under the mean serum human IGFBP-3 concentration-time curves (AUC) and dose level are presented below.

#### Original material

Nominal dose level (mg/kg)	Achieved dose level ratio		C <sub>max</sub> ratio		AUC <sub>32</sub> ratio	
	Males	Females	Males	Females	Males	Females
1	1	1	1	1	1	1
10	9.5	9.6	6.8	6.7	6.1	7.1
30	27.8	27.8	7.0	9.1	7.4	10.7

#### New material

Nominal dose level (mg/kg)	Achieved dose level ratio		C <sub>max</sub> ratio		AUC <sub>32</sub> ratio	
	Males	Females	Males	Females	Males	Females
1	1	1	1	1	1	1
10	9.8	9.8	4.2	4.2	5.0	4.7
30	30.5	30.5	5.0	5.4	6.4	6.3

Group	Nominal dose level (mg/kg)	C <sub>max</sub> (ng/mL)		T <sub>max</sub> (hours)		AUC <sub>32</sub> (ng·h/mL)		t <sub>1/2</sub> (hours)	
		Males	Females	Males	Females	Males	Females	Males	Females
1 Original material	1	2410	2050	4	4	24900	17000	5.2 <sup>a</sup>	3.8 <sup>a</sup>
2 Original material	10	16300	13700	4	4	151000	120000	4.8	4.3 <sup>b</sup>
3 Original material	30	16800	18600	1	2	185000	182000	4.0	4.4
4 New material	1	3350	3270	4	4	30000	25100	5.6 <sup>b</sup>	5.4 <sup>a</sup>
5 New material	10	14200	13800	2	4	150000	117000	4.6	4.8
6 New material	30	16900	17500	2	2	193000	159000	4.2	5.7

<sup>a</sup> Value should be regarded as an estimate since terminal rate constant could not be adequately determined (period over which t<sub>1/2</sub> was calculated was less than twice the half-life itself)

<sup>b</sup> Value should be regarded as an estimate since terminal rate constant could not be adequately determined (regression coefficient was <0.95 and fraction of variance accounted for <0.90)

#### 4. Conclusion:

Following single subcutaneous bolus administration, the rate and extent of systemic exposure of rats to rhIGF-I/rhIGFBP-3 appeared to be characterized by dose-dependent (non-linear) kinetics. Increasing the dose above 1 mg/kg resulted in serum concentrations lower than those expected from a linear relationship, which is consistent with the possibility of a capacity limited process for the systemic availability of rhIGF-I/rhIGFBP-3, although an increase in systemic clearance at the higher doses would have a similar effect.

Following administration of rhIGF-I/rhIGFBP-3 at dose levels of 10 and 30 mg/kg, the rate (C<sub>max</sub>) and extent (AUC) of systemic exposure of rats to human IGF-I and human IGFBP-3 in the new material was similar to those indices of exposure of the original material, batch number DPO204. Based on the data obtained from the studies, the sponsor concluded that the change in the manufacturing process/site used for the test material is not considered to have any clinical or toxicological significance with respect to the overall safety profile of the material, which the reviewer agrees.

#### **Title: Toxicokinetics Study of rhIGF/IGFBP-3 in Cynomolgus Monkeys**

1. Purpose: To define the serum pharmacokinetics and urinary excretion of rhIGF-I/IGFBP-3 following a single intravenous administration to cynomolgus monkeys, including hypoglycemic response following test article administration. This study was performed by — for Celtrix Pharmaceuticals, Inc., Santa Clara, CA.

#### 2. Methods:

The test articles for this study were rhIGF-I, rhIGFBP-3, and a complex of these two peptides. Eighteen young adult male cynomolgus monkeys were randomly allocated into six groups of three monkeys each as shown below. The monkeys in each of the six treatment groups received a single intravenous administration of one of the following test articles and dosages: the peptide complex at 1, 10, 25, and 100 mg/kg, rhIGF-I at 2.0 mg/kg, or rhIGFBP-3 at 7.6 mg/kg. Serial blood specimens were collected prior to dosing (3 samples) and 5, 10, 15, 30, 45, 60 min, 2, 4, 8, 16, 24, 48, 72, and 96 hours post-dosing. Each monkey was observed at least twice daily for overt signs of toxicity and changes in general behavior and appearance during the study. Furthermore, the animals were closely evaluated for signs of hypoglycemic shock during and after dosing. Body weights were measured at time of assignment to dose groups, just prior to dosing, and at the completion of the in-life phase.

*Appears This Way  
On Original*

Group Number	Animal Numbers (Males)	Dose Level (mg/kg)	Test Article	Treatment Regimen
1	101, 102, 103	1	complex <sup>a</sup> : low dose	Single intravenous
2	201, 202, 203	10	complex: mid dose	Single intravenous
3	301, 302, 303	25	complex: high dose	Single intravenous
4	401, 402, 403	100	complex: very high dose	Single intravenous
5	501, 502, 503	2.0	rhIGF-I	Single intravenous
6	601, 602, 603	7.6	rhIGFBP-3	Single intravenous

a. Complex = rhIGF-I/IGFBP-3.

### 3.Results:

There were no remarkable abnormal clinical observations recorded for any animal in Groups 1, 2, 3 (complex; 1, 10, and 25 mg/kg, respectively) and 6 (7.6 mg/kg rhIGFBP-3). The clinical observations for Groups 4 (100 mg/kg complex) and 5 (2.0 mg/kg rhIGF-I) were consistent with acute hypoglycemia (i.e., lethargy/inactivity followed by unresponsiveness/recumbency) and corresponded with marked reduction of serum glucose levels. These clinical indications were occasionally accompanied by pupil dilation and tremors that were also attributed to acute hypoglycemia. Following the oral administration of 50 percent dextrose, all animals appeared normal within 20 minutes or less and remained normal for the remainder of the study. Please see Fig. 1 and 2 for the effects of the complex (Fig. 1) and rhIGF-1 and rhIGFBP (Fig. 2) below. An inspection of the two figures demonstrated that the rate of decreases in serum glucose after 100 mg/kg of the complex was much slow that that after 2 mg/kg doses of rhIGF-1 alone. rhIGFBP-3 had no effect on serum glucose as seen in many previous pharmacokinetics of rhIGF-1 alone or in combination with rhIGFBP-3. Data on rhIGF-1 or rhIGFBP concentrations were not provided.

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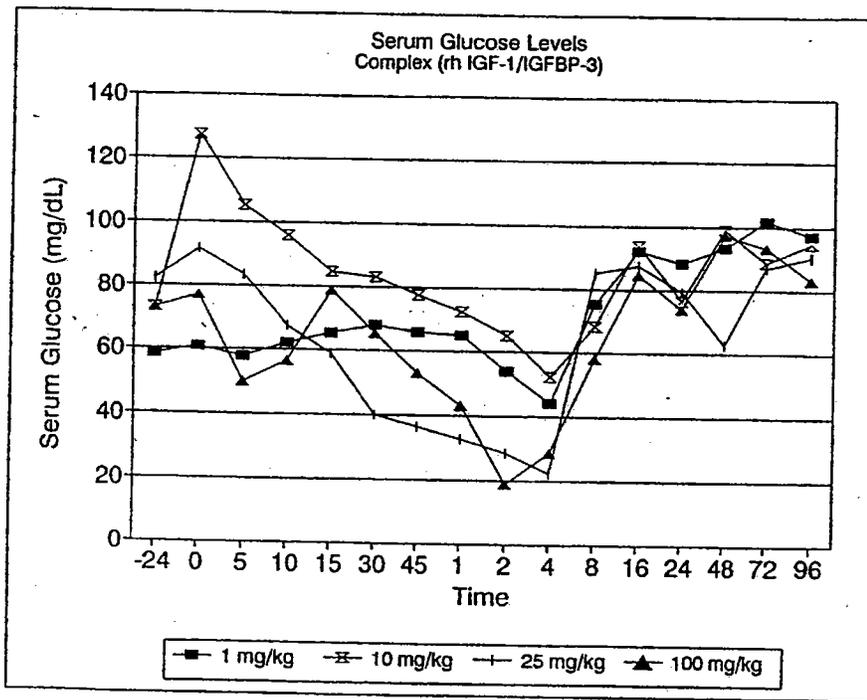


Figure 1. Serum Glucose Levels (Groups 1-4)

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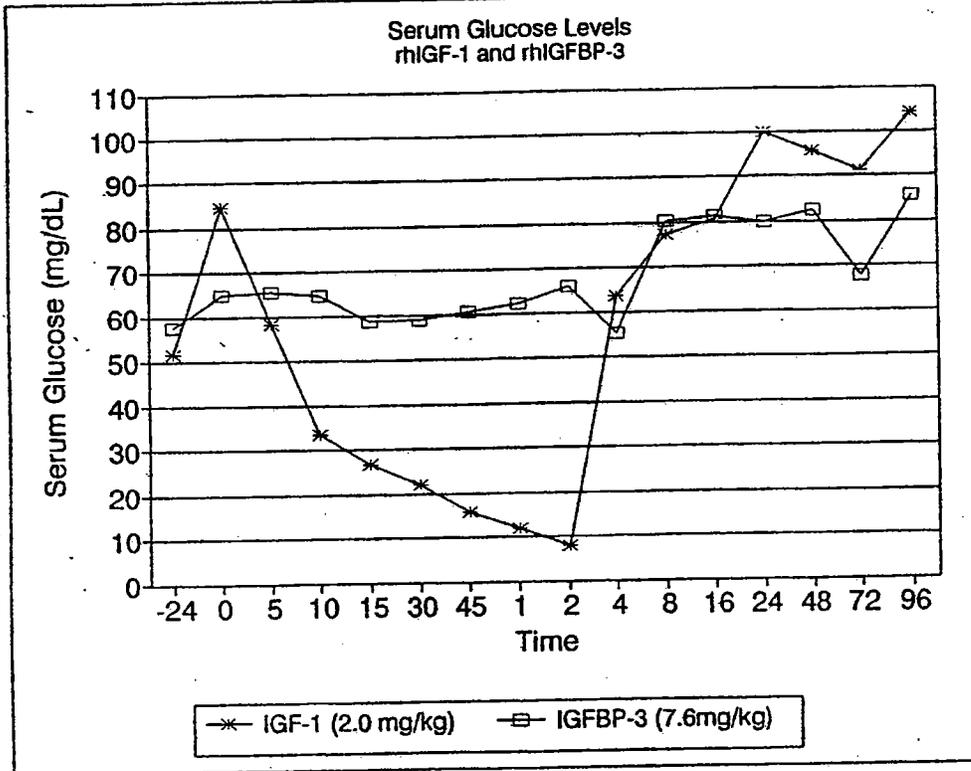


Figure 2. Serum Glucose Levels (Groups 5-6)

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**Title: PK profiles of IGF-1 and IGF-1/IGFBP-3 following their administration in pigs  
4.2.2.2.a.6**

1. Purpose: To define the serum pharmacokinetics of rhIGF-I/IGFBP-3 following a single intravenous and subcutaneous administration to pigs. This study was performed by \_\_\_\_\_ for Celtrix Pharmaceuticals, Inc., Santa Clara, CA.
2. Methods: Human recombinant IGF-1 and IGFBP-3 were obtained from \_\_\_\_\_. IGF-1 and IGFBP-3 complex was prepared one day prior to administration. Two pigs were implanted with indwelling catheters. One pig was dosed IGF-1 and IGFBP-3 at a dose of 0.2 mg/kg intravenously and another one was dosed with IGF-1 and IGFBP-3 at a dose of 1 mg/kg subcutaneously. Following 3-day washout period, the pigs were crossed-over for the route of administration. Blood samples were taken at 0.25h before treatment and at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, and 48 hours after administration of IGF-1 alone or IGF-1 and IGFBP-3 complex. IGF-1 and IGFBP-3 analyses were carried out by radioimmunoassay \_\_\_\_\_ and pharmacokinetic parameters were calculated using log-linear trapezoidal rule.
3. Results: The mean serum concentrations of IGF-1 of two pigs were approximately 150 ng/ml before any treatment while the baseline IGFBP-3 values were little less than 20 ng/ml in pigs. Intravenous administration of IGF-1 and IGFBP-3 (0.2 mg/kg) produced C<sub>max</sub> level of 7 µg/ml in 15 minutes while subcutaneous administration of IGF-1 and IGFBP-3 (1 mg/kg) produced 2 µg/ml in 30 minutes in the first pig (#58). The values in another pig (#61) were quite different in terms of C<sub>max</sub> and T<sub>max</sub> as shown (Table 1). The animal individual variability was clear in AUC, clearance rate, and steady state distribution volume after an intravenous administration.

Pharmacokinetic parameters of IGFBP-3 after an intravenous and subcutaneous administration are summarized in Table 2. C<sub>max</sub> was the highest (\_\_\_\_\_/ml) in 15 minutes after subcutaneous administration while animal#61 had C<sub>max</sub> value after 6 hours as a T<sub>max</sub> in the study. t<sub>1/2</sub> was about twice longer after subcutaneous administration in IGF-1 while the t<sub>1/2</sub> values in IGFBP-3 was reduced after subcutaneous administration, compared to the value after an intravenous administration. The mean residence time of IGF-1 alone and with IGFBP-3 complex significantly increased after the subcutaneous administration, compared to the intravenous administration, which might justify the use of subcutaneous administration route.

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Table 1: Pharmacokinetic parameters of IGF-1 after bolus injection of IGF-1+IGFBP-3 complex.

PARAMETER	ROUTE			
	IV		SC	
	PIG #58	PIG #61	PIG #58	PIG #61
T max (hours)				
C max (µg/ml)				
t 1/2 (hours)				
AUC (pig/µl) hour				
MRT (hours)				
Cl (l/hour)				
V dss (liter)				

Table 2: Pharmacokinetic measurements of BP3 after bolus injection of IGF-1+IGFBP-3 complex.

PARAMETER	IV		SC	
	PIG #58	PIG #61	PIG #58	PIG #61
	T max (hours)			
C max (µg/ml)				
t 1/2 (hours)				
AUC (µg/ml) - hours				
MRT (hours)				
Cl (liter/hours)				
V dss (liter)				

**Title: Pharmacokinetics of rhIGF-1 in the rats (4.2.2.2.b.1)**

1. Purpose: To document pharmacokinetics of IGF-1 after their several doses of intravenous and subcutaneous administrations in rats.

2. Methods: The source of rh-IGF-1 was — (Batch#:36295, of which purity was only —, where the study was performed. Male rats (40-50 days old and weighed 222-273 g) received IGF-1 at doses of 0.25, 3 and 16 mg/kg intravenously or 2 and 16 mg/kg subcutaneously. Each treatment included 12 rats randomly divided into 3 groups of 4 rats. Blood samples (0.3 ml) were collected from a tail vein at 0, 5, 10, 15, 30, 45, 60, 90, 120, 240, 480 and 600 minutes randomly so that no rats were subjected to bleed more than 4 times.

3. Results: The sponsor provided the following table as results of the study after 2 mg/kg iv and 16 mg/kg subcutaneously. The results do not offer any interpretation of the pharmacokinetic parameters of IGF-1 in rats since there were several PK data in this species. The title of the study appears to be dealt with both the rhIGF-1 and rhIGFBP-3. However, there were no data as to the complex, but only the data with IGF-1 alone.

Pharmacokinetic parameters for IGF-1 in the rat after 2 intravenous and 2 subcutaneous doses.

Parameter	Dose 2 mg/kg	Dose 16 mg/kg
Given dose (median) i.v.	2.43 mg/kg	18.90 mg/kg
Given dose (median) s.c.	2.72 mg/kg	18.38 mg/kg
Clearance (Dose i.v./Area under curve (AUC) i.v.)	4.9 ml/kg/min	4.9 ml/kg/min
AUC i.v. dose	496 µg/ml x min	3885 µg/ml x min
AUC s.c. dose	592 µg/ml x min	3140 µg/ml x min
AUC residual i.v. dose	3.2%	<1%
AUC residual s.c. dose	15.9%	<1%
Bioavailability		
<u>AUC s.c./dose s.c.</u>		
AUC i.v./dose i.v.	107%	83%
C <sub>max</sub> s.c. dose	2.5 µg/ml	16.9 µg/ml
T <sub>max</sub> s.c. dose	20 min	30 min
t <sub>½</sub> declining phase s.c. dose	215 min	100 min

**Title: Pharmacokinetics of rhIGF-1 in the dog following intravenous injection of 0.5 mg/kg and subcutaneous administration of 0.5 and 2.5 mg/kg**

1. Purpose: To study the pharmacokinetics of rhIGF-1 in the dogs at a dose of 0.5 mg/kg intravenously and subcutaneously. In addition, the sponsor also studied the pharmacokinetics of rhIGF-1 in the dogs at a dose of 2.5 mg/kg subcutaneously only. The study was conducted by

2. Methods:

Four female beagle dogs (age: 2- 3 years old, 12.4 kg) were used. Five ml blood samples were collected at -15, 0, 15, 30, 60, 90, 120, 150 180, 210 240, 270, 300, 360, 480 and 600 minutes after the treatment. The sponsor only determined the serum concentrations as a function of time after drug administration.

3. Results:

In two dogs (#249), the serum concentrations from -15 and 0 minutes increased gradually as the time of samplings were increased up to 5 hours after an intravenous administration. From that point the serum concentrations decreased up to approximately 1440 minutes. Serum

concentrations were significantly reduced after a subcutaneous injection of 0.5 mg/kg IGF-1 as expected.

**Title: Recombinant Human IGF-1 PK/PD study in Cynomolgus monkeys: Phase I- Pharmacokinetic Study.**

1. Purpose and Methods: The objective of this study was to compare the kinetic profiles of IGF-1 in monkey following a single dose administration of the material by intravenous bolus injection, intravenous infusion and subcutaneous bolus injection, respectively. Four monkeys/sex were rotated to receive either 0.1 or 0.5 mg/kg IGF-1 in intravenous bolus or subcutaneous bolus injection. After the washout period they also received 0.5 mg/kg IGF-1 either subcutaneous bolus injection or intravenous infusion over a 2-hour period. Serial blood sampling (0, 2, 5, 10, 15, 30, 45, 90, 120, 240, 480, and 600 minutes ) was obtained on each day of treatment prior to dosing and periodically up to 10 h after dosing to measure "total IGF-1" concentrations in serum.

2. Results: Following an intravenous injection of 0.1 mg/kg IGF-1, the Cmax was observed in a matter of 2 minutes, which was also true after 0.5 mg/kg dose as shown two tables below. Cmax was revealed approximately after 90 to 120 minutes after the subcutaneous administration. Intravenous infusion over 2 hours in restrainer also produced Cmax in 90 to 120 minutes after the treatment. Table 7 summarized all pharmacokinetic parameters in cynomolgus monkeys after IGF-1 treatment. Tmax, t<sub>1/2</sub>, Cmax, and AUC were all expected from the previous experiments in different species.

Dose: 0.1 mg/kg, intravenous bolus

Animal No	Serum concentration of "total" IGF-1, µg/ml											
	0	2	5	10	15	30	45	90	120	240	480	600
1												
2												
5												
6												

Dose: 0.5 mg/kg, intravenous bolus

Animal No	Serum concentration of "total" IGF-I, $\mu\text{g/ml}$											
	Minutes											
	0	2	5	10	15	30	45	90	120	240	480	600
3												
4												
7												
8												

Dose: 0.1 mg/kg, subcutaneous bolus

Animal No	Serum concentration of "total" IGF-I, $\mu\text{g/ml}$									
	Minutes									
	0	10	25	40	60	90	120	240	480	600
1										
2										
5										
6										

Dose: 0.5 mg/kg, subcutaneous bolus

Animal No	Serum concentration of "total" IGF-I, $\mu\text{g/ml}$									
	Minutes									
	0	10	25	40	60	90	120	240	480	600
3										
4										
7										
8										

Dose: 0.5 mg/kg, intravenous infusion (120 minutes)

Animal No	Serum concentration of "total" IGF-I, $\mu\text{g/ml}$											
	Minutes											
	0	90	120	125	130	135	150	165	210	240	360	600
1												
2												
5												
6												

Summary. Table 7 Pharmacokinetic parameters in cynomolgus monkeys following administration of rhIGF-1

Treatment	Monkey no.	t1/2,alfa (min)	t1/2,beta (min)	t1/2 (min)	Cmax (ug/ml)	Tmax (min)	AUC (0-6h) F (%) <sup>*</sup>
0.1 mg/kg, IV bolus	1						
	2						
	5						
	6						
0.5 mg/kg, IV bolus	3						
	4						
	7						
	8						
0.1 mg/kg, SC bolus	1						
	2						
	5						
	6						
0.5 mg/kg, SC bolus	3						
	4						
	7						
	8						
0.5 mg/kg, IV inf. (2 hrs)	1						
	2						
	5						
	6						
MEAN, 0.1 mg/kg, IV	6	623		1.26		91	
MEAN, 0.5 mg/kg, IV	10	417 (n=3)		8.23		386	
MEAN, 0.1 mg/kg, SC			1326	0.41	240	195	
MEAN, 0.5 mg/kg, SC			705	0.74	205	374	
MEAN, 0.5 mg/kg, IV INF	22	734		1.15		321	
MEAN F, 0.1 and 0.5 mg/kg						160	

<sup>\*</sup>) F, bioavailability, is calculated as (AUCsc(0-6 hours) / AUCiv(0-6 hours) X 100) for each monkey at each dose level.

<sup>\*\*</sup>) Cmax = Serum concentration at 2 minutes.

nd-not determined

## 2.6.6 TOXICOLOGY

### 2.6.6.1 Overall toxicology summary

The single dose toxicity of intravenously administered rhIGF-I/rhIGFBP-3 has been studied in rats and monkeys at doses up to 200 mg/kg. Except for the expected decreases in blood glucose, there were no treatment-related indications of toxicity. Similar studies conducted with free rhIGF-I in mice and rats at doses up to 80 mg/kg produced no signs of toxicity. In monkeys, subcutaneous administration of 1 mg/kg was well tolerated with no remarkable signs of toxicity.

Repeat dose toxicity studies with rhIGF-I/IGFBP have been conducted for up to 13 weeks in rats and monkeys with doses of 10 to 30 mg/kg/day, which give exposures 1 to 4 times the average human dose, based on body surface area comparison. A 13-Week mouse study and 26-Week toxicology studies in rats and monkeys were conducted with IGF-1 alone. The 26-Week toxicology study in rat was carried out at a high dose of 10 mg/kg/day, which provides an exposure 1 times the average human dose, based on body surface area comparison. The 26-Week toxicology study in monkeys was performed with IGF-1 alone at a high dose of 1 mg, which gives an exposure 0.2 times the average human dose, based on body surface area comparison.

In general, results of the studies with IGF-1 alone were similar to those observed in the repeat dose studies conducted with rhIGF-1/rhIGFBP-3. For example, in mice, administration of rhIGF-1 at doses up to 10 mg/kg/day for 13 weeks resulted in increased body weights and an increased weight of the thymus. In rats, rhIGF-1 administration at doses up to 10 mg/kg/day for 26 weeks resulted in increased body weights and an increased incidence of reactive hyperplasia of the submandibular lymph nodes. Similarly, an increase in body weights was observed in monkeys following 26 weeks of treatment with rhIGF-1 at doses up to 1 mg/kg/day.

The repeat dose toxicity of rhIGF-1/IGFBP has been studied in rats and monkeys for up to 90 days. In rats, daily subcutaneous administration of rhIGF-1/rhIGFBP-3 at doses up to 30 mg/kg/day resulted in dose-dependent increases in body weights and an increase in the weight of the lymphoid tissues, particularly the spleen, thymus and lymph nodes. Similar results were obtained following 90 consecutive days of rhIGF-1/rhIGFBP-3 administration to monkeys at doses up to 10 mg/kg/day. In both species, the increased weight of the lymphoid tissues correlated with mild lymphoid hyperplasia with an expansion of the T-cell components. The dual energy X-ray absorptiometry studies in monkeys showed an increase in lean body mass in all treated females and an increase in whole body bone mineral density in high dose males at the end of the treatment period. All animals treated with rhIGF-1/rhIGFBP-3 for 90 days developed antibodies directed against the rhIGFBP-3 portion of the complex. However, results of an in vitro neutralizing assay indicated that the antibodies did not neutralize the mitogenic effects of rhIGF-1/rhIGFBP-3.

Results of three cytogenetic studies indicate that rhIGF-1 did not increase the frequency of chromosomal aberrations at concentrations up to 5000 µg/mL. In addition, rhIGF-1 did not increase the number of revertant colonies in a gene mutation test in bacteria at any concentration tested. No genotoxicity studies have been conducted with the rhIGF-1/rhIGFBP-3 complex.

Results of one 2 year carcinogenicity study (publication in abstract form) indicate that treatment with rhIGF-1 at doses of 4 mg/kg/day in female rats and 10 mg/kg/day in male rats causes an increased incidence of mammary neoplasms. However, administration of rhIGF-1 at doses up to 10 mg/kg/day for 14 days to mice bearing either Lewis lung or HT-29 colorectal carcinomas had no effect on primary tumor growth or metastases. No studies to assess the carcinogenic potential of rhIGF-1/rhIGFBP-3 have been conducted.

No studies to assess the reproductive or developmental toxicity of rhIGF-1/rhIGFBP-3 have been conducted. However, the reproductive and developmental toxicity of free rhIGF-1 have been studied in both rats and rabbits by the subcutaneous route of administration. In neonatal rats, administration of rhIGF-1 at doses up to 5 mg/kg/day for 14 days produced no signs of toxicity. In addition, administration of rhIGF-1 at doses up to 10 mg/kg/day to adult male and female rats produced no treatment-related effects on fertility or on growth and development of the fetuses. Ten mg/kg/day in rats are equivalent to an exposure 1.2 times the average human dose, based on body surface area comparison.

In contrast, administration of rhIGF-1 at doses of 1.25 mg/kg/day to rabbits (equivalent to 0.3 times the average human dose, based on body surface area comparison) on days 6-18 of gestation produced a number of treatment-related effects, including an increased incidence of abortion, an

increase in post-implantation loss, a reduction in the number of viable fetuses and an increase in fetal skeletal abnormalities. Since the toxicological profiles of rhIGF-I and rhIGF-I/rhIGFBP-3 are similar where data are available for comparison, one would not expect the reproductive and developmental effects of rhIGF-I/rhIGFBP-3 to be significantly different than those observed with rhIGF-I alone.

In a local tolerance study of rhIGF-I/rhIGFBP-3 in rats, subcutaneous administration of the test article at a dose of 100 mg/kg/day for 14 days resulted in a diffuse, mild to marked chronic-active inflammatory reaction involving the subcutaneous tissues, which was reversible following discontinuation of dosing. In pigs, subcutaneous administration of 4 mg rhIGF-I produced no microscopic changes in the skin or subcutaneous tissues apart from those associated with needle insertion. A direct comparison of the results from the two drug products is not straightforward owing to the differences in administered dose and duration of dosing. However, it is safe to say that the toxicological profiles of rhIGF-I/rhIGFBP-3 and rhIGF-I are qualitatively similar despite the prolonged plasma level after the administration of the binary complex.

### 2.6.6.3 Repeat-dose toxicity:

#### **Title: Toxicity study of recombinant Human IGF-1 in Cynomolgus monkeys. PK evaluation of serum concentration 4.2.2.2.b.4**

1. Purpose: To determine the pharmacokinetic parameters of IGF-1 in a 4 week toxicity study of rh-IGF-1 in Cynomolgus monkey. The toxicity study was performed at \_\_\_\_\_

2. Methods: Monkeys were given IGF-1 daily for 30 days as intravenous bolus injections during 20 seconds and as continuous infusions during 120 minutes. The bolus doses were 0.25 and 0.50 mg/kg and the infusion doses 0.25, 0.50 and 1.00 mg/kg. Blood samples were collected at time 0 just before the injection or at start of infusion and then at the time points 5, 15, 30, 120 and 240 minutes. In the infusion experiments the time points except time 0 refer to time after stopping the infusion. The blood samples were collected Days 1 and 30 in the study.

#### 3. Results:

When IGF-1 was administered by continuous infusion no differences in IGF-1 concentration on Day 0 (C<sub>0</sub>) were seen between day 1 and day 30 at the three dose levels of 0.25, 0.5 and 1 mg/kg. When IGF-1 was administered as bolus doses, the increase in C<sub>max</sub> was almost proportional to the increase in dose. It appears that there were two phases in declining of the serum concentrations of IGF-1:  $\alpha$  and  $\beta$  phases. For the  $\alpha$  phase, the half-life was about 1.5 minutes and the  $\beta$  phase the half-lives were in the range 265-367 minutes.

Bolus dose 0.25 mg/kg

Monkey No.	Day 1	Day 30	T 1/2 Day 1		T 1/2 Day 30	
	Cmax	Cmax	$\alpha$	$\beta$	$\alpha$	$\beta$
14						
15						
31						
32						
$\bar{x} \pm SD$	3.2 $\pm$ 0.9	3.7 $\pm$ 1.3	1.3 $\pm$ 0.1	265 $\pm$ 80	1.1 $\pm$ 0.5	367 $\pm$ 94

Bolus dose 0.50 mg/kg

Monkey No.	Day 1	Day 30	T 1/2 Day 1		T 1/2 Day 30	
	Cmax	Cmax	$\alpha$	$\beta$	$\alpha$	$\beta$
16						
17						
33						
34						
$\bar{x} \pm SD$	6.0 $\pm$ 0.7	5.1 $\pm$ 0.7	1.4 $\pm$ 0.4	265 $\pm$ 80	1.3 $\pm$ 0.0	267 $\pm$ 42

**Title: 13-Week repeat dose subcutaneous toxicity study of rhIGF-1/IGFBP-3 in rats**

Sponsor's ID #/study#: — 97-5038-R-TX/H-98-1035/H-98-1072

Sponsor's original title: 13-Week repeat dose subcutaneous toxicity study of rhIGF-1/IGFBP-3 in rats

Documents: Module 4; volume 6, page 1-321

Study Number. — 97-5038-R-TX/H-98-1035/H-98-1072

Conducting laboratory: —

Date of study initiation: Jan. 2000

GLP compliance: Yes

QA Report: Yes (x) No ()

**Methods:****Dosing information**

- Species: SD rats
- #/sex/group or time point: Total 50 rats/sex (10 to 15 rats/sex/group). Group 1 was a placebo control group. Groups 2, 3, and 4 had low, mid and high dose groups. Groups 1 and 4 had 15 rats/sex while 10 rats/sex were assigned for groups 2 and 3, respectively. The 5 rats/sex in the groups 1 and 4 were used as recovery groups after 4-week treatment.
- Age: 6 weeks
- Weight: Male: 236-304 g; Female 179-234 g
- Satellite groups used for recovery: 5 rats/sex for groups 1 and 4.
- Dosage groups in administered units: 0, 1, 10 and 30 mg/kg/day
- Route, form, volume, and infusion rate (if i.v.): Subcutaneous

Drug, lot#, radiolabel (if applicable), and % purity: rhIGF-1/IGFBP-3 Lot# DP9704 and DP9606

Formulation/vehicle: Inactive ingredients are commonly used in pharmaceutical products in the U.S.A. such as 50 mM sodium acetate, 105 mM sodium chloride, pH 5.5, as listed under "Clinical formulation (and components)".

Times at which Observations are made:

- Clinical signs: Once daily
- Body weights: Days -3, 1, and then weekly
- Food consumption: Days -8 to -1 and then daily
- Ophthalmoscopy: Day -5 and 5 to 8 day before termination
- EKG: NA
- Hematology: Day 92 for the main and Day 120 for the recovery animals
- Clinical chemistry: Day 92 for the main and Day 120 for the recovery animals
- Urinalysis: Day 90 prior to termination in metabolic cages.
- Gross pathology: At the time of death or sacrifice
- Organs weighed: At the time of death, sacrifice, or necropsy
- Histopathology: At the time of death or sacrifice
- Toxicokinetics: Analyses were performed on Days 0, 26, and 88 at 1, 2, 4, 6, 8, 12, and

24 hours after the dose.

#### RESULTS:

- Clinical signs and mortality: There were no deaths and no significant clinical signs were observed in all animals.
- Body weights: High-dose males were significantly heavier than control males on Day 22, Day 43, and Day 57 to Day 85. High-dose females were significantly heavier than control females on Day 8 and from Day 22 to Day 99. Other statistically significant differences in body weights and mean body weight are summarized below.

**Mean Body Weights (g)**

Males													
Treatment Group	Day 8	Day 22	Day 29	Day 36	Day 43	Day 50	Day 57	Day 64	Day 71	Day 78	Day 85	Day 92	Day 99
0 mg/kg	294	361	384	414	437	456	474	488	499	514	524	519	529
1 mg/kg	298	363	383	415	433	452	470	475	487	508	517	--	--
10 mg/kg	309	385	410	442	460	479	499	516	527	543	550	--	--
30 mg/kg	311	392*	417	450	477*	496	522*	547*	563*	587*	596*	572	585
Females													
0 mg/kg	210	241	250	259	269	279	286	292	294	302	308	304	320
1 mg/kg	217	240	254	266	272	281	293	300	298	304	309	--	--
10 mg/kg	218	252	263	272	278	305	304	308	313	325	328	--	--
30 mg/kg	225*	263*	277*	288*	302*	318*	328*	344*	352*	361*	367*	378^	382^

\*Statistically different from vehicle-treated concurrent controls,  $p \leq 0.05$ , using a Dunnett's test.

^Statistically different from vehicle-treated concurrent controls,  $p \leq 0.05$ , using a Wilcoxon test

Food consumption: Group mean food consumption values in the control animals were not significantly different from the LD and Mid Dose groups. However, high-dose males (Group 4)

consumed more food than control males during Week 5 and from Week 8 to Week 14. High-dose females consumed more than control females during Week 3, Week 4, and Week 7 to Week 12. A summary of statistically significant differences in food consumption is detailed below.

**Group Mean Food Consumption (g/day)**

Males												
Treatment Group	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12	Week 13	Week 14
0 mg/kg	28	28	28	28	28	27	27	26	29	29	27	27
1 mg/kg	29	28	29	28	28	28	27	27	28	28	28	--
10 mg/kg	28	29	30	28	28	29	28	28	30	30	29	--
30 mg/kg	30	30	31*	30	30	30*	31*	31*	32*	34*	33*	32*
Females												
0 mg/kg	21	22	21	21	21	21	21	20	22	22	22	22
1 mg/kg	22	23	23	21	22	23	22	21	23	23	22	--
10 mg/kg	23	23	22	22	22	22	22	23	24	24	22	--
30 mg/kg	23*	24*	23	22	24*	24*	25*	25*	25*	25*	25	25

\*Statistically different from vehicle-treated concurrent controls,  $p \leq 0.05$ , using a Dunnett's test.

^Statistically different from vehicle-treated concurrent controls,  $p \leq 0.05$ , using a Wilcoxon test.

- Ophthalmoscopy: The eyes of all rats in groups 1 and 4 were examined by a certified ophthalmologist. Rats with ophthalmic abnormalities prior to dosing were excluded from the study. There were no remarkable abnormalities in ophthalmic findings, though there were a few findings which were not related to the treatment as shown below.

**Table 3. Summary of Ophthalmic Examinations<sup>a</sup>**

Treatment Group	Animal ID/ Sex	Findings <sup>b</sup>	
		Pre-Dose	Termination
0 mg/kg	114M	Normal	Corneal epithelial fibrosis (exposure keratopathy) (O.U.)
	156F	Normal	Horizontal band of choroidal thinning inferior to optic disk (O.S.)
	164F	Normal	Mild corneal epithelial fibrosis (exposure keratopathy) (O.U.)
30 mg/kg	412M	Normal	Corneal epithelial fibrosis (exposure keratopathy) (O.U.)
	415M	Normal	Mild corneal epithelial fibrosis (exposure keratopathy) (O.U.)

<sup>a</sup>All animals not reported were normal for both examinations.

<sup>b</sup>O.S. = left eye, O.U. = both eyes

• Electrocardiography: NA

• Hematology: Most of hematological parameters in the treated-animals were not remarkably different from those of the control group as shown in Tables 4 and 5 below. In males, hemoglobin values were reduced at the HD group during the recovery phase, which was not seen in females. However, nucleated RBC counts were reduced in both males and females at the HD groups during the recovery phase.

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Table 4. Hematology Results

Treatment-phase Necropsy									
Males									
Treatment Group		Red Blood Cells (10E6/mm <sup>3</sup> )	Hemoglobin (g/dL)	Hematocrit (%)	Mean Corpuscular Volume (fL)	Mean Corpuscular Hemoglobin (pg)	Mean Corpuscular Hemoglobin Concentration (g/dL)	Platelets (10E6/mm <sup>3</sup> )	Reticulocytes (10E3/mm <sup>3</sup> )
0 mg/kg	Mean	8.5	15.6	46	53.9	18.3	33.9	1204	272
	SD	0.5	0.9	3	1.4	0.4	0.6	307	59
	N	10	10	10	10	10	10	10	10
1 mg/kg	Mean	8.8	16.1	48	54.4	18.2	33.5	1337	241
	SD	0.4	0.7	2	2.1	0.6	0.4	150	44
	N	10	10	10	10	10	10	10	9
10 mg/kg	Mean	8.8	16.0	47	54.2	18.3	33.8	1271	263
	SD	0.6	1.0	3	1.4	0.7	0.5	230	71
	N	10	10	10	10	10	10	10	10
30 mg/kg	Mean	8.4	15.9	46	54.9	19.0	34.7	1381	298
	SD	0.9	0.8	5	1.4	1.8	3.4	275	68
	N	10	10	10	10	10	10	10	9
Females									
Treatment Group		Red Blood Cells (10E6/mm <sup>3</sup> )	Hemoglobin (g/dL)	Hematocrit (%)	Mean Corpuscular Volume (fL)	Mean Corpuscular Hemoglobin (pg)	Mean Corpuscular Hemoglobin Concentration (g/dL)	Platelets (10E6/mm <sup>3</sup> )	Reticulocytes (10E3/mm <sup>3</sup> )
0 mg/kg	Mean	8.1	15.7	47	57.6	19.4	33.8	1210	211
	SD	0.4	0.7	2	1.8	0.5	0.6	199	38
	N	9	9	9	9	9	9	9	9
1 mg/kg	Mean	8.3	15.9	47	56.8	19.1	33.6	1157	212
	SD	0.4	0.6	2	1.6	0.4	0.8	101	35
	N	9	9	9	9	9	9	9	9
10 mg/kg	Mean	8.3	16.1	48	57.6	19.4	33.8	1280	199
	SD	0.4	0.7	1	1.6	0.6	0.8	165	42
	N	10	10	10	10	10	10	10	10
30 mg/kg	Mean	8.1	16.0	47	58.4	19.8	34.0	1201	217
	SD	0.3	0.3	1	1.8	0.7	0.6	110	35
	N	10	10	10	10	10	10	10	10

\*Statistically different from vehicle-treated concurrent controls,  $p \leq 0.05$ , using a Dunnett's test  
 \*Statistically different from vehicle-treated concurrent controls,  $p \leq 0.05$ , using a Dunn's test

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Table 4 (cont'd). Hematology Results

Recovery-phase Necropsy									
Males									
Treatment Group		Red Blood Cells (10E9/mm <sup>3</sup> )	Hemoglobin (g/dL)	Hematocrit (%)	Mean Corpuscular Volume (fL)	Mean Corpuscular Hemoglobin (pg)	Mean Corpuscular Hemoglobin Concentration (g/dL)	Platelets (10E9/mm <sup>3</sup> )	Reticulocytes (10E3/mm <sup>3</sup> )
0 mg/kg	Mean	8.6	16.5	48	56.0	19.2	34.2	1346	208
	SD	0.7	0.6	3	2.0	1.2	1.2	264	50
	N	5	5	5	5	5	5	5	5
30 mg/kg	Mean	8.3	15.6 <sup>*</sup>	46	54.9	18.7	34.0	1124	193
	SD	0.3	0.4	1	1.3	0.5	0.5	366	41
	N	5	5	5	5	5	5	5	5
Females									
Treatment Group		Red Blood Cells (10E9/mm <sup>3</sup> )	Hemoglobin (g/dL)	Hematocrit (%)	Mean Corpuscular Volume (fL)	Mean Corpuscular Hemoglobin (pg)	Mean Corpuscular Hemoglobin Concentration (g/dL)	Platelets (10E9/mm <sup>3</sup> )	Reticulocytes (10E3/mm <sup>3</sup> )
0 mg/kg	Mean	8.1	15.8	47	57.5	19.8	34.1	1072	183
	SD	0.5	0.6	2	1.7	0.5	0.6	171	14
	N	5	5	5	5	5	5	5	5
30 mg/kg	Mean	8.1	15.8	47	57.9	19.6	33.9	1135	179
	SD	0.3	0.7	2	1.8	0.3	0.9	106	27
	N	5	5	5	5	5	5	5	5

\*Statistically different from vehicle-treated concurrent controls,  $p \leq 0.05$ , using a Dunnett's test

\*Statistically different from vehicle-treated concurrent controls,  $p \leq 0.05$ , using a Wilcoxon test

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Table 5. Differential Cell Counts

Treatment-phase Necropsy								
Males								
Treatment Group		White Blood Cells (10E6/mm <sup>3</sup> )	Nucleated RBC Count (nRBC/100 WBC)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
0 mg/kg	Mean	9.4	17.0	6.8	87.5	3.6	0.7	1.4
	SD	2.8	1.2	2.1	4.0	1.2	0.8	1.2
	N	10	10	10	10	10	10	10
1 mg/kg	Mean	8.9	16.7	7.7	87.4	3.2	0.6	1.1
	SD	2.9	1.2	2.0	2.4	1.3	0.5	1.2
	N	10	10	10	10	10	10	10
10 mg/kg	Mean	9.1	16.6	7.6	88.6	2.7	0.4	0.7
	SD	2.7	1.1	5.0	4.9	2.0	0.5	1.0
	N	10	10	10	10	10	10	10
30 mg/kg	Mean	11.3	16.4	10.0	84.0	3.5	1.0	1.4
	SD	3.4	1.2	3.8	4.4	1.5	1.1	1.1
	N	10	10	10	10	10	10	10
Females								
Treatment Group		White Blood Cells (10E6/mm <sup>3</sup> )	Nucleated RBC Count (nRBC/100 WBC)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
0 mg/kg	Mean	7.6	15.6	6.4	85.9	4.7	1.2	1.9
	SD	2.1	0.5	3.8	4.4	1.5	2.0	1.1
	N	9	9	9	9	9	9	9
1 mg/kg	Mean	6.9	14.9	6.2	88.4	3.7	0.6	1.1
	SD	2.2	1.0	3.4	5.4	2.3	0.4	0.8
	N	9	9	9	9	9	9	9
10 mg/kg	Mean	5.7	15.3	6.6	87.5	4.8	0.3	0.8
	SD	1.8	1.1	2.7	5.0	2.3	0.7	1.1
	N	10	10	10	10	10	10	10
30 mg/kg	Mean	6.6	15.2	6.7	88.2	3.2	0.5	1.4
	SD	2.5	1.0	2.4	3.4	1.9	0.6	1.1
	N	10	10	10	10	10	10	10

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Table 5 (cont'd). Differential Cell Counts

Recovery-phase Necropsy								
Males								
Treatment Group		White Blood Cells (10E6/mm <sup>3</sup> )	Nucleated RBC Count (nRBC/100 WBC)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
0 mg/kg	Mean	8.3	17.6	10.1	82.7	5.4	0.7	1.1
	SD	2.1	1.2	2.3	2.5	3.0	0.8	1.1
	N	5	5	5	5	5	5	5
30 mg/kg	Mean	8.5	16.7 <sup>A</sup>	7.1	88.6 <sup>A</sup>	2.9	0.5	0.9
	SD	5.0	0.4	2.1	2.0	1.1	0.6	1.3
	N	6	6	5	5	5	5	5
Females								
Treatment Group		White Blood Cells (10E6/mm <sup>3</sup> )	Nucleated RBC Count (nRBC/100 WBC)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
0 mg/kg	Mean	5.7	15.4	7.8	87.2	4.3	0.2	0.6
	SD	3.0	0.3	1.8	2.3	0.8	0.2	1.0
	N	5	5	5	5	5	5	5
30 mg/kg	Mean	4.2	14.0 <sup>A</sup>	9.2	86.6	4.0	0.2	0.3
	SD	3.0	1.2	2.2	2.9	1.4	0.4	0.6
	N	5	5	5	5	5	5	5

<sup>A</sup>Statistically different from vehicle-treated concurrent controls,  $p \leq 0.05$ , using a Wilcoxon test

• Clinical chemistry: There were no treatment related changes in serum glucose levels in males and females even after the high dose. This lack of effects on glucose was also observed during the recovery phase (Please see the two Tables below). In HD females, creatinine, total protein, albumin, and calcium were significantly decreased compared to the control group. Albumin was also decreased in MD group. It appears that the values were within normal reference ranges so that they are not considered to be toxicologically significant.

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Table 7. Serum Chemistry Results

		Treatment-Phase Necropsy																			
		Males										Females									
Treatment Group		Na (mEq/L)	K (mEq/L)	Cl (mEq/L)	GLU (mg/dL)	BUN (mg/dL)	CRE (mg/dL)	PROT (g/dL)	ALB (g/dL)	GLOB (g/dL)	A/G	Ca (mg/dL)	PHOS (mg/dL)	CK (U/L)	LDH (U/L)	AST (U/L)	ALT (U/L)	ALP (U/L)	GGT (U/L)	CHOL (mg/dL)	TRIG (mg/dL)
0 mg/kg	Mean	140	6.7	102	220	16.5	0.4	6.3	3.1	3.2	0.97	10.3	8.7	203	538	111	89	78	0	67	70
	SD	2	2.9	1	54	2.1	0.1	0.3	0.1	0.2	0.03	0.4	1.9	127	495	49	128	14	0	18	43
	N	9	9	9	9	9	9	9	8	8	8	8	8	8	8	8	8	8	9	8	8
1 mg/kg	Mean	140	7.3	103	255	17.7	0.4	6.2	3.0	3.2	0.94	10.1	9.6	251	324	100	58	79	0	63	52
	SD	1	3.2	3	83	5.1	0.1	0.4	0.2	0.2	0.04	0.8	2.4	219	187	39	23	13	0	17	41
	N	10	10	10	10	10	10	9	9	9	9	9	9	9	9	9	10	10	9	10	9
10 mg/kg	Mean	141	7.4	103	285	15.3	0.4	6.4	3.1	3.3	0.94	10.3	9.2	328	695	97	55	67	0	66	59
	SD	6	3.3	5	91	2.5	0.0	0.5	0.2	0.3	0.05	1.0	2.0	324	1058	29	26	8	0	17	22
	N	8	8	8	7	7	7	7	7	6	6	6	6	6	6	6	7	7	7	6	7
30 mg/kg	Mean	140	6.7	103	263	16.4	0.4	6.3	3.0	3.3	0.94	10.1	9.2	135	174	81	41	70	0	64	43
	SD	1	2.4	2	37	5.6	0.1	0.3	0.1	0.2	0.06	0.7	1.4	51	105	18	11	14	0	13	19
	N	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
0 mg/kg	Mean	138	7.9	103	182	15.5	0.5	6.6	3.5	3.1	1.14	10.4	9.5	358	290	103	52	49	0	80	37
	SD	1	3.6	2	37	3.3	0.1	0.4	0.2	0.3	0.10	0.6	2.2	734	451	46	30	16	0	10	10
	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
1 mg/kg	Mean	139	5.2	104	169	18.7	0.4	6.4	3.4	3.0	1.15	9.7	7.7	276	213	97	54	34	0	69	33
	SD	1	2.6	2	37	5.1	0.1	0.5	0.3	0.3	0.15	0.4	1.6	245	86	68	54	12	0	13	9
	N	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
10 mg/kg	Mean	138	6.3	102	213	13.7	0.4	6.2	3.2*	2.9	1.10	10.0	8.6	182	243	94	47	44	0	61	37
	SD	2	3.3	2	83	2.9	0.1	0.4	0.2	0.2	0.07	0.7	1.8	55	111	33	25	20	0	9	16
	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
30 mg/kg	Mean	139	4.9	105	209	13.2	0.4*	6.0*	3.1*	2.9	1.08	9.5*	8.0	203	237	75	31	33	0	53	28
	SD	1	1.4	2	48	2.7	0.0	0.5	0.3	0.2	0.09	0.7	1.1	74	137	26	14	7	0	14	6
	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

\*Statistically different from vehicle-treated concurrent controls,  $p \leq 0.05$ , using a Dunnett's test  
 \*Statistically different from vehicle-treated concurrent controls,  $p \leq 0.05$ , using a Dunnett's test

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Table 7 (cont'd). Serum Chemistry Results

		Recovery-phase Necropsy																		
		Males																		
Treatment Group	Na (mEq/L)	K (mEq/L)	Cl (mEq/L)	GLU (mg/dL)	BUN (mg/dL)	CRE (mg/dL)	PROT (g/dL)	ALB (g/dL)	GLOB (g/dL)	A/G	Ca (mg/dL)	PHOS (mg/dL)	CK (U/L)	LDH (U/L)	AST (U/L)	ALT (U/L)	ALP (U/L)	GGT (U/L)	CHOL (mg/dL)	TRIG (mg/dL)
0 mg/kg	Mean	139	7.4	103	228	16.1	6.0	3.0	3.1	0.97	9.9	8.8	318	693	118	65	67	0	52	38
	SD	2	2.6	2	92	3.0	0.4	0.2	0.3	0.10	0.7	1.9	250	779	48	39	13	0	17	18
	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
30 mg/kg	Mean	137	7.7	103	270	15.4	6.0	2.9	3.1	0.93	9.8	7.9	171	243	81	45	78	0	57	50
	SD	3	2.9	2	28	2.2	0.1	0.2	0.3	0.04	0.6	1.5	122	274	31	17	6	0	9	20
	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		Females																		
Treatment Group	Na (mEq/L)	K (mEq/L)	Cl (mEq/L)	GLU (mg/dL)	BUN (mg/dL)	CRE (mg/dL)	PROT (g/dL)	ALB (g/dL)	GLOB (g/dL)	A/G	Ca (mg/dL)	PHOS (mg/dL)	CK (U/L)	LDH (U/L)	AST (U/L)	ALT (U/L)	ALP (U/L)	GGT (U/L)	CHOL (mg/dL)	TRIG (mg/dL)
0 mg/kg	Mean	140	5.0	104	203	14.1	6.6	3.6	3.0	1.21	9.5	6.4	166	345	148	90	31	0	70	42
	SD	2	2.6	2	13	1.6	0.6	0.3	0.3	0.07	0.7	1.2	48	105	68	59	5	0	20	13
	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
30 mg/kg	Mean	140	5.1	104	209	13.4	6.6	3.5	3.1	1.18	9.5	6.2	186	324	74	42	23	0	84	49
	SD	1	2.3	1	32	1.3	0.1	0.2	0.2	0.12	0.3	1.3	60	188	11	14	7	0	32	12
	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

• Urinalysis: No toxicologically significant findings were noted among the groups treated with the drug and in control group in the study.

• Organ weight: The absolute organ weight of heart, kidneys, lung, pituitary gland and spleen increased significantly at the HD male groups as the body weight also increased from the control 508 to 584 g. The low dose and mid-dose had no effect on the absolute organ weights of any organs in males (Table 9). The absolute organ weight of kidneys increased in the MD and HD groups in females. Similar increases were also observed in lungs, thymus, ovaries, and spleen in the MD groups in female groups. The body weight of females at a dose of 30 mg/kg was not statistically different from the control females.

The elevated organ weights returned to normal in both males and females at the end of recovery phase except the kidney weight in the HD female group. In females, the liver weight increased during the recovery phase, although it was not elevated in the main study. If the absolute organ weight data are expressed based on body weight ratio (%), only spleen and thymus weight ratios at the HD were still significant in both males and females. Based on organ to brain weight ratio (%), the ratios in heart, kidneys, lung, pituitary gland, spleen, and thymus were still higher in the HD group, compared to the control in males. In females, organ to brain weight ratios in kidneys, lung, thymus, ovaries and spleen increased significantly at the HD females.

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Table 9. Absolute Organ Weights (g)<sup>a</sup>

Treatment-Phase Necropsy												
Males												
Treatment Group	Terminal Body Weight (g)	Adrenal Gland	Brain	Heart	Kidneys	Liver	Lungs	Pituitary Gland	Spleen	Thymus	Thyroid Gland	Testes
0 mg/kg	Mean	608	2.217	1.435	3.673	14.833	1.616	0.0124	0.861	0.358	0.0268	1.638
	SD	64	0.0138	0.079	0.575	2.814	0.194	0.0031	0.138	0.077	0.0053	0.209
1 mg/kg	Mean	495	2.154	1.540	3.678	14.506	1.576	0.0135	0.888	0.335	0.0247	1.614
	SD	55	0.0057	0.207	0.320	1.581	0.131	0.0026	0.153	0.094	0.0039	0.085
10 mg/kg	Mean	528	2.213	1.589	3.888	15.320	1.724	0.0147	0.999	0.380	0.0281	1.834
	SD	54	0.0143	0.091	0.199	2.767	0.144	0.0023	0.207	0.100	0.0073	0.679
30 mg/kg	Mean	584 <sup>*</sup>	2.239	1.847 <sup>*</sup>	4.462 <sup>*</sup>	16.798	1.923 <sup>*</sup>	0.0168 <sup>*</sup>	1.140 <sup>*</sup>	0.597	0.0314	1.740
	SD	72	0.0062	0.113	0.229	3.087	0.227	0.0035	0.173	0.088	0.0068	0.094

Females												
Treatment Group	Terminal Body Weight (g)	Adrenal Gland	Brain	Heart	Kidneys	Liver	Lungs	Pituitary Gland	Spleen	Thymus	Thyroid Gland	Ovaries
0 mg/kg	Mean	289	2.078	1.155	2.224	8.429	1.207	0.0176	0.616	0.239	0.0192	0.114
	SD	26	0.0177	0.091	0.255	0.737	0.075	0.0019	0.092	0.042	0.0054	0.018
1 mg/kg	Mean	290	2.056	1.021	2.272	8.043	1.164	0.0154	0.597	0.267	0.0251	0.098
	SD	31	0.0115	0.077	0.098	0.191	0.089	0.0032	0.079	0.087	0.0060	0.020
10 mg/kg	Mean	307	2.049	1.106	2.618 <sup>^</sup>	8.761	1.261	0.0189	0.711	0.299	0.0232	0.125
	SD	29	0.0150	0.095	0.098	0.203	0.075	0.0039	0.074	0.064	0.0065	0.092
30 mg/kg	Mean	347	2.115	1.184	3.021 <sup>^</sup>	9.238	1.415 <sup>*</sup>	0.0187	0.835 <sup>*</sup>	0.452 <sup>*</sup>	0.0252	0.151 <sup>*</sup>
	SD	34	0.0109	0.101	0.496	1.040	0.107	0.0023	0.127	0.090	0.0044	0.023

<sup>a</sup>For the post-treatment necropsy, n = 10 (n = 9 for 1 mg/kg females). For the recovery necropsy, n = 5.  
<sup>\*</sup>Statistically different from vehicle-treated concurrent controls, P ≤ 0.05, using a Dunnett's test  
<sup>^</sup>Statistically different from vehicle-treated concurrent controls, P ≤ 0.05, using a Dunnett's test

87-5038-R-TX/R-98-1035/H-98-1072  
 13-Week Repeat Dose Subcutaneous Toxicity Study of rhIGF-1/IGFBP-3 in Rats  
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Table 9 (cont'd). Absolute Organ Weights (g)\*

Recovery-Phase Necropsy												
Males												
Treatment Group	Terminal Body Weight (g)	Adrenal Gland	Brain	Heart	Kidneys	Liver	Lungs	Pituitary Gland	Spleen	Thymus	Thyroid Gland	Testes
0 mg/kg	Mean	532	2.258	1.585	4.026	14.422	1.700	0.0153	0.849	0.284	0.0288	1.782
	SD	46	0.179	0.192	0.363	0.947	0.198	0.0030	0.056	0.073	0.0082	0.119
30 mg/kg	Mean	576	2.281	1.599	4.354	15.772	1.803	0.0148	1.008	0.267	0.0321	1.583
	SD	50	0.069	0.221	0.492	1.422	0.281	0.0011	0.161	0.054	0.0143	0.199

Females												
Treatment Group	Terminal Body Weight (g)	Adrenal Gland	Brain	Heart	Kidneys	Liver	Lungs	Pituitary Gland	Spleen	Thymus	Thyroid Gland	Ovaries
0 mg/kg	Mean	305	2.101	1.060	2.217	8.057	1.143	0.0184	0.544	0.243	0.0256	0.0825
	SD	28	0.077	0.106	0.181	0.702	0.028	0.0047	0.023	0.031	0.0070	0.0226
30 mg/kg	Mean	361	2.135	1.219	2.640 <sup>a</sup>	10.035 <sup>a</sup>	1.211	0.0206	0.686	0.218	0.0835	0.0972
	SD	37	0.0113	0.122	0.275	1.614	0.087	0.0050	0.085	0.108	0.1118	0.0260

\*For the post-treatment necropsy, n = 10 (n = 9 for 1mg/kg females). For the recovery necropsy, n = 5.

<sup>a</sup>Statistically different from vehicle-treated concurrent controls, p ≤ 0.05, using a Dunnett's test

<sup>b</sup>Statistically different from vehicle-treated concurrent controls, p ≤ 0.05, using a Wilcoxon test

Gross pathology: In some injection sites for the administration of IGF-1 with or without IGFBP there were discolored spots. There were evidence of hyperkeratosis with diffuse, mild hemorrhage or moderate inflammation and mild fibrosis. At times there were focal sites with 0.5 cm subcutis as summarized below.

**Incidence of Histopathologic Findings for All Study Animals**

Celtrix Pharmaceuticals, Inc.

13-Week Repeat Dose Subcutaneous Toxicity Study of rhIGF-1/IGFBP-3 in Rats

PROJECT NUMBER: - 7-5038-R-TX/H-98-1035/H-98-1072 SPECIES: Rat

Printed on 03-09-2000.

Tissue/ Diagnosis/ Modifier(s)	Group 1		Group 2		Group 3		Group 4		Group 5	
	M	F	M	F	M	F	M	F	M	F
<u>Administration Site</u>	( 10)	( 10)	( 10)	( 10)	( 10)	( 10)	( 10)	( 10)	( 5)	( 5)
Congestion	0	0	1	1	0	0	0	0	0	0
Mild	0	0	1	1	0	0	0	0	0	0
Fibrosis	9	8	6	4	4	4	8	10	5	1
Trace	2	2	2	2	0	1	1	0	0	1
Mild	7	6	2	2	2	3	1	4	5	0
Moderate	0	0	2	0	2	0	6	6	0	0
Foreign body	0	1	1	0	0	0	0	0	0	0
Hemorrhage	4	4	2	4	2	2	2	0	0	0
Trace	1	0	1	1	1	0	0	0	0	0
Mild	1	4	1	2	0	1	0	0	0	0
Moderate	2	0	0	1	1	1	2	0	0	0
Hyperkeratosis	8	2	9	1	9	4	10	5	5	4
Trace	6	0	6	0	2	2	10	0	0	3
Mild	2	2	2	1	6	2	0	5	4	1
Moderate	0	0	1	0	1	0	0	0	1	0
Infiltration, eosinophilic	0	1	0	0	1	0	0	0	0	0
Mild	0	1	0	0	0	0	0	0	0	0
Moderate	0	0	0	0	1	0	0	0	0	0
Inflammation, chronic	5	6	5	5	5	7	6	5	0	0
Trace	3	3	1	2	0	1	1	1	0	0
Mild	2	3	1	2	3	5	0	3	0	0
Moderate	0	0	3	1	2	1	5	1	0	0
Inflammation, granulomatous	0	1	0	0	0	0	0	0	0	0
Trace	0	1	0	0	0	0	0	0	0	0
Inflammation, necrotic	0	0	1	0	0	1	2	0	0	0
Mild	0	0	0	0	0	1	1	0	0	0
Moderate	0	0	1	0	0	0	1	0	0	0
Within Normal Limits	1	2	0	1	0	2	0	0	0	1

• Histopathology:

The major changes in histopathology are limited to bone (femur), liver, nasal tissues, pancreas and thymus. The mild increases in cortical bone of femur were identified in 8 rats out of 10 in the HD male group. The effects reversed after 4-week wash-out period in males. The LD or MD had no effects in males, although one female rat had a mild increase in cortical bone as shown below. The high dose had no effects in females, thus, it appears that IGF-1/IGFBP had little or

no effect on femur cortical growth in females. Acute hepatic inflammation and hepatic centrilobular hypertrophy were observed in several male rats at the HD. The MD had no clear effects in males, although one female had hepatic cytoplasmic alteration. All the hepatic effects reversed returned toward normal after the recovery period. There were increased incidences of cortical bone growth in nasal tissues in males. That is, there were two and 5 male rats in the MD and HD groups, respectively, had the finding, which was reversed after 4-week recovery period. Mild lymphoid hyperplasia in nasal tissues was observed in a female rat at the HD group.

Trace to mild degree of chronic inflammation in pancreas was observed in the control as well as the HD group animals. Traces to moderate degrees of thymic congestion were also observed in several treated rats. Several females had thymic congestion at doses of 1 and 30 mg/kg/day. A few male rats also had thymus congestion at 1 and 10 mg/kg/day, although there were positive findings at the HD in males. Trace to mild hyperplasia in thymus was noted  $\geq 10$  mg/kg/day in both males and females, although the effects were reversible after 4-week recovery period. It appears that the mild hyperplasia in both male and female rats was IGF-1/IGFBP dose related.

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Major Histopathological Findings in 13-Week Subcutaneous Toxicity Study of rhIGF-1/IGFBP-3 in Rats@										
Tissues/Diagnosis/Modifiers	Control		1 mg/kg		10mg/kg		30mg/kg		Recovery	
	M	F	M	F	M	F	M	F	M	F
Bone, Femur, cortical bone increased, Trace	0	0	0	2	1	1	0	0	0	1
Bone, Femur, cortical bone increased, Mild	0	0	0	0	0	1	8	0	0	1
Bone, trabacular, increased, Trace	0	0	1	2	0	0	0	0	0	0
Bone, trabacular, increased, Mild	0	0	0	0	0	2	0	0	0	0
Cecum, hyperplasia, interfollicular zone, Trace	0	0	0	0	0	0	0	0	1	0
Cecum, hyperplasia, interfollicular zone, Mild	0	0	0	0	0	0	1	0	0	0
Eye, keratitis, acute, Severe	0	0	0	1	0	0	0	0	0	0
Heart, fibrosis, Trace	0	0	0	0	0	0	1	0	0	0
Heart, fibrosis, Mild	0	0	0	0	0	0	1	0	0	0
Ileum, congestion, Trace	0	0	0	0	0	0	0	1	0	1
Ileum, congestion, Mild	0	0	0	0	0	0	0	1	0	1
Jejunum, hyperplasia, cortex, Mild	0	0	0	0	0	0	1	0	0	0
Kidney, pyelitis, acute, Moderate	0	0	0	0	0	0	0	1	0	0
Liver, cytoplasmic alteration, clear, Mild	0	0	0	0	0	1	0	0	0	0
Liver, inflammation, acute, Trace	0	0	0	0	0	0	2	0	0	0
Liver, hypertrophy, centrilobular, Trace	0	0	0	0	0	0	1	0	0	0
Lung, inflammation, granulomatous, Trace	0	0	0	0	0	0	1	0	0	0
Lymph Node, mesenteric, congestion, Mild	1	0	0	1	0	0	0	1	0	1
Lymph Node, mesenteric, Moderate	0	0	0	1	0	0	0	0	0	0
Nasal tissues, cortical bone, increased, Trace	0	0	0	0	2	0	5	0	0	0
Nasal tissues, hyperplasia, lymphoid, Mild	0	0	0	0	0	0	0	1	0	0
Nasal tissues, inflammation, acute, Moderate	0	0	0	1	0	0	0	0	0	0
Nasal tissues, inflammation, chronic, Trace	0	0	0	0	0	0	0	0	0	1
Pancreas, infiltration, lymphocytic, Trace	0	0	0	0	0	0	1	0	0	0
Pancreas, inflammation, chronic, Trace	2	0	0	0	0	0	0	0	0	0
Pancreas, inflammation, chronic, Mild	0	0	0	0	0	0	1	0	0	0
Pancreas, inflammation, fibrotic, Mild	2	0	0	0	0	0	2	1	0	0
Thymus, congestion, Trace	0	0	1	0	0	0	0	0	0	0
Thymus, congestion, Mild	0	0	1	1	2	0	0	3	1	0
Thymus, congestion, Moderate	0	0	0	0	0	0	0	1	0	0
Thymus, hyperplasia, Trace	1	0	0	0	3	2	1	1	0	0
Thymus, hyperplasia, Mild	0	0	0	0	2	3	9	8	0	0
Thyroid, fibrosis, Trace	0	0	0	0	0	0	1	0	0	0

@ All groups had 10 rats at the beginning except the recovery groups, which had 5 rats.

#### Summary and Conclusion:

Subcutaneous administration of rhIGF-1/IGFBP-3 daily at the top dose of 10 mg/kg/day for 91 days was reasonably well tolerated. It was associated with increased body weight gain and increased food consumption in the high-dose group. There were histologically correlated increases in organ weights in the kidney, thymus, and spleen. Histologically, there was a mild increase in the T-cell component in the thymus and spleen and possibly in the lymph nodes. In many organs in male and female rats, it appears that there was mild hyperplasia that was rhIGF-

1/IGFBP-3 dose-related. This may be a part of the extension of the intrinsic action of rhIGF-1/IGFBP-3.

There was also a mild increase in the incidence of chronic glomerular nephritis both at the terminal sacrifice and at the recovery sacrifice. Test article-related changes were not observed in most of all tissues of recovery sacrifice rats. There may be an increases cortical bone in the femurs of Group 4 recovery sacrifice female rats. Based on histopathologic findings, a dose of 1 mg/kg may be considered a NOEL (No Observable Effects Level) dose, while 10 mg/kg is considered NOAEL (No Observable Adverse Effects Level) doses. The NOAEL dose is equivalent to an exposure 8 times the average human dose, based on body surface area comparison

**Title: 90-Day repeat dose subcutaneous toxicity study of rhIGF-1/IGFBP-3 in Rats: Rat Anti-rh IGF-1/IGFBP-3 Antibody Report 4.2.3.2.a.4/Study 07-5038-R-TX)**

1. Purpose: To determine potential immune response was generated to daily subcutaneous doses of rhIGF-1/IGFBP-3 over ninety days in male and female rats. The study was carried out by the sponsor was Celtrix Pharmaceuticals Inc.

2. Methods: One hundred Sprague Dawley rats was divided into one of the following dosage groups: vehicle (30 animals), 1 mg/kg rhIGF-I/IGFBP-3 (20 animals), 10 mg/kg rhIGF-I/IGFBP-3 (20 animals), or 30 mg/kg rhIGF-I/IGFBP-3 (30 animals). Ten rats from the vehicle group and ten from the 30 mg/kg rhIGF-I/IGFBP-3 group were maintained for an additional 28 day non-treated recovery period. For the assay of anti-rhIGF-I/IGFBP-3 antibodies, serum samples were assayed for antibodies against rhIGF-I/IGFBP-3 using an enzyme linked immunoabsorbent assay (ELISA). To provide a positive control, five Sprague-Dawley rats were immunized with rhIGF-I/IGFBP-3, and heparinized plasmas from these rats were pooled, aliquoted and frozen at -80°C. This control was titered from 1:640 to 1:327680 using serial dilutions. A pool of normal Sprague-Dawley rat plasma was diluted 1:20 and used as a negative control. A sample was considered to be negative if OD < 0.070, while it was considered to be positive if it had an OD ≥ 0.070. Western blots and neutralizing bioassay were also carried in this study.

3. Results:

One female rat in the 1 mg/kg/day group died. All ten males and nine out of ten females in the vehicle group were negative for antibodies to rhIGF-I/IGFBP-3 (Table 1). All rats in the 1, 10 and 30 mg/kg dosage groups developed antibody titers to rhIGF-I/IGFBP-3. After the recovery period, all five males and four out of five females in the vehicle group were negative, whereas 4/5 males, and 4/5 females in the 30 mg/kg group still had detectable antibody titers to rhIGF-I/IGFBP-3 (Table 2).

The highest titers were found in the lowest dosage group (1 mg/kg). There were no significant differences between males and females in the titers. The OD correlates to the number of cells present. There does not appear to be any significant neutralization of the complex due to the antibodies in rat serum. For each plate,

there is no inhibition of the mitogenic effect of complex on the cells when serum from a rat that received placebo is compare to serum from a rat that received complex (Table 3).

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Section 4.2.3.2.a.2

**Table 1**  
**Rat Antibody Titers to rhIGF-IGFBP-3 as Measured by ELISA In Treated Rats**

Dose	Animal #	Sex	Titer*	Animal #	Sex	Titer
0 mg/kg	101	M	Neg**	151	F	Neg
	102	M	Neg	152	F	Neg
	103	M	Neg	153	F	Neg
	104	M	Neg	154	F	Neg
	105	M	Neg	155	F	Neg
	106	M	Neg	156	F	Neg
	107	M	Neg	157	F	Neg
	108	M	Neg	158	F	Neg
	109	M	Neg	159	F	Neg
	110	M	Neg	160	F	1:160
			Mean			Mean
		Titer = 0			Titer=1:16	
1 mg/kg	201	M	1:10240	252	F	1:5120
	202	M	1:2560	253	F	1:10240
	203	M	1:5120	254	F	1:2560
	204	M	1:5120	255	F	1:10240
	205	M	1:2560	256	F	1:10240
	206	M	1:2560	257	F	1:5120
	207	M	1:40960	258	F	1:10240
	208	M	1:10240	259	F	1:5120
	209	M	1:2560	260	F	1:1280
	210	M	1:10240			
			Mean			Mean
		Titer=1:9216			Titer=1:6684	
10 mg/kg	301	M	1:1280	351	F	1:80
	302	M	1:2560	352	F	1:320
	303	M	1:160	353	F	1:1280
	304	M	1:5120	354	F	1:2560
	305	M	1:1280	355	F	1:2560
	306	M	1:1280	356	F	1:2560
	307	M	1:2560	357	F	1:1280
	308	M	1:1280	358	F	1:160
	309	M	1:1280	359	F	1:1280
	310	M	1:1280	360	F	1:640
			Mean			Mean
		Titer=1:1808			Titer=1:1272	
30 mg/kg	401	M	1:320	451	F	1:1280
	402	M	1:2560	452	F	1:1280
	403	M	1:1280	453	F	1:2560
	404	M	1:1280	454	F	1:1280
	405	M	1:640	455	F	1:320
	406	M	1:2560	456	F	1:640
	407	M	1:1280	457	F	1:320
	408	M	1:640	458	F	1:640
	409	M	1:2560	459	F	1:640
	410	M	1:640	460	F	1:1280
			Mean			Mean
		Titer=1:1376			Titer=1:1024	

\* Titer is defined as the last dilution in which the OD is  $\geq 0.070$ \*\* A sample (diluted 1:20) is considered negative when the OD is  $< 0.070$ 

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TABLE 4  
Rat Antibody Titers to rhIGF-I/IGFBP-3 as Measured by ELISA in Recovery Group Rats

Dose	Animal #	Sex	Titer*	Animal #	Sex	Titer
0 mg/kg Recovery Group	111	M	Neg**	161	F	Neg
	112	M	Neg	162	F	Neg
	113	M	Neg	163	F	Neg
	114	M	Neg	164	F	1:40
	115	M	Neg	165	F	Neg
			Mean Titer=0			Mean Titer=1:8
30 mg/kg Recovery Group	411	M	1:160	461	F	1:160
	412	M	1:320	462	F	1:1280
	413	M	1:320	463	F	1:160
	414	M	Neg	464	F	1:1280
	415	M	1:160	465	F	Neg
			Mean Titer=1:192			Mean Titer=1:576

Table 3  
Neutralizing Antibody Bioassay  
The Effects of Rat-Anti rhIGF-I/IGFBP-3 Antibodies on Human Osteosarcoma (MG63) Cell Proliferation

Plate Number	Animal Number	Dosage mg/kg	rhIGF-I/IGFBP-3 at 50 ng/mL mixed with Rat Serum at the following dilutions:**					rhIGF-I/IGFBP-3 at 100 ng/mL mixed with Rat Serum at the following dilutions:				
			1:40	1:80	1:160	1:320	1:640	1:40	1:80	1:160	1:320	1:640
			Optical Density					Optical Density				
1	101	0	0.908	0.677	0.578	0.550	0.533	0.997	0.761	0.625	0.604	0.604
2	207	1	0.901	0.704	0.614	0.560	0.531	0.912	0.763	0.660	0.623	0.609
3	102	0	0.619	0.496	0.430	0.394	0.393	0.680	0.552	0.475	0.452	0.439
4	302	10	0.710	0.507	0.441	0.432	0.422	0.750	0.563	0.479	0.482	0.495
5	103	0	0.882	0.671	0.568	0.534	0.540	1.092	0.801	0.665	0.629	0.644
6	402	30	0.948	0.702	0.604	0.553	0.540	1.023	0.793	0.688	0.626	0.638
7	159	0	0.759	0.582	0.500	0.464	0.462	0.771	0.602	0.528	0.471	0.479
8	253	1	0.740	0.536	0.474	0.434	0.423	0.776	0.555	0.526	0.465	0.449
9	356	10	0.810	0.599	0.487	0.456	0.463	0.861	0.625	0.523	0.471	0.503
10	451	30	0.832	0.593	0.515	0.481	0.487	0.844	0.608	0.538	0.498	0.528

\* Human osteosarcoma cells proliferation is increased in the presence of rhIGF-I/IGFBP-3, with the OD correlating to the number of cells. This bioassay was done to test whether rat antibodies would neutralize the effect of complex on these cells, which would result in lower ODs.

\*\*Fixed concentrations of complex were added to varying dilutions of rat serum and incubated, followed by addition of cells.

**Title: 90-Day Repeat Dose Subcutaneous Toxicity Study of rhIGF-1/IGFBP-3 in Monkeys (Section 4.2.3.2.a.4)**

Sponsor's ID #/study#: N057646A

Sponsor's original title: 90-Day repeat dose subcutaneous toxicity study of rhIGF-1/IGFBP-3 in monkeys

Study Number: N057646A

Document: Module 4, vol. 8, p1-294

Conducting laboratory: —

Date of study initiation: Oct. 1997

GLP compliance: Yes

QA Report: Yes (x) No ( )

Methods:

Dosing information

- Species: Cynomolgus Monkey
- #/sex/group or time point: 6 monkeys/sex/group were used. Group 1 was a placebo control group. Groups 2 and 3 were assigned to the monkeys for the low and high doses. Monkeys in Groups 2 and 3 had rhIGF-1/IGFBP-3 subcutaneously at doses of 1 and 10 mg/kg for 90 days. Two monkeys/sex from Groups 1 and 3 were used as recovery animal after a 28-day wash-out period.
- Age: 6 weeks
- Weight: Male: 2.3-3.4 kg; Female 2.2-2.8 kg
- Satellite groups used for recovery: 2 monkeys/sex for groups 1 and 3.
- Dosage groups in administered units: 0, 1, 10 mg/kg/day
- Route, form, volume, and infusion rate (if i.v.): Subcutaneous

Drug, lot#, radiolabel (if applicable), and % purity: rhIGF-1/IGFBP-3 lot was DP9604 and DP9606

Formulation/vehicle: Inactive ingredients are commonly used in pharmaceutical products marketed in the U.S.A. such as 50 mM sodium acetate, 105 mM sodium chloride, pH 5.5, as listed under "Clinical formulation (and components)" above.

Times at which Observations are made:

- Clinical signs: Twice daily
- Body weights: At randomization and then weekly thereafter
- Food consumption: Days -8 to -1 and then daily
- Ophthalmoscopy: Prior to initiation of dosing, on Day 84 and on Day 115 (males) and Day 114 (females)
- EKG: Not evaluated.
- Hematology: Day -9(males), Day -8(females), and on Days 48, 91 (interim necropsy) and 118 (final necropsy). Blood samples were obtained from the femoral vein.
- Clinical chemistry: Day -9(males), Day -8(females), and on Days 48, 91 (interim necropsy) and 118 (final necropsy). Blood samples were obtained from the femoral vein.

- Urinalysis: Day -8(males), Day -7(females), and on Days 48, 91 (interim necropsy) and 118 (final necropsy).
- Gross pathology: At the time of death or sacrifice
- Organs weighed: At the time of death, sacrifice, or necropsy
- Histopathology: At the time of death or sacrifice
  
- Toxicokinetics: Blood samples were obtained prior to dosing and at 2, 4, 8, and 24 hours after the dose on Days 1 and 90.

**RESULTS:**

- Clinical signs and mortality: There were no deaths and no significant clinical signs that were related to the test article. In some animals, there were incidences of digestive system observations such as soft feces, emesis, diarrhea, and low food consumption, which may not be directly related to the treatment.
  
- Body weights and food consumption: In males, the mean body weights of groups 2 and 3 animals were not changed significantly after the treatment (Table 2). The mean body weights of groups 2 and 3 female monkeys increased significantly after the treatment (Table 2), and returned to the normal after 4-week recovery period. The significant increases in mean body weight in Group 2 and 3 female monkeys were pronounced from the fourth week of the treatment. It appears that group-mean food consumption values in the control animals were not significantly different from those values in group 2 and 3.

**Table 2. Mean Body Weights (kg)**

Treatment Group		Day								
		64	71	78	84	90	98	106	113	117
<b>Males</b>										
0 mg/kg	Mean	3.23	3.26	3.30	3.22	3.33	3.14	3.21	3.27	3.25
	SD	0.40	0.42	0.40	0.40	0.39	--	--	--	--
	N	6	6	6	6	6	2	2	2	2
1 mg/kg	Mean	3.21	3.23	3.29	3.17	3.32	3.52	3.62	3.64	3.63
	SD	0.37	0.35	0.38	0.34	0.39	--	--	--	--
	N	6	6	6	6	6	2	2	2	2
10 mg/kg	Mean	3.37	3.41	3.46	3.40	3.55	3.49	3.56	3.52	3.48
	SD	0.52	0.55	0.55	0.56	0.55	--	--	--	--
	N	6	6	6	6	6	2	2	2	2
Treatment Group		Day								
<b>Females</b>										
0 mg/kg	Mean	2.57	2.60	2.61	2.56	2.61	2.70	2.67	2.68	2.59
	SD	0.08	0.08	0.10	0.09	0.10	--	--	--	--
	N	6	6	6	6	6	2	2	2	2
1 mg/kg	Mean	2.80*	2.83*	2.87*	2.77*	2.85*	3.02	3.04	3.06	3.00
	SD	0.17	0.16	0.16	0.15	0.17	--	--	--	--
	N	6	6	6	6	6	2	2	2	2
10 mg/kg	Mean	2.94*	2.95*	2.98*	2.94*	3.03*	3.10	3.08	3.10	2.86
	SD	0.15	0.12	0.13	0.13	0.11	--	--	--	--
	N	6	6	6	6	6	2	2	2	2

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• Ophthalmoscopy: The eyes of all monkeys in groups 1 and 4 were examined by a certified ophthalmologist. Two monkeys had ophthalmic problems prior to the initiation of the study, which were followed to the termination of the study as summarized (Table 3). It appears that the two incidences were not affected by the test article and there were no other remarkable ophthalmic findings in the study.

Table 3. Summary of Remarkable Ophthalmic Findings<sup>a</sup>

Treatment Group	Animal ID/Sex	Finding <sup>b</sup>	
		Predose	Termination
1 mg/kg	211/F	Multiple patches of depigmented retina located nasolateral to optic disc. Probable retinal scars (O.S.).	Small, focal pigmentation changes in the retina in the area nasolateral to the optic disc (O.S.).
10 mg/kg	304/M	Old laceration wound to lower eyelid (O.S.).	Slight swelling along lower eyelid - possible old scar also (O.S.).

• Electrocardiography: NA

• Hematology: Most of hematological parameters in the 1 mg/kg group were not remarkably different from those of the control group (Table 4). In the 10 mg/kg group, the mean values of red blood cells, hemoglobin and hematocrit were elevated significantly on Day 91 in males, which were reversed after 4-week recovery period. There were no such changes in female monkeys (Table 4).

Table 4. Hematology Results

	Day	0 mg/kg			1 mg/kg			10 mg/kg		
		Mean	SD	N	Mean	SD	N	Mean	SD	N
<b>Males</b>										
Red Blood Cells (10 <sup>6</sup> /μL)	-9	6.41	0.63	6	6.46	0.36	6	6.55	0.60	6
	48	6.26	0.61	6	6.42	0.24	6	6.68	0.57	6
	91	5.98	0.50	6	6.03	0.17	6	6.88*	0.40	6
	118	6.46	—	2	6.41	—	2	7.48	—	2
Reticulocytes (% of RBC)	-9	0.5	0.3	6	0.5	0.4	6	0.3	0.2	6
	48	0.3	0.3	6	0.3	0.2	6	0.3	0.2	6
	91	0.2	0.1	6	0.4	0.2	6	0.3	0.4	6
	118	0.3	—	2	0.2	—	2	0.0	—	2
Hemoglobin (g/dL)	-9	13.7	0.6	6	14.0	1.2	6	13.8	1.1	6
	48	13.1	0.8	6	13.4	0.5	6	13.7	0.8	6
	91	12.1	0.5	6	12.2	0.6	6	13.6*	0.6	6
	118	12.6	0.6	2	13.5	0.2	2	14.2	0.4	2
Methemoglobin (g/dL)	-9	0.1	0.0	6	0.1	0.0	6	0.1	0.0	6
	48	0.1	0.0	6	0.1	0.0	6	0.1	0.0	6
	91	0.2	0.0	6	0.2	0.0	6	0.2	0.0	6
	118	0.2	—	2	0.1	—	2	0.2	—	2
Hematocrit (%)	-9	41.2	2.2	6	42.4	3.1	6	41.1	3.0	6
	48	40.0	2.0	6	41.8	1.2	6	42.9	2.8	6
	91	38.2	1.8	6	38.7	1.2	6	43.3*	1.7	6
	118	39.0	—	2	41.5	—	2	44.3	—	2
Mean Corpuscular Volume (fL)	-9	64.5	3.4	6	65.6	2.4	6	62.9	2.8	6
	48	64.1	3.2	6	65.2	2.5	6	64.4	2.4	6
	91	64.1	2.8	6	64.2	2.3	6	63.0	2.3	6
	118	60.8	—	2	64.8	—	2	59.2	—	2

\* Statistically different from concurrent vehicle control at p<0.05.

Table 4. Hematology Results

	Day	0 mg/kg			1 mg/kg			10 mg/kg		
		Mean	SD	N	Mean	SD	N	Mean	SD	N
Females										
Red Blood Cells (10 <sup>6</sup> /μL)	-8	6.06	0.32	6	5.94	0.31	6	5.90	0.36	6
	48	6.05	0.57	6	6.19	0.25	6	6.29	0.24	6
	91	5.70	0.64	6	5.66	0.36	6	5.99	0.35	6
	118	6.23	--	2	5.89	--	2	6.01	--	2
Reticulocytes (% of RBC)	-8	0.4	0.2	6	0.2	0.1	6	0.4	0.3	6
	48	0.3	0.2	6	0.2	0.1	6	0.5	0.3	6
	91	0.6	0.6	6	0.4	0.2	6	0.4	0.3	6
	118	0.1	--	2	0.5	--	2	0.1	--	2
Hemoglobin (g/dL)	-8	12.9	0.2	6	12.5	0.6	6	12.1	0.8	6
	48	12.4	0.9	6	12.6	0.6	6	12.7	0.6	6
	91	11.4	1.0	6	11.3	0.7	6	11.7	0.7	6
	118	13.2	--	2	11.8	--	2	11.8	--	2
Methemoglobin (g/dL)	-8	0.1	0.0	6	0.1	0.1	6	0.1	0.0	6
	48	0.1	0.0	6	0.1	0.0	6	0.1	0.0	6
	91	0.1	0.0	6	0.1	0.0	6	0.1	0.0	6
	118	0.1	--	2	0.1	--	2	0.1	--	2
Hematocrit (%)	-8	39.1	1.1	6	38.1	1.5	6	37.3	1.8	6
	48	38.7	2.8	6	39.8	1.1	6	40.1	1.3	6
	91	36.4	2.9	6	36.4	1.8	6	37.9	2.0	6
	118	40.3	--	2	36.1	--	2	36.5	--	2
Mean Corpuscular Volume (fL)	-8	64.6	2.6	6	64.2	3.4	6	63.4	2.5	6
	48	64.2	2.8	6	64.4	3.7	6	63.9	2.1	6
	91	64.0	3.1	6	64.4	4.2	6	63.3	2.2	6
	118	64.8	--	2	61.4	--	2	60.7	--	2

\* Statistically different from concurrent vehicle control at p≤0.05.

• Clinical chemistry: There were no treatment related changes in differential White Blood Cell Counts in males and females in all groups. Prothrombin time was significantly increased in the 10 mg/kg male and female monkeys after 48 and 91 day-treatment (Table 6). There were some sporadic changes without clear patterns. For examples, Sorbitol dehydrogenase activity was reduced in males in 1 and 10 mg/kg/day groups and 10 mg/kg/day group only in females, which was reversed after 4-week recovery period. Gamma Glutamyl Transferase (IU/L) activity was elevated in HD groups in both males and females. Urea nitrogen levels were reduced both in males and females. All these changes were reversed after 4-week recovery study as shown below.

Table 6. Coagulation Results

	Day	0 mg/kg			1 mg/kg			10 mg/kg		
		Mean	SD	N	Mean	SD	N	Mean	SD	N
<b>Males</b>										
Prothrombin Time (sec)	-9	10.4	0.2	6	10.3	0.3	6	10.4	0.2	6
	48	10.5	0.4	6	10.6	0.3	6	11.1*	0.3	6
	91	10.3	0.3	6	10.4	0.4	6	11.2*	0.2	6
	118	10.2	--	2	10.4	--	2	10.4	--	2
Activated Partial Thromboplastin Time (sec)	-9	21.0	1.6	6	20.8	2.3	6	20.1	0.9	6
	48	20.5	1.3	6	19.9	1.0	6	20.9	0.8	6
	91	20.1	1.6	6	19.7	1.4	6	21.4	1.6	6
	118	19.7	--	2	19.9	--	2	20.7	--	2
<b>Females</b>										
Prothrombin Time (sec)	-8	10.0	0.3	6	10.2	0.1	6	9.9	0.3	6
	48	10.2	0.5	6	10.5	0.3	6	10.8*	0.2	6
	91	9.9	0.4	6	10.2	0.3	6	10.7*	0.2	6
	118	10.0	0.1	2	9.9	0.1	2	10.2	0.0	2
Activated Partial Thromboplastin Time (sec)	-8	19.6	1.7	6	19.5	0.7	6	19.2	2.2	6
	48	19.4	2.2	6	19.7	0.8	6	20.2	1.5	6
	91	18.9	1.3	6	19.1	1.1	6	19.9	1.9	6
	118	20.1	--	2	18.8	--	2	18.9	--	2

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Effects of IGF-1/IGFBP-3 on Serum Chemistry in 90-Day Toxicity Study with 4-Week Recovery in Monkeys#							
Sex		Male			Female		
Dose (mg/kg/day)		0	1	10	0	1	10
Sorbitol Dehydrogenase (IU/L)	-9@	12	10	10	10	7	11
	48	8	7	5	9	9	8
	91	13	7*	4*	12	9	6*
	118#	6	4	5	7	6	6
Alanine Aminotransferase (IU/L)	-9	42	44	62*	50	52	58
	48	41	42	60*	48	48	69
	91	70	72	68	57	57	77
	118	44	47	49	56	50	77
Gamma Glutamyl Transferase (IU/L)	-9	211	196	222	136	161	151
	48	180	173	224	118	143	160*
	91	173	158	228*	106	122	137*
	118	194	158	162	113	127	105
Blood Urea Nitrogen (mg/dL)	-9	22	21	23	17	15	19
	48	21	20	18*	21	20	17*
	91	21	21	18	20	17	14*
	118	21	23	18	18	16	18

#Each group had 6 animals except on Day 118 (recovery study), which had 2 monkeys. @Indicate the day when the test was performed in males. In females, each test was performed one day earlier.

- Urinalysis: No toxicologically significant findings were noted among the groups treated with the drug and in control group in the study.
- Organ weight: As expected, there were dose-dependent increases in group mean absolute organ weight of spleen and thymus in male monkeys (Table 10a). Similar patterns of increases in female group mean absolute organ weight were also observed in liver, kidney, lung, heart,

adrenal gland and pituitary gland (Table 10a). There were also dose-related increases in female body weight, although that was not significant in male monkeys. Thus, group mean values for spleen-to-body weight, and spleen-to-brain weight values were increased in males. The statistically significant changes in organ weights in both males and females returned toward normal values after 4-Week recovery period (Table 10b).

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Table 10a. Absolute Organ Weights, Grams (Day 91 Sacrifice)

Interim Necropsy - Day 91														
Males														
Treatment Group	Terminal Body Weight (kg)	Liver	Kidney	Lung	Heart	Epididymis	Prostate	Testis	Spleen	Thymus	Adrenal Gland	Thyroid Gland	Pituitary Gland	Brain
0 mg/kg	Mean	3.34	71.58	16.82	17.24	12.52	1.57	1.26	8.82	6.06	4.97	0.40	0.056	68.93
	SD	0.35	8.28	3.17	1.24	0.80	0.89	0.82	7.99	0.49	1.59	0.12	0.003	9.48
	N	4	4	4	4	4	4	4	4	4	4	4	4	4
1 mg/kg	Mean	3.13	63.75	15.30	18.61	11.51	1.69	1.27	9.02	8.38	6.68	0.39	0.045*	68.50
	SD	0.39	9.52	3.15	2.45	1.80	1.21	1.29	10.15	2.69	1.52	0.12	0.004	2.53
	N	4	4	4	4	4	4	4	3	4	4	4	4	4
10 mg/kg	Mean	3.45	72.85	19.54	22.89	12.42	2.14	2.78	9.78	11.57*	8.27*	1.03	0.054	70.62
	SD	0.66	10.36	3.28	4.62	2.52	1.83	3.64	11.57	1.68	0.81	0.20	0.025	4.84
	N	4	4	4	4	4	4	4	4	4	4	4	4	4
Females														
Treatment Group	Terminal Body Weight (kg)	Liver	Kidney	Lung	Heart	Ovary	Spleen	Thymus	Adrenal Gland	Thyroid Gland	Pituitary Gland	Brain		
0 mg/kg	Mean	2.50	56.32	12.23	14.48	9.49	0.85	5.52	0.66	0.34	0.037	62.80		
	SD	0.09	4.38	1.41	2.16	0.36	0.36	0.93	0.03	0.14	0.007	2.90		
	N	4	4	4	4	4	4	4	4	4	4	4		
1 mg/kg	Mean	2.74*	59.6	13.44	16.83	9.22	0.99	5.25	0.70	0.41	0.043	67.12		
	SD	0.14	3.46	1.20	3.04	0.83	0.37	0.28	0.08	0.11	0.012	5.17		
	N	4	4	4	4	4	4	4	4	4	4	4		
10 mg/kg	Mean	2.90*	68.13*	17.46*	20.19*	11.83*	1.43	9.30	1.08*	0.49	0.056*	64.55		
	SD	0.12	6.98	2.27	2.92	1.59	0.74	2.19	0.08	0.18	0.008	1.71		
	N	4	4	4	4	4	4	4	4	4	4	4		

Table 10b. Absolute Organ Weights, Grams (Day 118 Sacrifice)

Final Necropsy - Day 118													
Males													
Treatment Group	Terminal Body Weight (kg)	Liver	Kidney	Lung	Heart	Epithymus	Prostate Glands	Spleen	Thymus	Adrenal Gland	Thyroid Gland	Pituitary Gland	Brain
0 mg/kg	Mean	64.60	14.41	18.55	11.17	11.17	1.09	6.61	7.89	0.55	0.45	0.051	76.19
	SD	--	--	--	--	--	--	--	--	--	--	--	--
	N	2	2	2	2	2	2	2	2	2	2	2	2
1 mg/kg	Mean	64.33	15.47	18.17	13.03	12.71	2.15	8.89	3.55	0.76	0.30	0.057	69.51
	SD	--	--	--	--	--	--	--	--	--	--	--	--
	N	2	2	2	2	2	2	2	2	2	2	2	2
10 mg/kg	Mean	64.99	15.00	18.51	13.23	1.08	1.10	8.35	4.80	0.85	0.53	0.049	69.02
	SD	--	--	--	--	--	--	--	--	--	--	--	--
	N	2	2	2	2	2	2	2	2	2	2	2	2
Females													
Treatment Group	Terminal Body Weight (kg)	Liver	Kidney	Lung	Heart	Ovary	Spleen	Thymus	Adrenal Gland	Thyroid Gland	Pituitary Gland	Brain	
0 mg/kg	Mean	50.49	11.47	17.12	9.59	1.22	5.94	6.17	0.55	0.39	0.056	67.00	
	SD	--	--	--	--	--	--	--	--	--	--	--	
	N	2	2	2	2	2	2	2	2	2	2	2	
1 mg/kg	Mean	58.38	14.19	15.03	11.13	0.89	8.21	4.56	0.71	0.40	0.061	65.53	
	SD	--	--	--	--	--	--	--	--	--	--	--	
	N	2	2	2	2	2	2	2	2	2	2	2	
10 mg/kg	Mean	61.64	13.97	16.61	9.20	1.17	6.64	3.94	0.93	0.51	0.042	60.04	
	SD	--	--	--	--	--	--	--	--	--	--	--	
	N	2	2	2	2	2	2	2	2	2	2	2	

. Gross pathology: One monkey in the HD male group and one female monkey in the control group had discoloration in brain. In some injection sites for the administration of IGF-1/IGFBP there were discolored spots on Day 91 necropsy, which disappeared after 4-week recovery period. There were no remarkable findings in other animals.

• Histopathology:

Histopathologic evaluations of preserved tissues collected at necropsy were performed by \_\_\_\_\_ for Celtrix Pharmaceuticals, Inc., Santa Clara, CA. Following 90 days of treatment, possible treatment related changes were noted microscopically in lymphoid tissue, bone, and at the injection site. Inflammation at the injection sites was reduced following the recovery period (Please see a table below). It appears that the changes in the lymphoid tissue and bone were considered to be related to the pharmacological effects of the test article rather than to toxic effects. The changes in the bone should be interpreted cautiously in view of small group size and the uncertain age and nutritional status of the monkeys. In general, histopathological effects of the test article were expected based on IGF-1 action without remarkable new findings as documented below.

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Major Histopathological Findings in 13-Week Subcutaneous Toxicity Study of rhIGF-1/IGFBP-3 in Monkeys@										
Tissues/Diagnosis/Modifiers	Control		1 mg/kg		10 mg/kg		Cont-Rec		10-Rec	
	M	F	M	F	M	F	M	F	M	F
Bone, Femur, cortical bone increased, Trace	1	1	4	1	0	2	1	0	1	0
Bone, Femur, cortical bone increased, Mild	1	1	4	2	0	2	1	0	1	0
Bone, trabacular, increased, Trace	1	0	1	0	2	2	0	0	0	0
Bone, trabacular, increased, Mild	0	0	0	0	1	1	0	0	1	0
Cecum, hyperplasia, interfollicular zone, Trace	0	1	1	3	3	0	2	0	0	0
Cecum, inflammation, acute, Trace	0	1	1	2	1	3	0	0	0	0
Heart, inflammation, granulomatous, Trace	0	0	0	0	0	1	0	0	0	0
Heart, inflammation, granulomatous, Mild	0	0	0	0	0	1	0	0	0	0
Heart, pericarditis, Moderate	0	0	0	0	0	0	1	0	0	0
Ileum, hyperplasia, interfollicular, Trace	0	2	0	0	0	0	0	1	1	1
Ileum, hyperplasia, interfollicular, Mild	0	0	1	0	0	0	0	0	0	0
Injection site/subcutis, infiltration-eosinophilic, Trace	0	0	0	0	0	1	0	0	0	0
Injection site/subcutis, infiltration-eosinophilic, Mild	0	0	0	2	2	2	0	0	0	0
Injection site, subcutis, infiltration-eosinophilic, Moderate	0	0	0	1	0	0	0	0	0	0
Kidney, fibrosis, Trace	0	1	1	0	0	0	0	0	0	0
Kidney, granuloma, Trace	0	0	0	0	1	1	0	0	0	0
Kidney, mineralization, Mild	0	0	0	1	0	0	0	0	0	0
Liver, cytoplasmic alteration, clear, Mild	0	0	0	0	1	0	0	0	0	0
Liver, fibrosis, Mild	0	0	1	1	0	0	0	0	0	0
Liver, hemorrhage, Moderate	0	0	0	1	0	0	0	0	0	0
Lung, fibrosis, Trace	0	0	0	0	0	0	0	1	0	0
Lung, inflammation, granulomatous, Trace	0	0	0	1	0	3	0	1	0	0
Lung, inflammation, granulomatous, Mild	0	0	0	0	0	1	0	0	0	0
Lymph Node, mandibular, interfollicular, hyperplasia, Mild	0	0	0	0	0	0	0	0	1	2
Spleen, congestion, Moderate	0	0	0	0	1	0	0	0	0	0
Spleen, fibrosis, Trace	0	0	0	0	0	0	1	0	0	0
Spleen, tangible body macrophages ↑, Trace	0	0	0	1	1	0	0	0	1	0
Thymus, cortical hyperplasia, Trace	0	0	0	0	4	1	0	0	0	0
Thymus, cortical hyperplasia, Mild	0	0	1	1	2	0	0	3	1	0
Thyroid, fibrosis, Mild	0	0	0	0	0	1	0	0	0	0
Urinary bladder, infiltration, lymphocytic, Trace	0	0	0	1	0	0	0	0	0	0

@All groups had 6 monkeys at the beginning except the recovery groups, which had 2. Cont-Rec and 10-Rec indicate recovery groups for the control and 10 mg/kg/day, respectively.

### Conclusion:

Following 90 days of treatment, the test article-related changes were increases mean body weights in the female treated animals. The changes were also included in an increase in lymphoid tissue organ weights in treated males and in female monkeys in the 10 mg/kg/day group. There were discolorations and possible macroscopic inflammation of the injection sites. Microscopically following the end of 90-Day of treatment, possible treatment-related changes were noted in bone, thymus, lymph nodes and spleen. The monkeys in the recovery groups did

not show any clear test article related changes in the body weights or organ weights due to presumable recovery during the recovery period. Microscopically, possible treatment-related changes were noted in the lymphoid tissues, bone and injection site, which were reversed in some animals after the recovery period.

Toxicokinetics of IGF-1 in a 90-Day Repeat Dose Subcutaneous Toxicity study of rhIGF-1/IGFBP-3 in Cynomolgus Monkeys (4.2.3.2.a.4): A toxicokinetic study was performed by (Study #057646A) in monkeys that were used for the main toxicology study above. The table below summarized the highlights of main pharmacokinetic parameters that were obtained from the study.

	Day 1				Day 90			
	AUC	Cmax	CL	Tmax	AUC	Cmax	CL	Tmax
<i>Females</i>								
1 mg/kg	12295	816	94	8	30406	1567	48	8
10 mg/kg	41297	2235	255	6	135696	6696	109	8
<i>Males</i>								
1 mg/kg	11805	710	88	8	42235	2341	33	8
10 mg/kg	44689	2761	228	3	200447	10652	68	6

Note: Parameters calculated using serum concentrations of IGF-I above baseline.  
Units for parameters are: AUC, ng-hr/mL; Cmax, ng/mL; CL, mL/hr/kg; Tmax, hr

Summary and Conclusion: Subcutaneous administration of rhIGF-1/IGFBP-3 daily at the top dose of 10 mg/kg/day for 91 days was reasonably well tolerated in most monkeys. It was associated with increased body weight gain in female monkeys, although the effects on mean absolute body weights were not significant in males. Histologically, there were slight to mild increases in cortical femoral and trabecular bone in both sexes. There was histopathological evidence of eosinophilic infiltration at the injection sites, particularly in males, which were reversed after a 4-Week recovery period. It appears that there was mild cortical hyperplasia in thymus in the treated animals.

According to the pharmacokinetic data provided by the sponsor, both AUC and Cmax were significantly greater after 90 days treatment, compared to those on Day one in both males and females. Naturally the clearance was significantly less in both males and females on Day 90. Tmax was 6 to 8 hours both in Days 1 and 90 in male and female monkeys. Based on histopathologic findings, a dose of 1 mg/kg may be considered a NOAEL (No Observable

Adverse Effects Level) doses, which will be approximately 2 times clinical exposures with the MRHD based on body surface comparison.

**Additional Study:**

**Title: Anti-rhIGF-1/IGFBP-3 Antibody Report: 90-Day Repeat Dose Subcutaneous Toxicity Study of rhIGF-1/IGFBP-3 in Cynomolgus Monkeys (In this 90-day monkey study immunogenicity study was performed)**

Sponsor's original title: Anti-rhIGF-1/IGFBP-3 Antibody Report: 90-Day Repeat Dose Subcutaneous Toxicity Study of rhIGF-1/IGFBP-3 in Cynomolgus Monkeys

Documents: Module 4; volume 8, page 252-294

Study Number: N057646A

Conducting laboratory: \_\_\_\_\_

Sponsor: Celtrix Pharmaceuticals, Inc.

Date of study initiation: Sept. 1998

GLP compliance: Yes

QA Report: Yes (x) No ( )

**Methods:**

Frozen monkey (6/sex) sera from \_\_\_\_\_ were used. Antibodies against rhIGF-1/IGFBP-3 were assayed using an enzyme linked immunosorbent assay (ELISA). Serum samples were analyzed by Western blots to determine whether the antibodies measured in the ELISA were directed against the IGF-I or the IGFBP-3 component of the rhIGF-1/IGFBP-3. Controls include the following:

goat anti-human IGF-I antibody \_\_\_\_\_, with a rabbit anti-goat IG-HRP \_\_\_\_\_ and rabbit anti-human IGFBP-3 antibody \_\_\_\_\_, with goat anti-rabbit IG-HRP \_\_\_\_\_

For neutralizing bioassay, the IGF-I bioassay provides a method for assaying the activity of IGF-I or rhIGF-1/IGFBP-3 which increases cell proliferation of human Osteosarcoma cells (MG63). Antibodies to either IGF-I or rhIGF-1/IGFBP-3 could potentially neutralize this activity.

**Results:**

All 12 monkeys in vehicle control male and female groups had no antibodies to rhIGF-1/IGFBP-3 (See Table below). All monkeys treated with 1 or 10 mg/kg/day developed antibody titers to rhIGF-1/IGFBP-3. In the recovery animals, antibodies were still present, although the titers of some monkeys were somewhat lower on Day 119 after recovery compared to Day 49. The antibody titers were similar among the 1 and 10 mg/kg dosage groups. The response is also similar between males and females. Results of the Western blots from gels run with rhIGF-1/IGFBP-3 show that the antibodies produced by the monkeys are against the IGFBP-3 portion of the rhIGF-1/IGFBP-3, rather than the IGF-I portion.

The results of the neutralization bioassay using rhIGF-1/IGFBP-3 correlate to the number of cells present (Table 2). For each plate, there is no inhibition of the mitogenic effect of rhIGF-1/IGFBP-3 on the cells when serum from the Pre-bleed is compared to serum from the Day 91

or Day 119 in any group. The optical density of the neutralization bioassay using IGF-I are shown in Table 4. Again, there is no inhibition of the mitogenic effect of the IGF-I on cells when Pre-serum is compared to Day 91 or Day 119 serum.

**Conclusion:**

All monkeys given daily subcutaneous injections of the vehicle did not develop a titer as measured by ELISA. However, all monkeys given daily subcutaneous injections of rhIGF-I/IGFBP-3 did develop antibodies to rhIGF-I/IGFBP-3. The Western blots indicate that the IGFBP-3 portion of the rhIGF-I/IGFBP-3 was immunogenic.

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Dose	MALES				FEMALES				Final Result				
	Animal #	Titer*		Final Result**	Animal #	Titer		Final Result					
0 mg/kg	101	Pre	1:320	Day 91	1:160	Negative	Pre	1:40	Day 91	1:20	Negative		
		1:40	1:40	1:40	1:20		1:20	neg					
		1:80	1:80	1:40	1:40		1:40	1:20	1:20	Negative			
		1:80	1:80	neg**	1:40		1:40	1:20	1:20	Negative			
	105	Pre	1:80	Day 49	1:80	Day 119	Negative	Pre	1:80	Day 49	1:160	Negative	
		1:80	1:80	1:80	neg	1:80		neg					
		1:80	1:80	1:80	1:80	1:80		1:160	1:160	Negative			
		1:80	1:80	1:80	neg	1:80		neg	1:160	Negative			
1 mg/kg	201	Pre	1:40	Day 49	1:10240	Day 91	Positive	Pre	1:80	Day 49	1:5120	Day 91	Positive
		1:40	1:40	1:5120	1:20480	1:160		1:1280					
		neg	1:1280	1:2560	1:2560	1:2560		1:10240	1:10240	Positive			
		1:40	1:40	1:5120	1:5120	1:20480		1:40960	1:40960	Positive			
	205	Pre	1:20	Day 49	1:119	Day 119	Positive	Pre	neg	Day 49	1:320	Day 119	Positive
		1:40	1:40	1:5120	1:2560	1:320		1:320					
		1:40	1:10240	1:10240	1:10240	1:5120		1:10240	1:10240	Positive			
		1:40	1:40	1:5120	1:5120	1:5120		1:10240	1:10240	Positive			
10 mg/kg	301	Pre	neg	Day 49	1:20480	Day 91	Positive	Pre	neg	Day 49	1:2560	Day 91	Positive
		neg	neg	1:40960	1:81920	1:2560		1:2560					
		neg	1:5120	1:10240	1:10240	1:10240		1:40960	1:40960	Positive			
		1:40	1:40	1:5120	1:2560	1:20480		1:20480	1:20480	Positive			
	305	Pre	1:20	Day 49	1:119	Day 119	Positive	Pre	neg	Day 49	1:5120	Day 119	Positive
		neg	neg	1:20480	1:5120	1:5120		1:320					
		neg	1:40960	1:40960	1:40960	1:5120		1:5120	1:5120	Positive			
		neg	1:40960	1:40960	1:40960	1:5120		1:5120	1:5120	Positive			

\*A titer is defined as the last dilution in which the OD is ≥ 0.100.

\*\*An individual monkey is considered positive for antibodies when the titer increases 2 fold from the "Pre" sample; an individual sample (diluted 1:20) is considered negative when the OD < 0.100.

effect of monkey antibodies on the activity of rhIGF-I/IGFBP-3 on osteosarcoma cells

Plate #	Animal #	Dosage mg/kg	Time Point	Complex (50 ng/mL) + serum at the following dilutions:							CONTROLS:*		
				1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	anti-IGF-I	anti-IGFBP3
1	102	0	Pre	1.007	0.803	0.706	0.624	0.557	0.556	0.541	0.553	0.224	0.702
			Day 91	0.785	0.686	0.640	0.598	0.546	0.549	0.551	0.548		
2	112	0	Pre	0.988	0.705	0.653	0.571	0.511	0.499	0.511	0.530	0.214	0.875
			Day 91	0.852	0.638	0.601	0.544	0.524	0.511	0.527	0.530		
3	103	0	Pre	0.879	0.497	0.501	0.484	0.482	0.455	0.468	0.438	0.257	0.474
			Day 91	0.666	0.583	0.520	0.498	0.482	0.476	0.476	0.467		
4	113	0	Pre	0.554	0.435	0.528	0.528	0.494	0.475	0.472	0.482	0.272	0.567
			Day 91	0.416	0.611	0.551	0.519	0.485	0.494	0.493	0.490		
5	202	1	Pre	0.884	0.823	0.673	0.580	0.545	0.535	0.551	0.543	0.220	0.642
			Day 91	0.865	0.787	0.704	0.632	0.584	0.547	0.550	0.572		
6	211	1	Pre	0.899	0.829	0.711	0.591	0.508	0.494	0.498	0.510	0.214	0.618
			Day 91	0.832	0.847	0.761	0.638	0.573	0.538	0.545	0.523		
7	206	1	Pre	0.692	0.548	0.486	0.470	0.449	0.446	0.448	0.433	0.245	0.455
			Day 119	0.797	0.808	0.594	0.521	0.474	0.456	0.456	0.437		
8	214	1	Pre	0.619	0.569	0.551	0.534	0.507	0.489	0.484	0.480	0.256	0.540
			Day 91	0.532	0.844	0.631	0.657	0.582	0.552	0.529	0.512		
9	302	10	Pre	0.249	0.827	0.748	0.622	0.551	0.542	0.532	0.551	0.209	0.640
			Day 91	0.919	0.860	0.814	0.787	0.720	0.687	0.655	0.607		
10	313	10	Pre	0.968	0.764	0.762	0.833	0.550	0.566	0.551	0.547	0.217	0.634
			Day 91	1.078	0.991	0.933	0.854	0.762	0.677	0.620	0.590		
11	306	10	Pre	0.898	0.595	0.498	0.457	0.445	0.429	0.426	0.433	0.255	0.473
			Day 119	0.843	0.896	0.575	0.543	0.480	0.457	0.462	0.442		
12	314	10	Pre	0.992	0.695	0.598	0.565	0.519	0.500	0.504	0.507	0.262	0.536
			Day 91	0.844	0.836	0.757	0.759	0.681	0.628	0.581	0.543		

\*The complete set of controls for each plate is shown in Table 3.

Table 4. - effect of monkey antibodies on the activity of IGF-I on sarcoma cells

Plate #	Animal #	Dosage mg/kg	Time Point	IGF-I (20 ng/mL) + serum at the following dilutions:					CONTROLS:*			
				1:40	1:80	1:160	1:320	1:640	Cells	Cells + IGF-I	Cells + IGF-I + anti-IGF-I	Cells + IGF-I + anti-IGFBP3
1	103	0	Pre	0.558	0.618	0.577	0.559	0.554	0.258	0.512	0.283	0.537
			Day 91	0.898	0.619	0.582	0.566	0.578				
	206	1	Pre	0.687	0.589	0.558	0.523	0.538				
			Day 119	0.794	0.641	0.623	0.578	0.635				
2	306	10	Pre	0.741	0.617	0.572	0.549	0.530	0.245	0.513	0.265	0.524
			Day 119	0.733	0.690	0.800	0.582	0.541				
	113	0	Pre	0.601	0.585	0.560	0.529	0.537				
			Day 91	0.671	0.610	0.566	0.540	0.553				
3	214	1	Pre	0.743	0.649	0.605	0.558	0.556	0.238	0.511	0.276	0.538
			Day 91	0.772	0.660	0.813	0.794	0.749				
	314	10	Pre	0.703	0.661	0.800	0.875	0.793				
			Day 91	0.755	0.651	0.673	0.645	0.633				

\*All antibodies diluted 1:40

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**Title: 13-Week repeat dose subcutaneous toxicity study of rhIGF-1 in Mice (Section 4.2.3.2.b.1)**

Sponsor's ID #/study#: N614-Q1374/Pharmacia 9650014

Sponsor's original title: 13-Week repeat dose subcutaneous toxicity study of rhIGF-1/IGFBP-3 in mice

Study Number: N614-Q1374

Document: Module 4, vol. 9, page1-280

Conducting laboratory: Toxicology and Safety Assessment. — for  
Pharmacia S.p.A. under GLP and OECD guideline

Date of study initiation: Jan. 9, 1995

GLP compliance: Yes

QA Report: Yes (x) No ()

Methods:

Dosing information

- Species: — CD-1 Br mice
- #/sex/group or time point: 30 rats/sex/group. Group 1 was a placebo (10 mM phosphate solution) control group. Groups 2, 3, and 4 had low, mid and high dose groups.
- Age: 5 weeks
- Weight: Male: 24-32 g; Female 20-26 g
- Satellite groups used for toxicokinetics or recovery: 5 rats/sex for groups 1 and 4.
- Dosage groups in administered units: 0, 0.4, 2 and 10 mg/kg/day
- Route, form, volume, and infusion rate (if i.v.): Subcutaneous

Drug, lot#, radiolabel (if applicable), and % purity: rhIGF-1, batch #59108-51/IGFBP-3

Formulation/vehicle: Inactive ingredients are commonly used in pharmaceutical oral tablets marketed in the U.S.A. as listed under "Clinical formulation (and components)".

Times at which Observations are made:

- Clinical signs: Once daily
- Body weights: Days 1, and then weekly
- Food consumption: Days 1 and then weekly
- Ophthalmoscopy: NA
- EKG: NA
- Hematology: Days 43 and 92
- Clinical chemistry: Day 92
- Urinalysis: NA
- Gross pathology: At the time of death or sacrifice
- Organs weighed: At the time of death, sacrifice, or necropsy
- Histopathology: At the time of death or sacrifice
- Toxicokinetics: Days 0, 26, and 88 at 1, 2, 4, 6, 8, 12, and 24 hours after the dose

**RESULTS:**

- Clinical signs and mortality:

Deaths occurred between Days 9 and 44 in one female at 2 mg/kg/day and in six females at 10 mg/kg/day. No gross or histopathological evidence of toxicity was observed in decedents. It appears that some of the deaths were hypoglycemia as an extension of pharmacological action, although some deaths were clearly due to technical errors such as an excess of ether anesthesia. During Weeks 2 to 6 a marked depression due to hypoglycemia occurred in a few animals given the high dose of rhIGF-I. This treatment-related finding characterized by individual differences in frequency, appeared about two to three hours after the treatment. The signs usually disappeared within thirty minutes of administration of oral glucose. A case of marked depressive inactivity was also seen in one female at 2 mg/kg/day at Week 11.

• Body weights:

A slight increase in body weight gain was observed in all treated groups throughout the treatment period in comparison to controls. At the end of the study (Day 91) the increase in mean body weight of treated groups ranged from about 5 % to 10% in males and from about 10% to 15 % in females with scant dose relationship. Group mean body weights in male and female mice are summarized below in two tables below.

rhIGF-I: 13-WEEK SUBCUTANEOUS TOXICITY STUDY IN MICE

TABLE: 6.3

Exp. N614 - Q1374

GROUP DOSE (mg/Kg/day): Control 1 2 3 4

BODY WEIGHTS (g)

Group(s)		Day of Study											
		-4	1	8	15	22	29	36	42	50	57	64	71
Male Animals													
1	(N)	30	30	30	30	30	30	30	30	17	17	17	17
	Means	24.3	28.7	31.1	34.0	36.0	37.7	38.9	39.7	40.7	41.1	41.3	42.0
	SDEVs	1.03	1.34	2.04	2.16	2.36	2.45	2.62	2.68	2.39	2.63	3.22	3.14
2	(N)	30	30	30	30	30	30	30	30	17	17	17	17
	Means	23.9	28.6	31.6	34.6	36.8	38.7	40.3	41.0	42.8	43.2	43.6	44.4
	SDEVs	1.24	1.54	2.11	2.37	2.61	2.60	2.53	2.86	2.92	2.68	3.07	3.35
3	(N)	30	30	30	30	30	30	30	30	17	17	17	17
	Means	23.7	28.1	31.5	35.0	37.0	39.2	40.8	41.2	42.1	42.3	42.9	43.7
	SDEVs	1.61	1.64	2.08	2.58	3.31	3.54	3.80	3.73	3.98	4.12	4.39	4.04
4	(N)	30	30	30	30	30	30	30	30	17	17	17	17
	Means	24.3	28.9	32.2	35.4	38.2*	40.1*	42.1*	43.1*	43.3	44.6*	44.5*	45.2*
	SDEVs	1.28	1.40	2.05	2.58	2.99	3.29	3.55	3.60	3.57	3.71	3.79	3.93
Female Animals													
1	(N)	30	30	30	30	30	30	30	30	17	17	17	17
	Means	22.2	24.0	24.8	26.1	29.0	29.6	30.4	31.2	31.1	31.8	31.7	31.9
	SDEVs	1.04	1.00	1.33	1.56	1.75	1.74	2.39	2.02	2.42	2.26	2.12	2.41
2	(N)	30	30	30	30	30	30	30	30	17	17	17	17
	Means	22.0	23.7	24.9	26.6	28.6	30.0	31.3	32.7*	33.5*	33.9	34.1*	34.0
	SDEVs	0.88	1.17	1.39	1.62	2.31	2.02	2.06	2.50	2.41	3.34	2.55	2.33
3	(N)	30	30	30	30	30	30	29	29	16	16	16	16
	Means	22.4	24.0	24.7	27.5*	29.6	30.8*	32.8*	33.6*	34.3*	34.5*	34.5*	35.6*
	SDEVs	1.07	1.26	1.35	1.23	1.70	2.05	1.77	1.59	2.62	2.98	2.77	2.67
4	(N)	30	30	30	28	28	28	27	26	13	13	13	13
	Means	22.5	24.2	25.5	27.5*	29.0	30.8	32.0*	32.5	34.0*	34.3	34.5*	35.1*
	SDEVs	0.69	0.96	0.89	1.30	1.64	2.01	2.16	2.40	3.10	3.20	2.65	3.17

\* = mean value of group was significantly different from control at p = 0.05 with Dunnett's test of significance

Exp. N614 - 01374

FIG-1: 13-WEEK SUBCUTANEOUS TOXICITY STUDY IN MICE

TABLE

GROUP : 1 2 3 4

DOSE (mg/Kg/day): Control 0.4 2 10

BODY WEIGHTS (g)

Group(s)	Day of study		
	78	85	91
Male Animals			
1	(N) 17 Means 42.8 SDEVs 3.41	17 43.4 3.65	17 42.6 3.97
2	(H) 17 Means 44.7 SDEVs 3.38	17 45.1 3.33	16 44.2 3.55
3	(H) 17 Means 44.2 SDEVs 3.94	17 44.5 3.83	16 44.8 3.59
4	(H) 17 Means 45.8* SDEVs 3.86	17 46.8* 3.98	17 46.3* 4.16
Female Animals			
1	(N) 17 Means 32.5 SDEVs 2.25	17 32.7 2.20	17 32.0 2.35
2	(H) 17 Means 35.5* SDEVs 3.92	17 36.2* 3.27	17 35.3* 3.27
3	(H) 16 Means 36.0* SDEVs 2.89	16 37.2* 3.26	16 36.4* 3.82
4	(H) 13 Means 35.7* SDEVs 2.86	13 37.8* 3.48	12 35.9* 3.30

\* = mean value of group was significantly different from control at p = 0.05 with Dunnett's test of significance

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#### Food consumption:

A slight increase in group mean food consumption was seen in treated female groups. This increase ranged from 10% to 16% in comparison to controls on Day 91. No significant differences in mean water consumption were observed between treated and control groups. The increases in water consumption sometimes seen in females given 2 and 10 mg/kg/day were considered not to be toxicologically meaningful because they were occasional and related to individual variations.

- Ophthalmoscopy: Not performed.
- Electrocardiography: Not performed.
- Hematology:

Large untyped cells increased in the 0.4 mg/kg/day male group on Day 43. The cell numbers were also increased in the 2 and 10 mg/kg/day male groups on Day 92. Percent platelet distribution width was reduced from 52 to 46% on Day 92 in the 0.4 mg/kg/day male group. Neutrophils counts were significantly increased in the 2 mg/kg/day male group on Day 92. Mean corpuscular hemoglobin concentration was increased on Day 43 in the 10 mg/kg/day female

group. It appears that the changes were random and not dose-dependent. Thus, the reviewer believes that the changes have no clinically relevant since they are not the test article-related events.

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13-Week Repeat Dose Subcutaneous Toxicity Study of rhIGF-1/IGFBP-3 in Rats  
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Table 4 (cont'd). Hematology Results

Recovery-phase Necropsy									
Males									
Treatment Group		Red Blood Cells (10E6/mm <sup>3</sup> )	Hemoglobin (g/dL)	Hematocrit (%)	Mean Corpuscular Volume (fL)	Mean Corpuscular Hemoglobin (pg)	Mean Corpuscular Hemoglobin Concentration (g/dL)	Platelets (10E9/mm <sup>3</sup> )	Reticulocytes (10E3/mm <sup>3</sup> )
0 mg/kg	Mean	8.8	18.5	48	56.0	19.2	34.2	1346	208
	SD	0.7	0.6	3	2.0	1.2	1.2	264	50
	N	5	5	5	5	5	5	5	5
30 mg/kg	Mean	8.3	15.6 <sup>A</sup>	46	54.9	18.7	34.0	1124	193
	SD	0.3	0.4	1	1.3	0.5	0.5	366	41
	N	5	5	5	5	5	5	5	5
Females									
Treatment Group		Red Blood Cells (10E6/mm <sup>3</sup> )	Hemoglobin (g/dL)	Hematocrit (%)	Mean Corpuscular Volume (fL)	Mean Corpuscular Hemoglobin (pg)	Mean Corpuscular Hemoglobin Concentration (g/dL)	Platelets (10E9/mm <sup>3</sup> )	Reticulocytes (10E3/mm <sup>3</sup> )
0 mg/kg	Mean	8.1	15.8	47	57.5	19.6	34.1	1072	183
	SD	0.5	0.6	2	1.7	0.5	0.6	171	14
	N	5	5	5	5	5	5	5	5
30 mg/kg	Mean	8.1	15.8	47	57.9	19.6	33.9	1135	179
	SD	0.3	0.7	2	1.9	0.3	0.9	106	27
	N	5	5	5	5	5	5	5	5

<sup>A</sup>Statistically different from vehicle-treated concurrent controls,  $p \leq 0.05$ , using a Dunnett's test

<sup>B</sup>Statistically different from vehicle-treated concurrent controls,  $p \leq 0.05$ , using a Wilcoxon test

- Clinical chemistry:

In males, alanine amino transferase was reduced from the control value 36 IU/L to 21, 16, and 17 at IGF-1 doses of 0.4, 2, and 10 mg/kg/day, respectively. The value decreased also in female mice in the 10 mg/kg/day group only. In females, the glucose level was elevated at the HD group from control 155 to 198 mg/dL, in spite of the anticipated hypoglycemic action of the test article. There were no other changes in clinical chemistry in both males and females. It appears that the changes were not toxicologically relevant because the nature of changes was random and not dose-dependent.

- Urinalysis: Not performed.

- Organ weight: Male and female body weights on Day 43 and 92 were not affected the treatment, although the female body weights increased in 13-week toxicology study in monkey as presented above. The absolute organ weight of spleen and liver increased significantly in the 2 and 0.4 mg/kg/day groups, respectively, in male mice on Day 43. Female organ weights were not altered at any doses on Day 43. On Day 92, spleen weight increased in the 10 mg/kg/day male group. The weights of thymus increased in all treated male groups. In females, kidney weights increased significantly at the LD and HD dose groups. Thymus weights increased only

in the LD and MD female groups, not in the HD female group. Several changes in organ weight were not dose-related as shown below.

Organ Weights in 13- Week Toxicology Study after Subcutaneous IGF-1 Administration in Mice on Day 43@								
Sex	Male				Female			
Dose#	Control	0.4	2	10	Control	0.4	2	10
Body Wt.	38	41	41	43	32	32	33	31
Spleen	0.12	0.15	0.17*	0.15	0.16	0.18	0.17	0.14
Kidney	0.64	0.74	0.68	0.72	0.45	0.45	0.43	0.41
Liver	1.95	2.28*	2.12	2.21	1.60	1.79	1.74	1.41
Thymus	0.05	0.05	0.05	0.04	0.05	0.05	0.05	0.05
Heart	0.20	0.23	0.22	0.22	0.17	0.17	0.17	0.16
Organ Weights in 13- Week Toxicology Study after Subcutaneous IGF-1 Administration in Mice on Day 92@								
Body Wt.	44	45	46	49	33	36	37	37
Spleen	0.13	0.13	0.15	0.17*	0.17	0.18	0.18	0.22
Kidney	0.77	0.76	0.76	0.76	0.44	0.48*	0.47	0.49*
Liver	2.34	2.34	2.26	2.29	1.70	1.80	1.81	1.82
Thymus	0.04	0.05*	0.05*	0.05*	0.05	0.07*	0.06*	0.06
Heart	0.24	0.21	0.25	0.24	0.18	0.19	0.18	0.18

@Represent means of 12 to 11 mice and unit was in gram. #Dose in mg/kg/day. \*p<0.05.

. Gross pathology: Not remarkable.

• Histopathology:

The histopathology results observed in mice treated with IGF-1 in 13-week toxicology study have limited usefulness. According to the reviewer's observation the sponsor examined limited numbers of animals' organs in the control and in the HD (10 mg/kg/day) groups. For example, in the final examination, the sponsor did not examine uterus. Only one ovary in the control group, three ovaries in the 2 mg/kg/day and one ovary in the 10 mg/kg/day were examined as shown in a table below.

In general, IGF-1 had no remarkable histopathological effects since the control and the HD groups behaved similarly, although the numbers of organ examined were limited without clear histological details. It appears that there were limited effects on mouse bone system. The major changes in histopathology were bone (femur), liver, nasal tissues, pancreas and thymus in rats and monkeys as reviewed previous 13-week toxicity studies with IGF-1 and its BP-3 may be secondary to the prolonged half-life of IGF-1 when complexed with the binding protein.

Major Histopathological Findings in 13-Week Subcutaneous Toxicity Study of rhIGF-1 in Mice@								
Sex	Male				Female			
Dose#	Control	0.4	2	10	Control	0.4	2	10
Adrenal, not remarkable	0	0	3/3	0	0	0	0	0
Adrenal cortical cell vacuolation	0	0	0	0	0	0	0	0
Bone Marrow, not remarkable	12/12	0	0	12/12	12/12	0	0	9/12
Kidney, not remarkable	12/12	0	0	12/12	11/12	0	0	9/9
Kidney congestion	0	0	0	0	0	0	0	0
Kidney tubular dilation	0	0	0	0	1	0	0	0
Liver, not remarkable	10/12	0	0	11/12	8/12	0	0	8/9
Liver, congestion	0	0	0	0	0	0	0	0
Liver, chronic inflammation	0	0	0	0	1	0	0	1
Liver, extramedullary hematopoiesis	2	0	0	1	3	0	0	0
Liver, necrosis	0	0	0	0	0	0	0	1
Mesenteric lymph node, not remark	11/11	0	0	11/12	11/12	0	0	9/9
Mesenteric lymph node, lymphocele(s)	0	0	0	1	0	0	0	0
Mesenteric lymph node, histiocytosis	0	0	0	0	1	0	0	0
Submax. lymph node, not remarkable	5/10	0	0	11/12	9/11	0	0	4/8
Submax. lymph node(only one)	5	0	0	1	2	0	0	4
Ovaries, not remarkable	0	0	0	0	0/1	0	0/3	0/1
Ovaries, hemorrhage	0	0	0	0	0	0	1	0
Ovaries, dilation of the bursa	0	0	0	0	1	0	2	1
Ovaries, follicular cyst(s)	0	0	0	0	0	0	1	0
Spleen, not remarkable	2/12	0	0	1/12	0/12	0	0	0/9
Spleen, extramedullary hematopoiesis	10	0	0	11	12	0	0	9
Spleen, lymphoid depletion	0	0	0	0	0	0	0	0
Spleen, histiocytosis	2	0	0	0	0	0	0	0
Spleen, hemosiderosis	0	0	0	0	1	0	0	1
Seminal vesicles	0	0	0	0	0	0	0	0
Thymus, not remarkable	12/12	0	0	12/12	11/12	0	0	9/9
Thymus, branchial cyst(s)	0	0	0	0	1	0	0	0
Uterus	0	0	0	0	0	0	0	0

@There were 9 to 12 animals in each group and 0 to 12 animals were used in each group. Values indicate the number of positive animals over total numbers of examined mice. 0 indicates no animal was examined. #Dose in mg/kg/day.

Summary and conclusion: A 13-Week toxicology study with IGF-1 only was performed in mice at doses of 0.4, 2, and 10 mg/kg/day. There were many deaths in the HD female mice due to hypoglycemia as a secondary action of IGF-1 pharmacological actions. Increases in thymus weight at all doses in both sexes and in spleen weight of high dose males could be related to a possible stimulation of cell growth. Potential IGF-1 effects on immune system have not characterized because its effects on lymphocyte subsets, cytokine levels, and histological examination have not been completed. In particular, there were no histological changes in the lymph nodes. It appears that the test article, rhIGF-I is a species- specific protein acting as a growth factor but also exerting various metabolic effects. Based on clinical chemistry data and histopathologic findings, a dose of 2 mg/kg may be considered a NOAEL (No Observable Adverse Effects Level) doses. At NOAEL, clinical exposures with the MRHD would be approximately 1 based on body surface area

**Title: Determination of antibodies against rh-IGF-1 in rat serum after repeated iv administration (Section 4.2.3.2.b.4)**

1. Purpose: To determine antibodies against yeast derived recombinant human IGF-1 in serum samples from Sprague Dawley (SD) (SD) BR) rats. The study was performed at

2. Methods: Six rats/sex/group received rhIGF-1 intravenously daily at doses of 0.25, 2 and 16 mg/kg/day for 29-32 days. Serum samples were diluted with buffer, polyethyleneglycol (PEG), and extragammaglobulin to ensure an effective precipitation. Precipitation of antibody-bound tracer from the incubation mixture is achieved by a new addition of PEG. The radioactivity of the antibody-bound IGF-1 tracer in the pellet is determined in a gamma counter for 60 seconds.

Calculations: A serum pool from untreated male rats was used as a negative reference. Samples with counts per minute (CPM) level lower than the respective mean value plus 10 SD were considered not significantly different from the negative reference level and are reported as not detectable (ND).

3. Results and Conclusion: Repeated daily intravenous administration of rhIGF-1 for four weeks did not result in any antibody development measurable in the species under the experimental conditions as shown in two tables below.

Animal no.	Sex	Dose (mg/kg/day)	1 month treatm.	1 month treatm. + 3 days treatm.-free	1 month treatm. + 1 month treatm.-free
11	M	0	-	ND.	ND.
12	M	0	-	ND.	ND.
13	M	0	-	ND.	ND.
14	M	0	-	ND.	-
15	M	0	-	ND.	-
16	M	0	-	ND.	-
75	F	0	-	ND.	ND.
76	F	0	-	ND.	ND.
77	F	0	-	ND.	ND.
78	F	0	-	ND.	-
79	F	0	-	ND.	-
80	F	0	-	ND.	-
27	M	0.25	-	ND.	ND.
28	M	0.25	-	ND.	ND.
29	M	0.25	-	ND.	ND.
30	M	0.25	-	ND.	-
31	M	0.25	-	ND.	-
32	M	0.25	-	ND.	-
91	F	0.25	-	ND.	ND.
92	F	0.25	-	ND.	ND.
93	F	0.25	-	ND.	ND.
94	F	0.25	-	ND.	-
95	F	0.25	-	ND.	-
96	F	0.25	-	ND.	-
43	M	2	-	ND.	ND.
44	M	2	-	ND.	ND.
45	M	2	-	ND.	ND.
46	M	2	-	ND.	-
47	M	2	-	ND.	-
48	M	2	-	ND.	-
107	F	2	-	ND.	ND.
108	F	2	-	ND.	ND.
109	F	2	-	ND.	ND.
110	F	2	-	ND.	-
111	F	2	-	ND.	-
112	F	2	-	ND.	-

N.D. = not detectable

- = no sample taken

Section 4.2.3.2.b.4

Table 1 (cont). anti-rhIGF-1 in rat serum

7 (7)

Animal no.	Sex	Dose (mg/kg/day)	1 month treatm.	1 month treatm. + 3 days treatm.-free	1 month treatm. + 1 month treatm.-free
59	M	16	-	ND.	ND.
60	M	16	-	ND.	ND.
61	M	16	-	ND.	ND.
62	M	16	-	ND.	-
63	M	16	-	ND.	-
64	M	16	-	ND.	-
123	F	16	-	ND.	ND.
124	F	16	-	ND.	ND.
125	F	16	-	ND.	-
126	F	16	-	ND.	-
127	F	16	-	ND.	-
128	F	16	-	ND.	-

N.D. = not detectable

- = no sample taken

**Title: 26-Week repeat dose subcutaneous toxicity study with IGF-1 only in rats with 8 week recovery period**

Study No: — Project#450724/Document:20821F/Project# 1530' — Report#7895

Amendment # 080; Vol. #3a; and pages#001-530

Sponsor: Kabi Pharmacia AB, Stockholm, Sweden

Conducting laboratory and location:

Date of study initiation: March 2, 1992

GLP compliance: Yes

QA- Report Yes (x) No ()

#### METHODS:

Species/strain: Rat/Sprague-Dawley

#/sex/group or time point: 10/sex/group

Age: 4 weeks

Weight: Males (75-82 g); females (51-58 g)

Dosage groups in administered units: 0, 0.4, 2.0, and 10 mg/kg/day/sex/group

Route, form, volume, and infusion rate: Subcutaneous

Drug, lot#, radiolabel, and % purity: Not available

Formulation/vehicle: Clear, sterile, aqueous solution containing appropriate amounts of rhIGF-1, phosphate buffer (10 mM), and sodium chloride (145 mM, pH 5.9).

Observations and times:

Clinical signs: daily

Body weights: weekly

Food consumption: Weekly

Ophthalmoscopy: pretreatment, during Weeks 14 and 25

Hematology: pretreatment, during Weeks 7, 13 and 26. All surviving animals were sampled at Week 34

Clinical chemistry: pretreatment, during Weeks 7, 13 and 26. All surviving animals were sampled at Week 34.

Urinalysis: pretreatment, during Weeks 8, 12 and 25

Rectal temperature: pretreatment, 2, 4 and 6 h post dose during Weeks 2, 13 and 24 of dosing

Gross pathology: necropsy

Organs weighed: necropsy

Histopathology: necropsy

## RESULTS:

Clinical signs and mortality: There were 4 male and 17 female deaths in the HD group. One male and one female from the low and intermediate dose groups died prematurely. The deaths of some animals were due to eye damage resultant from the orbital bleeds and one male in the HD group died of lymphoma. The causes of the death were not clearly established except hypoglycemia in the HD group. The test article -induced hypoglycemia was likely the cause of the death since some of the animals were saved by the administration of 20% dextrose. Observed clinical signs were shallow breathing, salivation, staining around muzzle, and chromodacryorrea.

Body weights (weekly): There were increases in body weight in both males and females as summarized in a table below.

Time in week	Dose of rhIGF-1 (mg/kg/day) in Male and Female Rats@							
	Male				Female			
	0	0.4	2.0	10	0	0.4	2.0	10
Pretrial	128	129	127	127	91	90	91	90
1	252	255	253	255	163	160	164	163
4	375	382	377	398*	228	224	236	238
8	470	484	478	514*	271	270	287*	288*
12	532	555	543	600*	294	300	317*	325*
20	614	648	644	719*	326	332	356*	379*
24	648	688*	692*	774*	341	350	374*	394*
26	661	702*	703*	794*	343	354	382*	401*
%/Control		108	109	129		106	118	129

@The unit of bodyweight is in gram. \*p<0.05, compared to the control.

Food consumption (daily): There was a slight increase in total food consumed in the HD group both at Weeks 26 and 34 (14% and 16%, respectively) in males. In the LD and HD male groups, the total food consumption was not significantly different from the control group. In females, there was approximately 12% increase in total food consumed in the HD group both at Weeks 26 and 34. The parameter in other female groups was not remarkable.

Rectal temperatures: Not remarkable.

Ophthalmoscopy: Not remarkable.

Hematology:

In males, there was a slight (3%) increase in hematocrit in the MD and HD groups while white blood cell counts were reduced (14%) in the LD group at Week 7, which were not observed at Week 13. The findings were observed again at Week 26. In females, the increase in hematocrit in the HD group was 3%, which was significant at Weeks 7 and 13. At Week 26, there was an increase in lymphocytes (24%) and eosinophils (38%) in the HD group. Neutrophils were reduced in the LD (42%) and MD (48%) groups at Week 26, which appeared to be not drug dose dependent.

Clinical chemistry:

Glucose concentrations were reduced in both sex at all dose groups at Weeks 7, 13 and 25. In males, at Week 7, there was an increase in phosphate in all dose groups which received rhIGF-1 (11%, low dose; 11% intermediate dose and 14% in HD group). At Week 13, the increases in phosphate were 10, 11 and 17%, respectively, for the LD, MD and HD groups, and 16 and 20% in the MD and HD dose group at Week 26. The increases in phosphate were also observed in female MD and HD groups at Weeks 13 and 26. In addition, there was an increase in total bilirubin in LD (44%), MD (78%) and HD (67%) groups. Additionally, a decrease in insulin in the low (55%) and intermediate (49%) dose groups was observed.

Urinalysis:

In males, urine volume was increased in the HD group (60%), which may be due to reductions in glucose, osmolarity, sodium, potassium and chloride at Weeks 7 and 13. The urine volume was not different from the control group at Weeks 26 and 34 in males. In females, the volume of urine was increased at Weeks 7, 13 and 26 in the HD group with comparable changes in osmolarity, and sodium and potassium chloride, although the change reversed by Week 34.

Organ weights:

There were increases in many organ weights, which may be related to the increases in body weight gain as presented previously. The changes in organ weight were recalculated to reflect the body weight differences therefore only the data after correction for final body weight are summarized below. The weight changes returned to normal after 8-week recovery period except the weight of kidney and lung in males. In females, thymus weight was not reversible after an 8-week recovery period as indicated below. The bodyweight corrected, non-reversible increases in organ weight in the 3 organs was not correlated with histopathological changes in those organs as reviewed subsequently.

Tissues	Dose of rhIGF-1 (mg/kg/day) in Male and Female Rats at Week 26@							
	Male				Female			
	0	0.4	2.0	10	0	0.4	2.0	10
Body Wt.	657	698	693	793*	341	344	373*	393*
Adrenal	0.064	0.065	0.069	0.069	0.076	0.079	0.077	0.075
Brain	2.26	2.27	2.26	2.30	2.02	2.00	2.03	1.99
Heart	2.04	2.01	2.02	1.96	1.25	1.28	1.25	1.24
Kidney	4.30	4.46	4.35	4.51	2.48	2.49	2.54	2.50
Kidney <sup>&amp;</sup>	4.28			5.06*	2.41			2.51
Liver	24.10	23.62	22.04*	21.88*	11.87	12.06	11.73	11.54
Lung	2.12	2.19*	2.22	2.23	1.57	1.62	1.68	1.75
Lung <sup>&amp;</sup>	2.09			2.38*	1.59			1.83
Spleen	1.08	1.08	1.18*	1.16	0.68	0.70	0.68	0.68
Thymus	0.24	0.25	0.29	0.32	0.24	0.28*	0.27	0.31*
Thymus <sup>&amp;</sup>	0.16			0.20	0.17			0.23*
Prostate	0.85	0.96	0.95	1.14*				

@The unit of bodyweight is in gram. \*p<0.05, compared to the control. <sup>&</sup> Indicates values after 8-Week recovery.

Gross necropsy finding: In males, there was an enlargement of the submandibular lymph node: 3 out of 21 animals in the HD group and 1 out of 24 in the LD group and there were no cases in the control and ID groups. There were no remarkable intergroup differences in females. In prematurely dead animals the incidence was one out of 4 HD males and 4 out of 17 HD females.

Histopathology: At necropsy glycogen content was increased in male liver, which was observed in females. Significant hyperplasia in submandibular lymph node in male and female rats was also noted, which was not drug dose dependent. In the MD group of females, 6 rats had cardiomyopathy which was observed in prematurely dead animals. Cystic ducts in the thymus were observed in the LD female group, which was not significantly increased in the ID and HD group females. The major positive histopathological findings were summarized in a table below. There were minor changes in subcutaneous hemorrhage and inflammation at injection sites in the HD group.

Histopathological Findings in 6-Month Toxicity Study in Rats									
Organ and Findings	Animal Status	Males							
		Survivors				Decedents			
		Dose@	0	0.4	2.0	10.0		0.4	2.0
Liver, Glycogen		2			14*		0	0	0
Submandibular LN, Hyperplasia		0	1	13*	13*		0	0	0
		Females							
Heart, Cardiomyopathy		1	2	6*	2		0	0	8
Submandibular LN, Hyperplasia		0	1	4*	3*		0	0	5
Thymus, Cystic Duct(s)		0	7*	2	2		0	1	0

@The unit was in mg/kg/day. \*p<0.05 and LN stands for lymph node.

**Conclusion:**

At the high dose (10 mg/kg/day) premature death due to drug-induced sustained hypoglycemia was observed in both sexes. Increases in body weight gain, food consumption and phosphate concentration were observed in both sexes with a decrease in glucose concentration in the HD group. Histopathological study indicates that there was an increase in reactive hyperplasia of the submandibular lymph node in both sexes. The above findings were also confirmed in the intermediate dose group. The low dose did not produce adverse effects except for increases in body weight gain and phosphate in males. Thus, NOAEL in rats dosed for 26 weeks appeared to be 2 mg/kg/day, although the increase in body weight gain and phosphate was noted at the LD group. Human dose would be  $2 \text{ mg/kg} \times 0.2 \times 20 = 8 \text{ mg/M}^2$ , while that of rat would be  $2 \text{ mg/kg} \times 6 = 12 \text{ mg/M}^2$ . Thus, therapeutic exposure ratio would be under 2, based on body surface area comparison.

**Title: Analysis of rhIGF-1 and anti-IGF-1 antibodies in serum after a 26-Week subcutaneous toxicity study in rats with 8 week recovery period (Section 4.2.3.2.b.7)**

1. Purpose: The purpose of this study (#450-724-XFI) was to characterize antibodies after 26-week toxicology study in male rats. This study was performed at Kabi Pharmacia, Stockholm, Sweden under GLP.
2. Methods: Approximately 6-7 weeks old Sprague-Dawley rats (males, 150 g and females, 110 g) of both sexes were used. 30 rats/sex/group were used for the control and HD groups, while 25 rats/sex/group were used for the LD and MD groups. They received IGF-1 subcutaneously daily at doses of 0, 0.4, and 2, and 10 mg/kg/day for 26 weeks. Five rats/sex from the control and HD groups were retained after the dosing period for a further 8-week recovery study. Blood samples were collected from 5 rats/sex/group after 2-hour treatment on Day 1(plasma), Day 4 (serum), Week 14 (serum) and Week 25(serum), respectively.  
  
A competitive radioimmunoassay was used for the determination of IGF-1 and anti-IGF-1 antibody in samples. Immunoglobulins were separated from endogenous IGF-1 and IGF1BP by PEG precipitation. The immunoglobulins precipitate was dissolved and  $^{125}\text{I}$ -IGF-1 was added subsequently for RIA procedures. Anti-IGF-1 antibody titer was calculated by dividing the sample count per minute (CPM) with the CPM of the positive control serum (rabbit). Samples yielding a quotient (titer)  $\geq 1$  were considered anti-IGF-1 positive.
3. Results: A positive antibody titer was found in only one rat in the MD (2 mg/kg/day IGF-1) group at 26 weeks as shown below. In all rats of both sexes, anti-IGF-1 titers were not significantly increased (NSI) under the present experimental conditions. The CPM ratios were 4.86 in the positive female rat (#178) in the MD dose group. This is notable since rats treated with IGF-1:IGF1BP-3 displayed significant antibody responses after 3 months of treatment.

TABLE 11  
Results of measurement of antibodies to rhIGF-I in serum.

26 Week Subcutaneous (Bolus) Toxicity Study in Rats with 8 Week Recovery Period.

STUDY NO: 450-724-XF1

ANALYTE: Serum anti-IGF-I

Dates of analysis 921209-921216/TAVI

Animal No	Sex	Dose mg/kg/day	Sampling date	Anti-IGF-I titre	BXFI-No 1992-	Sampling date	Anti-IGF-I titre	BXFI-No 1992-
166	F	2	920529	N.S.I.	5174	920824	N.S.I.	5304
167	F	2				920024	N.S.I.	5305
168	F	2	920529	N.S.I.	5175	920824	N.S.I.	5306
169	F	2				920824	N.S.I.	5307
170	F	2				920824	N.S.I.	5308
171	F	2	920529	N.S.I.	5176	920824	N.S.I.	5309
172	F	2				920824	N.S.I.	5310
173	F	2				920824	N.S.I.	5311
174	F	2				920824	N.S.I.	5312
175	F	2				920824	N.S.I.	5313
176	F	2				920824	N.S.I.	5347
177	F	2				920824	N.S.I.	5348
178	F	2				920824	4.86	5349
179	F	2				920824	N.S.I.	5350
180	F	2				920824	N.S.I.	5351
181	F	2				920824	N.S.I.	5352
182	F	2	920 21	N.S.I.	5177	920824	N.S.I.	5353
183	F	2				920824	N.S.I.	5354
184	F	2				920824	N.S.I.	5355
185	F	2				920824	N.S.I.	5356
186	F	2				920824	N.S.I.	5357
187	F	2				920824	N.S.I.	5358
188	F	2				920824	N.S.I.	5359
189	F	2	920529	N.S.I.	5178	920824	N.S.I.	5360
190	F	2				920824	N.S.I.	5361

N.S.I. = Not Significantly Increased

APPEARS THIS WAY  
ON ORIGINAL

Animal no.	Sex	Dose (mg/kg/day)	1 month treatm.	1 month treatm. + 3 days treatm.-free	1 month treatm. + 1 month treatm.-free
11	M	0	-	ND.	ND.
12	M	0	-	ND.	ND.
13	M	0	-	ND.	ND.
14	M	0	-	ND.	-
15	M	0	-	ND.	-
16	M	0	-	ND.	-
75	F	0	-	ND.	ND.
76	F	0	-	ND.	ND.
77	F	0	-	ND.	ND.
78	F	0	-	ND.	-
79	F	0	-	ND.	-
80	F	0	-	ND.	-
27	M	0.25	-	ND.	ND.
28	M	0.25	-	ND.	ND.
29	M	0.25	-	ND.	ND.
30	M	0.25	-	ND.	-
31	M	0.25	-	ND.	-
32	M	0.25	-	ND.	-
91	F	0.25	-	ND.	ND.
92	F	0.25	-	ND.	ND.
93	F	0.25	-	ND.	ND.
94	F	0.25	-	ND.	-
95	F	0.25	-	ND.	-
36	F	0.25	-	ND.	-
43	M	2	-	ND.	ND.
44	M	2	-	ND.	ND.
45	M	2	-	ND.	ND.
46	M	2	-	ND.	-
47	M	2	-	ND.	-
48	M	2	-	ND.	-
107	F	2	-	ND.	ND.
108	F	2	-	ND.	ND.
109	F	2	-	ND.	ND.
110	F	2	-	ND.	-
111	F	2	-	ND.	-
112	F	2	-	ND.	-

N.D. = not detectable

- = no sample taken

**Title: Determination of antibodies against rhIGF-1 in Cynomolgus monkey serum during repeated iv injection (Section 4.2.3.2.b.11)**

1. Purpose: To determine antibodies against yeast derived rh-IGF-1 in serum samples from Cynomolgus monkeys. This study was conducted at \_\_\_\_\_ (Project#640374).
2. Methods: Groups of monkeys (3 monkeys/sex/group) were given daily for 30 days as intravenous bolus injection (20 seconds) or as continuous intravenous infusion (120 minutes). The bolus doses were 0.25 and 0.50 mg/kg/day and the infusion doses were 0.25, 0.50 and 1.00 mg/kg/day. Samples were also obtained from group of recovery animals (2 monkeys/sex/group) on the 28th day of the treatment-free period. To obtain a negative reference, serum samples were collected from all animals before treatment. The mean value was 516 cpm with a standard deviation of 109 cpm. Samples with a cpm level lower than that mean value  $\pm$  10 standard deviations are considered not significantly different from the negative reference level and is reported as not detectable (N.D.).
3. Results: Individual values are given in Table 1. Repeated daily intravenous injections of rhIGF-I for four weeks did not result in any antibody development measurable with this method.

**APPEARS THIS WAY  
ON ORIGINAL**

Animal no.	Sex	Mode of adm.	Dose mg/kg/day	1 month treatm.	1 month treatm. + 1 month treatm.-free
1	M	Infusion	0	N.D.	-
2	M	Infusion	0	N.D.	-
3	M	Infusion	0	N.D.	N.D.
18	F	Infusion	0	N.D.	-
19	F	Infusion	0	N.D.	-
20	F	Infusion	0	N.D.	N.D.
12	M	Bolus	0	N.D.	-
13	M	Bolus	0	N.D.	-
29	F	Bolus	0	N.D.	-
30	F	Bolus	0	N.D.	-
4	M	Infusion	0.25	N.D.	-
5	M	Infusion	0.25	N.D.	-
21	F	Infusion	0.25	*	-
22	F	Infusion	0.25	N.D.	-
10**	M	Infusion	0.25	N.D.	-
14	M	Bolus	0.25	N.D.	-
15	M	Bolus	0.25	N.D.	-
31	F	Bolus	0.25	N.D.	-
32	F	Bolus	0.25	N.D.	-
6	M	Infusion	0.5	N.D.	-
7	M	Infusion	0.5	N.D.	-
8	M	Infusion	0.5	N.D.	N.D.
23	F	Infusion	0.5	N.D.	-
24	F	Infusion	0.5	N.D.***	-
25	F	Infusion	0.5	N.D.	N.D.
16	M	Bolus	0.5	N.D.	-
17	M	Bolus	0.5	N.D.	-
33	F	Bolus	0.5	N.D.	-
34	F	Bolus	0.5	N.D.	-
9	M	Infusion	1	N.D.	-
11	M	Infusion	1	N.D.	N.D.
26	F	Infusion	1	N.D.	-
27	F	Infusion	1	N.D.	-
28	F	Infusion	1	N.D.	N.D.

N.D. = Not detectable

\* Died on day 3 of the study

\*\* Animal no. 10 was treated with 1mg/kg/day during days 1 to 5.

From day 6 the animal was assigned to the low dose group (0.25mg/kg/day)

\*\*\* Sacrificed on day 22 of the study.

**Title: Recombinant human insulin-like growth factor-1 (rhIGF-1) 26-week subcutaneous (bolus) toxicity study in cynomolgus monkeys**

Study No: — Project#643815/Report#7437  
Amendment # 080: Vol. #2, and page # 1-348  
Conducting laboratory and location: —  
Sponsor: Kabi Pharmacia AB, Stockholm, Sweden  
Date of study initiation: May 1990  
GLP compliance: Yes  
QA- Report Yes (x) No ( )

**METHODS:**

Species/strain: Cynomolgus monkey  
#/sex/group or time point: 5/sex/group; 2/sex for control and HD groups for 8-week recovery study.  
Dosage groups in administered units: 0, 0.1, 0.3 or 1.0 mg of IGF-1 alone /kg/day for 26 weeks  
Route, form, volume, and infusion rate: Subcutaneous (bolus)  
Drug, lot#, radiolabel, and % purity: Not available  
Formulation/vehicle: Clear, sterile, aqueous solution containing appropriate amounts of rhIGF-1, phosphate buffer (10 mM), and sodium chloride (145 mM, pH 5.9).

**Observations and times:**

Clinical signs: daily  
Body weights: weekly  
Food consumption: Daily  
Ophthalmoscopy: pretreatment, during weeks 7, 13 prior to necropsy  
EKG: pretreatment, during weeks 7, 13 prior to necropsy  
Hematology: pretreatment, during weeks 7, 13 prior to necropsy  
Clinical chemistry: pretreatment, during weeks 7, 13 prior to necropsy  
Urinalysis: pretreatment, during weeks 7, 13 prior to necropsy  
Rectal temperature: pretreatment, during weeks 7, 13 prior to necropsy  
Gross pathology: necropsy  
Organs weighed: necropsy  
Histopathology: necropsy

**RESULTS:**

Clinical signs and mortality: All HD monkeys except one animal in Weeks 13 and 26 had hypoglycemic shock, which was reversed by glucose iv. One HD animal was found dead on Day 7, which might be due to drug-induced hypoglycemia.

Body weights: There were significant increases in body weight gain in the HD animals of both sexes as summarized in a table below.

Time in week	Dose of rhIGF-1 (mg/kg/day) in Male and Female Monkeys@							
	Male				Female			
	0	0.1	0.3	1.0	0	0.1	0.3	1.0
0	2.6	2.6	2.6	2.5	2.4	2.3	2.4	2.4
4	2.5	2.6	2.7	2.7	2.4	2.4	2.4	2.6
8	2.6	2.7	2.8	2.9	2.5	2.5	2.5	2.7
12	2.7	2.8	2.9	3.0	2.5	2.6	2.6	2.8
16	2.7	2.9	3.0	3.1	2.5	2.6	2.6	2.9
20	2.8	3.0	3.1	3.3	2.6	2.7	2.7	3.0
24	2.9	3.2	3.3	3.5	2.6	2.8	2.7	3.0
Mean Gain	0.2	0.4	0.6	0.9*	0.1	0.3	0.2	0.7*

@The unit of bodyweight is in kg of 6 to 7 determinations. \*p<0.001, compared to the control.

Food consumption: No treatment related changes were observed.

Ophthalmology: There were no treatment related abnormalities.

Electrocardiography: There were no treatment related abnormalities

Rectal temperature: There was a reduction of 0.2 to 0.4°C at 2-4 hours post dose of IGF-1 in the HD group animals on Day 7 and in Weeks 13 and 26 as indicated below.

Time in Week (hour)*	Dose of rhIGF-1 (mg/kg/day) in Male and Female Monkeys@							
	Male				Female			
	0	0.1	0.3	1.0	0	0.1	0.3	1.0
1 (0)	38.6	38.6	38.8	39.0	39.0	38.9	39.0	38.7
1 (4)	38.7	38.6	38.7	35.9	38.5	38.8	38.7	36.7
7 (0)	38.8	38.8	39.1	39.1	38.8	37.8	39.0	38.9
7 (4)	38.6	38.6	38.9	38.7	38.7	38.8	38.8	38.8
13 (0)	38.9	38.9	39.0	38.9	38.7	38.8	39.1	38.7
13 (4)	38.9	39.0	39.0	36.0	38.6	38.7	38.8	36.4
26 (0)	38.3	38.6	38.5	38.5	38.4	38.5	38.6	38.6
26 (4)	38.7	38.6	38.8	36.8	38.8	39.0	38.6	36.4

@The unit of rectal temperature is centigrade of 5 to 7 determinations. \*The numbers in parentheses indicate hours after drug administration in indicated week.

Hematology: No drug-related hematologic changes were noted after the treatment of IGF-1 alone.

Clinical chemistry: There were no remarkable drug-treatment related changes except a dose dependent transient reduction in plasma glucose, which occurred at 2-4 hour post dose.

Urinalysis: Urine volume and creatinine clearance were increased with a reduction in specific gravity and osmolarity of urine. There were no changes indicative of diuretic effect after the test article treatment, although fluid intake was increased in both sex at 1 mg/kg groups.

Organ Weights: There were no treatment related changes in organ weights.

Gross and Histopathology: No drug-related gross or histopathological changes were observed in macroscopic findings at any dose groups.

Conclusion: It appears that the maximum tolerated dose was 1 mg/kg/day because hypoglycemic shock was observed at this dose. NOAEL may be at or near 0.3 mg/kg/day in monkeys treated for 26 weeks, although the drug-induced hypoglycemia was an extension of drug's pharmacodynamic effects. Human dose would be  $2 \text{ mg/kg} \times 0.2 \times 20 = 8 \text{ mg/M}^2$ , while that of monkey is  $0.3 \text{ mg/kg} \times 12 = 3.6 \text{ mg/M}^2$ . Thus, therapeutic exposure ratio would be under 0.5, based on body surface area comparison.

### **Analysis of anti-IGF-1 Antibodies in Monkey Serum after Treatment with rhIGF-1 for 26 Weeks**

#### **Background:**

In this study, analysis of anti-IGF-1 antibodies in monkey serum after treatment with rhIGF-1 for 26 weeks were performed as summarized below. This specific study number was 643815-XFI as a part of the main study above. The study was performed at

#### **Methods:**

Five monkeys/sex/group received rhIGF-1 subcutaneous at doses of 0, 0.1, 0.3 or 1.0 mg/kg/day for 26 weeks. This study included 2 additional monkeys/sex for the control and high dose groups for an 8-week recovery study. Blood samples were collected into plain tubes from all animals pretrial and once during Weeks 13 and 26 of the study and on completion of the recovery period. The determination of anti-IGF-I antibodies was performed at Quantitative Immunology, Kabi Pharmacia Peptide Hormones R&D, Stockholm, Sweden.

The immunoglobulins are separated from the endogenous IGF-I and its binding proteins by a polyethylene glycol (PEG) precipitation. Then the immunoglobulin precipitate is dissolved and a  $^{125}\text{I}$  labelled truncated IGF-I tracer is added. After overnight incubation antibody-bound tracer is precipitated by a new addition of PEG-solution. Finally, the radioactivity in the precipitate is counted and the titer of the serum sample calculated. The anti-IGF-I antibody titer is calculated by dividing the sample cpm with the cpm of the positive control serum (rabbit). Samples yielding a quotient (titer) of  $\geq 1$  are considered anti-IGF-I positive. The intra-assay and inter-assay coefficients of variation for the positive control were 4.7 and 14.6%, respectively.

#### **Results:**

Anti-IGF-1 antibodies were detected in one monkey (MD group) out of 34 animals that were treated with rhIGF-1. The data from individual animals in the four groups are summarized in 4 tables. There were relevant data for the recovery study of 8 weeks.

SUBJECT	SAMPLE	DOSE (mg/kg)	TITER
1	Pretrial		N.S.I
	week 13	0	N.S.I
	week 26	0	N.S.I
2	Pretrial		N.S.I
	week 13	0	N.S.I
	week 26	0	N.S.I
3	Pretrial		N.S.I
	week 13	0	N.S.I
	week 26	0	N.S.I
4	Pretrial		N.S.I
	week 13	0	N.S.I
	week 26	0	N.S.I
5	Pretrial		N.S.I
	week 13	0	N.S.I
	week 26	0	N.S.I
6	Pretrial		N.S.I
	week 13	0	N.S.I
	week 26	0	N.S.I
	Recovery		N.S.I
7	Pretrial		N.S.I
	week 13	0	N.S.I
	week 26	0	N.S.I
	Recovery		N.S.I
25	Pretrial		N.S.I
	week 13	0	N.S.I
	week 26	0	N.S.I
26	Pretrial		N.S.I
	week 13	0	N.S.I
	week 26	0	N.S.I
27	Pretrial		N.S.I
	week 13	0	N.S.I
	week 26	0	N.S.I
28	Pretrial		N.S.I
	week 13	0	N.S.I
	week 26	0	N.S.I
29	Pretrial		N.S.I
	week 13	0	N.S.I
	week 26	0	N.S.I

N.S.I: Not Significantly Increased from the positive control

SUBJECT	SAMPLE	DOSE (mg/kg)	TITER
30	Pretrial		N.S.I
	week 13	0	N.S.I
	week 26	0	N.S.I
	Recovery		N.S.I
31	Pretrial		N.S.I
	week 13	0	N.S.I
	week 26	0	N.S.I
	Recovery		N.S.I
8	Pretrial		N.S.I
	week 13	0,1	N.S.I
	week 26	0,1	N.S.I
9	Pretrial		N.S.I
	week 13	0,1	N.S.I
	week 26	0,1	N.S.I
10	Pretrial		N.S.I
	week 13	0,1	N.S.I
	week 26	0,1	N.S.I
11	Pretrial		N.S.I
	week 13	0,1	N.S.I
	week 26	0,1	N.S.I
12	Pretrial		N.S.I
	week 13	0,1	N.S.I
	week 26	0,1	N.S.I
32	Pretrial		N.S.I
	week 13	0,1	N.S.I
	week 26	0,1	N.S.I
33	Pretrial		N.S.I
	week 13	0,1	N.S.I
	week 26	0,1	N.S.I
34	Pretrial		N.S.I
	week 13	0,1	N.S.I
	week 26	0,1	N.S.I
35	Pretrial		N.S.I
	week 13	0,1	N.S.I
	week 26	0,1	N.S.I
36	Pretrial		N.S.I
	week 13	0,1	N.S.I
	week 26	0,1	N.S.I

N.S.I: Not Significantly Increased from the positive control

SUBJECT	SAMPLE	DOSE (mg/kg)	TITER
13	Pretrial		N.S.I
	week 13	0,3	N.S.I
	week 26	0,3	N.S.I
14	Pretrial		N.S.I
	week 13	0,3	N.S.I
	week 26	0,3	N.S.I
15	Pretrial		N.S.I
	week 13	0,3	N.S.I
	week 26	0,3	N.S.I
16	Pretrial		N.S.I
	week 13	0,3	N.S.I
	week 26	0,3	N.S.I
17	Pretrial		N.S.I
	week 13	0,3	N.S.I
	week 26	0,3	N.S.I
37	Pretrial		N.S.I
	week 13	0,3	N.S.I
	week 26	0,3	N.S.I
38	Pretrial		N.S.I
	week 13	0,3	N.S.I
	week 26	0,3	N.S.I
39	Pretrial		N.S.I
	week 13	0,3	N.S.I
	week 26	0,3	N.S.I
40	Pretrial		N.S.I
	week 13	0,3	N.S.I
	week 26	0,3	2
41	Pretrial		N.S.I
	week 13	0,3	N.S.I
	week 26	0,3	N.S.I
18	Pretrial		N.S.I
	week 13	1,0	N.S.I
	week 26	1,0	N.S.I
19	Pretrial		N.S.I
	week 13	1,0	N.S.I
	week 26	1,0	N.S.I
20	Pretrial		N.S.I
	week 13	1,0	N.S.I
	week 26	1,0	N.S.I

N.S.I: Not Significantly Increased from the positive control

SUBJECT	SAMPLE	DOSE (mg/kg)	TITER
21	Pretrial		N.S.I
	week 13	1,0	N.S.I
	week 26	1,0	N.S.I
22	Pretrial		N.S.I
	week 13	1,0	N.S.I
	week 26	1,0	N.S.I
23	Pretrial		N.S.I
	week 13	1,0	N.S.I
	week 26	1,0	N.S.I
	Recovery		N.S.I
24	Pretrial		N.S.I
	week 13	1,0	N.S.I
	week 26	1,0	N.S.I
	Recovery		N.S.I
42	Pretrial		N.S.I
	week 13	1,0	N.S.I
	week 26	1,0	N.S.I
43	Pretrial		N.S.I
	week 13	1,0	N.S.I
	week 26	1,0	N.S.I
44	Pretrial		N.S.I
	week 13	1,0	N.S.I
	week 26	1,0	N.S.I
45	Pretrial		N.S.I
	week 13	1,0	N.S.I
	week 26	1,0	N.S.I
46	Pretrial		N.S.I
	week 13	1,0	N.S.I
	week 26	1,0	*
47	Pretrial		N.S.I
	week 13	1,0	N.S.I
	week 26	1,0	N.S.I
	Recovery		N.S.I
48	Pretrial		N.S.I
	week 13	1,0	N.S.I
	week 26	1,0	N.S.I
	Recovery		N.S.I

\*no sample

N.S.I: Not Significantly Increased from the positive control

#### 2.6.6.4. GENOTOXIC STUDY

**Title: Study to evaluate the chromosome damaging potential of recombinant Human IGF-1 by its effects on cultured human lymphocytes using an in vitro cytogenetic assay (Section 4.2.3.3.b.1)**

1. Purpose: To evaluate the clastogenic potential of recombinant human IGF-1 by examining its effects on the chromosomes of the lymphocytes of a single human donor. Testing facility: This study (# → 10/HLC(1)) was carried at \_\_\_\_\_ under GLP.

2. Methods: Cultured human lymphocytes were incubated with rhIGF-1 (Batch#50528R) at final concentrations of 104, 160, 245, 377, 580, 893, 1373, 2113, 3250 and 5000 µg/ml in the presence and absence of S-9. Based on preliminary experiments, the sponsor selected 5000 µg/ml as the top dose. The positive control agents were methylmethanesulfonate (75 and 100 µg/ml as final concentration) and cyclophosphamide (12.5 and 25 µg/ml as final concentration). Metabolic activation system (S-9 fraction) was obtained from male Wistar rats, which were treated with Aroclor 1254.

A single female donor (who was not suspected of any virus infection nor had been exposed to high levels of radiation or hazardous chemicals) was used. Approximately 30 ml of the peripheral blood was drawn on the day before culture initiation. After completion of scoring and decoding of each slide the cells were divided into one of three categories: 1) cells with structural aberrations including gaps, 2) cells with structural aberrations excluding gaps, and 3) polyploidy, endoreduplicated and hyperdiploid cells.

#### 3. Results:

In duplicate control 100 cells there were 5 cells that have chromosomal aberrations with gaps. IGF-1 doses of 2113, 3250 and 5000 µg/ml did not increase significantly the numbers of cells that had aberrations including and excluding gaps. Structural aberrations observed both in the absence and presence of S-9 are summarized in Tables 1 and 2. Both methylmethanesulfonate (MMS, 100 µg/ml) and cyclophosphamide (CPA, 25 µg/ml) significantly increased the number of positive cells that have aberrations including and excluding gaps in both the absence and presence of S-9.

4. Conclusion: It appears that rh-IGF-1 was not able to induce structural chromosome aberrations in human lymphocytes in the absence or presence of rat liver S-9.

Metaphase Analysis in vitro

TABLE 1

Data for IGF-1 in the absence of S-9

Cells with structural aberrations

Donor sex: FEMALE

Treatment	Replicate	No of cells scored	No of cells with abs. inc. gaps	No of cells with abs. ex. gaps	Significance +	Mitotic Index
Solvent	A	100	0	0		4.9
	B	100	5	1		3.1
Totals	A+B	200	5	1		4.0
2113 µg/ml	A	100	2	0		3.9
	B	100	4	0		3.3
Totals	A+B	200	6	0	NS	3.6
3250 µg/ml	A	100	8	0		4.5
	B	100	9	1		4.2
Totals	A+B	200	17	1	NS	4.4
5000 µg/ml	A	100	5	0		4.5
	B	100	5	1		4.7
Totals	A+B	200	10	1	NS	4.6
100 µg/ml MMS	A	25	12	12		
	B	25	13	12		
Totals	A+B	50	25	24	ND	

+ Statistical significance (Appendix 5)

Numbers in bold typeface exceed historical negative control ranges (Appendix 6).

NS = not significant  
ND = not determinedAPPEARS THIS WAY  
ON ORIGINAL

Metaphase Analysis in vitroTABLE 2

Data for IGF-1 in the presence of S-9

Cells with structural aberrations

Donor sex: FEMALE

Treatment	Replicate	No of cells scored	No of cells with abs. inc. gaps	No of cells with abs. ex. gaps	Significance +	Mitotic Index
Solvent	A	100	0	0		4.0
	B	100	1	0		3.6
Totals	A+B	200	1	0		3.8
2113 µg/ml	A	100	3	1		2.8
	B	100	3	0		3.3
Totals	A+B	200	6	1	NS	3.1
3250 µg/ml	A	100	4	1		3.7
	B	100	7	3		2.5
Totals	A+B	200	11	4	p <0.05	3.1
5000 µg/ml	A	100	3	1		3.3
	B	100	6	1		2.5
Totals	A+B	200	9	2	NS	2.9
25 µg/ml CPA	A	25	14	11		
	B	25	11	9		
Totals	A+B	50	25	20	ND	

+ Statistical significance (Appendix 5)

Numbers in bold typeface exceed historical negative control ranges (Appendix 6).

NS = not significant

ND = not determined

**Title: Study to evaluate the chromosome damaging potential of recombinant Human IGF-1 by its effects on human peripheral blood lymphocytes using an in vitro cytogenetic assay (4.2.3.3.b.2)**

1. Purpose: To evaluate the clastogenic potential of recombinant human IGF-1 by examining its effects on the chromosomes of the lymphocytes of a single human donor. Testing facility: This study (# — 10/HLC(2)) was carried at

under GLP.

2. Methods: Cultured human lymphocytes were incubated with rhIGF-1 (Batch#53728) at final concentrations of 588, 840, 1201, 1715, 2450, 3500, and 5000 µg/ml in the presence and absence of S-9. Based on preliminary experiments, the sponsor selected 5000 µg/ml as the top dose. The positive control agents were methylmethanesulfonate (12.5, 25 and 50 µg/ml as final concentrations) and cyclophosphamide (12.5 and 25 µg/ml as final concentrations). Metabolic activation system (S-9 fraction) was obtained from male Wistar rats, which were treated with Recolor 1254.

A single female donor (who was not suspected of any virus infection nor had been exposed to high levels of radiation or hazardous chemicals) was used. Approximately 60 ml of the peripheral blood was drawn on the day before culture initiation. After completion of scoring and decoding of each slide the cells were divided into one of three categories: 1) cells with structural aberrations including gaps, 2) cells with structural aberrations excluding gaps, and 3) polyploidy, endoreduplicated and hyperdiploid cells.

3. Results: In duplicate control 100 cells there were no cells that had chromosomal aberrations with and excluding gaps. IGF-1 at doses 2450, 3500, and 5000 µg/ml did not increase significantly the numbers of cells that had aberrations including and excluding gaps (Table 1). Methylmethanesulfonate (MMS, 50 µg/ml) significantly increased the number of positive cells that have aberrations including and excluding gaps in the absence of S-9. In the presence of S-9 fraction cell numbers in the control increased to 3 cells that had aberrations including gaps (Table 2). The positive cell numbers did not increase significantly in the presence of any doses of IGF-1.

4. Conclusion: It appears that rh-IGF-1 was not able to induce structural chromosome aberrations in human peripheral blood lymphocytes in the absence or presence of rat liver S-9.

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Metaphase Analysis in vitro

TABLE 1

Data for IGF-1 in the absence of S-9

Cells with structural aberrations, 20 hour sampling time:

Donor sex: Female

Treatment	Replicate	No of cells scored	No of cells with abs. inc. gaps	No of cells with abs. excl. gaps	Significance +	Mitotic Index (mean)
Solvent	A	100	0	0		9.6
	B	100	0	0		10.1
	<b>Totals</b>	<b>200</b>	<b>0</b>	<b>0</b>		<b>(9.9)</b>
2450 µg/ml	A	100	1	0		7.6
	B	100	1	0		8.6
	<b>Totals</b>	<b>200</b>	<b>2</b>	<b>0</b>	NS	<b>(8.1)</b>
3500 µg/ml	A	100	4	0		5.3
	B	100	3	2		6.7
	<b>Totals</b>	<b>200</b>	<b>7</b>	<b>2</b>	NS	<b>(6.0)</b>
5000 µg/ml	A	100	2	1		5.2
	B	100	1	0		5.3
	<b>Totals</b>	<b>200</b>	<b>3</b>	<b>1</b>	NS	<b>(5.3)</b>
50 µg/ml MMS	A	25	17	15		
	B	25	18	16		
	<b>Totals</b>	<b>50</b>	<b>35</b>	<b>31</b>	ND	

+ Statistical significance (Appendix 5a)

NS = not significant

ND = not determined

Numbers in bold typeface exceed historical negative control ranges (Appendix 6).

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ON ORIGINAL

Metaphase Analysis in vitroTABLE 2

Data for IGF-1 in the presence of S-9

Cells with structural aberrations, 20 hour sampling time:

Donor sex: Female

Treatment	Replicate	No of cells scored	No of cells with abs. inc. gaps	No of cells with abs. excl. gaps	Significance +	Mitotic Index (mean)
Solvent	A	100	1	1		3.9
	B	100	2	0		3.1
	Totals	200	3	1		(3.5)
2450 µg/ml	A	100	0	0		8.6
	B	100	1	0		4.7
	Totals	200	1	0	NS	(6.6)
3500 µg/ml	A	100	0	0		7.1
	B	100	1	1		8.1
	Totals	200	1	1	NS	(7.6)
5000 µg/ml	A	100	0	0		7.8
	B	100	3	0		6.2
	Totals	200	3	0	NS	(7.0)
12.5 µg/ml CPA	A	25	7	4		
	B	25	9	8		
	Totals	50	16	12	ND	

+ Statistical significance (Appendix 5a)

NS = not significant

ND = not determined

Numbers in bold typeface exceed historical negative control ranges (Appendix 6).

APPEARS THIS WAY  
ON ORIGINAL

**Title: Study to evaluate the chromosome damaging potential of recombinant Human IGF-1 by its effects on rat peripheral blood lymphocytes treated in vivo and cultured in vitro (Section 4.2.3.3.b.3)**

1. Purpose: To evaluate the clastogenic potential of recombinant human IGF-1 by examining its effects on the chromosomes of rat peripheral blood lymphocytes treated in vivo and cultured in vitro. Testing facility: This study (# 10' — was carried at — for Kabi Pharmacia, Stockholm, Sweden under GLP.

2. Methods: Ten-week old Sprague Dawley CD rats/sex (male 320 g; female 231 g) were given rh-IGF-1 (Batch#63242-51) subcutaneously at doses of 0, 5, 10, and 20 mg/kg IGF-1 or 20 mg/kg cyclophosphamide as a positive control(5/sex). The rats were bled by cardiac puncture approximately 6 hours after the treatment. Approximately 4 mls of blood or phosphate buffer in saline as a control were processed. Two and a half hours prior to harvest, colchicine was added to give a final concentration of approximately 1 µg/ml to arrest dividing cells in metaphase. Lymphocytes were kept in fixative in the refrigerator before slides were prepared but slides were not made on the day of harvest to ensure cells were adequately fixed.

After completion of scoring and decoding of each slide the cells were divided into one of three categories: 1) cells with structural aberrations including gaps, 2) cells with structural aberrations excluding gaps, and 3) polyploidy, endoreduplicated and hyperdiploid cells. The assay was considered valid if the following criteria were met:

- 1) at least 8 animals out of each group of 10 (excluding positive controls) were available for analysis
- 2) the positive control chemical induced a clear and statistically significant increase in the incidence of cells with structural aberrations.

The test chemical was to be considered as clearly positive in this assay if statistically significant increases in the proportion of cells with structural aberrations occurred at one (or more) concentration. Increased incidences of gaps or increased number of structural aberrations not exceeding the normal range or occurring only at very high or very toxic concentrations were likely to be concluded as "equivocal" or "probably of no biological importance". Cells with exchange aberrations or cells with greater than one aberration occur very infrequently in negative control cultures. Their appearance was therefore to be considered biologically significant.

3. Results: Mitotic indices of cultures from animals receiving negative control or IGF-1 at doses of 5, 10 and 20 mg/kg were not significantly different in males or females as shown below.

Chromosome aberration analysis shows that there were 11 cells that have chromosomal aberrations including gaps in the control males. Seven cells with aberrations excluding gaps were observed in males. In females, the positive cell numbers were significantly reduced, compared to the males. IGF-1 at a dose of 20 mg/kg did not change the numbers of cells, although the total

cells scored were comparable (Table 1). Animals that received cyclophosphamide (20 mg/kg) had significantly ( $P < 0.001$ ) higher cells with aberrations with or without gaps.

Statistical analysis of aberration data from the cells with structural aberrations excluding gaps in the vehicle, rh-IGF-1 or cyclophosphamide treatment groups were summarized in Appendix 4 below. It is safe to conclude that recombinant human IGF-1 given subcutaneously at a dose level of 20 mg/kg was not able to induce chromosome aberrations in rat peripheral blood lymphocytes treated in vivo and cultured in vitro.

Mitotic index determinations were as follows:

	<u>Negative controls</u>		<u>5 mg/kg</u>		<u>10 mg/kg</u>		<u>20 mg/kg</u>	
	Animal #	MI(%)	Animal #	MI(%)	Animal #	MI(%)	Animal #	MI(%)
Males	1131	4.8	1125	4.0	1116	6.4	1132	1.6
	1115	5.2	1123	3.4	1117	0.2	1111	3.8
	1128	8.0	1133	4.0	1118	2.8	1129	3.6
	1134	3.8	1121	10.0	1119	8.6	1113	4.0
	1114	5.2	1122	5.4	1112	3.4	1124	1.4
Females	1141	4.2	1144	3.4	1157	2.2	1142	4.4
	1137	1.6	1155	5.4	1149	2.4	1146	5.0
	1138	4.2	1145	3.8	1160	4.8	1151	3.8
	1153	4.0	1139	3.0	1140	1.8	1136	5.4
	1150	3.0	1156	5.8	1148	2.0	1143	5.8
Mean		4.4		4.8		3.5		3.9

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**TABLE 1****Summary of test chemical, positive and vehicle control data**

Kill time: 6 hours

Treatment	Sex	Cells scored	Cells with abs. inc. gaps	Cells with abs. excl. gaps	Significance +	Mitotic Index (Mean)
Vehicle	M	500	11	7		5.4
	F	499	3	1		3.4
	Totals	999	14	8		4.4
20 mg/kg rhIGF-1	M	500	11	5		2.9
	F	500	7	4		4.9
	Totals	1000	18	9	NS	3.9
20 mg/kg CPA	M	283	83	80		
	F	375	82	76		
	Totals	658	165	156	p ≤ 0.001	

+ Statistical significance (Appendix 4)

NS = not significant

Numbers highlighted exceed historical negative control ranges (Appendix 5)

See Appendix 1 for abbreviations

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**APPENDIX 4****Statistical analysis of aberration data****Cells with structural aberrations excluding gaps**

Treatment (mg/kg)	Cells	Aberrant cells	Proportion	Heterogeneity $\chi^2$	Contingency $\chi^2$
Vehicle	999	8	0.008	12.22	0.002, NS
rhIGF-1, 20	1000	9	0.009		
CPA, 20	658	156	0.237		

NS = not significant

**Title: Gene Mutation test in bacteria: rh-IGF-1(4.2.3.3.b.4)**

1. Purpose: To evaluate the genotoxic potential of rh-IGF-1 (Batch# 11768-51) by a gene mutation assay (reverse mutation). This study (N440-Q1408) was conducted at Pharmacia Spa, Toxicology and Safety Assessment under OECD guidance for Biopharmaceuticals.

**2. Materials and Methods:**

The mutagenesis study was conducted according to protocol# 440M-Q1408 dated 2/9/1995 according to EEC Directive 92/69 (1992) and Japanese Guidelines for Toxicity Studies of Drugs (1989) and GLP regulations. The rhIGF-1 batch was provided as a 10 mg/mL solution, which was diluted with 10 mM phosphate buffer before the test. The test article was checked for any antibacterial effect at the various concentrations, with or without metabolic activation. The background lawn of the control plates was compared with that of the plates containing the test article at different concentrations. Any concentrations giving a definite reduction (at least 50%) in the background lawn compared with the control were considered toxic.

Liver homogenate fraction (S9) was prepared from CrI:CD(SD)BR male rats that were given a single intraperitoneal injection of 500 mg/kg of Aroclor 1254. On the fifth day of induction, the rats were sacrificed to prepare the fraction in metabolic activation mixture. The negative control agent that was used for rhIGF-1 placebo was 10 mM phosphate butter (Batch# DSQ274). The list of positive control agents were:

Positive controls	Supplier	Lot
2-nitrofluorene (2-NF)	/	03364
9-aminoacridine (9-AA)		84C-0189
2-aminoanthracene (2-AAN)		A3880-0
2-acetylaminofluorene (2-AAF)		030478
Benzo(a)pyrene (B(a)P)		101847
Sodium azide (SA)		-
Methyl methanesulfonate (MMS)		JA07267

The following strains of Salmonella typhimurium, auxotrophic for histidine, were obtained from \_\_\_\_\_

STRAIN	MUTATION IN THE HISTIDINE OPERON	OTHER CHARACTERISTICS
TA 1535	missense* mutation in the his G gene	rfa ΔuvrB
TA 100	like TA 1535	rfa ΔuvrB pKM 101
TA 1537	frameshift** mutation in the his C gene	rfa ΔuvrB
TA 98	frameshift** mutation in the his D gene	rfa ΔuvrB pKM 101

The following strain of Escherichia coli, auxotrophic for tryptophan, was obtained from \_\_\_\_\_

STRAIN	MUTATION IN THE TRYPTOPHAN OPERON	OTHER CHARACTERISTICS
WP2uvrA	missense* mutation in the trp E gene	ΔuvrA

3. Results: Mutagenic activity was tested with control (placebo) and positive agents in the presence of metabolic activation with a series of rh-IGF-1 dilution (Tables 1 and 2). The two tables show triplicate assay of 4 strains of *S. typhimurium* and one strain of *E. coli*. IGF-1 at concentrations up to 2000 µg/plate did not increase revertants in all strains. However, the positive agents increased significantly them, which indicate the tester strains responded. The second experiments under similar conditions confirmed the accuracy of the first experiment (Tables 5 and 6).

4. Summary and Conclusion: rhIGF-I was tested in the pre-incubation test at concentrations between 125 and 2000 µg/plate, with and without metabolic activation, on four *S. typhimurium* strains. TA 1535, TA 1537, TA 98 and TA 100 and the WP2 uvrA strain of *E. coli*. rhIGF-I did not induce statistically significant increases in the mean number of revertants/plate, at any of the concentrations tested with and without metabolic activation. Positive agents, however, increased the revertants significantly, which indicates the validity of assay systems. It is safe to conclude that the IGF-1 batch was not genotoxic in vitro test systems.

TABLE 1 - Direct mutagenic activity of rhIGF-I in 4 strains of Salmonella typhimurium and 1 strain of Escherichia coli.  
Revertants/plate after 48h at 37°C.  
First experiment (440M-Q1408)

Compound	$\mu\text{g}/\text{plate}$	<u>S. typhimurium</u>				<u>E. Coli</u>	
		TA 1535	TA 1537	TA 98	TA 100	WP2 uvrA	
Control (Placebo)	-	23	9	60	106	30	
		23	17	46	85	31	
		18	17	40	89	23	
		25	22	42	112	21	
		25	12	48	122	26	
		18	18	53	113	27	
rhIGF-I	125.00	26	20	52	122	26	
		18	9	55	111	14	
		26	19	49	83	22	
	250.00	19	11	40	104	11	
		26	9	40	104	20	
		22	16	57	105	13	
	500.00	20	18	52	89	22	
		19	15	48	99	23	
		19	11	44	111	24	
	1000.00	20	12	39	99	21	
		27	16	34	89	27	
		27	12	47	92	21	
	2000.00	20	7	34	88	11	
		25	8	42	88	25	
		26	7	39	83	24	
	SA	5.00	1588			1343	
			1579			1265	
			1793			1361	
9-AA	70.00		1430				
			1621				
			1713				
2-NF	10.00			567			
				716			
				702			
MMS	2.50 ( $\mu\text{L}/\text{pl.}$ )					502	
						454	
						487	

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TABLE 2 - Mutagenic activity with metabolic activation of rhIGF-I in 4 strains of Salmonella typhimurium and 1 strain of Escherichia coli. Revertants/plate after 48h at 37°C. First experiment (440M-Q1408)

Compound	$\mu\text{g}/\text{plate}$	<u>S. typhimurium</u>				<u>E. Coli</u>	
		TA 1535	TA 1537	TA 98	TA 100	WP2 uvrA	
Control (Placebo)	-	15	19	42	81	26	
		30	11	42	93	28	
		26	14	48	94	25	
		11	14	37	114	33	
		29	15	57	105	31	
		15	13	48	118	24	
rhIGF-I	125.00	13	19	45	106	45	
		24	18	41	122	42	
		27	13	42	106	25	
	250.00	18	14	44	81	24	
		19	18	46	100	29	
		13	17	51	93	24	
	500.00	24	16	53	101	35	
		15	15	37	88	34	
		24	17	47	104	27	
	1000.00	15	14	46	108	39	
		22	9	57	121	34	
		19	18	38	93	29	
	2000.00	19	15	39	102	31	
		17	17	40	85	31	
		23	11	53	88	35	
	2-AAN	5.00	241	212	2417		
			236	212	2604		
			249	205	2692		
2-AAN	10.00				3105	527	
					2934	458	
					2823	468	
B( $\alpha$ )P	5.00			301	368		
				242	348		
				397	363		
2-AAF	50.00			1613			
				1449			
				1428			

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TABLE 5 - Direct mutagenic activity of rhIGF-I in 4 strains of Salmonella typhimurium and 1 strain of Escherichia coli. Revertants/plate after 48h at 37°C.  
Second experiment (440M-Q1408)

Compound	$\mu\text{g}/\text{plate}$	<u>S. typhimurium</u>			<u>E. Coli</u>		
		TA 1535	TA 1537	TA 98	TA 100	WP2 uvrA	
Control (Placebo)	-	20	16	29	99	27	
		11	8	30	101	29	
		13	12	41	113	40	
		9	12	40	103	27	
		12	14	33	94	34	
		9	13	39	104	35	
rhIGF-I	125.00	13	8	40	94	35	
		8	15	41	105	31	
		12	12	23	112	46	
	250.00	16	9	31	119	34	
		9	13	39	108	36	
		14	9	44	95	36	
	500.00	8	13	41	84	45	
		9	15	32	106	34	
		14	9	43	108	37	
	1000.00	13	11	27	110	34	
		12	12	30	107	42	
		9	9	45	99	29	
	2000.00	12	9	41	107	34	
		9	13	38	96	24	
		14	14	38	88	26	
	SA	5.00	1304			1009	
			1458			1054	
			1476			1134	
9-AA	70.00		1157				
			1133				
			1299				
2-NF	10.00			645			
				584			
				637			
MMS	2.50 ( $\mu\text{L}/\text{pl.}$ )					417	
						457	
						436	

TABLE 6 - Mutagenic activity with metabolic activation of rhIGF-I in 4 strains of Salmonella typhimurium and 1 strain of Escherichia coli. Revertants/plate after 48h at 37°C. Second experiment (440M-Q1408)

Compound	$\mu\text{g}/\text{plate}$	<u>S. typhimurium</u>			<u>E. Coli</u>	
		TA 1535	TA 1537	TA 98	TA 100	WP2 uvrA
Control (Placebo)		16	18	48	117	24
		12	18	36	102	34
		13	16	38	115	29
		12	15	40	93	25
		14	19	44	92	37
		15	12	48	121	29
rhIGF-I	125.00	17	14	36	118	27
		8	17	44	92	53
		13	17	39	99	37
	250.00	9	12	37	107	27
		17	17	31	124	30
		13	14	43	101	35
	500.00	12	12	37	112	33
		14	16	40	124	39
		12	16	41	106	25
	1000.00	14	12	36	94	41
		12	11	36	112	30
		19	13	42	106	36
	2000.00	18	14	45	105	22
		12	13	38	101	30
		15	12	40	125	26
2-AAN	5.00	198	200	2618		
		213	189	2477		
		209	192	2553		
2-AAN	10.00				2751	555
					2868	666
					2889	636
B( $\alpha$ )P	5.00			291	364	
				287	356	
				325	410	
2-AAF	50.00			1320		
				1314		
				1525		

#### 2.6.6.5 Carcinogenicity (Section 4.2.3.4)

The carcinogenic potential of rhIGF-I/rhIGFBP-3 has not been evaluated in 2-year rodent bioassays. In mouse 3T3 fibroblasts, rhIGF-I was shown to increase mitogenic activity in a dose-

dependent manner. Two non-clinical studies to determine the mitogenic potential of the active moiety, rhIGF-I were conducted by Pharmacia and are presented in Section 4.2.3.4.b.1 and 2 below.

**Title: Study of the effect of rhGH and IGF-1 on the growth of primary tumor and lung metastases from Lewis Lung carcinoma (Section 4.2.3.4.b.1)**

1. Purpose: To determine the effects of repeated administration (s.c.) of rhGH and rhIGF-1 on the growth of murine Lewis Lung carcinoma that were implanted intramuscularly. In addition, the sponsor also wished to evaluate effects of rhGH and rhIGF-1 on the number of spontaneous metastases from the primary tumor.

Document: Source: Volume 16: Pages 1-26/Pharmacia document 95 50 179

Testing Facility: This study (9550179) was conducted at Pharmacia Spa, Toxicology and Safety Assessment ( ) under OECD guidance for Biopharmaceuticals, Stockholm, Sweden.

2. Materials and Methods: The followings are the list of drug compounds with specific batch numbers that were used on indicated experimental date.

Compound	Batch	Exp.date
rhGH (Genotropin): 4IU	56697-51	01/01/96
Placebo for rhGH 4IU	42551-51	01/01/96
rhIGF-1(2mg/ml)	59114B51	01/11/95
Placebo for rh IGF-1(5mg/ml) DSQ260		01/12/95

Murine Lewis Lung carcinoma was maintained by serial s.c. passages every 15 days in C57BL mice. Cells were prepared as single cell suspensions from solid tumors by mincing and treatment with 0.25% trypsin. Cell viability was examined by trypan blue exclusion and the concentration adjusted to  $10^6$  cells/mL. Tumor cells (0.1 mL/mouse) were injected intramuscularly in the right leg of 3-4 weeks old female C57BL mice (body weight: 20-23 g). Mice bearing Lewis Lung murine carcinoma (17- 19 mice/group) received daily s.c. administration of either control saline, rhGH or rhIGF-1 placebo and rhGH or rh-IGF-1 for 17 days as shown below.

Group	Treatment	Dose
2	rhGH Placebo	
3	rhIGF-1 Placebo	
4	rhGH	0.4 IU/kg
5	rhGH	2 IU/kg
6	rhGH	10 IU/kg
7	rhIGF-1	0.4 mg/kg
8	rhIGF-1	2 mg/kg
9	rhIGF-1	10 mg/kg

### 3. Results:

Effects of three doses of rh-GH on tumor weight and metastasis are summarized in Table 7.1 below. The table also presents the number of mice died during the treatment based on total number of mice at the beginning. No animal died in the saline control group. One or two mice died in other groups. Growth hormone at doses of 0.4, 2, or 10 IU/kg had no effects on tumor weight or median metastasis number. In Table 7.2 the effects of rh-IGF-1 were analyzed on Day 17 as the case of Growth Hormone. There were one and two mice died in place and 10 mg/kg group, respectively. IGF-1 did not increase either tumor weight nor median metastasis numbers.

A post-mortem examination was carried out on mice killed terminally and those which were found dead during the study. In most mice, including those receiving the placebo, the primary tumor appeared as a firm grayish mass in the left thigh. The size was 20 to 30 mm in diameter and was well encapsulated. The masses frequently had a soft center, with transparent fluid oozing from the cut surface. A moderate enlargement of the spleen was observed in most mice, irrespective of the differences in treatment. This finding was considered to be a reactive change, secondary to the presence of the tumor. No treatment-related changes were seen in the remaining organs examined. In particular, no metastases of the primary tumor were seen in organs other than the lungs.

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## 7.1 Effect of rhGH on Lewis Lung carcinoma

GROUPS	Tumor weight at day 17 ( $\pm$ SD) (gr)	Median metastasis number (range)	N° mice dead during the treatment/Total number of mice
Saline	3.99 $\pm$ 0.51	14(1-31)	0/17
Placebo rhGH	3.4 $\pm$ 0.95	13(4-40)	2/18
rhGH 0.4 UI/kg	3.6 $\pm$ 0.8	11(0-38)	1/17
2	3.61 $\pm$ 1.66	7(0-74)	1/18
10	3.76 $\pm$ 1.18	9(1-40)	1/19

## 7.2 Effect of rhIGF on Lewis Lung carcinoma

GROUPS	Tumor weight at day 17 ( $\pm$ SD) (gr)	Median metastasis number (range)	N° mice dead during the treatment/Total number of mice
Saline	3.99 $\pm$ 0.51	14(1-31)	0/17
Placebo rhIGF	3.34 $\pm$ 0.75	8(2-24)	1/18
rhIGF 0.4 mg/kg	3.6 $\pm$ 0.8	9(2-64)	0/18
2	3.61 $\pm$ 1.66	7.5(2-13)	0/16
10	3.76 $\pm$ 1.18	10(1-40)	2/17

**Title:** Study of the effect of rhGH and IGF-1 on the growth of HT-29 human colon carcinoma implanted in nude mice (Section 4.2.3.4.b.2)

**Source:** Volume 16: Pages 1-45/Pharmacia document 95 50 177

1. Purpose: To determine the effects of repeated administration (s.c.) of rhGH and rhIGF-1 on the growth of human colon adenocarcinoma (HT-29) that was implanted into athymic mice HT-29. The sponsor selected implanted athymic mice tumor model because HT-29 cells produce IGF-1 in vitro, proliferate in the presence of IGF-1, express IGF-I receptors and their growth is inhibited by the treatment with antibodies against IGF-1 receptors.

Testing Facility: This study (9550177) was conducted at Pharmacia Spa, Toxicology and Safety Assessment ( ) under OECD guidance for Biopharmaceuticals.

Materials and Methods: For in vivo studies, the following drugs were used as indicated batches and date.

Drug	Batch	Date
rhGH: 4IU	BATCH: 51884-51	EXP 1995-02-01
Placebo for rhGH	BATCH: 76492-51	EXP 1995-03-01
rhIGF-1 : 2 mg/ml	BATCH: 59114B51	EXP 1995-11-01
Placebo for rhIGF-1	BATCH: DSO 260	EXP 1995-12-0.1

Tumors were excised and fragments (2x2 mm) implanted s.c. into the left flank of male Swiss CD-1 mice. The mice were 4 to 6 weeks old weighing 20-25g were employed, of which colony is routinely tested for the absence of antibodies to a panel of pathogens including mouse hepatitis and Sendai virus. Tumor growth was evaluated on Days 4, 9, 12, 17, 20, and 25 from tumor implant. Mean tumor growth was estimated by the following formula:  $(d^2 \times D) / 2$  here d=minor tumor diameter while D = major tumor diameter). Tumor growth (%) was calculated according to the following formula:  $100 \times (\text{mean tumor weight of treated group}) / (\text{mean tumor weight of control group})$ . The study was designed as presented below.

Groups	Treatment	Dose	Animal Numbers
1	Saline		1-2
2	rhGH Placebo		13-24
3	rhGH	0.4 IU/Kg	25-36
4	rhGH	2 IU/Kg	37-48
5	rhGH	10 IU/Kg	49-60
6	rh IGF-1 Placebo		61-72
7	rh IGF-1	0.4 mg/kg	73-84
8	rh IGF-1	2 mg/kg	85-96
9	rh IGF-1	10 mg/kg	97-108

Results: Mean tumor weights of HT-29 human colon carcinoma in control group were increased as a function of days after tumor implantation as shown in Table 6.1 below. rhGH placebo and rhGH at doses of 0.4, 2 and 10 IU/kg did not change significantly the rate of tumor growth. Rh-IGF-1 at doses of 0.4, 2, and 10 mg/kg also had no significant effect on mean tumor weight of HT 29 Human colon carcinoma (Table 6.2). Thus, the reviewer is in agreement with the sponsor who concluded that both rhGH and rhIGF-1 had no effect on HT human colon carcinoma in athymic mice. However, in this study the sponsor did not test the combination of the two hormones or IGF-1BP.

**Table 6.1 HT29 Human Colon Carcinoma: treatment with rhGH**

GROUP <sup>1)</sup>	MEAN TUMOR WEIGHT (g) <sup>2)</sup>					
	day 4	day 9	day 12	day 17	day 20	day 25
1 Saline	0.07 ± 0	0.12 ± 0.03	0.19 ± 0.06	0.34 ± 0.13	0.53 ± 0.24	0.78 ± 0.28
2 Placebo rhGH	0.07 ± 0.02	0.11 ± 0.02	0.17 ± 0.04	0.31 ± 0.07	0.45 ± 0.14	0.65 ± 0.15
3 rhGH 0.4 IU/Kg	0.07 ± 0.02	0.11 ± 0.03	0.18 ± 0.06	0.33 ± 0.12	0.48 ± 0.17	0.67 ± 0.26
4 rhGH 2 IU/Kg	0.07 ± 0.02	0.11 ± 0.03	0.19 ± 0.06	0.30 ± 0.09	0.46 ± 0.17	0.66 ± 0.22
5 rhGH 10 IU/Kg	0.07 ± 0.02	0.12 ± 0.05	0.18 ± 0.08	0.31 ± 0.13	0.48 ± 0.24	0.68 ± 0.34

Tumor fragments were implanted s.c. on day 0 Treatment was performed s.c. daily from day 4 to day 18

1) 12 mice / group

2) Tumor weight  $d^2 \times D / 2 \pm SD$

**Table 6.2 HT29 Human Colon Carcinoma : treatment with IGF1**

GROUP <sup>1)</sup>	MEAN TUMOR WEIGHT (g) <sup>2)</sup>					
	day 4	day 9	day 12	day 17	day 20	day 25
1 Saline	0.07 ± 0	0.12 ± 0.03	0.19 ± 0.06	0.34 ± 0.13	0.53 ± 0.24	0.78 ± 0.28
6 Placebo IGF1	0.07 ± 0.02	0.12 ± 0.04	0.18 ± 0.07	0.33 ± 0.16	0.49 ± 0.23	0.80 ± 0.45
7 IGF1 0.4 mg/Kg	0.07 ± 0.02	0.12 ± 0.04	0.17 ± 0.05	0.30 ± 0.09	0.50 ± 0.16	0.70 ± 0.24
8 IGF1 2 mg/Kg	0.07 ± 0.02	0.11 ± 0.03	0.17 ± 0.08	0.32 ± 0.21	0.47 ± 0.31	0.63 ± 0.34
9 IGF1 10 mg/Kg	0.07 ± 0.02	0.13 ± 0.05	0.18 ± 0.08	0.3 ± 0.1	0.47 ± 0.21	0.67 ± 0.33

Tumor fragments were implanted s.c. on day 0 Treatment was performed s.c. daily from day 4 to day 18

1) 12 mice / group

2) Tumor weight  $d^2 \times D / 2 \pm SD$

### Reproductive and development toxicity (Section 4.2.3.5)

No studies to determine the reproductive and developmental toxicity potential with rhIGF-1/IGFBP-3 have been conducted. The following 5 studies were conducted by Pharmacia to determine the reproductive and developmental toxicity potential of rhIGF-1 alone.

**Title: rhIGF-1: Study on fertility and pre- and post-natal development in the rat (Section 4.2.3.5.b.2)**

Study Nr.: — 026 and 93, — .026/0560

Documents: Vol. 17, pages 1-116

Purpose: To assess the effects of subcutaneous administration of rhIGF-1 upon the reproductive performance of male and female rats. An additional objective of this study is to find the suitable doses for use in main fertility and peri- and post-natal studies.

Methods: Approximately 9 to 11 weeks old virgin male and female Sprague Dawley rats (337 to 402 g for males and 213 to 275 g for females) were used. Five rats/sex/group were acclimated prior to mating on Day 1. Males were terminated on Day 5 while the females were sacrificed on Day 17 post coitum. Different group of females were maintained till Day 4 post partum. The batch of placebo and rhIGF-1 were DsQ 188 and 52827-5, respectively. Control (5 males and 15 females) group received placebo. Three treated group (5 males and 15 females) received rhIGF-1 subcutaneously at doses of 0.4, 2 and 10 mg/kg/day for 15 days.

Observation: Parental:

Clinical Signs: Daily

Body weight: Males were weighted at twice weekly. Females were weighed twice weekly until mating was detected, on Days 0, 3, 6, 10, 14, 17 and 20 post coitum and on Days 1 and 4 post partum.

Food consumption: Weekly

Assessment of reproductive performance:

On the fifteenth day of treatment, males and the five females were paired on a one-to-one basis. Each morning following pairing, a vaginal smear was prepared from each female and examined for the presence of spermatozoa. The day on which evidence of mating was found was designated Day 0 of gestation. All females that were paired with treated males with different doses of IGF-1.

Maternal examination: On Day 14 after mating the females were killed and examined macroscopically for evidence of any potential effects of treatment.

Post-natal phase: The remaining females in each group were allowed to deliver their young and rear their offspring till Day 4 post partum. All offspring were observed on Day 1 for live (or dead), sexes and bodyweights of live offspring.

Terminal observations: Males were killed following successful mating to examine the reproductive organs, which were weighed and retained. For females and litters, on Day 4 post partum, females were killed for macroscopic examination and the number of implantation sites in dams.

Results:

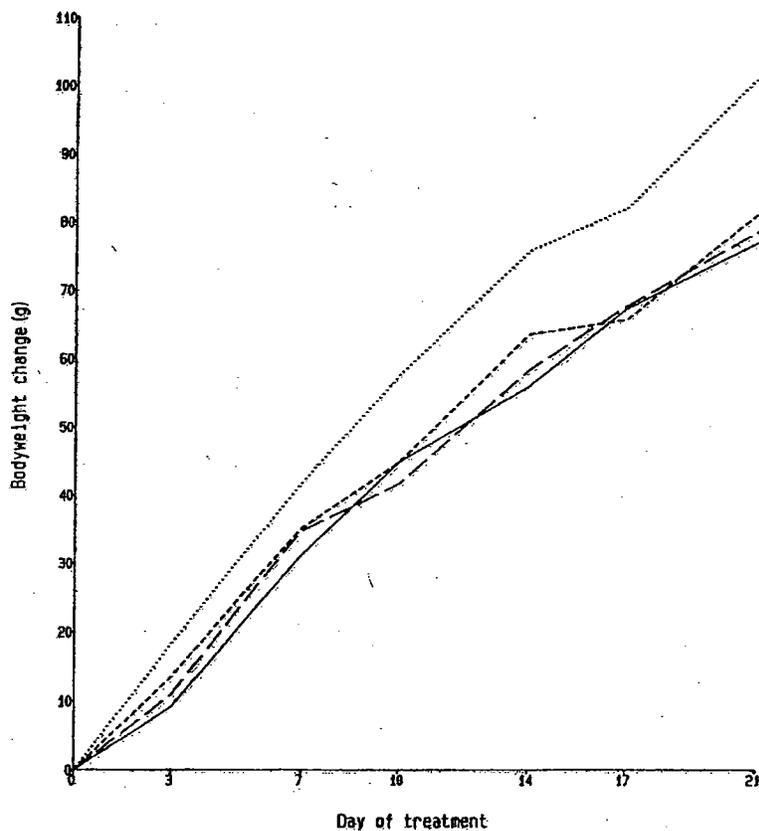
Clinical signs and mortality: No animals died and there were no remarkable treatment-related signs or appearances.

Body weight: Bodyweight gain of males in the HD (10 mg/kg/day) group 4 was increased, compared to the control group. Males in the LD and MD doses (0.4 and 2 mg/kg/day) had similar body weight gain as the control (Fig. 1). In the case of females, there were IGF-1 dose-dependent increases in body weight gain (Fig.2).

FIGURE 1

Bodyweight change of males

- Group 1 : Control
- Group 2 : rhIGF-1 : 0.4 mg/kg/day
- Group 3 : rhIGF-1 : 2.0 mg/kg/day
- Group 4 : rhIGF-1 : 10.0 mg/kg/day



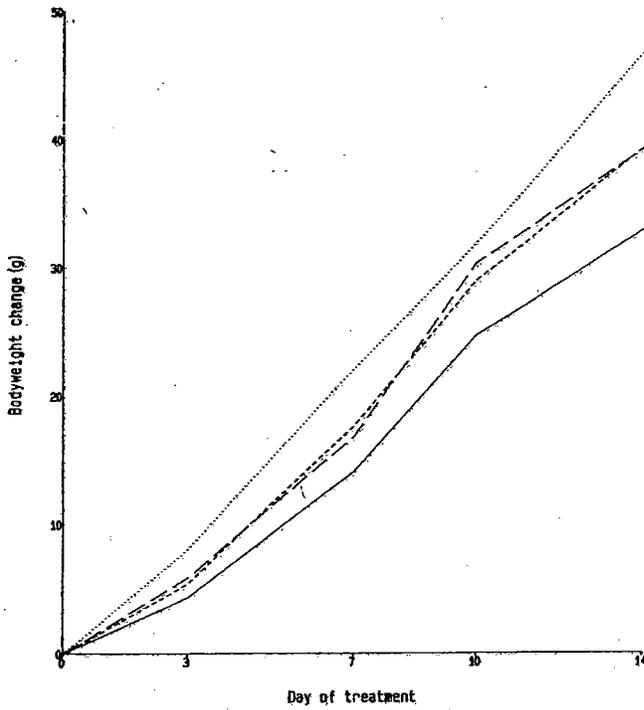
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FIGURE 2

Bodyweight change of females before pairing

- Group 1 : Control
- Group 2 : rhIGF-1 : 0.4 mg/kg/day
- Group 3 : rhIGF-1 : 2.0 mg/kg/day
- Group 4 : rhIGF-1 : 10.0 mg/kg/day



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Food consumption: Not remarkable except the food intake during lactation period in females.

Estrous cycles, mating performance and fertility:

All females exhibited regular estrous cycles of 4 or 5 days during the treatment. The majority of matings occurred at the first estrous during paring. One female in the HD group (10 mg/kg/day), which was paired with an untreated stock male, failed to mate. Fertility indices were slightly lower in the LD and MD groups (Table 7).

**TABLE 7**

**Mating performance and fertility.**

Group : 1 2 3 4  
 Compound : Control ----- rhIGF-1 -----  
 Dosage (mg/kg/day) : 0 0.4 2.0 10.0

Group and sex	Paired with (group and sex)	Number paired	Number mating	Number achieving pregnancy	Percentage mating	Conception rate (%)	Fertility index (%)
1 M	1 F	5	5	5	100	100	100
2 M	2 F	5	5	4	100	80	80
3 M	3 F	5	5	4	100	80	80
4 M	4 F	5	5	5	100	100	100
1 F	1 M	5	5	5	100	100	100
2 F	2 M	5	5	4	100	80	80
3 F	3 M	5	5	4	100	80	80
4 F	4 M	5	5	5	100	100	100
1 F	UM	10	10	9	100	90	90
2 F	UM	10	10	10	100	100	100
3 F	UM	10	10	8	100	80	80
4 F	UM	10	9	8	90	89	80

UM Untreated stock males.

Necropsy finding on Day 14 after mating: There were no abnormalities that were considered to be related to the treatment. Corpora lutea count, implantations, and total viable young were not significantly different in all groups. The total resorptions were higher in the LD (0.4 mg/kg/day) group in comparison to the control due to slight high resorption in early phase. Per cent of implantation loss was slight high in the LD group in post-natal period, which was not IGF-1 dose dependent (Table 12).

TABLE 12

Group mean litter data - females killed on Day 14 of gestation

Group : 1 2 3 4  
 Compound : Control ----- rhIGF-1 -----  
 Dosage (mg/kg/day) : 0 0.4 2.0 10.0

Group	Number of pregnant animals	Mean SD	Corpora lutea count Mean SD	Implantations Mean SD	Viable young Total Mean SD	Resorptions			Implantation loss (%)	
						Early	Late	Total	Pre-	Post-
1	9	Mean SD	16.6 2.4	15.0 3.7	14.2 4.2	0.67 0.82	0.11 0.33	0.78 0.88	11.2	5.2
2	10	Mean SD	17.8 2.6	16.2 3.9	14.1 5.6	1.90 1.38	0.20 0.45	2.10 1.45	9.0	13.0
3	8	Mean SD	17.4 4.2	16.5 5.8	15.4 5.4	1.13 1.06	0.00 0.00	1.13 1.06	5.7	6.8
4	8	Mean SD	18.8 2.3	19.0 2.3	17.1 2.0	1.75 1.32	0.13 0.35	1.88 1.37	1.3	9.9
Background control* (23 studies)										
Mean			17.9	16.3	15.4	0.92	0.02	0.94	9.4	5.8
Low			17.0	15.3	14.0	0.38	0.00	0.38	5.7	2.3
High			19.0	17.7	17.0	1.47	0.14	1.47	15.3	9.5

SD Standard deviation.

\* Based on females killed on Day 20 of gestation.

Post-natal phase (females allowed to litter):

Gestation length was within the expected range of 22 to 23 days and parturition and gestation index was not significantly different among groups. Litter size and their survival were also comparable in the control and treated groups. Sex ratio was similar in the control as well as treated groups. Body weight of male and female offspring was not affected by the IGF-1 treatment.

Terminal examination: Macroscopic examination of offspring that died before termination revealed absence of milk in the stomach. Necropsy of offspring killed on Day 4 post partum revealed no abnormalities that might be related to the treatment. Macroscopic examination of females that allowed to litters on Day 4 necropsy revealed no significant findings. Necropsy of males revealed no macroscopic findings that might be related to the treatment.

Conclusion:

Subcutaneous treatment with rhIGF-1 at doses up to 10 mg/kg/day did not produce remarkable adverse effects upon the general condition and reproductive performance of male and female

rats. The treatment also did not affect significantly the growth and viability of their offspring up to Day 4 of age. Based on the findings documented above, the reviewer agrees that IGF-1 will not likely produce reproductive toxicity, although the sponsor has not tested the study with IGF-1/IGFBP together. The top dose of 10 mg/kg/day in rats is equivalent to an exposure 1.2 times the average human dose, based on body surface area comparison.

**Title: rhIGF-1: An investigation of the effects on neonatal development in the rat (4.2.3.5.b.2)**

Document: Volume 17, Page 1-293

Study: Pharmacia & Upjohn (Document 9620859); Schedule# ( — 030); Report# 95 — 030/1006

Study facility: — conduct the study under United Kingdom GLP principle with the OECD guidelines in 1996.

Sponsor: toxicology and Safety Assessment, Pharmacia AB, Sweden

**Materials and Methods:**

rhIGF-1 in 5 mg/ml sterile solution per vial (Batch #10450-51, 61664-51 and 62001-51) was used for this study. Adult virgin male and female Sprague-Dawley rats were used. At the commencement of treatment the male weight ranged 191 to 225 (6-7 weeks old) and the females were 10-11 weeks old (body weight = 211 to 244 g). Twenty two rats/sex/group were assigned to control group or treated groups as shown below.

Group	Treatment	Dosage (mg/kg/day)	Number of animals		Animal numbering	
			M	F	M	F
1	Control (Placebo)	0	22	22	1001-1022	1089-1110
2	rhIGF-1	0.4	22	22	1023-1044	1111-1132
3	rhIGF-1	2.0	22	22	1045-1066	1133-1154
4	rhIGF-1	10.0	22	22	1067-1088	1155-1176

The animals were dosed daily by the subcutaneous route in the interscapular region at a volume of 2 ml/kg. Control animals received the placebo saline solution (Batch# DSQ260). Males were dosed for 71 days before pairing, throughout the mating period, and up to termination after necropsy of the females. Females were dosed for 15 days before pairing, throughout pairing for mating, gestation and lactation to Day 20 of lactation.

**Observations: Parental (Fo)**

**Clinical signs and mortality: Daily**

Body weight: Males were weighed weekly until termination while females were weighed twice weekly until mating was detected and subsequently, on Days 0, 3, 6, 10, 17, and 20 of gestation.

Food consumption: Weekly until the animals were paired for mating. The parameter was checked for females on Days 0-2, 3-5, 6-9, 10-12, 13-16 and 17-19 of gestation.

Mating procedure: Males and females were paired on a one-to-one basis. Each morning following pairing, a vaginal smear was prepared from each female. The day when evidence of mating was established was designated Day 0 of gestation.

Postnatal observations: From Day 20 post coitum, females were inspected three times each weekday and twice daily at weekends for the onset and progress of parturition. Bodyweight of each female was recorded on Days 1, 4, 7, 11, 14, 18 and 21 of lactation. Maternal food consumption was checked on Days 1-3, 4-6, 7-10, 11-13, 14-17 and 18-20 of lactation.

Postnatal observation (Offspring): All offspring were examined after birth (Day 1) and number of live and dead offspring, litter weight and sex ratio were determined. Mortality, clinical signs, and litter size were checked daily. On Day 4 postpartum, litters containing more than 8 litters were reduced to 8 by random culling, leaving, wherever possible, 4 males and 4 females in each litter. Offspring's bodyweights were checked on Days 1, 4, 7, 11, 14, 18, 21 and 28 post partum.

Physical development of the offspring was assessed collectively (Pinna unfolding, hair growth, tooth eruption, and eye opening) or individually (vaginal opening and preputial separation). Auditory and visual function was checked on Day 25 post partum. Water maze and neuromuscular function was also examined on Day 27 post partum.

Development and reproductive performance of F1 generation: At five weeks of age, 20 offsprings/sex/group were selected from each group, using random number tables within litters to form the F1 generation. The reproductive performance of progeny derived from control and treated Fo generation animals was evaluated as described under Fo generation.

Necropsy examinations: The Fo females were sacrificed after weaning or total litter death. Any female failing to produce a viable litter by Day 25 post coitum was killed for macroscopic examination. After successful littering of the females, the males were killed for examination (See the result section).

F1 females were killed on Day 14 of gestation for macroscopic examination for implantation sites, resorption sites, and number and distribution of fetuses (See the results section for details).

### **Results: Fo generation**

Clinical signs and mortality: The general clinical signs of the treated animals were similar to that of the control. The only signs after dosing such as hypoactivity, prostration, hypothermia and convulsion were attributed to hypoglycemia due to IGF-1 treatment. A total of 9 rats (3 males and 6 females) died in the HD group (10 mg/kg/day) as a direct result of hypoglycemic shock-induced by the treatment.

Bodyweight: There were no statistical differences between the control and groups 2 and 3 in Fo male rats. However, 7 week-treatment with IGF-1 at a dose of 10 mg/kg/day increased the bodyweight significantly ( $P < 0.01$ ) for entire duration of 14 weeks. In females before pairing (Fo) significant increases in body weight were observed in the MD and HD groups 7 days after the treatment. There were no typical patterns in group mean bodyweights in females during gestation and lactation because the values were not clearly dependent on IGF-1 treatment duration or its doses.

Food and water consumption: Not remarkable.

Mating performance, estrus cycle, and fertility: 95% of treated rats had regular estrous cycles of 4 to 5 days duration so that IGF-1 treatment did not affect estrous cycle. Pre-coital interval in the control and treated animal was comparable, being 1-4 days (91%) and 5-8 (5%). Mating occurred at the first estrus with the exception of two females which received 10 mg/kg/day. One female was acyclic before mating and the other mated at the second estrus. Conception rates and fertility indices were 100% in all groups as shown below.

Mating performance and fertility (Fo-F<sub>1</sub>)

Group	:	1	2	3	4
Compound	:	Control	rhIGF-1		
Dosage (mg/kg/day)	:	0	0.4	2.0	10.0

Group and sex	Number paired	Number mating	Number achieving pregnancy	Percentage mating	Conception rate (%)	Fertility index (%)
1M	20	20	20	100	100	100
2M	22	22	22	100	100	100
3M	22	22	22	100	100	100
4M	19 <sup>o</sup>	19	19	100	100	100
1F	22	22	22	100	100	100
2F	22	22	22	100	100	100
3F	22	22	22	100	100	100
4F	22	22	22	100	100	100

<sup>o</sup> Excludes one male killed *in extremis* at pairing and one male killed for humane reasons after four nights in pairing.

Necropsy findings: Macroscopic findings on surviving females were examined on Day 21 of lactation and of males after 14-week treatment were not different from the control and treated group.

Organ weight: In males, absolute organ weights in prostate and epididymides increased in the HD group after 15-week treatment. There were not other changes in the LD or MD dose groups.

Fo general -post-natal phase: Sex ratio, gestation length, gestation index, number of live litters born, litter sizes and offspring survival indices were not significantly different between the control and treated groups. Absolute bodyweight of offsprings on day 1 of lactation and subsequent weights gains to Day 28 of lactation were similar in all groups. The rate of physical development, hair growth, tooth eruption, eye opening in both sexes was not affected by parental

treatment with IGF-1. Preputial separation in males and vaginal opening in females were also not affected the treatment. Auditory and visual responses, locomotor activity, water maze performance and neuromuscular function were not altered by the treatment.

F1 generation: There were no deaths in F1 animals and no treatment-related effects on general conditions were observed. Initial bodyweights for males at 5-week old and subsequent bodyweight gain up to termination were not affected by the treatment. Bodyweights and bodyweight gains before pairing and during gestation (up to Day 14 of gestation) were also similar in all female groups.

Mating performance and fertility: Number of pregnancy, conception rate and fertility index were slightly reduced in the HD group, compared to the LD and MD groups as shown in Table 22 below, although the values in the control group were also slightly reduced.

TABLE 22

## Mating performance and fertility (F1-F2)

Group	:	1	2	3	4
Compound	:	Control	----- rhIGF-1 -----		
Dosage (mg/kg/day)	:	0	0.4	2.0	10.0

Dosing restricted to F<sub>0</sub> generation.

Group and sex	Number paired	Number mating	Number achieving pregnancy	Percentage mating	Conception rate (%)	Fertility index (%)
1M	20	19	17	95	89	85
2M	20	20	20	100	100	100
3M	20	20	19	100	95	95
4M	20	19	16	95	84	80
1F	20	20	18	100	90	90
2F	20	20	20	100	100	100
3F	20	20	19	100	95	95
4F	20	19	16	95	84	80
<b>Background control (40 studies)</b>						
<b>Males</b>	<b>Mean</b>			<b>98.9</b>	<b>96.3</b>	<b>95.2</b>
	<b>Low</b>			<b>95</b>	<b>85</b>	<b>85</b>
	<b>High</b>			<b>100</b>	<b>100</b>	<b>100</b>
<b>Females</b>	<b>Mean</b>			<b>100.0</b>	<b>96.3</b>	<b>96.3</b>
	<b>Low</b>			<b>100</b>	<b>85</b>	<b>85</b>
	<b>High</b>			<b>100</b>	<b>100</b>	<b>100</b>

Necropsy findings for females: Macroscopic observations on Day 14 of gestation in 4-group animals revealed that there were no treatment-related abnormalities. The number of females in the HD group (10 mg/kg/day to Fo generation) achieving a successful pregnancy was slightly below that of the control group (Table 24). It appears that the numbers of pregnant females in the LD and MD groups were slight better than those in the control, which is not clear for its scientific basis. Mean number of corpora lutea, implantations, total viable young, resorptions, and the extent of pre- and post-implantation losses demonstrated no clear indication of IGF-1 effects on the Fo generation.

TABLE 24

Uterine examination - group mean values for females killed on Day 14 of gestation (F1-F2)

Group	:	1	2	3	4
Compound	:	Control	rhIGF-1		
Dosage (mg/kg/day)	:	0	0.4	2.0	10.0

Dosing restricted to Fo generation.

Group	Number of pregnant animals		Corpora lutea count	Implantations	Viable young Total	Resorptions			Implantation loss (%)	
						Early	Late	Total	Pre-	Post-
1	18	Mean	16.7	15.6	14.9	0.61	0.06	0.67	6.7	4.3
		SD	3.9	3.6	3.5	0.78	0.24	0.82		
2	20	Mean	17.8	16.4	15.7	0.70	0.00	0.70	7.9	4.3
		SD	2.1	2.1	2.3	0.84	-	0.84		
3	19	Mean	17.3	16.1	14.9	1.11	0.00	1.11	7.0	6.9
		SD	1.9	2.1	1.9	1.05	-	1.05		
4	16	Mean	16.4	15.4	14.4	0.94	0.06	1.00	6.1	6.5
		SD	3.0	2.7	3.3	0.97	0.25	1.00		

SD Standard deviation.

Macropathology of males: There were several macroscopic findings at necropsy of F1 males. Several rats in the LD and MD groups had incidences of hairloss on head or head scabs. Four rats in the HD group had either an abrasion on dorsal thorax or abnormalities in the left testis (Table 25). Left testis and/or left epididymis were changed in color or reduced in size. The events were not IGF-1 dose related since there were no such incidences in the LD and MD groups.

TABLE 25

Macropathology - summary of findings for males (F<sub>1</sub>)

Group : 1 2 3 4  
 Compound : Control ----- rhIGF-1 -----  
 Dosage (mg/kg/day) : 0 0.4 2.0 10.0

Dosing restricted to F<sub>0</sub> generation.

Group:	1	2	3	4
Number of animals examined:	20	20	20	20
Number of animals with observations:	0	3	2	4
<b>Observations: animals affected<sup>a</sup></b>				
Slight hairloss on head	0	2	1	0
A few scabs on head	0	2	1	0
Abrasion on dorsal thorax	0	0	0	1
Unilateral renal cavitation	0	0	1	0
Left testis flaccid and reduced in size	0	0	0	2
Left testis blue	0	0	0	1
Left epididymis reduced in size	0	0	0	2
Left epididymis blue	0	0	0	1
Testes flaccid	0	0	0	1

<sup>a</sup> One animal may have more than one observation.

## Summary and Conclusion:

IGF-1 was administered subcutaneously at doses of 0.4, 2 and 10 mg/kg/day to male and female CD (Sprague Dawley t origin) rats before pairing throughout mating and during the pre- and post-natal periods including organogenesis. There were deaths in two males and six females which had received 10 mg/kg/day. The animals showed typical signs of hypoglycemia before deaths. Glucose administration did not save all hypoglycemic animals. There were no other clear IGF-1 related-adverse clinical effects.

As expected, increased bodyweight gains were observed before pairing for males and for females which had received the HD (10 mg/kg/day). During the gestation and lactation periods bodyweight gains were similar across the four groups. Mating performance and fertility were not clearly affected by the treatment. Gestation length for females which received 2 or 10 mg/kg/day was longer by a half day when compared to the control group. Litter survival, growth, development and reproductive capacity were not compromised by the treatment. The HD (10 mg/kg/day) was considered to be the MTD in view of the deaths at this dose and NOAEL for fertility and reproductive effects was 10 mg/kg/day (8X MRHD based on body surface area comparison).

**Title: rhIGF-1: Teratology study in the rat (Section 4.2.3.5.b.4)**

Study No. : KAB/015 — report#:91/KAB015/0872)

Document: Vol. 20, pp 1-127

Testing Facility: This study was performed at  
under OECD guidance.

Sponsor: Kabi Pharmacia AB, Stockholm Sweden.

Purpose: To assess the effects of subcutaneous administration of rhIGF-1 during the organogenesis phase of gestation upon the progress and outcome of pregnancy in the rats. This report is the final report that is stored in the archives of —

#### Materials and Methods:

Rh-IGF-1: Batch# was 53678, which was colorless solution (5 mg/ml).

Placebo: Batch# DsQ57 (Phosphate saline buffer) was used for the control group and for the dilution of the LD and MD preparation.

Animals: Virgin female rats (CD: Sprague Dawley) whose bodyweight ranged 211 to 263 g (approximately 10-11 weeks old) were used. Females were paired on a one-to-one basis with stock males of the same strain. A vaginal smear was prepared from each female and examined for the presence of spermatozoa.

The day on which a sperm positive vaginal smear or at least three copulation plugs were found was designated Day 0 of gestation.

Treatment: 32 animals/group were given rhIGF-1 subcutaneously at doses of 0, 0.4, 2, and 10 mg/kg/day from Day 6 to Day 15 post coitum. Control animals received the vehicle (Placebo solution).

Maternal observations: Mortality and clinical signs were examined daily. Bodyweight was recorded on Days 0, 3, 6 to 18 inclusive and 20 post coitum. Food consumption was recorded on Days 0-2, 3-5, 6-8, 9-11, 12-15, 16-17 and 18-19 post coitum. Five satellite females from each group had 1 ml blood samples taken at 0.5 and 6 hours after treatment on Days 6 and 15 post coitum for hematologic study. The other five satellite females had blood samples taken at 2 and 24 hours after dose administration.

Terminal study: On Day 20 post coitum the females were killed to examine number of corpora lutea in each ovary, number of implantation sites, number of resorption sites and number and distribution of live and dead fetuses in each uterine horn. Each fetus was weighed, sexed and examined for any external abnormalities. Individual placental weights and abnormalities were examined.

#### Results:

Maternal observations: There were no deaths except one female in the control satellite group was killed for humane reasons on Day 12 post coitum due to blood sampling mistake. Food consumption, bodyweight and mean bodyweight were not affected significantly by the treatment. There were the test article-dose dependent decreases in blood glucose on Day 15 post coitum 30 minutes after the administration, which returned toward normal in 6-hour samples (Table 16).

TABLE 16

Group mean plasma glucose levels (mg%)  
of satellite females on Day 15 post coitum

Group : 1 2 3 4  
Compound : Control ----- rh16F-1 -----  
Dosage (mg/kg/day) : 0 0.4 2.0 10.0

Group		Hours after dosing*		
		0.5	2.0	6.0
1	Mean	114	124	123
	SD	5	7	5
	n	4	5	4
2	Mean	121	125	127
	SD	2	9	3
	n	5	5	5
3	Mean	96	127	120
	SD	14	13	4
	n	5	5	5
4	Mean	86	85	127
	SD	26	13	8
	n	5	5	5

\* First five animals per group sampled at 0.5 and 6.0 hours after dosing; second five animals per group sampled 2.0 hours after dosing.

SD Standard deviation  
n Number of animals.

Teratology phase: The results of macroscopic examination at necropsy of females on Day 20 post coitum indicate 2 to 5 animals from each group had some abnormalities. However, they are the test article-dose dependent (Table 8). Numbers of corpora lutea, implantations, resorptions and live implantations were not different in the control and treated groups (Table 9).

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TABLE 8

Summary of observations at necropsy of females on Day 20 of gestation

Group	1	2	3	4
Compound	Control	rhIGF-1		
Dosage (mg/kg/day)	0	0.4	2.0	10.0
Group:	1	2	3	4
Number of animals examined:	22	22	22	22
Number of animals with observations:	3	5	2	4
<b>Observations:</b>				
Staining on head	1	1	0	0
Hair-loss on fore-limbs	1	0	0	0
Xiphoid cartilage protruding 90° from rib cage	0	0	1	0
Small haemorrhage at internal dose site (<100 mm <sup>2</sup> )	2	4	1	2
Pale area on cranial surface of median liver lobe	0	0	0	1
Pale striation on caudal surface of left liver lobe	0	0	0	1

**Litter observations:**

Corpora lutea	Mean	17.3	17.1	16.6	17.8
	SD	1.1	1.9	2.0	1.8
Total number of implantations	Mean	16.4	16.7	15.4	16.3
	SD	1.0	2.1	1.4	2.4
Resorptions	Mean	1.56	1.20	0.80	1.90
	SD	1.25	1.10	0.89	1.38
Live implantations	Mean	14.9	15.5	14.6	14.4
	SD	0.9	2.0	1.6	3.4
% pre-implantations loss		6.3	2.9	7.2	8.9
% post-implantation loss		9.5	7.2	5.2	11.7

SD Standard deviation.

‡ Excludes one female killed for humane reasons : see Table 1.

Fetal evaluation: Fetus examination at necropsy revealed a number of anomalies in all groups without any clear treatment related pattern (Table 12). A small number of fetuses had more unusual abnormalities. For examples, one fetus in the LD group had a gross cardiovascular anomaly. Another fetus from the same litter had agenesis of the median lung lobe. One fetus in the MD group died with edematous appearance and one fetus in the HD group had a domed like head. The isolated nature and group distribution of these abnormalities suggest that IGF-1 treatment did not likely result in the changes. There were no clear external and internal anomalies in the treated group, although there were questionable changes (without clear dose dependency) in hydronephrosis  $\geq 2$  mg/kg/day.

TABLE 12

## Summary of foetal observations at necropsy

Group	:	1	2	3	4
Compound	:	Control	-----	rhIGF-1	-----
Dosage (mg/kg/day)	:	0	0.4	2.0	10.0

Group:	1	2	3	4	Control data	
<u>External examination</u>						
Number of foetuses (litters) examined:	334(22)	323(22)	322(22)	341(22)	12469(875)	43
Number of male : female foetuses:	174:160	173:150	169:153	166:175	foetuses	studies
<u>Observations: % incidence<sup>†</sup> (number of litters)</u>					Mean	Study ranges
Large foetus (more than 4.10 g)	9.3(12)	13.0(12)	12.4(12)	8.5(8)	3.16	0.0-16.4
Small foetus (less than 2.80 g)	0.0(0)	0.6(2)	0.6(2)	0.6(2)	3.56	0.0-12.7
Pale foetus	0.0(0)	0.0(0)	0.3(1)	0.0(0)	0.06	0.0- 0.6
Shiny skin, domed head, apparent absence of right eye	0.0(0)	0.0(0)	0.0(0)	0.3(1)	#	#
Dark red line down centre of palate	0.0(0)	0.3(1)	0.0(0)	0.0(0)	#	#
Subcutaneous haemorrhage on right side of neck	0.3(1)	0.0(0)	0.0(0)	0.0(0)	0.02	0.0- 0.6
Large placenta (more than 0.70 g)	2.7(4)	5.6(8)	3.7(6)	2.6(5)	1.95	0.0-17.1
Small placenta (less than 0.35 g)	0.0(0)	0.3(1)	0.0(0)	0.3(1)	0.55	0.0- 1.73
Placenta rimmed with green, and amniotic fluid tinged green	0.0(0)	2.2(1)	0.0(0)	0.0(0)	#	#

<sup>†</sup> One foetus may have more than one observation.

# No previous record in background control data.

## Summary and conclusion:

Subcutaneous administration of IGF-1 at doses of 0.4, 2 and 10 mg/kg/day to female rats from day 6 to Day 15 post coitum was performed to detect its potential teratologic effects. There was one death in the control group due to accidental blood sampling and no test article-associated clinical signs were observed. Bodyweight, food and water consumption were not significantly different in the four groups. IGF-1 induced hypoglycemia was evident without leading to deaths. There were no other clear IGF-1 related-adverse effects in maternal examinations, litter responses, and fetus external and internal examinations in teratology phase. NOAEL appears to be 10 mg/kg/day, although there were inconsistent findings of the test article on hydronephrosis. The dose gives approximately 8 times MRHD, based on body surface area comparison.

**Title: rhIGF-1: Teratology study in the rabbit (Section 4.2.3.5.b.5)**

Document: Vol: 20, pp1-114

Sponsor: Kabi Pharmacia AB, Stockholm, Sweden

Sponsor's ID #/study#: KAB/018/rhIGF-1(Kabi Pharmacia document# 20882F; Amended report# 92/KAB018/0030) —  
Conducting laboratory: —  
Date of study initiation: March 18, 1993  
GLP compliance: Yes  
QA Report: Yes (x) No ( )

Methods:

- IGF-1 batch# 64538-51
- Placebo batch#DsQ57 (Saline buffer)
- Species: sexually mature virgin female New Zealand white rabbits. They were 18-26 weeks old, whose body weights ranged 3.3-5 kg. On Day 6 after insemination animals were divided into four groups. 16-20 rabbits/group received IGF-1 subcutaneously at doses of 0, 0.2, 0.5 or 1.25 mg/kg/day from Day 6 to Day 18 of gestation. Four rabbits/group received the same treatment and served as a satellite group. On Day 29 of gestation, females were killed to allow examination of their uterine contents.

Observations:

Maternal signs, mortality, and bodyweight: Daily  
Food consumption: Daily from Day 1-5, Day 6-22 and Day 24-28  
Plasma glucose (Females in Groups 1 to 4 only): 2 hour post-dosing  
Terminal study: Litter responses, number of corpora lutea in each ovary, implantation sites and resorption sites including live and dead fetuses were counted on Day 29 after insemination. In addition, external, internal and skeletal examinations were performed on Day 29 of gestation.

Results:

Maternal responses: Basically there were no differences in all control and treated animals except a few rabbits had hypoglycemic reactions, as expected. Mean bodyweight gains in the treated animals were slightly increased initially, but returned to the control levels. Thus, the effects of IGF-1 on body weight and food intake in Fo females were not remarkable. Plasma glucose level decreased in the HD (1.25 mg/kg/day) group 2-hours after the treatment.

Litter responses: Increases in early resorptions and post-implantation loss were observed in all treated groups. The HD (1.25 mg/kg/day) group had statistical significance ( $p < 0.01$ ) as shown below. As a result of the marked increase in post-implantation loss in the HD group, a reduction in the number of viable fetuses was recorded at a dose of 1.25 mg/kg/day, which suggests the MTD as presented below.

TABLE 8

## Group mean litter data - females killed on Day 29 of gestation

Group : 1 2 3 4  
 Compound : Control ----- rhIGF-1 -----  
 Dosage (mg/kg/day) : 0 0.2 0.5 1.25

Group	Number of pregnant animals		% Abortion <sup>a</sup> and total litter loss	Corpora lutea count	Implantations	Viable young			Resorptions			Implantation loss (%)	
	Mean	SD				M	F	Total	Early	Late	Total	Pre-	Post-
1	16	Mean SD	6.3	10.3 1.8	8.2 2.2	4.0 1.8	3.6 1.9	7.6 2.1	0.3 0.5	0.3 0.6	0.6 0.8	20.6	7.3
2	14	Mean SD	0.0	10.5 2.1	9.6 2.6	4.6 1.7	3.5 1.1	8.1 1.6	0.9 1.0	0.6 0.8	1.6 1.3	9.4	16.3
3	17	Mean SD	5.9	10.3 1.7	8.8 1.9	4.1 1.9	3.1 1.1	7.3 1.9	1.1 1.0	0.5 0.7	1.6 1.3	14.0	17.7
4	19	Mean SD	21.1	10.2 1.7	8.9 2.1	3.2 2.5	2.3 2.1	5.5 3.4	3.3** 1.8	0.1 0.4	3.4 1.8	13.1	38.3**
Background control (24 studies)													
Mean			0.3	10.5	8.7	4.0	3.7	7.7	0.6	0.5	1.1	16.7	12.0
Low			0.0	9.2	7.2	2.9	2.7	6.2	0.2	0.1	0.5	6.5	6.1
High			16.7	11.6	10.7	4.8	4.5	9.4	1.3	1.3	1.8	28.0	17.7

The means are derived only from animals that survived to term and bore viable young.

<sup>a</sup> See Appendix 2. SD Standard deviation.

\*\* Significantly different from Controls, P<0.01 (Mann-Whitney U-test).

Fetal evaluation: Evaluation of group mean fetal and placental weight in the control and treated groups indicates there were no statistically significant differences. Fetal evaluation at necropsy revealed a number of anomalies in all groups. The majority of the anomalies were similar to the previous documented historical data of this strain of rabbits in the testing laboratories. It appears that there were no treatment-related changes in the anomalies (Tables 10 and 11) in general organs and bony structures. Thus, this study with IGF-1 alone did not show that IGF-1 produced any remarkable abnormalities, although the HD group had seemingly high incidence in anomalies of the anterior fontanelle with reduced the right frontal bone. There were no clear treatment-related anomalies in internal organs and structures.

TABLE 10

## Summary of foetal observations at necropsy

Group : 1 2 3 4  
 Compound : Control ----- rhIGF-1 -----  
 Dosage (mg/kg/day) : 0 0.2 0.5 1.25

Group:	1	2	3	4	Control data	
Number of foetuses (litters) examined:	114(15)	113(14)	116(16)	82(15)	2495 foetuses	24 studies
<u>Observations: % incidence<sup>♠</sup> (litters)</u>					Mean	Study range
Abnormal foetus	0.0(0)	0.0(0)	0.0(0)	1.2(1)	0.16	0.0- 1.0
White spot in centre of eyes	0.0(0)	0.0(0)	0.9(1)	0.0(0)	0.08	0.0- 0.8
Prominent capillary in centre of palate	0.9(1)	0.0(0)	0.0(0)	0.0(0)	0.04	0.0- 0.9
Thymus gland haemorrhagic	0.0(0)	0.9(1)	0.0(0)	0.0(0)	0.08	0.0- 1.2
Abnormal heart and major vessels	0.9(1)	0.0(0)	0.0(0)	0.0(0)	0.08	0.0- 0.9
Clear serous fluid in abdominal cavity	0.9(1)	0.9(1)	0.0(0)	0.0(0)	0.12	0.0- 1.7
Pale areas on liver	0.0(0)	0.0(0)	0.0(0)	1.2(1)	0.28	0.0- 2.8
Liver pale	4.4(1)	3.5(1)	0.0(0)	0.0(0)	0.20	0.0- 4.4
Accentuated lobular pattern of liver	4.4(1)	6.2(2)	0.0(0)	3.7(1)	0.24	0.0- 4.4
Liver friable	0.0(0)	3.5(1)	0.0(0)	0.0(0)	#	#
Liver thickened and granular	0.9(1)	0.0(0)	0.0(0)	0.0(0)	0.04	0.0- 0.9
Gall bladder variants	6.1(7)	16.8(9)	12.1(10)	8.5(4)	8.62	0.0-16.1
Gas in stomach	1.8(2)	1.8(2)	1.7(2)	4.9(2)	0.80	0.0- 3.1
Small foetus (less than 32.0 g)	15.8(6)	8.0(4)	7.8(4)	19.5(3)	8.10	0.0-15.8
Clotted blood around placenta	0.9(1)	0.0(0)	0.0(0)	0.0(0)	0.24	0.0- 1.8
Pale placenta	0.0(0)	0.0(0)	0.0(0)	1.2(1)	0.12	0.0- 1.4

♠ One foetus may have more than one observation.

# No previous record in background control data.

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ON ORIGINAL

TABLE 11

Summary of foetal observations at skeletal examination

Group	1	2	3	4
Compound	Control	rhIGF-1		
Dosage (mg/kg/day)	0	0.2	0.5	1.25

Group:	1	2	3	4	Control data
Number of foetuses (litters) examined:	78(15)	78(14)	81(16)	56(14)	1661 24 foetuses studies

Observations: % foetal incidences<sup>φ</sup> (number of litters) Mean Study ranges

Head	1	2	3	4	Mean	Study ranges
Extra small anterior fontanelle, negligible, size of suture lines only	1.3(1)	0.0(0)	3.7(3)	0.0(0)	0.90	0.0-3.9
Small anterior fontanelle	79.5(14)	76.9(14)	84.0(16)	69.6(11)	53.58	0.0-86.3
Medium anterior fontanelle	17.9(7)	23.1(9)	12.3(6)	28.6(6)	44.91	0.0-76.6
Large anterior fontanelle	1.3(1)	0.0(0)	0.0(0)	1.8(1)	0.48	0.0-1.4
Anterior fontanelle extended anteriorly	0.0(0)	0.0(0)	0.0(0)	1.8(1)	0.18	0.0-1.3
Anterior fontanelle extended laterally into frontal bones	0.0(0)	0.0(0)	0.0(0)	1.8(1)	#	#
Anterior fontanelle asymmetrical	2.6(2)	5.1(3)	0.0(0)	8.9(3)	0.84	0.0-3.9
Posterior fontanelle enlarged	7.7(2)	5.1(3)	1.2(1)	3.6(1)	3.97	0.0-9.6
Incomplete ossification of interparietal bone	1.3(1)	0.0(0)	1.2(1)	0.0(0)	0.24	0.0-3.8
Interparietal bone reduced or reduced and cleft	2.6(2)	0.0(0)	0.0(0)	0.0(0)	#	#
Additional suture in parietal bone	1.3(1)	1.3(1)	0.0(0)	0.0(0)	0.90	0.0-4.5
Additional fissure in parietal bone	0.0(0)	1.3(1)	0.0(0)	0.0(0)	0.30	0.0-3.0
Right parietal bone fused to right frontal bone	0.0(0)	0.0(0)	1.2(1)	1.8(1)	0.42	0.0-1.8
Right frontal bone slightly reduced	0.0(0)	0.0(0)	0.0(0)	1.8(1)	#	#

<sup>φ</sup> One foetus may have more than one observation. # No record in background control data subset.

TABLE 11 - continued

Summary of foetal observations at skeletal examination

Group : 1 2 3 4  
 Compound : Control ----- rhIGF-1 -----  
 Dosage (mg/kg/day) : 0 0.2 0.5 1.25

Group:	1	2	3	4	Control data
Number of foetuses (litters) examined:	78(15)	78(14)	81(16)	56(14)	1661 foetuses studies
Observations: % foetal incidence <sup>φ</sup> (number of litters)					Mean Study ranges
Head - continued					
Additional suture in nasal bone	0.0(0)	1.3(1)	0.0(0)	0.0(0)	0.36
Irregular ossification of frontal suture	20.5(7)	9.0(6)	8.6(4)	8.9(5)	6.26
Frontal suture enlarged at fronto-nasal junction	1.3(1)	0.0(0)	0.0(0)	0.0(0)	0.06
Additional plaque of bone in frontal suture	1.3(1)	0.0(0)	0.0(0)	0.0(0)	0.30
Fronto-nasal suture enlarged	1.3(1)	0.0(0)	0.0(0)	0.0(0)	0.06
Hyoid body incompletely ossified	39.7(11)	42.3(13)	21.0(9)	21.4(9)	25.35
Hyoid cornua bent outwards	1.3(1)	1.3(1)	0.0(0)	0.0(0)	0.12
Incomplete ossification of 1st cervical vertebral centrum	2.6(1)	1.3(1)	1.2(1)	0.0(0)	0.84

φ One foetus may have more than one observation.

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TABLE 11 - continued

## Summary of foetal observations at skeletal examination

Group	1	2	3	4	Control data	
Compound	Control	rhIGF-1				
Dosage (mg/kg/day)	0	0.2	0.5	1.25		
Group:	1	2	3	4	2396	24
Number of fetuses (litters) examined:	114(15)	113(14)	116(16)	82(15)	foetuses	studies
Observations: % foetal incidence <sup>†</sup> (number of litters)					Mean	Study ranges
<u>Sternebrae and ribs</u>						
Incomplete ossification of 1 sternebra	31.6(10)	18.6( 9)	30.2(14)	23.2(10)	30.59	0.0-65.8
Incomplete ossification of 2 sternebrae	2.6( 2)	1.8( 2)	3.4( 4)	9.8( 4)	2.09	0.0- 5.0
Incomplete ossification of 3 sternebrae	2.6( 1)	0.9( 1)	0.0( 0)	1.2( 1)	0.25	0.0- 2.6
One or more sternebrae offset	2.6( 3)	5.3( 5)	5.2( 5)	2.4( 2)	3.26	0.0- 8.8
Two or more sternebrae fused	0.9( 1)	4.4( 5)	8.6( 6)	1.2( 1)	4.72	0.0-12.6
One omosternum ossified	1.8( 2)	0.9( 1)	0.0( 0)	0.0( 0)	0.71	0.0- 3.6
Small additional 6th sternebra between 5th and xiphisternum	0.0( 0)	0.9( 1)	3.4( 3)	1.2( 1)	3.17	0.0-10.9
Xiphisternum bifurcated	0.9( 1)	0.9( 1)	0.0( 0)	0.0( 0)	0.21	0.0- 1.6
Xiphisternum cleft or abnormally wide	0.9( 1)	0.9( 1)	0.0( 0)	1.2( 1)	0.25	0.0- 1.4
Ribs 12/12	41.2(11)	35.4(13)	57.8(15)	43.9(13)	46.12	0.0-66.0
Ribs 12/13	8.8( 8)	14.2(10)	15.5( 9)	15.9( 8)	12.81	0.0-21.3
Ribs 13/13	50.0(15)	50.4(14)	26.7(11)	40.2(12)	40.98	0.0-63.4
Short 13th rib or ribs	25.4(13)	23.9(13)	19.8(10)	18.3( 7)	20.66	0.0-27.1
Floating 13th rib or ribs	5.3( 6)	8.0( 7)	1.7( 2)	11.0( 7)	4.63	0.0-10.4

<sup>†</sup> One foetus may have more than one observation.

## Summary and Conclusion:

rhIGF-1 was administered subcutaneously at doses of 0, 0.2, 0.5 and 1.25 mg/kg/day to pregnant New Zealand White rabbits from Day 6 to 18 of gestation. On Day 29 of gestation, female rabbits were killed to evaluate the treatment effects on organogenesis by analysis of number of corpora lutea in each ovary, implantation sites and resorption sites including live and dead fetuses. The HD group had a high post-implantation loss, compared to the control group. The lower two doses had no remarkable detrimental maternal or fetus effects. Thus NOAEL was a 0.5 mg/kg/day, based on the fact that there was no significant increase in postimplantation loss. The dose give approximately 0.1 times the maximum human recommended dose (MRHD), based on body surface comparison. Based on the finding of a significant increase in post-implantation loss at 1.25 mg/kg/day the high dose must exceed the MTD, which resulted in approximately 2 times MRHD based on body surface area comparison.

**Title: A study of the effects of rhIGF-1 with and without glucose administration on pregnancy in the rabbit (Section 4.2.3.5.b.6)**

Document: Vol: 21, pp1-190/Pharmacia & Upjohn document#9620857

Sponsor: Pharmacia Toxicology and Safety Assessment, Stockholm, Sweden

Sponsor's ID #/study#: — .027/rhIGF-1(Report#: 94 — 27/0785)

Conducting laboratory:

Date of study initiation: Oct., 11, 1993

GLP compliance: Yes

QA Report: Yes (x) No ()

Methods and Materials:

rhIGF-1: Batch#52827-51, 54612-51 and 74168-51

Animals: Sexually mature virgin female New Zealand White rabbits. They were 15-23 weeks old and their bodyweights were approximately 3 to 5 kg.

Study design: 22 to 26 female rabbits/group received rhIGF-1 subcutaneously at doses of 0 (control), 0.5, 1.25, 1.25 + glucose, 2.5 + glucose mg/kg/day from Day 6 to Day 18 after insemination. Another 26 females received 2.5 IU/kg/day Insulin as a reference group. Control group received the saline vehicle at the same volume dosage and glucose was 505 (w/v) aqueous glucose solution, which was given by oral gavage three times each day of dosing; the first immediately following the rhIGF-1 administration, the second and third at one and three hours post-dosing. Glucose volume was 2.5 to 5 ml/kg, based on the individual bodyweight on that day.

Observation:

Clinical signs and bodyweight: Daily

Food consumption: Days 1-5 inclusive, Days 6-23 daily, and Days 24-28 inclusive

Plasma glucose: 2 hours after dosing. For the kinetic analysis of plasma glucose blood samples (0.5ml) were taken on Days 6 and 12 after insemination at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, and 6 hours after dosing.

Litter responses: On Day 29 after insemination the females were killed and numbers of corpora lutea in each ovary and implantation sites, resorption sites and live and dead fetuses were determined.

Results:

Maternal mortality and signs: Total 5 rabbits two from Group 3 (rhIGF-1 1.25 mg/kg/day) and three from Group 6 (Insulin: 2.5 IU/kg/day) group as shown (Table 1). There were one to three abortions in every group except the Insulin group.

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TABLE 1

## Disposition of animals

Group	Treatment	Dosage (mg/kg/day)	Dosage (IU/kg/day)			
1	Control	0	-			
2	rhIGF-1	0.50	-			
3	rhIGF-1	1.25	-			
4	rhIGF-1 + Glucose	1.25	-			
5	rhIGF-1 + Glucose	2.50	-			
6	Insulin	-	2.50			

Group:	1	2	3	4	5	6
Total number inseminated:	26	22	26	22	22	26
Deaths	0	0	1*	0	0	1*
Terminated	0	0	1+	0	0	2+
Not pregnant	11	7	9	8	5	6
Abortion	2	1	1	1	3	0
Total litter loss	0	1	0	0	0	0
Pregnant at term with viable young	13	13	14	13	14	17

\* Includes females that were killed *in extremis*.  
 + Females terminated following hypoglycaemic shock.

Maternal bodyweight and food consumption: Group 2 (LD: 0.5 mg/kg/day) had no effects on this parameter. There were significant increases in bodyweight gain in group 3 for the entire duration except Days 0-6 and 24-28 of gestation. Some animals in Group 4, 5 and 6 also gained weight in the middle of treatment duration. It appears that the magnitude of maternal body weight gain was in IGF-1- dose dependent. Food consumption in 0.5 mg/kg/day (group 2) was similar to that of the control group. In other groups food consumption was elevated in different time of gestation without clear patterns.

Plasma glucose level: On Day 18 of gestation plasma glucose concentrations (See Table 4) at two hours post-dosing were significantly reduced in Group 3 and 6, which may explain the deaths as presented above. As indicated, the animals in groups 4 and 5 had glucose administration.

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ON ORIGINAL

TABLE 5

Plasma glucose levels (mmol/l)  
Day 18 after insemination - group mean values

Group	Treatment	Dosage (mg/kg/day)	Dosage (IU/kg/day)	Sampling point	
				Pre-dose	Two hours after post-dosing
1	Control	0	-		
2	rhIGF-1	0.50	-		
3	rhIGF-1	1.25	-		
4	rhIGF-1 + Glucose	1.25	-		
5	rhIGF-1 + Glucose	2.50	-		
6	Insulin	-	2.50		
1	Mean	7.8		7.9	
	SD	0.6		0.7	
	n	13		13	
2	Mean	7.9		7.2	
	SD	0.6		1.0	
	n	13		13	
3	Mean	7.9		4.5 <sup>c</sup>	
	SD	0.2		1.6	
	n	14		14	
4	Mean	7.6		7.0	
	SD	1.0		1.3	
	n	13		13	
5	Mean	7.8		7.1	
	SD	0.6		1.3	
	n	14		14	
6	Mean	8.2		4.7 <sup>c</sup>	
	SD	0.7		1.3	
	n	17		17	

SD Standard deviation.

n Number of animals.

Significant when compared with vehicle Controls: c -  $p < 0.001$   
(Student's t-test).

Litter statistics: Table 6 has the summary of mean litter data Day 29 of gestation. Percent abortion was 7 to 13 % except the Group 6 (insulin 2.5 IU/kg/day). Corpora lutea counts and

implantations in the five treated groups were comparable to the control. Total viable litter numbers were comparable in all groups except group 3, which was significantly reduced due to increased resorptions. In this group, per cent of implantation loss was significantly elevated at doses of 0.5 and 1.25 mg/kg/day (Table 6), which is consistent with the teratology study in rabbits as presented before. It is also true that the hypoglycemia-induced by the IGF-1 played an important role in implantation loss, although the percentage of in other groups such as 5 and 6 was also elevated.

TABLE 6

Group mean litter data - females killed on Day 29 of gestation

Group	Treatment	Dosage (mg/kg/day)	Dosage (IU/kg/day)	Number of pregnant animals <sup>a</sup>	% Abortion and total litter loss <sup>a</sup>	Corpora lutea count	Implant- ations	Viable young			Resorptions			Implantation loss (%)	
								M	F	Total	Early	Late	Total	Pre-	Post-
1	Control	0	-	15	13.3	13.0 2.0	10.7 2.4	5.2 1.3	4.9 2.4	10.1 2.3	0.2 0.5	0.4 0.6	0.6 0.8	17.8	5.8
2	rhIGF-1	0.50	-	15	13.3	12.1 2.3	10.5 3.7	3.5 2.0	4.8 2.0	8.4 3.1	1.5 1.2	0.5 0.7	2.1 1.4	15.0	19.9
3	rhIGF-1	1.25	-	15	6.7	11.2 2.8	9.8 3.1	3.0 1.8	2.9 2.2	5.9 <sup>b</sup> 3.2	3.4 1.9	0.4 0.7	3.9 2.0	13.3	39.4 <sup>c</sup>
4	rhIGF-1 + Glucose	1.25	-	14	7.1	13.4 2.8	12.2 3.2	6.2 2.3	4.8 1.6	11.0 3.5	0.8 0.9	0.5 0.7	1.2 1.1	8.6	10.1
5	rhIGF-1 + Glucose	2.50	-	17	17.6	11.1 2.7	9.8 3.4	4.5 2.7	3.5 1.8	8.0 3.8	1.4 1.2	0.4 0.7	1.8 1.3	12.7	18.2 <sup>b</sup>
6	Insulin	-	2.50	17	0.0	11.0 2.1	9.9 2.3	4.4 1.6	4.1 2.1	8.5 3.0	1.1 1.0	0.4 0.6	1.4 1.2	10.2	14.3 <sup>b</sup>
Background control (30 studies)															
Mean					3.6	11.9	9.7	4.5	4.0	8.4	0.7	0.6	1.3	17.2	12.9
Low					0.0	9.3	6.5	2.8	2.6	5.7	0.1	0.2	0.5	4.3	4.8
High					13.3	14.3	12.3	5.4	5.1	9.8	0.9	1.6	2.8	30.6	22.7

<sup>a</sup> Includes females that did not survive to term with viable young.

SD Standard deviation.

Significant when compared with vehicle Controls: b - p&lt;0.01 (Mann-Whitney test - two-tailed).

Fetus evaluation: Six abnormal fetuses from 6 different litters were found at terminal necropsy on Day 29 of gestation. Three of these (one each from Groups 2 and 3, and four from group 4) had cleft palate. The incidences in group 4 exceeded the control data of 30 studies (Table 8). Some fetuses in group 4 had abnormalities in head, which appears to be related to the treatment (2.5 mg/kg/day). In this group, 40 fetuses have small in size (less than 20 g), which appears to be significant, compared to the control (8) or insulin group (6). A small number of other abnormalities was recorded, which occurred at incidences previously recorded in this strain of rabbit from the laboratories. Other anomalies such as enlarged heart, and abnormal major

vessels, were observed, but the incidences were all within the range of the control data. However, it would be important to evaluate the effects of IGF-1 and IGFBP-3 together on fetus development if the sponsor seeks an approval for indications which include women of child bearing potential.

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ON ORIGINAL**

TABLE 8  
Necropsy - summary of foetal observations

Group	Treatment	Dosage (mg/kg/day)	Dosage (IU/kg/day)	1	2	3	4	5	6	Control data
1	Control	0	-	131(13)	109(13)	83(14)	143(13)	112(14)	144(17)	3642 foetuses
2	rhIGF-1	0.50	-							30 studies
3	rhIGF-1	1.25	-							
4	rhIGF-1 + Glucose	1.25	-							
5	rhIGF-1 + Glucose	2.50	-							
6	Insulin	-	2.50							

Observations: % incidence $\phi$ (litters)	Mean	Study ranges
Abnormal foetus I $\sigma$	0.0(0)	0.0(0)
Abnormal foetus II $\sigma$	0.0(0)	0.0(0)
Abnormal foetus III $\sigma$	0.0(0)	0.0(0)
Abnormal foetus IV $\sigma$	0.0(0)	0.0(0)
Abnormal foetus V $\sigma$	0.0(0)	0.0(0)
Abnormal foetus VI $\sigma$	0.0(0)	0.0(0)
Skin dark	0.0(0)	0.0(0)
Depression in anterior fontanelle	0.0(0)	0.0(0)
Snout and nares reduced	0.0(0)	0.0(0)

$\phi$  One foetus may have more than one observation.  
 $\sigma$  See key to Appendix 8.  
 $\#$  No previous record in background control data.

TABLE 8 - continued

## Necropsy - summary of foetal observations

Group	Treatment	Dosage (mg/kg/day)	Dosage (IU/kg/day)						
1	Control	0	-						
2	rhIGF-1	0.50	-						
3	rhIGF-1	1.25	-						
4	rhIGF-1 + Glucose	1.25	-						
5	rhIGF-1 + Glucose	2.50	-						
6	Insulin	-	2.50						

Group:	1	2	3	4	5	6	Control data	
Number of foetuses (litters) examined:	131(13)	109(13)	83(14)	143(13)	112(14)	144(17)	3642 foetuses	30 studies
<b>Observations: % incidence<sup>†</sup> (litters)</b>							<b>Mean</b>	<b>Study ranges</b>
Ablepharon	0.0(0)	0.0(0)	0.0(0)	1.4(2)	0.0(0)	0.0(0)	#	#
Slight brow ridge	0.0(0)	0.0(0)	1.2(1)	0.7(1)	0.0(0)	0.0(0)	0.19	0.0-3.1
Punctate dark area on palate	0.8(1)	0.0(0)	0.0(0)	0.0(0)	0.0(0)	0.0(0)	0.33	0.0-3.1
Upper incisors not visible	0.0(0)	0.0(0)	1.2(1)	0.0(0)	0.0(0)	0.0(0)	0.03	0.0-0.8
Cleft palate	0.0(0)	0.9(1)	1.2(1)	3.5(2)	0.0(0)	0.0(0)	0.05	0.0-2.0
Bilateral open eyes	0.0(0)	0.0(0)	0.0(0)	2.8(1)	0.0(0)	0.0(0)	#	#
Bilateral forelimb flexure	0.0(0)	0.0(0)	1.2(1)	1.4(2)	0.0(0)	0.0(0)	0.25	0.0-1.7
Bilateral forepaw twisted outwards	0.0(0)	0.0(0)	0.0(0)	0.7(1)	0.0(0)	0.0(0)	#	#
Rudimentary tail	0.0(0)	0.0(0)	0.0(0)	0.7(1)	0.0(0)	0.0(0)	0.03	0.0-0.6
Shortened tail	0.0(0)	0.0(0)	1.2(1)	0.0(0)	0.0(0)	0.0(0)	#	#
<b>Observations: % foetal incidence<sup>†</sup> (number of litters)</b>							<b>Mean</b>	<b>Study ranges</b>
<b>Head - continued</b>								
Posterior fontanelle enlarged	4.6(3)	0.0(0)	1.8(1)	1.0(1)	0.0(0)	0.0(0)	4.45	0.0-13.6
Incomplete ossification of supra-occipital bone	0.0(0)	0.0(0)	0.0(0)	3.1(2)	1.4(1)	0.0(0)	0.53	0.0- 5.8
Small interparietal bone	0.0(0)	1.4(1)	0.0(0)	3.1(2)	4.1(2)	0.0(0)	0.90	0.0- 3.8
Small discrete unossified area in parietal bone	0.0(0)	0.0(0)	1.8(1)	4.2(2)	0.0(0)	0.0(0)	0.86	0.0- 5.3
Suture in parietal bone	1.1(1)	0.0(0)	1.8(1)	1.0(1)	0.0(0)	2.1(2)	0.74	0.0- 4.1
Fissure in parietal bone	0.0(0)	1.4(1)	1.8(1)	0.0(0)	1.4(1)	0.0(0)	0.25	0.0- 1.3
Frontal suture enlarged at frontal-nasal junction	0.0(0)	0.0(0)	0.0(0)	1.0(1)	0.0(0)	0.0(0)	0.04	0.0- 1.9

† One foetus may have more than one observation.

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TABLE 8 - continued

Necropsy - summary of foetal observations

Group	Treatment	Dosage (mg/kg/day)	Dosage (IU/kg/day)	1	2	3	4	5	6	Control data	
1	Control	0	-	131(13)	109(13)	83(14)	143(13)	112(14)	144(17)	3642 foetuses	
2	rhIGF-1	0.50	-							30 studies	
3	rhIGF-1	1.25	-								
4	rhIGF-1 + Glucose	1.25	-								
5	rhIGF-1 + Glucose	2.50	-								
6	Insulin	-	2.50								
Group:											
Number of foetuses (litters) examined:				131(13)	109(13)	83(14)	143(13)	112(14)	144(17)	3642 foetuses	30 studies
<u>Observations: % incidence<sup>φ</sup> (litters)</u>											
Agenesis of median lung lobe				0.0(0)	0.0(0)	1.2(1)	0.0(0)	0.0(0)	0.0(0)	0.0(0)	0.08 #
Heart enlarged				0.0(0)	0.0(0)	0.0(0)	0.7(1)	0.0(0)	0.0(0)	0.0(0)	0.0-1.4 #
Abnormal major vessels I <sup>σ</sup>				0.0(0)	0.0(0)	0.0(0)	0.7(1)	0.0(0)	0.0(0)	0.0(0)	0.03 #
Abnormal major vessels II <sup>σ</sup>				0.0(0)	0.0(0)	0.0(0)	0.0(0)	0.9(1)	0.0(0)	0.0(0)	#
Punctate, clear fluid-filled cyst on caudal surface on left median liver lobe				0.0(0)	0.0(0)	0.0(0)	0.0(0)	0.0(0)	0.0(0)	0.0(0)	#
Pale area(s) on liver lobe(s)				0.0(0)	0.0(0)	0.0(0)	0.0(0)	1.8(2)	0.0(0)	0.0(0)	0.19 #

φ One foetus may have more than one observation.  
 σ See key to Appendix 8.  
 # No previous record in background control data.

TABLE 8 - continued  
Necropsy - summary of foetal observations

Group	Treatment	Dosage (mg/kg/day)	Dosage (IU/kg/day)	1	2	3	4	5	6	Control data
1	Control	0	-							
2	rhIGF-1	0.50	-							
3	rhIGF-1	1.25	-							
4	rhIGF-1 + Glucose	1.25	-							
5	rhIGF-1 + Glucose	2.50	-							
6	Insulin	-	2.50							
Group:				131(13)	109(13)	83(14)	143(13)	112(14)	144(17)	3642 foetuses
	Number of foetuses (litters) examined:									30 studies
	Observations: % incidence <sup>φ</sup> (litters):									Mean Study ranges
	Dark, pedunculate body on visceral surface of right median liver lobe adjacent to gall bladder	3.8(4)	4.6(5)	1.2(1)	4.9(5)	0.0(0)	0.0(0)	0.9(1)	0.0(0)	5.55 #
	Gall bladder variants	0.8(1)	0.0(0)	0.0(0)	0.0(0)	0.0(0)	0.0(0)	0.0(0)	0.0(0)	0.0-20.7 #
	Small amount of clotted blood attached to stomach	0.0(0)	0.0(0)	0.0(0)	1.4(2)	0.0(0)	0.0(0)	1.8(2)	1.4(1)	0.0-5.9 #
	Gas in stomach	0.0(0)	0.0(0)	1.2(1)	0.0(0)	0.0(0)	0.0(0)	0.0(0)	0.0(0)	
	Unilateral agenesis of kidney and ureter	0.0(0)	0.0(0)	0.0(0)	0.0(0)	0.0(0)	0.0(0)	0.0(0)	0.0(0)	0.11
	Unilateral renal cavitation	0.0(0)	1.8(2)	0.0(0)	0.0(0)	0.0(0)	0.0(0)	0.0(0)	0.0(0)	0.0-1.1

φ One foetus may have more than one observation.  
# No previous record in background control data.

TABLE 8 - continued  
Necropsy - summary of foetal observations

Group	Treatment	Dosage (mg/kg/day)	Dosage (IU/kg/day)	1	2	3	4	5	6	Control data	
1	Control	0	-								
2	rhIGF-1	0.50	-								
3	rhIGF-1	1.25	-								
4	rhIGF-1 + Glucose	1.25	-								
5	rhIGF-1 + Glucose	2.50	-								
6	Insulin	-	2.50								
Group:				131(13)	109(13)	83(14)	143(13)	112(14)	144(17)	3642 foetuses	30 studies
Number of foetuses (litters) examined:											
Observations: % incidence <sup>φ</sup> (litters)										Mean	Study ranges
Clotted blood around urinary bladder				0.8(1)	0.0(0)	1.2(1)	0.0(0)	1.8(1)	0.0(0)	0.38	0.0-5.6
Clear fluid-filled punctate cyst on surface of kidney (unilateral)				0.0(0)	0.0(0)	0.0(0)	0.0(0)	0.0(0)	0.7(1)	#	#
Unilateral elongated and cranially displaced ovary <sup>†</sup>				0.0(0)	0.0(0)	2.4(1)	0.0(0)	0.0(0)	0.0(0)	#	#
Persistent posterior cardinal vein				0.0(0)	0.9(1)	1.2(1)	0.0(0)	0.9(1)	0.0(0)	#	#
Small foetus (less than 32.0 g)				7.6(5)	14.7(6)	16.9(5)	40.6(11)	23.2(8)	6.3(4)	15.8	0.0-28.6
Clotted blood around placenta				0.0(0)	1.8(1)	0.0(0)	0.0(0)	0.0(0)	0.0(0)	#	#
Pale placenta				0.0(0)	0.0(0)	0.0(0)	0.7(1)	0.9(1)	0.0(0)	#	#
Placenta thickened				0.0(0)	0.0(0)	0.0(0)	0.0(0)	0.9(1)	0.0(0)	#	#

φ One foetus may have more than one observation.  
# No previous record in background control data.  
+ Calculated with respect to number of female foetuses.

### Summary and Conclusions:

To test the potential organogenesis effects of IGF-1 16 to 20 sexually mature pregnant New Zealand White rabbits received IGF-1 subcutaneously from Day 6 to Day 18 gestation at doses of 0 (control), 0.5, 1.25 and 2.5 mg/kg/day. The control group received saline buffer in an equal volume of the treated groups. For the MD and HD groups 50% (W/v) aqueous glucose solution by oral gavage was given three times each day of dosing to prevent potential hypoglycemia. The last group 6 rabbits received insulin (2.5 IU/kg/day) as reference controls at the same volume-dosage during the same treatment period.

As expected, rhIGF-1 or Insulin administration induced hypoglycemia, which forced to kill five animals. Hypoglycemia-induced by the treatment with rhIGF-1 or Insulin resulted in an increased incidence of post-implantation loss. But, it is clear that IGF-1 alone also produced the effect as demonstrated by the study with IGF-1 and glucose combination groups. Exposure to rhIGF-1 in utero, with or without glucose, or Insulin, produced a number of effects on the fetuses. Thus the fetal effects described above may represent a response to the fluctuating glucose levels during organogenesis.

The bodyweights of fetuses from females treated with rhIGF-1 at 1.25 mg/kg/day supplemented with glucose were lower than those of the Controls. This data was corroborated with the finding of an increased incidence of small fetuses in all IGF-1 treated groups. Furthermore, at necropsy a marginal increase in the incidence of cleft palate was observed in these fetuses. This, in part, was confirmed after examination of the fetal skeletons. In this group, and in the groups treated with rhIGF-1 at 1.25 mg/kg/day and 2.50 mg/kg/day with glucose, a number of fetal abnormalities were also evident in bone examination. These changes were not seen in the fetuses treated in utero with Insulin. It is possible; therefore, that the changes described for the animals treated with IGF-1 at doses of 1.25 and 2.5 mg/kg/day probably represent a response to treatment. It appears that NOAEL is 0.5 mg/kg/day based on post-implantation loss, which is 0.8X MHRD based on body surface comparison.

#### 2.6.6.7 Local tolerance

##### **Title: 14-day subcutaneous irritation study of rhIGF-1/IGFBP-3 in SD rats (Section 4.2.3.6.a.1)**

Document: Vol: 21, pp1-86

Battelle Study#:N057645A

Sponsor: Celtrix Pharmaceuticals, Inc. Santa Clara, CA

Conducting laboratory: \_\_\_\_\_

Date of study initiation: October 14, 1996

GLP compliance: Yes

QA Report: Yes (x) No ()

Purpose: To characterize the potential irritation elicited by the twice daily repeated

subcutaneous administration of rhIGF-I/IGFBP-3 for fourteen days in rats. In addition, the reversibility of any toxic effects was evaluated following a 14-day recovery period.

Methods and Materials: The lot numbers of IGF-1/IGFBP-3 and vehicle control were:

- IGF-1/IGFBP-3: Lot# DP9502
- Vehicle control: Lot#DP9503

Total twenty male Sprague-Dawley rats (approximately 6 weeks old), with body weights of 213-236 g were used. Five male SD rats/group received a twice-daily subcutaneous injection of rhIGF-1/IGFBP-3 at doses of 0 (control vehicle) or 100 mg/kg/day for 14 days. Satellite animals were 5 males/group for the control and treated groups, which were killed on Day 29 after 2-week recovery period.

**Observation:**

Mortality and clinical signs: Twice a day

Body weight: On Day -2, Days 1, 7, 14, and 28

Hematology: On Day 15 and on Day 29 (satellite animals)

Draize evaluations of local irritation site: Daily. Please see a table below for scoring skin irritation.

Clinical chemistry: On Day 15 for interim necropsy and on Day 29 for final necropsy

Gross and histopathological evaluations: Injection sites of all animals.

Necropsy: On Day 15 and Day 29 for satellite animals.

Histopathology: Graded using a semiquantitative scale of 1(minimal), 2(mild), 3(moderate) and 4 (marked) changes.

Statistics: Not performed for Draize scores between groups.

**Draize Scale for Scoring Primary Skin Irritation<sup>1</sup>**

Evaluation of Skin Reactions	Score
<b>Erythema and eschar formation:</b>	
No erythema	0
Very slight erythema	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
<b>Edema formation:</b>	
No edema	0
Very slight edema	1
Slight edema (edges of area well-defined by definite raising)	2
Moderate edema (raised approximately 1.0 mm)	3
Severe edema (raised more than 1.0 mm extending beyond the area of exposure)	4

Other Dermal Effects: B = Blanching      Sc = Scab  
 F = Fissuring      T = Thickening  
 N = Necrosis      U = Ulceration  
 S = Epidermal Scaling

<sup>1</sup> From: Draize, J. H., "Dermal Toxicity." *Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics*, The Association of Food and Drug Officials of the United States, 46-59, 1959.

**Results:**

**Mortality and clinical signs:** No animals died in the duration of the study. Slight edema was observed at the dose administration site of three 100 mg/kg/day rats for 3 days from Days 8 to Day 10. Other clinical signs were not remarkable. The Draize score for the edema for three days was 0.2, which is far less than 1 (very slight edema).

**Body Weights:** There were no differences in body weight values between the control and treated groups for the duration of the study as shown below (Table 3).

**Table 3. Body Weights (g)**

Treatment Group		Day 2	Day 1	Day 7	Day 14	Day 21	Day 28
Vehicle Control	Mean	206.92	228.43	269.63	322.44	371.34	410.98
	SD	5.19	6.10	11.92	21.15	31.14	39.04
	(N)	10	10	10	10	5	5
100 mg/kg/day	Mean	206.38	225.43	275.75	332.21	370.38	406.10
	SD	5.50	6.56	7.11	17.20	34.92	42.41
	(N)	10	10	10	10	5	5

**Clinical Pathology and serum chemistry:** There were no differences between the control and treated groups in all hematology parameters except reticulocytes on Day 15, which were increased from 2 to 3.2% while they were reduced from 1.9 to 1.4% on Day 29, respectively. The values were still in the range of sponsor's historical values, so that the changes were of no significance. There were no apparent test article-related effects in the serum chemistry values on Days 15 and 29.

**Necropsy:** Rat #205 (100 mg/kg/day treated rat on interim necropsy on Day 15) had a 35 x 35 mm, dark discoloration and mild subcutaneous edema involving the skin of the injection site. This gross lesion was considered to be the result of inadvertent puncture of a blood vessel with resultant inflammation. There were no other treatment-related abnormalities.

**Histopathology:** All 5 control and treated rats that were sacrificed on Day 15 had chronic inflammation in subcutaneous tissues. There were hemorrhage and serum pocket in subcutaneous tissue in one rat in the treated group. On Day 29 after 14-day recovery inflammation was still present in both the control and treated groups and all 5 treated rats had subcutaneous fibrosis as shown below (Table 8).

Table 8. Summary of Microscopic Observations

(Interim Necropsy (Day 15))			
Notes: Animals = interim sacrifice 1		Animals Affected	
Controls from group(s): 1		Males	
	Animal sex:		
	Dosage group:	Ctls	100 mg/kg/day
Tissues With Diagnoses	No. in group:	5	5
SKIN-SOA .....Number examined:		5	5
INFLAMMATION, CHRONIC-ACTIVE, SUBCUTANEOUS TISSUE		5	5
HEMORRHAGE, SUBCUTANEOUS TISSUE		0	1
SERUM POCKET		0	1
FIBROSIS, SUBCUTANEOUS TISSUE		0	0
GRANULOMA, DERMIS, FOCAL		0	0

(Final Necropsy (Day 29))			
Notes: Animals = final sacrifice only		Animals Affected	
Controls from group(s): 1		Males	
	Animal sex:		
	Dosage group:	Ctls	100 mg/kg/day
Tissues With Diagnoses	No. in group:	5	5
SKIN-SOA .....Number examined:		5	5
INFLAMMATION, CHRONIC-ACTIVE, SUBCUTANEOUS TISSUE		3	5
HEMORRHAGE, SUBCUTANEOUS TISSUE		0	0
SERUM POCKET		0	0
FIBROSIS, SUBCUTANEOUS TISSUE		0	5
GRANULOMA, DERMIS, FOCAL		0	1

### Summary and Conclusion:

The purpose of this 14-day toxicity study was to evaluate the potential local toxicity of IGF-1/IGFBP-3 in rats. Five male SD rats/group received twice-daily subcutaneous injections of rhIGF-1/IGFBP-3 at doses of 0 (control vehicle) or 100 mg/kg/day for 14 days. Satellite animals were 5 males/group for the control and treated groups, which were killed on Day 29 after 2-week recovery period.

There were increases in body weights when treated animals were compared to controls on Day 7 and 14, although there was a considerable variability in the individual body weights lends question to the effect of treatment on body weights. There were no test article-related changes in any hematology or serum chemistry parameter. Very slight edema was observed at the dose site of 3/10 in the rat treated for 3 days. No observations of erythema/eschar were recorded for any animals, and other clinical observations were considered to be incidental to treatment. Based on microscopic examination of the dose administration site, rhIGF-1/IGFBP-3 administered twice-daily for 14 days at 100 mg/kg/day resulted in a diffuse, mild to marked, chronic-active inflammatory reaction involving the subcutaneous tissues of 5/5 rats sacrificed at the end of the treatment phase on Day 15. rhIGF-1/IGFBP-3 administered twice-daily for 14 days at 100 mg/kg/day resulted in a diffuse, mild to marked, chronic-active inflammatory reaction involving the subcutaneous tissues, which was not reversible following the two-week recovery period. NOAEL must be less than 100 mg/kg/day in this study. Considering clinical subcutaneous dose (2 mg/kg/day), the total dose of IGF-1 (100 mg/kg x 0.225 kg) is approximately 25 % of human local dose, based on 50 kg subjects.

**Title: A study on the local subcutaneous toxicity of rhIGF-1 in pigs (Section 4.2.3.6.b.1)**

Document: Vol: 21, pp1-20

Document#: Scientific report 8996406; Project#530

Battelle Study#:N057645A

Sponsor \_\_\_\_\_

Conducting laboratory: \_\_\_\_\_

Date of study initiation: July 05, 1989

GLP compliance: Yes

QA Report: Yes (x) No ()

**Purpose:** To characterize the potential irritation elicited by the subcutaneous administration of rhIGF-I/IGFBP-3 in pigs because the subcutaneous tissue structure of pigs is similar to that of man.

**Methods and Materials:**

rhIGF-1: Batch No.DSO33 (1 mg/ml in ampoules containing 4 ml)

Animals: Two crossbred SPF pigs (Danish Landrace x Yorkshire LYY, body weight 21 and 25 kg).

Treatment: The animals were given rhIGF-1 subcutaneously at doses of 0 (Control) and 4 mg per injection sites. The control pig received 0.9% saline in an equal volume of the tested article (4 ml). The injection sites were the left and right sides of the thoracic and lumbar regions of the animals. For the first pig the test article injections were performed on Day 1, 3, and 4 from the thoracic to lumbar area and autopsy was performed on Day 5. For the second pig rhIGF-1 was injected daily at a dose of 4 mg on thoracic area. The same dose was given to lumbar sites on Day 2 and 3. To avoid potential hypoglycemic effect of the test article, the test article injections were performed with an interval of some hours on Day 2 and 3. In this animal saline was injected on Day 3 and autopsy was performed on Day 5.

Observation: Daily for the reaction to the treatment and for the injection site examination.

Necropsy: The subcutaneous tissues at each injection site and the underlying muscle were inspected for macro- and microscopic changes.

Results: The two pigs tolerated the treatment well in the beginning. Pig #2 had hypoglycemic crisis about 4 hours after the first injection on Day 1. Glucose was given which alleviated the condition. The procedure was repeated again on Day 3 and the animal died on Day 4. Necropsy was performed immediately after the death.

Macroscopic and microscopic findings: There were some incidences related to needle lesion, which might cause local hemorrhage because they used 19G needle as shown below (please see the table). There were no differences between the saline control sites and the test article-treated sites. The sponsor concluded that there were no local detrimental effects of IGF-1 in pigs, which the reviewer agrees.

## rhIGF-1 - local subcutaneous toxicity

## Microscopic findings in pig No. 1

Injection day	Preparation injected	Findings			
		Left side		Right side	
		Skin and subcutis	Muscle	Skin and subcutis	Muscle
1	4 ml, rhIGF-1	No changes	No changes	No changes	No changes
3	4 ml, rhIGF-1	Needle canal	Needle canal	No changes	No changes
4	4 ml, rhIGF-1	Needle canal	No changes	Needle canal	Small focal eosinophil infiltration
3	4 ml, 0.9% NaCl	No changes	Needle lesion	Needle canal	Needle lesion

## 2.6.6.8. Special toxicology study

**Title: Autoradiographic distribution of IGF-1 in male mouse (4.2.2.3.b.1)**

1. Purpose: To determine distribution patterns of radiolabeled IGF-1 in whole body of male mouse. This study was carried out at

2. Methods: Human recombinant IGF-1 prepared from the (Batch 2038-4) was labeled with  $^{125}\text{I}$ . The  $^{125}\text{I}$ -IGF (3.12  $\mu\text{Ci}$ ) was injected intravenously via a tail vein into four adult male C57BL mice (mean weight 22 g). The mice were killed 1 min, 5 min, 1 and 5 hours after injection and the whole animals were rapidly frozen. The frozen blocks were sectioned for whole-body autoradiography (20, 60 and 100  $\mu\text{m}$ ) onto tape in a the cry sections were put on X-ray film for autoradiography.

3. Results: At times 1 and 2 minutes the highest concentration of radioactivity was observed in the kidney and thyroid gland. Blood, too, showed a high level of radioactivity. Several other organs contained radioactivity, although slightly less. The spleen contained most radioactivity in the red pulp, the highest concentration found in the border between red and white pulp. No staining of lymph nodes was seen. At 5 minutes staining was also seen in organs mentioned above, and also some further, stomach content, bone marrow, cerebrospinal fluid and melanin of the eye. Urinary bladder and duct were highly labelled.

At 30 minutes labeling of stomach content was pronounced as well as the kidney cortex, and at 60 minutes the major labelled "spots" were thyroid gland, stomach and urinary bladder, although other organs were still labelled but to a lesser extent. No labeling of cerebrospinal fluid could be detected. At 300 minutes only weak autoradiograms were obtained, with highest radioactivity again found in thyroid, stomach and urinary bladder. Uptake of radioactivity in salivary glands and a specific structure in the nasal cavity was observed.

4. Conclusion: <sup>125</sup>I-labeled rhIGF-I was distributed throughout the body of adult male mice. Several tissues which had high labeling may reflect rich vascularization since blood contained high radioactivity levels. A high labeling in some other organs like kidney may reflect as receptor binding. The high labeling of thyroid probably reflects an accumulation of <sup>125</sup>I-iodine.

#### 2.6.6.9. Discussion and Conclusions

The sponsor did not perform Safety and Pharmacodynamic Drug Interaction studies. The repeat dose toxicity of rhIGF-I/rhIGFBP-3 has been studied in rats, and monkeys for up to 90 days. In rats, daily subcutaneous administration of rhIGF-I/rhIGFBP-3 at doses up to 30 mg/kg/day resulted in dose-dependent increases in body weights and an increase in the weight of the lymphoid tissues, particularly the spleen, thymus and lymph nodes. Similar results were obtained following 90 consecutive days of rhIGF-I/rhIGFBP-3 administration to monkeys at doses up to 10 mg/kg/day. Dual energy X-ray absorptiometry studies in monkeys showed an increase in lean body mass in all treated females and an increase in whole body bone mineral density in high dose males at the end of the treatment period. Repeat dose toxicity studies with free rhIGF-I have been conducted for up to 13 weeks in mice and up to 26 weeks in rats and monkeys. In general, results of these studies are similar to those observed in the repeat dose studies conducted with rhIGF-I/rhIGFBP-3.

In 13-Week toxicology studies in rats and monkeys, 10 mg/kg/day dose resulted in exposures 1 and 2 times, respectively, the maximum recommended human dose, based on body surface area comparison. No formal studies to assess the genotoxic, carcinogenic or reproductive and developmental toxicity of rhIGF-I/rhIGFBP-3 have been conducted. Based on the available nonclinical data generated with rhIGF-I/rhIGFBP-3 combined with the nonclinical toxicology studies with rhIGF-I, it is safe to say that the toxicology profiles of rhIGF-I alone and those of the rhIGF-I/rhIGFBP-3 binary complex are not drastically different. In conclusions, there were no remarkable preclinical pharmacology and toxicology concerns with IGF-I/IGFBP-3. Most observations were anticipated pharmacologic effects of IGF-I.

2.6.6.10 Tables and Figures: Please see the tables and figures that were presented under individual studies.

Unresolved toxicology issues (if any): None.

#### Final Recommendations:

Pharmacology and toxicology data support approval of this NDA for severe growth hormone insensitivity syndrome (GHIS). The submitted data were derived largely from studies with IGF-

1 alone rather than the IGF-1/IGFBP-3 binary complex. Teratology studies in rabbits indicates IGF-1 increased the postimplantation loss at a dose of 1.25 mg/kg/day. In this study NOAEL was 0.5 mg/kg/day, which gives an exposure ratio of 0.2X MRHD, based on body surface area comparison.

#### Suggested labeling:

##### Carcinogenesis, Mutagenesis, Impairment of Fertility:

Long term animal studies for the evaluation of carcinogenicity have not been performed with iPlex (rhIGF-1/rhIGFBP-3). The genotoxic potential of iPlex has not been assessed. rhIGF-1 tested negative for genotoxic potential in the Ames test and in chromosomal aberration assays conducted with human lymphocytes or rat peripheral lymphocytes. Animal fertility studies have not been performed with iPlex. Effects of rhIGF-1 on fertility and reproductive performance were assessed in male and female rats administered 0.4, 2 and 10 mg/kg/day, subcutaneously (0.4, 2 and 10 times clinical exposures with the MRHD based on body surface area). rhIGF-1 had no effects on mating, fertility, or reproductive performance in rats.

##### Pregnancy: Teratogenic Effects: Pregnancy Category C

Animal reproduction studies have not been conducted with iPlex™ [mecasermin rinfabate (rDNA origin) injection]. Effects of rhIGF-1 on embryofetal development were assessed in rats and rabbits.

Subcutaneous administration of 0.2, 0.5, or 1.25 mg/kg/day rhIGF-1 to pregnant rats during organogenesis had no effects on embryofetal development (0.2, 0.5, and 1.25 times therapeutic exposures with MRHD based on body surface area). Subcutaneous administration of 0.2, 0.5 or 1.25 mg/kg/day rhIGF-1 to rabbits during organogenesis resulted in an increased incidence of fetal loss but no fetal anomalies. Increased early resorptions were observed in rabbits treated with 1.25 mg/kg and increased preimplantation loss was observed (1.25 and 2.5 times exposures equivalent to 0.2 times MRHD based on body surface area).

A second rabbit embryofetal development study was conducted to determine the role of hypoglycemia in rhIGF-1 mediated fetal loss. Rabbits were administered subcutaneous doses of 0, 0.5, and 1.25 mg/kg/day rhIGF-1, 1.25 or 2.5 mg/kg rhIGF-1 plus glucose supplementation, or 2.5 IU/kg/day insulin. A comparable degree of hypoglycemia was observed in rabbits treated with 1.25 mg/kg rhIGF-1 alone or 2.5 IU/kg insulin. Animals treated with 0.5 mg/kg rhIGF-1 or rhIGF-1 plus glucose maintained normal glucose levels. Similar to the initial rabbit study, an increase in early fetal resorptions was observed in rabbits treated with 1.25 mg/kg/day rhIGF-1 (2 times MRHD based on body surface area). This finding was not observed in insulin-treated rabbits despite a comparable degree of drug-induced hypoglycemia. A dose-related increase in postimplantation loss was observed in all rhIGF-1 treated groups ( $\geq 0.5$  times MRHD based on body surface area). While the incidence of fetal loss was somewhat reduced in glucose

supplemented rabbits, it was not clearly attributable to drug-induced hypoglycemia since significant fetal loss was still observed in normoglycemic rhIGF-1 treated rabbits.

Nursing Mothers:

It is not known whether iPlex™ [mecasermin rinfabate (rDNA origin) injection] is excreted in human milk. Because many drugs are excreted in human milk,

Signatures (optional):

Reviewer Signature \_\_\_\_\_

Supervisor Signature \_\_\_\_\_ Concurrence Yes \_\_\_ No \_\_\_

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/s/

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Herman Rhee  
9/12/2005 10:02:11 AM  
PHARMACOLOGIST

Jeri El Hage  
9/12/2005 11:26:02 AM  
PHARMACOLOGIST



## Memorandum

**Date:** September 7, 2005

**From:** Jeri El Hage, Ph.D, Supervisory Pharmacologist, DMEP

**Subject:** NDA 21-884, Iplex, IGF-1: IGF BP-3

**To:** File, NDA 21-884

This NDA includes substantial preclinical toxicity data for IGF-1 alone but limited data for the to-be-marketed IGF-1: IGF BP-3 drug product as described below. The sponsor has been advised that the 13-week bridging toxicity studies with IGF-1:BP-3 are adequate to support approval for the orphan indication due to the limited patient population. However, if approval is sought for other indications additional toxicology studies including carcinogenicity evaluations of IGF-1:BP-3 may be recommended.

**Toxicology data for IGF-1 only.**

1. Genotoxicity – Ames assay, multiple chromosomal aberrations assays with human lymphocytes and rat peripheral lymphocytes were conducted , all with negative results.
2. Mitogenicity – studies to assess the effects of IGF-1 on growth of Lewis lung carcinoma or HT-29 human colon carcinoma implanted in mice were performed. IGF-1 had no effects on tumor growth or metastases.
3. General toxicity – 13-week subcutaneous (SC) toxicity study in mice and 26-week sc toxicity studies in rats and monkeys were conducted. The anticipated pharmacological effects of dose-related hypoglycemia, weight gain, and bone growth were observed. In addition, lymphoid hyperplasia was observed in rats and monkeys.
4. Reproductive toxicity – fertility and peri/postnatal development study in rats and embryofetal development studies in rats and rabbits were conducted. No effects on fertility, reproductive performance, or embryofetal development were observed in rats. IGF-1 increased early resorptions and postimplantation loss in rabbits. These effects were not clearly secondary to drug-induced hypoglycemia since they occurred in rabbits supplemented with glucose to reverse hypoglycemia and the incidence was greater in IGF-1 treated rabbits ( with and without glucose supplementation) than the incidence in insulin treated rabbits displaying comparable decreases in blood glucose levels .

**Toxicology data for IGF-1 : IGF BP-3 complex ( to be marketed product)**

Thirteen week bridging toxicology studies with the IGF-1: BP 3 product were conducted in rats and monkeys. The toxicity profiles of the IGF-1: IGF binding protein 3 complex was not qualitatively different from IGF -1 alone. The addition of the binding protein significantly increased plasma half-life ( from 15 minutes to > 12 hours). In addition, the hypoglycemic effects of high doses of IGF-1 were minimized in animals treated with the complexed IGF-1 compared to IGF-1 alone, probably due to lower concentrations of free IGF-1 after administration of the complex. The most significant difference between the IGF-1 alone and IGF-1:BP-3 were the antibody responses observed in both species.

In the 6-month sc rat toxicity study with IGF-1 alone , a single mid-dose rat (n = 1/150) displayed a minimal antibody response, while all rats treated with IGF-1:BP 3 for 3 months (n = 60/60) displayed significant antibody titers (non- neutralizing). Similar results were obtained in monkeys. Namely, one mid-dose monkey (1/34) treated with IGF-1 alone for 6 months displayed a positive antibody response, while all IGF-1:BP-3 treated monkeys ( n = 24/24) displayed positive antibody titers after 3 months of

treatment. Antibody titers were modestly decreased in both rats and monkeys after a 28-day drug free recovery period. The sponsor states that the antibodies appeared to be generated to the IGF-1 :BP 3 portion of the molecule. The antibodies in monkeys were also non-neutralizing ( as measured by IGF-1 activity in an *in vitro* mitogenicity assay).

Potential differences in the immunogenicity of IGF-1 alone vs the IGF-1 :BP 3 complex should be carefully assessed clinically. An immunogenic response to the recombinant IGF-1 binding protein 3 may occur in patients since the recombinant protein produced in E. coli is not glycosylated like the endogenous binding protein.

Suggested Labeling Revisions:

### **Carcinogenesis, Mutagenesis, Impairment of Fertility**

Long term animal studies for the evaluation of carcinogenicity have not been performed with iPlex (rhIGF-1/rhIGFBP-3). The genotoxic potential of iPlex has not been assessed. rhIGF-1 tested negative for genotoxic potential in the Ames test and in chromosomal aberration assays conducted with human lymphocytes or rat peripheral lymphocytes.

Animal fertility studies have not been performed with iPlex. Effects of rhIGF-1 on fertility and reproductive performance were assessed in male and female rats administered 0.4, 2 and 10 mg/kg/day, subcutaneously (0.2, 1 and 7 times clinical exposures with the MRHD based on body surface area). rhIGF-1 had no effects on mating, fertility, or reproductive performance in rats.

### **Pregnancy – Pregnancy Category C**

Animal reproduction studies have not been conducted with iPlex™ [mecasermin rinfabate (rDNA origin) injection]. Effects of rhIGF-1 on embryofetal development were assessed in rats and rabbits.

Subcutaneous administration of 0.2, 1, or 2 mg/kg/day rhIGF-1 to pregnant rats during organogenesis had no effects on embryofetal development (0.5, 1.5, and 4 times therapeutic exposures with MRHD based on body surface area).

Subcutaneous administration of 0.2, 0.5 or 1.25 mg/kg/day rhIGF-1 to rabbits during organogenesis resulted an increased incidence of fetal loss but no fetal anomalies. Increased early resorptions were observed in rabbits treated with 0.2 mg/kg and increased preimplantation loss was observed in all rhIGF-1 treated groups (exposures equivalent to 0.2, 0.5, and 1.25 times MRHD based on body surface area, respectively).

A second rabbit embryofetal development study was conducted to determine the role of hypoglycemia in rhIGF-1 mediated fetal loss. Rabbits were administered subcutaneous doses of 0, 0.5, and 1.25 mg/kg/day rhIGF-1, 1.25 or 2.5 mg/kg rhIGF-1 plus glucose supplementation, or 2.5 IU/kg/day insulin. A comparable degree of hypoglycemia was observed in rabbits treated with 1.25 mg/kg rhIGF-1 alone or 2.5 IU/kg insulin. Animals treated with 0.5 mg/kg rhIGF-1 or rhIGF-1 plus glucose maintained normal glucose levels.

Similar to the initial rabbit study, an increase in early fetal resorptions was observed in rabbits treated with 1.25 mg/kg/day rhIGF-1 (2 times MRHD based on body surface area). This finding was not observed in insulin-treated rabbits despite a comparable degree of drug-induced hypoglycemia. A dose-related increase in post-implantation loss was observed in all rhIGF-1 treated groups ( $\geq 0.5$  times MRHD based on body surface area). While the incidence of fetal loss was somewhat reduced in glucose supplemented rabbits, it was not clearly attributable to drug-induced hypoglycemia since significant fetal loss was still observed in normoglycemic rhIGF-1 treated rabbits.

Recommendation: Approval.

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/s/

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Jeri El Hage  
9/7/2005 05:23:19 PM  
PHARMACOLOGIST  
For inclusion in action package.

## NDA Filing Meeting Checklist

NDA # 21-883

DRUG: Mecasermin rinfabate(rhIGF-1/rhIGFBP-3)

Sponsor: Inmed, Inc.

### NONCLINICAL PHARMACOLOGY/TOXICOLOGY

ITEM	YES	NO	COMMENT
1) Does this section of the NDA appear to be organized (according to 21 CFR 314 and current guidelines for format and content) in a manner that would allow a substantive review to be completed?	X		All studies are in good order because most of studies were performed by — and Celtrix.
2) Is this section of the NDA indexed and paginated in a manner to enable a timely and substantive review?	X		
3) Is this section of the NDA sufficiently legible so that a substantive review can be done? Has the data been presented in an appropriate manner (consider tables, graphs, complete study reports, inclusion of individual animal data, appropriate data analysis, etc.)?	X		
4) Are all necessary and appropriate studies for this agent, including special studies/data requested by the Division during pre-submission communications/discussions, completed and submitted in this NDA?  (Please itemize the critical studies included and indicate any significant studies that were omitted from the NDA - e.g., safety pharm, genotox, reprotox, chronic tox, carcinogenicity)	X		Safety pharmacology; Pharmacokinetics; Immunogenicity; 2-week iv toxicity rat and monkey, 3-month sc rat and monkey toxicology studies with IGF 1:BP3; 6 month sc rat and monkey studies with IGF-1 only; Genotoxicity; Secondary carcinogenicity (lung metastases and human colon carcinoma implanted in nude mice); <b>No carcinogenicity studies</b> Seg I, II , III reprotoxicity and local tolerance studies in SD rats and pigs.

ITEM	YES	NO	COMMENT
5) Were the studies adequately designed (ie., appropriate number of animals, adequate monitoring consistent with the proposed clinical use, state-of-the art protocols, etc.)?	X		
6) If the formulation to be marketed is not identical to the formulation used in the toxicology studies (including the impurity profiles), has the sponsor clearly defined the differences and submitted reviewable supportive data (ie., adequate repeat studies using the marketed product and/or adequate justification for why such repetition would not be necessary)?	x		
7) Does the route of administration used in animal studies appear to be the same as the intended human exposure route? If not, has the sponsor submitted supportive data and/or an adequate scientific rationale to justify the alternative route?	X		
8) Has the proposed draft labeling been submitted? Are the appropriate sections for the product included and generally in accordance with 21 CFR 201.577? Is information available to express human dose multiples in either mg/m <sup>2</sup> or comparative serum/plasma AUC levels?	X		

ITEM	YES	NO	COMMENT
9) From a pharmacology/toxicology perspective, is this NDA fileable? If not, please state in item # 10 below why it is not.	X		
10) Reasons for refusal to file:			

Herman Rhee, Ph.D.

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Reviewing Pharmacologist

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Supervisory Pharmacologist

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Herman Rhee  
2/25/05 11:34:31 AM  
PHARMACOLOGIST

Jeri El Hage  
2/25/05 04:04:04 PM  
PHARMACOLOGIST