CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

BLA 125103/0

Pharmacology Review(s)

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:	**STN# 125103**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**0/0/04**
DRUG NAME:	**Palifermin**
INDICATION:	[redacted]
SPONSOR:	**Amgen**
DOCUMENTS REVIEWED:	**Toxicology**
REVIEW DIVISION:	**Division of Therapeutic Biological Internal Medicine Products (HFD-108)**
PHARM/TOX REVIEWER:	**Barbara J. Wilcox, Ph.D.**
PHARM/TOX SUPERVISOR:	**Martin D. Green, Ph.D.**
DIVISION DIRECTOR:	**Patricia Keegan, M.D.**
PROJECT MANAGER:	**Susan Giuliani**

Date of review submission to Division File System (DFS):

TABLE OF CONTENTS

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

The Biologics License Application STN# 125103 is approvable for the proposed indication based on the data contained in the non-clinical toxicology sections of the original submission. The toxicities of palifermin are primarily extensions of the pharmacologic activity of the product. The clinical treatment duration recommended in this application is limited and the patient population can be selected and monitored to avoid unreasonable risk.

B. Recommendation for nonclinical studies

Further non-clinical studies are recommended to address the potential for palifermin to promoted tumor progression.

C. Recommendations on labeling

Modifications to the labeling for palifermin are recommended. The statements that the maternal toxicity data '[ ] should be eliminated. The negative effects on the fetus were observed and whether those effects were due to maternal toxicity or direct effects of the drug on the fetus was not addressed in those studies. In addition, the statement [ ] should be eliminated. The absence of detectable palifermin in fetal blood may be a result of short half life or other factors not assessed in the study. Pregnancy Category C is recommended.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

This document contains only reviews of the non-clinical toxicology sections including single-dose toxicology, repeat-dose toxicology, genotoxicity, carcinogenicity and reproductive toxicology. The pharmacology, pharmacodynamics and pharmacokinetics and safety pharmacology were reviewed by Dr. Anita O'Connor in a separate document.

Non-clinical single-and repeat-dose toxicology studies were performed to evaluate the potential toxic effects of palifermin in a variety of animal models. The species studied included athymic nude mice, rats, rhesus and cynomolgus monkeys. Single doses were administered up to 30,000 ug/kg in rats and 50,000 ug/kg in monkeys. Repeated doses of up to 1000 ug/kg daily for up to 28 days were administered to rats and doses of 300 ug/kg daily for up to 28 days were administered to monkeys. The toxic effects observed were primarily extensions of the known pharmacologic activity of palifermin and included goblet cell hyperplasia, acanthosis and hyperkeratosis of the skin of various body regions and tongue in all species as well as involution of the thymus characterized as lymphoid depletion. Rats showed what appeared to be a higher sensitivity to the palifermin exposure with glomerulonephritis (rated mild to moderate),

centrilobular apoptosis of the liver and increased thyroid follicular size and number. In rats, doses of up to 1000 ug/kg, IV or SC, were administered daily for up to 28 days. Dose dependent effects included acanthosis of the skin, hyperplastic and hypertrophic changes in the GI tract epithelium and urinary bladder. Goblet cell hyperplasia was observed in all regions of the GI tract in the higher dose groups. Effects of the study drug on the kidney included mild to moderate increases in organ weight, mild to moderate glomerulonephritis and glomerulosclerosis accompanied by protein casts and thickening of the mesangial matrix at doses greater than 300 ug/kg.

Also observed in a dose dependent pattern was thymus gland involution and lymphoid depletion. Liver enlargement was also observed for the higher dose groups with increases in cholesterol, triglycerides, albumin, globulin and total protein. Microscopic changes of centrilobular necrosis was observed in the high dose group. Study drug effects were also seen in the thyroid of rats. Increases in the size and number of follicles were reported accompanied by periglandular fibrosis in some animals at doses 100 ug/kg.

The effects on the liver and kidney remained apparent, though at a lesser incidence and severity, at the end of the recovery period. The effects on the thyroid were observed with increased incidence after the recovery period.

In monkeys receiving doses of palifermin up to 300 ug/kg either SC or IV daily for 28 days, study drug related findings included acanthosis and hyperkeratosis of the skin (scalp, mammary area, gluteal region), hyperplasia of the mucosa of the tongue and esophagus, hyperplasia of goblet cells in all regions of the GI tract. One high dose monkey was sacrificed moribund. This animal showed significant weight loss and severe reduction in food consumption. Slight weight loss was noted for the surviving high dose mid-study but returned to amounts similar to control by the end of dosing. A decrease in mean RBC counts, hematocrit, and hemoglobin were noted at doses of $\leq$ 100 µg/kg/day. Slight decreases in hematocrit and hemoglobin were noted in females treated with 30 µg/kg/day. No treatment-related changes in hematology parameters were noted in groups treated with $\leq$ 10 µg/kg/day. The observed hematology changes were not apparent at the end of recovery.

Increased organ weight of the submandibular gland accompanied by elevation of serum amylase and hypertrophy of the acinar cells (rated slight) was noted for the high dose group. Involution of the thymus was also noted and did not resolve after the recovery period. These findings are thought to be primarily due to the pharmacological activity of the study drug and appeared to be largely reversible in the monkeys.

The toxicokinetic analysis showed that serum concentration time profiles of palifermin displayed an initial rapid decline followed by a plateau between 1 and 3 hours post-dose. The incidence of anti-drug antibodies increased with dose and dosing duration and appeared to increase palifermin serum concentration.

Studies to assess the potential for palifermin to induce genetic abnormalities were performed. The following assays were conducted: microchromosome reverse mutation assay, Salmonella/Escherichia coli Mutagenicity Assay, CHO/HGPRT Mutation Assay, Micronucleus genetic assay in mice. No genotoxic effects of palifermin were observed under the conditions of these studies.

Studies to evaluate the potential for palifermin to promote tumor growth were conducted *in vitro* and *in vivo*. A series of human tumor cell lines were studied to determine the presence and relative levels of the KGF receptor expression. In addition, xenograft models of tumor promotion were studied with human tumor cells implanted subcutaneously followed by treatment with palifermin or vehicle. The interpretation of the data are subject to debate. One tumor type ([redacted]) showed a statistically significant growth enhancement in a dose dependent manner when exposed to increasing doses of palifermin. The results also showed growth of one additional tumor cell line [redacted] to be enhanced by palifermin treatment. However, the sponsor states that this study did not show statistical significance. No toxicokinetics were performed to confirm exposure. Palifermin at higher doses appeared to inhibit growth of some cell lines.

A series of reproductive toxicology studies were performed in rats and rabbits to support this application covering segments one and two only. To determine potential effect of palifermin exposure on fertility parameters, doses of up to 1000 ug/kg/day were administered IV to male rats for two weeks prior to mating. For female fertility assessment, doses of up to 1000 ug/kg/day were administered IV for one week prior to mating and continued through gestational day 7. The results indicate that palifermin exposure at the high dose levels (1000 ug/kg/day) can have an adverse effect on fertility parameters including fertility reduction of pregnancy rate (the number of pregnant rats/number of rats in cohabitation). Female rats treated with 1000 µg/kg/day rHuKGF also had significant reductions in the litter averages for corpora lutea and implantations. An increase in embryonic deaths was noted at doses ≥ 300 ug/kg/day. For male rats, reductions in sperm counts were noted for rats receiving doses ≥ 300 ug/kg/day. Dose dependent microscopic changes related to male fertility included hyperplasia of the tubular epithelium in 100% of rats in the high dose group, necrotic germ cells and/or hypospermia in the epididymal tubules and signs of reduced secretory activity in the seminal vesicles and prostate

To address potential effects on embryo/fetal development, palifermin was administered IV during gestation in doses of up to 1000 ug/kg/day in rats and up to 500 ug/kg/day in rabbits. Dose dependent reduction in body weight gain was observed in rats with doses greater than 300 ug/kg/day and for in rabbits with doses ≥ 150 ug/kg/day. These dose groups also showed increased resorption of the conceptuses in these dose groups. In the rabbit study, increased postimplantation loss and a corresponding decrease in viable litter size were also noted in the 150 µg/kg/day group. Intrauterine growth and survival were unaffected by treatment with doses ≤ 50 µg/kg/day.

In rats, the 300 and 1000 ug/kg/day doses of the test article were associated with increases in embryo deaths evident as significant increases in nonviable embryos and percent nonviable embryos, significant reductions in the litter averages for viable embryos and litter sizes (the sum of viable and nonviable embryos) and increases in the number of dams with nonviable embryos.

In animals receiving 1000 µg/kg/day, mean fetal body weight was reduced and postimplantation loss was increased. No effects on embryo/fetal development were observed at doses up to 300 µg/kg/day in rats. For rabbits, no effects on reproductive parameters were noted at doses of 60 ug/kg/day or lower.

Toxicokinetic evaluation of palifermin in pregnant rats was performed. Doses of up to 1000 ug/kg IV were administered. Negligible amounts of palifermin were detected in fetal serum or amniotic fluid.

B. Pharmacologic activity

Keratinocyte growth factor is an endogenously secreted protein that binds to the keratinocyte growth factor receptor (FGFR2IIIb, a splice variant of the FGFR2 receptor). Binding of KGF to its receptor results in proliferation, differentiation, and upregulation of cytoprotective mechanisms (e.g, induction of antioxidant enzymes). The endogenous KGF is produced by mesenchymal cells and is upregulated in response to epithelial tissue injury.

In animal models, KGF receptor binding results in enhanced proliferation of epithelial cells in many tissues, including the tongue, buccal mucosa, and gastrointestinal tract. Of significance to this BLA, KGF can cause hyperplasia of the goblet cell population in the GI epithelium providing increased mucous secretion.

C. Nonclinical safety issues relevant to clinical use

In rats treated with palifermin at high doses daily for 28 days, findings were noted in liver, kidney and thyroid. These findings did not resolve completely after the recovery period. The thyroid findings actually increased after the recovery period. These toxicities were discussed with the clinical reviewer and no clinical correlations were noted. The dose levels and dosing duration used that resulted in the toxicities are significantly greater than the expected human exposure. Similar toxicities were not observed in monkeys receiving up to 300 ug/kg/day for 28-days.

There is some concern that exposure to palifermin may promote growth of non-hematologic tumors. This concern will be addressed in tumor promotion studies to be performed as part of a post-marketing commitment.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: BLA STN No. 125103
Review number:
Sequence number/date/type of submission:
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: Amgen, Inc.
Manufacturer for drug substance: Amgen, Inc.
Thousand Oaks, CA

Reviewer name: Barbara J. Wilcox, Ph.D.
Division name: Division of Biologic Internal Medicine Products
HFD #: 108
Review completion date: 12/15/04

Relevant INDs/NDAs/DMFs:
Drug:

Trade name: unknown
Generic name: palifermin
Code name:
Chemical name: recombinant human keratinocyte growth factor
CAS registry number:
Molecular formula/molecular weight: 16277.0 daltons

1 Page(s) Withheld

✓ § 552(b)(4) Trade Secret / Confidential

_____ § 552(b)(5) Deliberative Process

_____ § 552(b)(5) Draft Labeling

Indication:

[ ]

Clinical formulation:
Each vial of 6.25 mg palifermin is formulated with 50 mg mannitol, 25 mg sucrose, 1.94 mg L-histidine and 0.13 mg polysobate 20. When reconstituted with sterile water for injection, the final concentration of palifermin is 5 mg/ml at pH 6.5.

Route of administration:
Intravenous bolus injection.

Proposed use:

[ ]

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

Studies reviewed within this submission: Toxicology studies

Studies not reviewed within this submission: Pharmacology and pharmacodynamics studies are reviewed by Anita O'Connor, P.h.D.

2.6.2 PHARMACOLOGY

Not reviewed in this document. Pharmacology, pharmacodynamics, pharmacokinetics and safety pharmacology sections were reviewed by Dr. Anita O'Conner in a separate document.

2.6.2.1 Brief summary

2.6.2.2 Primary pharmacodynamics

Not reviewed in this document.

2.6.2.3 Secondary pharmacodynamics

Not reviewed in this document.

2.6.2.4 Safety pharmacology

2.6.2.5 Pharmacodynamic drug interactions

Not reviewed in this document.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Not included in this document. See review by Dr. Anita O'Connor

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

Not included in this document. Se review by Dr. Anita O'Connor

2.6.4.1 Brief summary

2.6.4.2 Methods of Analysis

2.6.4.3 Absorption

2.6.4.4 Distribution

2.6.4.5 Metabolism

2.6.4.6 Excretion

2.6.4.7 Pharmacokinetic drug interactions

2.6.4.8 Other Pharmacokinetic Studies

2.6.4.9 Discussion and Conclusions

2.6.4.10 Tables and figures to include comparative TK summary

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:
Palifermin was tested in single-dose and repeat dose studies in a variety of animal models including rats, athymic nude mice and rhesus monkeys.

In single-dose studies in rats and monkeys, doses up to 30,000 and 50,000 ug/kg were administered, respectively. Drug related effects included loss of apetite, weightloss, skin flushing, mucous cell hyperplasia in the GI tract, acanthosis of the tongue and involution of the thymus (monkeys). In monkeys, 50,000 ug/kg was lethal.

Repeated dose studies were performed in athymic nude mice, rats, rhesus and monkeys. The rat appeared to be a more sensitive model for palifermin induced toxicities than the monkey, although clear dose-related effects were seen in all specied tested.

In rats, doses of up to 1000 ug/kg, IV or SC, were administered daily for up to 28 days. Dose dependent effects included acanthosis of the skin, hyperplastic and hypertrophic changes in the GI tract epithelium and urinary bladder. Goblet cell hyperplasia was observed in all regions of the GI tract in the higher dose groups. Effects of the study drug on the kidney included mild to moderate increases in organ weight, mild to moderate glomerulonephritis and glomerulosclerosis accompanied by protein casts and thickening of the mesangial matrix.

In rats treated daily IV for 7 days both IV and SC, elevations of serum lipase, serum amylase, C-reactive protein levels were also noted after doses of 1000 ug/kg. When administered IV to rats daily for 28 days, multiple toxic effects were noted. Clinical pathology assessments revealed increases in total protein, globulin, albumin, calcium, cholesterol, and triglycerides at all doses (the lowest dose used was 30 ug/kg). Reduced RBC counts, decreased hematocrit and decreased hemoglobin were noted in rats that received 300 ug/kg or greater and increased reticulocytes and platelet counts were noted in rats receiving 100 ug/kg. These observations were made at day 15 of 28 day treatment and had returned to control levels by day 29.

Histopathological findings included centrolobular apoptosis of the liver (at 1000 ug/kg), lymphoid depletion of the liver (300 ug/kg or greater), increased size and number of thyroid follicles (300 ug/kg or greater). Hyperplastic/hypertrophic changes were observed in all regions of the GI tract after doses of 300 ug/kg or greater. Theses effects were observed at much lower severity or not observed after the 28-day recovery period. The histopathologic effects observed for the liver, thymus and thyroid remained apparent, although with lower severity for the liver, at the end of the recovery period.

Also observed in a dose dependent pattern was thymus gland involution and lymphoid depletion. Liver enlargement was also observed for the higher dose groups with increases in cholesterol, triglycerides, albumin, globulin and total protein. Microscopic changes of centrilobular necrosis was observed in the high dose group. Study drug effects were also seen in the thyroid of rats. Increases in the size and number of follicles were reported accompanied by periglandular fibrosis in some animals.

The effects on the liver and kidney were still observed, though at a lesser incidence and severity, at the end of the recovery period. The thyroid findings did not resolve during the recovery period, however, and appeared to increase in incidence.

In monkeys receiving doses of palifermin up to 300 ug/kg either SC or IV daily for 28 days, study drug related findings included acanthosis and hyperkeratosis of the skin (scalp, mammary area, gluteal region), hyperplasia of the mucosa of the tongue and esophagus, hyperplasia of goblet cells in all regions of the GI tract. Slight weight loss was noted for the high dose mid-study (weeks 2 and 3) but returned to amounts similar to control by the end of dosing. A decrease in mean RBC counts, hematocrit, and hemoglobin were noted at doses of $\geq$ 100 µg/kg/day. Slight decreases in hematocrit and hemoglobin were noted in females treated with 30 µg/kg/day. No treatment-related changes in hematology parameters were noted in groups treated with $\leq$ 10 µg/kg/day. The observed hematology changes were not apparent at the end of recovery. Clinical pathological findings included decreased total cholesterol and calcium, increased alpha-1 globulin, and reduced total protein and albumin. Phosphate was also decreased at doses $\geq$100 µg/kg/day during week 4. Amylase was increased, relative to controls, at doses $\geq$ 30 µg/kg/day. The increased amylase associated with increased submandibular gland weights and submandibular gland acinar cell hypertrophy. There were no alterations in serum chemistry parameters at doses $\leq$ 10 µg/kg/day.

Involution of the thymus with lymphoid depletion was noted in a dose dependent pattern. Increased organ weight of the submandibular gland accompanied by elevation of serum amylase and hypertrophy of the acinar cells (rated slight) was noted for the high

dose group. Most of these changes are thought to be due to the pharmacological activity of the study drug and appeared to be reversible.

The toxicokinetic analysis showed that serum concentration time profiles of palifermin displayed an initial rapid decline followed by a plateau between 1 and 3 hours post-dose. The incidence of anti-drug antibodies increased with dose and dosing duration and appeared to increase palifermin serum concentration.

Genetic toxicology:

Studies to assess the potential for palifermin to induce genetic abnormalities were performed. The following assays were conducted: microchromosome reverse mutation assay, Salmonella/Escherichia coli Mutagenicity Assay, CHO/HGPRT Mutation Assay, Micronucleus genetic assay in mice. No genotoxic effects of palifermin were observed under the conditions of these studies.

Carcinogenicity:

Studies to evaluate the potential for palifermin to promote tumor growth were conducted *in vitro* and *in vivo*. A series of human tumor cell lines were studied to determine the presence and relative levels of the KGF receptor expression. In addition, xenograft models of tumor promotion were studied with human tumor cells implanted subcutaneously followed by treatment with palifermin or vehicle. The interpretation of the data are subject to debate. One tumor type [ ], showed a statistically significant growth enhancement in a dose dependent manner when exposed to increasing doses of palifermin. The results also showed growth of one additional tumor cell line [ ] to be enhanced by palifermin treatment. However, the sponsor states that this study did not show statistical significance. No toxicokinetics were performed to confirm exposure. Palifermin at higher doses appeared to inhibit growth of some cell lines.

Reproductive toxicology:

A series of reproductive toxicology studies were performed in rats and rabbits to support this application covering segments one and two only. To determine potential effect of palifermin exposure on fertility parameters, doses of up to 1000 ug/kg/day were administered IV to male rats for two weeks prior to mating. For female fertility assessment, doses of up to 1000 ug/kg/day were administered IV for one week prior to mating and continued through gestational day 7. The results indicate that palifermin exposure at the high dose levels (1000 ug/kg/day) can have an adverse effect on fertility parameters including fertility reduction of pregnancy rate (the number of pregnant rats/number of rats in cohabitation). Female rats treated with 1000 µg/kg/day rHuKGF also had significant reductions in the litter averages for corpora lutea and implantations. An increase in embryonic deaths was noted at doses ≥ 300 ug/kg/day. For male rats, reductions in sperm counts were noted for rats receiving doses ≥ 300 ug/kg/day. Dose dependent microscopic changes related to male fertility included hyperplasia of the tubular epithelium in 100% of rats in the high dose group, necrotic germ cells and/or hypospermia in the epididymal tubules and signs of reduced secretory activity in the seminal vesicles and prostate

To address potential effects on embryo/fetal development, palifermin was administered IV during gestation in doses of up to 1000 ug/kg/day in rats and up to 500 ug/kg/day in rabbits. Dose dependent reduction in body weight gain was observed in rats with doses greater than 300 ug/kg/day and for in rabbits with doses ≥ 150 ug/kg/day. These dose groups also showed increased resorption of the conceptuses in these dose groups. In the rabbit study, increased postimplantation loss and a corresponding decrease in viable litter size were also noted in the 150 µg/kg/day group. Intrauterine growth and survival were unaffected by treatment with doses ≤ 50 µg/kg/day.

In rats, the 300 and 1000 ug/kg/day doses of the test article were associated with increases in embryo deaths evident as significant increases in nonviable embryos and percent nonviable embryos, significant reductions in the litter averages for viable embryos and litter sizes (the sum of viable and nonviable embryos) and increases in the number of dams with nonviable embryos.

In rats receiving 1000 µg/kg/day, mean fetal body weight was reduced and postimplantation loss was increased. No effects on embryo/fetal development were observed at doses up to 300 µg/kg/day in rats. For rabbits, no effects on reproductive parameters were noted at doses of 60 ug/kg/day or lower.

Toxicokinetic evaluation of palifermin in pregnant rats was performed. Doses of up to 1000 ug/kg IV were administered. Negligible amounts of palifermin were detected in fetal serum or amniotic fluid.

Special toxicology:
The antigenicity of palifermin was investigated using guinea pigs, mice and rats. Palifermin was administered alone and with adjuvant under a variety of dosing conditions. The results indicate that palifermin can produce anaphylactic-like reactions in guinea pigs, mice and weakly in rats.

2.6.6.2 Single-dose toxicity

Study title: Acute intravenous toxicity of rHuKGF in rats

Key study findings:
The purpose of this study was to investigate the range of toxicity of rHuKGF in male and female Sprague-Dawley rats following a single IV dose. Eight groups of rats were given a single IV dose of 0, 100, 1000, 10,000 or 30,000 ug/kg on day one and sacrificed on day 15. No in-life parameters showed a clear effect of study drug administration. A dose-related increase in serum globulin at day 15 for females in the 5,000, 10,000 and 30,000 groups but was not reported for male rats in the high dose group. Only the thymus gland was examined histologically. The glands removed showed a test article related increase in size but the microscopic examination revealed no remarkable pathology.

Study no.: Amgen # T-95-KGF-012; — M077-95
Volume #, and page #:
Conducting laboratory and location: [ ]

[redacted]

Date of study initiation: 12/01/95
GLP compliance: YES
QA report: yes (X) no ( )
Drug, lot #, and % purity: rHuKGF lot # 01195B5C, diluent lot # 02095B5
Methods

Doses: 0, 100, 300, 1000, 5000, 10000, 30000 µg/kg
Species/strain: Sprague-Dawley rats
Number/sex/group or time point (main study): 5/sex/group
Route, formulation, volume, and infusion rate: 2 ml/kg, IV injection via tail vein
Satellite groups used for toxicokinetics or recovery:
Age: 6-7 weeks
Weight (nonrodents only):
Unique study design or methodology (if any): Rats were injected with test article on study day 1 and sacrificed on study day 15.

The experimental design is summarized in the following figure provided by the sponsor:

Dose Levels and Number of Animals:

Group	Dose level (µg/kg)	Dose Vol.	No. of Animals Male	Female
1	KGF Placebo	2 ml/kg	5	5
2	100	2 ml/kg	5	5
3	300	2 ml/kg	5	5
4	1,000	2 ml/kg	5	5
5	5,000	2 ml/kg	5	5
6	10,000	2 ml/kg	5	5
7*	30,000	6 ml/kg	5	5
8*	KGF Placebo	6 ml/kg	5	5

* Groups 7 and 8 were added by amendment; these animals were received at [redacted] in a separate shipment and treated at a later date than the first set of rats.

Observation times and results

Mortality: Monitored twice daily on weekdays and once daily on weekends or holidays. No animal deaths occurred during this study.

Clinical signs: Monitored 1hr, 2hr and 4 hr post dose and daily thereafter. No significant clinical signs attributed to test article administration were reported.

Body weights: Body weights recorded at randomization, pre-dosing on day 1, day 8 and pre-necropsy on day 15. No statistically significant differences in body weight gain were

noted for any group. An apparent trend to a slight decrease in weight gain is noted for the high dose group, both males and females.

Food consumption: Food consumption was apparently not recorded.

Ophthalmoscopy: Not done.

EKG: Not done.

Hematology: The following parameters were analyzed for hematology:

- RBC count (RBC): Statistically significant increase in RBC and HGB is noted for group 6 males but was not present for the higher dose (group 7) males. Although some measures of corpuscular volume and hemoglobin content appeared reduced for female rats, there appears to be an increase noted for RBC measures and hematocrit for groups 3, 4 and 5 females.
- Hematocrit (HCT): Increase in HCT is reported for 3, 4 and 5 females.
- Hemoglobin (HGB): No differences from control are reported for any group
- Mean Corpuscular Volume (MCV):
- Mean Corpuscular Hemoglobin (MCH) MHC was reduced in group 4 and 5 males but was not present for groups 6 and 7.
- Mean Corpuscular Hemoglobin: Reductions in hemoglobin measures are reported for females in groups 3, 4, 5 and 6 but not group 7 compared to the respective controls.
- Concentration (MCHC) AN elevation was noted for group 2 males compared to control but reduction was observed in group 7 males. Although statistically significant, the differences are small (less than 3%).
- WBC count (WBC): a slight reduction is noted for groups 6 and 7 males but did not reach statistical significance.
- WBC differential counts: Differentials showed large variations but did not appear to be study drug related.
- Platelet count (PLC):
- Reticulocyte count (RET): An increase in reticulocytes counts is reported for groups 4 and 7 compared to their respective control groups.

In general, several differences from controls in hematologic measures are noted. They appear to be sporadic and inconsistent across dose groups. None appear to be consistent with an effect of the test article.

Clinical chemistry: Blood was collected via retro-orbital sinus or cardiac puncture. Samples were collected at sacrifice on day 15. Parameters examined are as follows:

- Alanine aminotransferase (ALT)
- Albumin (ALB)
- Alkaline phosphatase (ALP)
- Aspartate aminotransferase (AST)

- Bilirubin [total (TBI), direct (DBI), and indirect (IBI)]: Bilirubin measures appear to be reduced in all treated groups but the highest dose. However, all values were consistently near zero so biological significance is unclear.
- Blood urea nitrogen (BUN)
- Calcium (CAL)
- Chloride (U-IL)
- Cholesterol (CHO)
- Creatinine (WE)
- Glucose (GLU)
- Phosphorus (PHO)
- Potassium (POT)
- Protein, total (TPR)
- Sodium (SOD)
- Triglycerides (TRI)
- Albumin/globulin ratio (AGR)

No other differences from control are reported for clinical chemistry measures. None of the differences reported appear to indicate an effect attributable to test article administration.

Urinalysis: Not done.

Gross pathology: All body surfaces and orifices and organs were examined at necropsy. Gross lesions were retained for further examination. Tissue retained from this study was limited to thymus from the 30,000 ug/kg group and respective control group.

Organ weights (specify organs weighed if not in histopath table): Only the thymus from control and 30,000 ug/kg group was weighed. Thymus from the treated group averaged 52%-56% more than controls. The organs were visibly larger then the control organs.

Histopathology: Adequate Battery: yes ( ), no (X)—explain
Peer review: yes ( ), no ( )
Only the thymus from the 30,000 ug/kg group and control was retained for histological examination. The protocol does not provide an explanation of why the study was designed this way. Although the thymus glands from the treated group were visibly larger that control and weighed over 50% more, the histology appeared to be no different than normal.

Toxicokinetics: Not Done.

Study title: Acute subcutaneous toxicity of rHuKGF in rats.

Key study findings:
The purpose of this study was to evaluate the toxicity of rHuKGF following a single, subcutaneous injection. Both male and female rats were tested with 6 different doses

ranging from 100 ug/kg to 30,000 ug/kg. Some trends in hematology and clinical chemistry were observed but values did not fall outside normal limits for this species. (Dose related decrease in RBC up to 14% in females from dose groups higher than 300 ug/kg accompanied by reductions in other hemoglobin measures and increases in reticulocytes.

Rats were sacrificed 14 days after dosing. The thymus glands from the 30000 ug/kg group were visibly enlarged. The only tissue examined histologically was thymus of the high dose group. No remarkable findings are reported for the histopathology evaluation of the thymus gland.

Study no.: — # M078-95/ Amgen # T-95KGF-013
Volume #, and page #:
Conducting laboratory and location: [ ] [ ]

Date of study initiation: 12/01/95
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: KGF lot # 01195BC, Placebo lot # 04105D5
Concentrations of rHuKGF in the 100, 300, 1000, 5000, 10,000, and 30,000 ug/kg dose formulations were [ ] of the target concentrations.
No detectable rHuKGF was reported for the placebo dose formulation.
Note that the 300 ug group received less than half the intended dose.
Methods

Doses: 100, 300, 1000, 5000, 10,000, and 30,000 ug/kg
Species/strain: Sprague-Dawley rats, male and female
Number/sex/group or time point (main study): 5/sex/group
Route, formulation, volume, and infusion rate: Single subcutaneous dose on day 1 in a volume of 2 ml/kg for groups 1-6 and 6 ml/kg for group 7.
Satellite groups used for toxicokinetics or recovery:
Age: 6-7 weeks at initiation of the study.
Weight (nonrodents only): males: 171-211g; females: 140-182 g. Note that these rats are not fully adult.

Unique study design or methodology (if any): Study length: 15 days.

The study design (doses and group designations) are listed in the following table provided by the sponsor (See below):

Dose Levels and Number of Animals:

Group	Dose level (µg/kg)	Dose Vol.	No. of Animals Male	Female
1	KGF Placebo	2 ml/kg	5	5
2	100	2 ml/kg	5	5
3	300	2 ml/kg	5	5
4	1,000	2 ml/kg	5	5
5	5,000	2 ml/kg	5	5
6	10,000	2 ml/kg	5	5
7*	30,000	6 ml/kg	5	5

* Group 7 was added by amendment; these animals were received at — in a separate shipment and treated at a later date than the first set of rats.

Frequency/Route: Single subcutaneous injection on Day 1.

Day 1 = 12/01/95 for Groups 1-6.
Day 1 = 12/12/95 for Group 7.

Observation times and results

Mortality: Twice daily on weekdays and once daily on weekends and holidays. No unscheduled deaths occurred.

Clinical signs: Observations made at 1, 2 and 4 hours post-dosing and once daily thereafter.
No adverse clinical signs were reported.

Body weights: At randomization on days -1 or 1, on day 1 prior to dosing, day 8 and prior to necropsy on day 15.
No effects on body weight gain attributable to study drug administration were noted.

Food consumption: N/A

Ophthalmoscopy: Not Done.

EKG: Not Done.

Hematology: Blood samples for laboratory testing was collected at terminal sacrifice on day 15.

- RBC count (RBC): Elevated for group 4 and 5 males; reduced slightly for groups 5, 6 and 7 females.
- Hematocrit (HCT): Slightly elevated for group 4 and 5 males. Reduced in correlation with RBC levels for group 5, 6 and 7 females.
- Hemoglobin (HGB): Slightly elevated for group 4 and 5 males but differences not significant. Reduced for groups 5, 6 and 7 females.
- Mean Corpuscular Volume (MCV)
- Mean Corpuscular Hemoglobin (MCH): Slightly reduced for groups 4 and 5 males. Slightly elevated for group 7 females.
- Mean Corpuscular Hemoglobin Concentration (MCHC): Slightly reduced for group 4 and 5 males. Slightly elevated for group 7 females.
- WBC count (WBC)
- WBC differential counts
- [absolute band] neutrophil (ANB),
- segmented neutrophil (ANS)
- lymphocyte (ALY),
- monocyte (AMO): Reduced to 25% of control for group 4 males, reduced to 10% of control for group 7 males. (The data appear to show a dose related trend toward reduced MO for all doses, although only groups 4 and 7 reached statistical significance.) Reduction of monocyte counts to less than 10% of control was also noted for female rats in a dose dependent fashion although statistical significance was only achieved for group 7.
- eosinophil (AEO)
- basophil (ABA)
- Platelet count (PLC): Platelet count was modestly reduced for group 7 males. No observed effect for female rats.
- Reticulocyte count (RET): Female rats in groups 3, 4, 5, 6 and 7 showed statistically significant increase in RET levels. This effect appears to be study drug related.

Clinical chemistry: Blood samples for laboratory testing was collected at terminal sacrifice on day 15.

- Alanine, aminotransferase (ALT)
- Albumin (ALB)
- Alkaline phosphatase (ALP)
- Aspartate aminotransferase (AST)
- Bilirubin [total (TBI), direct (DBI), and indirect (IBI)]: Small increases in bilirubin measures are reported for the high dose group males; a slight increase in DBI is noted for group 7 females.
- Blood urea nitrogen (BUN)
- Calcium (CAL)
- Chloride (CHL): Small increase noted for group 7 males and females.
- Cholesterol (CHO): small increase for group 7 males

- Creatinine (CRE)
- Globulin (GLO)
- Glucose (GLU)
- Phosphorus (PHO)
- Potassium (POT)
- Protein, total (TPR)
- Sodium (SOD): A small increase is noted for group 7 males and females.
- Triglycerides (TRI)
- Albumin/globulin ratio (AGR)

Urinalysis: Not Done.

Gross pathology: Thymus glands from all rats in the high dose group were retained and weighed

Organ weights (specify organs weighed if not in histopath table): Thymus glands from all rats in the 30,000 ug/kg dose. Thymus glands from group 7 males were 47% larger than controls. Female thymus from group 7 females were 15% larger than controls.

Histopathology: Adequate Battery: yes ( ), no (X)—explain
Peer review: yes ( ), no (X)
The only tissue examined at necropsy and for histopathology was thymus from all rats in the 30,000 ug/kg group. No mention of whether or not the thymus from control rats were retained for comparison.
Pathology report states that all thymus samples appeared to be histologically within normal limits.

Toxicokinetics:
Not Done.

Study title: A single dose toxicity study of rHuKGF administered intravenously to Rhesus monkeys.

Key study findings:

The purpose of this study was to evaluate the toxicity of rHuKGF after IV administration in monkeys. Two doses were evaluated: 10,000 ug/kg and 50,000 ug/kg. No significant hematology or serum chemistry effects related to test article administration were noted. All animals showed reduced food intake for several hours post dosing. Reddening of the skin, both facial and systemic, is reported for both dose groups beginning within one day after dosing and continuing for up to 7 days. Severity and duration were increased for the high dose group.
No gross findings are noted at necropsy. However, test article related findings are apparent from histological examination. Those findings (rated very slight to slight) include: acanthosis of the tongue, and skin over the mammary glands and gluteal region, hypertrophy of the mucous producing cells in most regions of the GI tract.

Involution of the thymus gland rated moderate to severe is reported in a dose dependent manner. The biological significance of these findings is not clear since no control animals were included in this study. The sponsor concludes that the lethal dose for rHuKGF is over 50,000 ug/kg.

Study no.: — -39-29, Amgen study number 960098
Volume #, and page #:
Conducting laboratory and location: [redacted]

Date of study initiation: 8/28/96
GLP compliance: YES
QA report: yes (X) no ( )
Drug, lot #, and % purity: rHuKGF lot # 1101226A6A, diluent lot# 02D07226G6A

Methods

Doses: 50,000 ug/kg, 10,000 ug/kg No control group.
Species/strain: Rhesus monkey, 4 male and 4 female
Number/sex/group or time point (main study): 2/sex/group
Route, formulation, volume, and infusion rate: Intravenous administration at a rate of 5 ml/min.
Satellite groups used for toxicokinetics or recovery: not done
Age: 4-8 years old
Weight (nonrodents only): males: 5-10 kg, females: 4-6 kg
Unique study design or methodology (if any):

Observation times and results

Mortality: Twice daily from day 1-13. See clinical signs, below.

Clinical signs: Prior to dosing, frequently for 1 hour after dosing, hourly from 1-6 hours post dosing, twice daily for day 1-13.

- Both males and females in the 10,000 and 50,000 ug/kg groups showed loss of appetite immediately after dosing lasting up to 8 hours post dose.
- Reddening of the skin, both facial and systemic, is reported for both dose groups beginning within one day after dosing and continuing for up to 7 days. Severity and duration were increased for the high dose group.

Body weights: Once prior to dosing on day -1, day 0 prior to dosing, on days 1, 4, 8 and prior to necropsy. Animals in the 50,000 ug/kg group showed a small reduction in body weight gain in the days following inject. The effect was more severe for the female monkeys. The males returned to normal by day 2 but the females had not regained the starting weight by the final weight measurement.

Food consumption: Calculated on the day prior to dosing and daily during the observation period. Food consumption was reduced for the 50000 ug/kg group after

dosing and returned to normal by day 6. This effect was more marked for the female monkeys in the 50000 ug/kg group.

Ophthalmoscopy: Not done.

EKG: Not done.

Hematology: Samples taken once prior to dosing, day 5 and 13.

- erythrocyte count (RBC : [ ] detection method)
- leukocyte count (WBC : [ ] detection method)
- platelet count ([ ] detection method)
- hematocrit value ([ ] detection method)
- hemoglobin concentration ([ ] method)
- mean corpuscular volume (MCV)
- mean corpuscular hemoglobin (MCH)
- mean corpuscular hemoglobin concentration (MCHC)
- Reticulocyte count and differential leukocyte count
- coagulation

Small changes in some hematology parameters are noted but did not appear to show a clear dose relationship. No control animals are included in this study. The small changes in values for RBC and WBC most likely do not have biological significance.

Clinical chemistry: Samples taken one day prior to dosing, days 6 and 13.

- Aspartate aminotransferase (AST: [ ] method)
- Alaniue aminotransferase (AL [ ] method)
- Alkaline phosphatase (ALP: [ ] method)
- Lactate dehydrogenase (LDH: [ ] assay)
- r -Glutamyl transpeptidase (7 -GTP: [ ] method)
- Total bilirubin ([ ] bilirubin method)
- Total protein ([ ] method)
- Albumin ([ ] method)
- Total cholesterol [ ] method)
- Triglyceride [ ] method)
- Glucose ([ ] method)
- Blood urea nitrogen (BUN: [ ] method)
- Creatinine (— method)
- Uric acid ([ ] method)
- -norganic phosphorus ([ ] direct method)
- Calcium (Ca: — method)
- Sodium (Na: [ ] method)
- Potassium (K: [ ] method)
- Chloride (Cl- : [ ] method)

Globulin (calculation)

No test related changes in serum chemistry values are reported.

Urinalysis: Not done.

Gross pathology: See below. No macroscopic lesions were observed.

Organ weights (specify organs weighed if not in histopath table):
No organs were weighed. No macroscopic lesions were noted.

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes ( ), no (X)
The following tissues were examined histologically:
Heart, thoracic aorta, spleen, thymus, bone marrow and bone (femur and sternum), mesenteric lymph nodes, submandibular lymph nodes, tongue, lungs, bronchus, trachea, esophagus, stomach (fundus, cardia, pylorus), gall bladder, small intestine (duodenum, jejunum, ileum), pancreas, large intestine (cecum, colon, rectum), anus, liver, urinary bladder, kidneys, testes, penis (epithelial lining of ureter), seminal vesicles, epididymides, ovaries, prostate, vagina, uterus, pituitary, thyroids, cerebrum, parathyroids (as much as possible), brain stem, adrenals, sciatic nerve, cerebellum, lacrimal glands, spind cord (cervical, thoracic, lumbar), eyes (with optic nerve), tonsils, skin (gluteal, scalp), injection site, quadriceps muscle of the thigh, urethra, submandibular gland, mammary gland. Also examined were samples of buccal mucosa and skin from all animals.

- Thymus involution was recorded as severe for 4 of 4 high dose monkeys and 3 of 4 low dose monkeys. This may related to the test article but can not be clearly determined due to the lack of control group for this study.
- Slight inflammatory cell infiltration of the trachea is reported for all animals in this study.
- Acanthosis rated very slight for one of the high dose male animals and none of the low dose animals in the esophagus.
- Mucous cell hyperplasia of the stomach was rated moderate and very slight for the 4 of 4 high dose animals but was not reported for the lower dose monkeys
- Lymphocyte infiltration rated as very slight was noted for the stomach of 2 of 4 high dose animal, 1 of 4 noted for the lower dose animals.
- Goblet cell hyperplasia rated as very slight to slight is recorded for the jejunum, ileum, cecum, colon and rectum of 4 of 4 animals in the high dose group and not noted for the lower dose group.
- Very slight brown pigment is reported for 1 of 2 animals in each dose group
- Mononuclear cell infiltration in the liver rated very slight is reported for all animals in the high dose group and 3 of 4 in the lower dose group. Whether this is a test article related effect is not known since no control animals were included in this study.
- Slight hypertrophy of the zona fasciculate of the adrenal gland is reported for one of 2 high dose male animals, not noted for the lower dose animals or female high dose animals.

- Acanthosis of the skin over the mammary gland and gluteal region rated very slight is reported for one of 3 of 4 animals in the high dose group. Slight gluteal acanthosis is noted for 1 of 4 lower dose animals.
- Acanthosis of the tongue rated very slight is reported for the tongue of both male high dose animals. None of the female animals were found to display this effect.
- Slight acanthosis of the scalp is reported for 2 of 2 female high dose animals and 1 of 2 male high dose animals.

Toxicokinetics: Not Done.

Other:

2.6.6.3 Repeat-dose toxicity

Study title: *Maximum tolerated dosage determination of recombinant human keratinocyte growth factor, rHuKGF, in athymic nude mice.*

Key study findings:
The purpose of this non-GLP study was to determine a maximum tolerated dose (MTD) for recombinant human keratinocyte growth factor, rHuKGF, in athymic nude mice. The study was performed as a preliminary, dose-finding study for design of future tumor-promotion studies using human tumor xenografts. The mice showed a dose-related decrease in body weight over the duration of the treatment. An LD_{10} overall was estimated to be 4.8 mg/kg/dose. The value for female animals was 11.0 mg/kg/dose and that for males was 4.0 mg/kg/dose. Therefore, the maximum tolerated dose of rHuKGF, administered to male and female athymic nude mice for 3 consecutive days/week for six weeks is estimated to be 4.8 mg/kg/dose.

Study no.: 100929
Volume #, and page #:
Conducting laboratory and location: [redacted]

Date of study initiation:
GLP compliance: No.
QA report: yes ( ) no ( )
Drug, lot #, and % purity: Palifermin lot # 24010C9,

Methods

Doses: 0, 5, 10, 25 or 50 mg/kg
Species/strain: Athymic, NCR-nu mice supplied by [redacted]
Number/sex/group or time point (main study):
Route, formulation, volume, and infusion rate: IV route, 0.1 ml/10gm bodyweight for the 50 mg/kg/group, 0.1 ml/20gm body weight for all other groups.
Satellite groups used for toxicokinetics or recovery:
Age: 7 weeks
Weight (nonrodents only):

Unique study design or methodology (if any): Mice were injected on Monday, Wednesday and Friday each week for 6 weeks.

Observation times and results
The parameter used to determine the MTD for palifermin was mean survival time (MST) based upon death, morbidity or body weight loss of 20% or more.

Mortality:

- All animals in the high dose group were dead or euthanized by day 36 except one female that was sacrificed on day 41. Days for sacrifice were 12, 19, 26 (2), 29 (2), 36 and 41.

Clinical signs: N/A See above.

Body weights: A dose dependent decrease in body weights over time is reported. The body weights decreased during the week and showed a rebound over the weekend (treatment-free period).

Food consumption: N/A

Ophthalmoscopy: N/A

EKG: N/A

Hematology: N/A

Clinical chemistry: N/A

Urinalysis: N/A

Gross pathology:
Mice were sacrificed when body weight loss equaled 20% of original body weight. Limited autopsy was performed. Gross examination at necropsy revealed empty stomach and intestines, and blood observed within the thoracic cavity of treated mice. Similar observations were noted in both male and female mice. In one female (at the 10 mg/kg/dose level), blood was observed in the abdominal cavity. Pale kidneys were noted at autopsy in the majority of rHuKGF-treated mice, but no microscopic correlation could be made. No etiology for the bloody lungs was determined. However, this finding did not occur in control animals and may have been a result of study drug administration.

Organ weights (specify organs weighed if not in histopath table):

Histopathology: Adequate Battery: yes ( ), no ( )—explain
Peer review: yes ( ), no ( )
Limited histopathology was performed. However, tissue from mice from dose groups of 10mg/kg or greater showed moderate to severe hyperplasia of the nonglandular stomach mucosa and moderate to severe hyperkeratosis of the nonglandular stomach mucosa.

This finding represents an expected pharmacological effect of rHuKGF. The hyperkeratosis of the stomach and intestine may have formed physical obstructions that caused malnutrition, weight loss and death. No stomachs were examined in the lower dose groups so the incidence at lower doses could not be determined.

Mild to minimal congestion/pooling of blood in lungs of animals receiving 5 mg/kg/dose or greater was noted. It is not clear what the root cause was of the pulmonary congestion. The pathologist suggests that this finding may have been related to a lack of vascular integrity in the lungs, but was considered to be only a minor component of that finding. Hemorrhage may have originated within the mediastinum, or elsewhere. Hemorrhage in the lungs and thoracic cavity may have contributed to death in some mice. This finding was not observed in the control group. This finding may a result of study drug administration..

Study title: Maximum tolerated dosage determination of once-weekly intravenous recombinant human keratinocyte growth factor (rHuKGF) *in female athymic nude mice*

Key study findings: The purpose of this study was to determine the maximum tolerated dose of palifermin administered IV once per week for 6 weeks in athymic female nude mice. The stomach and tongue were used as target tissues for assessment of toxicity in addition to weight loss and general health. A range of 4 doses from 10 to 50 mg/kg were evaluated The MTD was determined to be 25 mg/kg/dose under the conditions of this study due to significant weight loss and death of one animal in the 50 mg/kg group.

Study no.: 101623
Volume #, and page #:
Conducting laboratory and location:

Date of study initiation:
GLP compliance: No.
QA report: yes ( ) no ( )
Drug, lot #, and % purity:

Methods

Doses: 0, 10, 15, 25, 50/mg/kg/dose
Species/strain: Female NCr-nu athymic nude mice
Number/sex/group or time point (main study): 8 females per group
Route, formulation, volume, and infusion rate: IV infusion
Satellite groups used for toxicokinetics or recovery: None
Age: Approximately 6 weeks.
Weight (nonrodents only): 16.3 to 21.2 at study initiation
Unique study design or methodology (if any): Test article was administered on Mondays for 6 weeks. Necropsy of all surviving mice on day 40.

Observation times and results

Mortality: Mice were observed daily for mortality and moribundity. One mouse receiving 50 mg/kg/wk was sacrificed moribund due to loss of greater than 20% original body weight. With this exception, the treatment was tolerated relatively well. Stomachs of the treated mice from the 25 and 50 mg/kg groups were significantly heavier than those from control animals. The tongues from treated animals showed slight thickening compared to control but this finding did not reach statistical significance. The approximate LD_{10} for this treatment regimen was 47.5 mg/kg/dose. The MTD for this treatment regimen in athymic nude mice was determined to be 25 mg/kg/dose.

Clinical signs: Cageside observation daily. With the exception of the single death in the high dose group, no overt toxicity is reported.

Body weights: Data collected twice weekly.

- The mean body weight changes over the duration of the study (Day 1-40) were + 5.7 g, + 6.3 g, + 4.4 g, + 3.8 g, and + 1.5 g, respectively, for dosages of 0, 10, 15, 25, and 50 mg/kg/injection. Mean body weights of mice receiving rHuKGF showed a general weight gain over the duration of the study; however, a dose-related suppression of weight gain compared to control is reported. Mean body weight gain in the high dose group was significantly lower than that of the control group. Two surviving mice in the high dose group lost approximately 5 and 18% body weight.

Food consumption: Not recorded.

Gross pathology: Necropsy was performed on all surviving mice on day 40. Stomach and tongue were retrieved, weighed and examined microscopically. No gross abnormalities are reported.

Organ weights (specify organs weighed if not in histopath table):
Stomach and tongue only. Stomach weights from the 25 and 50 mg/kg treatment groups were significantly greater than the placebo group (by 21 and 24%, respectively).

Histopathology: Adequate Battery: yes ( ), no ( )—explain
Peer review: yes ( ), no ( )
Tongue only. Tongue thickness was measured as an indicator of study drug activity/toxicity. The tongue thickness was not significantly greater in any treatment group compared to control. However, it is noted that some increase in thickness was noted (rated nominal) to confirm biological activity.

Study title: 28-day repeated SC dose toxicity study of rHuKGF in male and female rats

Key study findings:
The purpose of this study was to evaluate the toxicity of subcutaneous injection of palifermin in male and female rats when administered daily for 28 consecutive days. The doses used ranged from 30 to 1000 ug/kg/day. A subset of rats was designated as recovery group and was allowed to survive an additional 4 weeks after termination of

dosing. Hyperplastic/hypertrophic changes were present on Day 29 in the stomach, duodenum, jejunum, ileum, cecum, colon and rectum of both male and female rats in the 100, 300, and 1000 ug/kg/day groups. Dilation of the glandular mucosal crypts was the only microscopic change observed in rats in the 30 ug/kg/day group. The hyperplastic/hypertrophic gastrointestinal changes were not present in any of the treatment groups on Day 57.

Protein casts were present on Day 29 in male and female rats in groups 5 and 6, while urothelial hyperplasia was present on Day 29 in male rats in groups 5 and 6 and in female rats only in group 6. On Day 57, protein casts were present in male and female rats in groups 5 and 6, but urothelial hyperplasia was absent from all dose groups and both sexes. Glomerulosclerosis and glomerular hypertrophy were observed on Days 29 and 57 in all male and female rats in group 6, but not in any of the control rats examined (only rats in the control and high dose groups were examined for glomerular lesions using PAS staining methodology). No significant gender differences were observed for incidence or severity of these lesions.

Liver enlargement with centrilobular hepatocellular apoptosis was present on Day 29 in male and female rats in groups 5 and 6. The increase in liver size may be correlated with increases in total protein, albumin, globulin, calcium , cholesterol and triglycerides that was noted at all doses over 100ug/kg. On Day 57, hepatocellular apoptosis as an effect of palifermin was no longer clearly delineated in the females, and was only suggested as an effect in the males in group 6.

Thymic gland lymphoid depletion was present on Day 29 in male rats given 1000 ug/kg/day and in females receiving 300 and 1000 ug/kg/day. Thymic gland lymphoid depletion was not present on Day 57.

Thyroid follicles were increased in size and number on Day 29 in male rats given 100, 300, and 1000 ug/kg. May and in female rats given 300 and 1000 ug/kg/day. In addition, periglandular fibrosis was present on Day 29 in male and female rats receiving 1000 ug/kg/day. On Day 57, the increase in the number and size of thyroid follicles was present in male and female rats given 300 or 1000 ug/kg/day at the same incidence and severity as on Day 29. The periglandular fibrosis was still present on Day 57 in male and female rats receiving 1000 ug/kg/day; the incidence was significantly decreased, but the severity was similar to that observed on Day 29. Epithelial hyperplasia and hyperkeratosis was present on Day 29 in the tongue of male rats at the 300 and 1000 ug/kg/day levels, while female rats had the same lesion at the 1000 ug/kg/day level. Tongue epithelial hyperplasia and hyperkeratosis was not present at Day 57. Urinary bladder epithelial hyperplasia was present on Day 29 in male and female rats given 300 and 1000 ug/kg/day but was not present at Day 57.

The no-effect level for microscopic lesions in male and female rats appears to be less than 100 ug/kg/day under the conditions of this experiment.

Study no.: ~ # M015-95/ Amgen # T-95-KGF-001
Volume #, and page #:
Conducting laboratory and location:

Date of study initiation: 2/28/95
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: test article lot # 01195A5A, diluent lot # 02095B5, placebo lot # 02085B5

Methods

Doses: 0, 30, 100, 300 or 1000 ug/kg/day
Species/strain: Sprague-Dawley rats
Number/sex/group or time point (main study): 15/sex/group for main study;
Route, formulation, volume, and infusion rate: subcutaneous injections daily for 28 days
Satellite groups used for toxicokinetics or recovery: recovery groups 4-5/sex/group
Age: 7 weeks
Weight (nonrodents only):
Unique study design or methodology (if any):
The study design is illustrated in the table below, provided by the sponsor:

Group	Dose (µg/kg/day)	Total No. Animals	No. Animals Sacrificed	
			Day 29[a]	Day 57[b]
1 (Vehicle)	0	15♂/15♀	10♂/10♀	5♂/5♀
2 (Low dose)	30	15♂/15♀	10♂/10♀	5♂/5♀
3 (Lower mid dose)	100	15♂/15♀	10♂/10♀	5♂/5♀
4 (Upper mid dose)	300	15♂/15♀	10♂/10♀	5♂/5♀
5 (High dose)	1000	15♂/15♀	10♂/10♀	5♂/5♀

[a]5/sex/group started treatment on 03/23/95 (Day 1) and the remaining 5/sex/group started treatment on 03/24/95 (Day 1 for this subgroup).
[b]Started treatment on 03/23/95 (Day 1).

Observation times and results

Mortality: Twice daily on weekdays, once daily on holidays and weekends.

- One female rat was found dead on day 14 (#211, 300 ug/kg dose group). Moderate swelling of the right eye was noted on day 7 with marked exophthalmia. When found dead the eye was not found and the socket contained necrotic and hemorrhagic tissue. No other clinical signs were reported prior to death.
- One male rat from the 30 ug/kg dose group was euthanized on day 15 for humane reasons due to a markedly swollen hind leg. The sponsor hypothesizes that the

injury most likely occurred during restraint for dosing and is not related to the test article.

Clinical signs: Animals observed once daily for clinical signs.

- One male rat (#200 from the 300 ug/kg group) had a convulsion rated as moderate and lasting approximately one minute on day 43 (recovery).
- The eye swelling and exophthalmia is reported for one additional female in the 1000 ug/kg group and one control male. These findings are most likely due to injury during the blood sample collection procedure via retro-orbital sinus.

Body weights: Recorded prior to study initiation on day 1, then days 8, 15, 22, 29, 36, 43, 50, and 57 (weekly).

- Mean weekly body weights indicate that all groups gained weight at approximately similar rates during the main study. The male rats in all treated groups showed a significantly reduced weight gain on day 36 than controls. Mean body weight of the females from all treated groups showed a significant reduction in body weight gain on day 50 when compared to control values. The biological significance of this finding is not clear since both these findings occurred during the recovery period when active drug should be disappearing from the system. Total weight gains for both males and females were not significantly different from controls.

Food consumption: Measured at least 3X per week.

- Occasional reduced food intake was reported for the high dose male rats during the main study period.
- For the female rats, reduced food consumption was observed more regularly for the treated groups with increased incidence noted with increasing dose.
- Reduced food consumption appears to be a test article related effect but apparently, did not result in marked body weight gain differences among groups.

Ophthalmoscopy: Performed prior to study initiation, days 25 or 26 and day 55.

- No study drug related effects were noted either at the end of the treatment period or the 28-day recovery period.

EKG: Not Done.

Hematology: Blood samples were collected on days -3, 15, 29 and 57. Parameters assessed include the following:

- RBC count (RBC): The reductions in hemoglobin parameters (below) are not reflected in changes in RBC counts which remained unaffected.
- Hematocrit (HCT): Statistically significant reduction in HCT was noted for males and females in the 1000mg/kg group. For males, this was detected on day 15 and 29 but not day 57. For females, the reduction was not significant until day 29 but recovery was noted by day 57.
- Hemoglobin (HGB): Statistically significant reduction in HGB was noted for males and females in the 1000mg/kg group. For males, this reduction was apparent at both days 15 and 29 and recovery was observed by day 57.

- Mean corpuscular volume (MCV): The observed reductions in HGB and HCT is accompanied by reductions in other hemoglobin parameters including MCV, MCH.
- Mean corpuscular hemoglobin (MCI-I): See above.
- Mean corpuscular hemoglobin concentration (MCHC): See above.
- WBC count (WBC)
- WBC differential counts [absolute band neutrophils (ANB), segmented neutrophils (ANS), lymphocytes (ALY), monocytes AMO), eosinophils (AEO), and basophils (ABA)] Some statistically significant changes in WBC measures are reported: ANS increases on days 29 and 57 for the 1000 ug/kg male rats and on day 29 for the 30 ug/kg female rats, AMO increases on day 29 male rats from groups 3, 4, 5 and decreased on day 15 for female rats in the 100 ug/kg group. These findings do not appear to be dose-related as they were observed somewhat sporadically and were inconsistent among dose groups and timepoints.
- Platelet count (PLC): Dose-related increases in PLC were noted by day 15 for doses greater than or equal to 100 ug/kg/day. For male rats, the increase was 15% at the 100 ug dose, 29% at the 300 ug dose and 37% for the 1000 ug dose. This elevation continued through day 57 for the males, although statistical significance was observed only for the 300 ug dose at that time. For females, similar increases were noted at day 15, remained elevated on day 29 and had returned to control levels by day 57.
- Reticulocyte count (RET): Elevations in RET levels were reported by day 15 for male rats in the 300 and 1000 ug groups and for females in all groups at 100 ug or higher. Because the elevations were not clearly dose-related and were small and did not correlate with any changes in RBC levels, the RET biological significance of the RET findings is not clear.

Clinical chemistry: Blood samples were collected under C02 anesthesia on days -3, 15, 29 and 57. Parameters evaluated include the following:

- Alanine aminotransferase (ALT):Reduced on day 15 in a dose-related manner in male rats from groups 3, 4, and 5.
- Albumin(ALB): Increases in ALB were observed in male and female rats in all groups receiving 100 ug/kg or greater. Increases were noted on day 15 for male and female rats and values remained elevated on day 29. (Male rat values: ALB increased 9%, 15%, and12% in the 100, 300, and 1000 ug/kg/day groups, respectively.) Values returned to control levels by day 57.
- Alkaline phosphatase (ALP): Reduced on day 15 for male rats in groups 3, 4, and 5. On day 29, only values for groups 4 and 5 were statistically significant, but the reduced values continued for all treated groups in a clearly dose-related manner. Reduced ALP values persisted through day 57. Statistical significance was apparent only for group 5 on day 57. For female rats, ALP values were reduced for groups 4 and 5 on day 15 and continued for group 5 on day 29.
- Aspartate aminotransferase (AST): Reduced in male animals from all treated groups on day 15 relative to control values. Values continued to be decreased on day 29 but were statistically significant for only groups 4 and 5. AST values for

male rats were similar to control values by day 57. For female rats, AST values were significantly reduced for groups 3, 4 and 5 on day 15 (18%, 28% and 38% for groups 3, 4, and 5, respectively.) Values for day 29 show a continued dose-dependent reduction for group 5.

- Bilirubin [total (TBI), direct (DBI), and indirect (IBI)]: TBI values are elevated for female rats on day 29 (approximately 2X).
- Blood urea nitrogen (BUN): BUN values were elevated for female rats from group 4 on day 15. On day 57, BUN values were reduced for group 5 females relative to control (20 for controls, 15 for group 5).
- Calcium (CAL): Increased relative to control in male rats in all treated groups on day 15 and 29. Values returned to control levels by day 57. For females, calcium values are increased on day 15 and 29 for groups 4 and 5. The increases are small but clearly dose dependent. By day 57, level were similar to control values.
- Chloride (CHL): Slightly reduced (statistically significant) relative to control for male and female rats in groups 4 and 5 on day 15. On day 29 values for treated male rats were also different with respect to control but the pattern was not consistent. Differences are small and not consistent (elevated for group 2 and 3, decreased for group 5. Values for female rats are similar; sporadic and not consistently dose-dependent.
- Cholesterol (CHO): Increased for both male and female rats. Values for male rats were elevated relative to control on days 15 and 29 for groups 3, 4, and 5. Values returned to control levels by day 57. For female rats, elevated values are reported for groups 4 and 5 on day 15 (approximately 1.8X and 3.6X for groups 4 and 5, respectively.) Values for day 29 for female rats remained elevated (1.6 and 3.2 X for groups 4 and 5, respectively.) Values for day 57 had returned nearly to control levels with only group 5 still showing a statistically significant increase.
- Creatinine (CRE): Increased for male rats on day 15 and 29 in groups 3, 4, and 5. Values were not different from control for male rats on day 57. Values for female rats were elevated on day 15 for groups 4 and 5 and a small elevation remains apparent for group 5 on day 29. The differences are very small so biological significance is not clear. On day 57, values for female rats had declined to less than control fro groups 4 and 5 (statistically significant for group 5)
- Globulin (GLO): Increases in ALB were observed in male and female rats in all groups receiving 100 ug/kg or greater. Increases were noted on day 15 for male rats and values remained elevated on day 29. (Male rat values: GLO increased 24%, 48%, and 76% in the 100,300, and 1000 ug/kg/day groups, respectively.) Values remained slightly elevated at day 57. Values for female rats returned to control values by day 57.
- Glucose (GLU): Blood glucose values are decreased for female rats in groups 4 and 5 on day 15.
- Phosphorus (PHO): No changes are noted for male rats. Female rats from group 5 showed a small but statistically significant decrease on day 15.
- Potassium (POT): Slightly reduced in group 5 males on day 15. Results for day 29 showed elevation for all treated groups but with statistical significance for only group 5. For females, statistically significant increases are noted for groups 4 and

5 on day 15. Values for day 29 show a continuing trend toward increased levels with group 5 reaching statistical significance. (4.6 for control vs. 5.6 for group 5 females.) Day 57 –similar to control levels.

- Protein, total (TPR): Increased in a dose-dependent manner by day 15 and remained elevated on day 29. (Increased 16% in 100 ug/kg/day males, 30% in 300 ug/kg/day males, and 43% in 1000 ug/kg/day males.) By day 57 the values had begun to decline but remained slightly elevated. Values for female rats showed similar changes on day 15 and 29 but results had returned to within normal limits by day 57.
- Sodium (SOD): Slightly reduced on day 15 for group 5 male and female rats. On day 29, elevated SOD levels are reported for groups 2 and 3 male rats. Values for female rats on day 29 show a small decrease in SOD relative to control. Values for male rats remained elevated for groups 3, 4 and 5 on day 57.
- Triglycerides (TRI). Elevated values for groups 4 and 5 male rats on days 15 and 29. Values for male rats on day 57 were similar to control. For females, a dose-related increase in triglycerides is also reported with a 5.5X increase for group 5 on day 15. Values for day 29 showed a 4.7X increase relative to control. Values for females on day 57 remained elevated (group 4: 1.9X, group 5: 1.7X)
- Albumin/globulin ratio (AGR): The changes in ALB and GLO values were accompanied by dose-related decreases in AGR on days 15 and 29 for both males and females. On day 57, values were normal with respect to control values.

Urinalysis: Not Done.

Gross pathology: 10 rats/sex/group will be sacrificed on day 29 and remaining animals on day 57. Tissues retained are as follows:

- Adrenal glands (pair)
- Aorta
- Bone marrow smear (femur)
- Bone and marrow, contralateral femur with
- femorotibial joint
- Buccal cavity
- Brain (fore-, mid-, and hindbrain)
- Cecum
- Cervix
- Colon
- Duodenum
- Esophagus
- Eyes, with optic nerve
- Cross lesions (including tissue masses and abnormal regional lymph nodes)
- Heart
- Ileum
- Injection site (skin from interscapular area)
- Jejunum
- Kidneys

- Larynx(pharynx examined at necropsy)
- Liver
- Lungs with bronchi
- Lymph nodes (mandibular and mesenteric)
- Mammary gland (to include nipple and surrounding tissue)
- Ovaries and oviduct
- Pancreas
- Pituitary gland
- Prostate
- Rectum
- Salivary gland
- Sciatic nerve
- Seminal vesicle
- Skeletal muscle
- Skin (abdominal; taken with mammary mgland)
- Spinal cord (thoracolumbar junction)
- Spleen
- Stomach
- Testes and epididymides
- Thymus
- Thyroid and parathyroids
- Tongue
- Trachea
- Urinary bladder
- Uterus
- Vagina

Organ weights (specify organs weighed if not in histopath table):

- Adrenal glands
- Brain
- Heart
- Kidneys: organ weights showed a trend toward increase on day 29 for male rats with a statistically significant increase for group 5 when expressed as percent body weight. Kidney weights from female rats were increased for groups 4 and 5 on day 29.
- Liver: increase for group 4 and 5 male rats and female on day 29 for absolute organ weight and as a percent of body weight.
- Lungs
- Pituitary (after fixation): No consistent changes.
- Spleen
- Testes with epididymides attached
- Thymus: Organ weight and percent of body weight reduced for male rats on day 29. Thymuses from female rats showed no change relative to control values.
- Ovaries

Liver weights were significantly increased for male animals in the 300 and 1000 ug/kg dose groups.

Thymus gland weight (mean) was significantly increased for males in the 1000 ug/kg group.

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes ( ), no ( )

Tissues from all animals in all groups will be examined microscopically.
The following findings are dose-dependent effects of the test article on day 29:

- Goblet cell hyperplasia of the cecum in 60% of male animals at 1000ug/kg , 10% of female rats in the 300 ug group, 80% of females in the 1000 ug group.
- Goblet cell hyperplasia of the colon in 60% of the male animals at 300 ug/kg and 90% at 1000ug/kg, 50% of females in the 300 ug group and 100% in the 1000 ug group.
- Goblet cell hyperplasia of the duodenum in 60% of male animals in the 1000 ug/kg group, 70% of female animals in the 1000 ug group.
- Hyperkeratosis and hyperplasia of the esophagus in 11% of male animals in the 1000ug/kg group. This finding was not reported for female rats.
- Goblet cell hyperplasia of the ileum: 50% of male animals in the 300 ug/kg group and 89% of male animals in 1000 ug/kg group, 50% of female rats in the 300 ug group and 90% in the 1000 ug group.
- Goblet cell hyperplasia of the jejunum: 40 of male animals in the 300 ug/kg group and 90% of male animals in the 1000ug/kg group, 50% of female rats in the 300 ug/kg group and 90% in the 1000 ug group.
- Urothelial hyperplasia observed in 10% of male animals in the 300 ug group and 20% of male animals in the 1000 ug group, 20% of female rats in the 1000 ug group.
- Glomerular hypertrophy observed in 100% of all animals from the 1000 ug/kg group rated as minimal to mild.
- Glomerulosclerosis in 100% of all animals in the 1000ug/kg group. This finding is described as increased mesangial matrix and rated as minimal to mild.
- Kidney: Protein cast was observed in 10% of male animals from the 300 ug/kg group and 60% of male animals from the 1000 ug, 20% of female rats in the 300 ug group and 90% in the 1000 ug group.
- Chronic inflammation and tubular regeneration are reported for the all treated groups but these findings do not reflect a dose dependent effect. Tubular regeneration is also reported for 1 of 10 male animals in the control group.
- Liver: extramedullary hematopoiesis observed in 30% of male animals in the 1000 ug/kg group, 10 of female rats in the 1000 ug group but was also present in 10% of female rats in the control group.
- Liver: apoptosis observed in 10% of male animals in the 300 ug/kg group and 90% of male animals in the 1000 ug/kg group, 50% of female animals in the 300 ug group and 100% in the 1000 ug group. This finding is described as focal centrolobular apoptosis and rated as minimal to mild.

- Lung hemorrhage was observed a t a high rate in all groups with no demonstrated dose relationship. This finding was observed in 70% of control animals.
- Goblet cell hyperplasia of the rectum observed in 10% of animals in the 300 ug/kg group and 63% of animals in the 1000 ug/kg group, 80% of female rats in the 1000 ug group.
- Skin at the injection site showed chronic inflammation at a rate of 20-30% in all treated groups. This finding was not reported for the control animals. Hemorrhage at the injection site was observed in 2 of 10 male animals in the 1000 ug/kg group and not in any other group. Similar reactions are reported for female rats but with lower incidence an less appearance of a relation ship to test article administration as injection site reactions were noted at a low rate in control females. Severity of injection site reactions was rated as generally minimal to mild.
- Spleen: extramedullary hematopoiesis was observed in 20% of male animals in the 1000 ug/kg group and lymphoid hyperplasia in 1 of 10 male animals in the 1000 ug/kg group. No findings are reported for female rats.
- Urinary bladder: hyperplasia observed in 20% of male animals in the 300 ug/kg group and 90% of male animals in the 1000 ug/kg group, 10 % of female rats in the 300 ug group and 100% in the 1000 ug group.
- Hyperplasia and hyperkeratosis of the tongue was observed for 30% of male animals in the 300 ug/kg group and 100% of male animals in the 1000 ug/kg group, 60% of female rats in the 1000 ug group. Rated as mild to moderate
- Thymus: lymphoid depletion is reported for 90% of male animals in the 1000ug/kg group, 20% of females in the 300 ug group and 30% in the 1000 ug group.
- Thyroid gland: increased follicular number and size is reported for 10% of male animals in the 100 ug/kg group, 30% in the 300 ug/kg group and 100% in the 1000ug/kg group, 10 % of female rats in the 300 ug group and 100% in the 1000 ug group. This finding is rated as severe. (Pathology report differentiates this finding from hyperplasia.) Thyroid fibrosis is reported for 90% of male animals in the 1000 ug/kg group, 40% of female rats in the 1000 ug group.
- Stomach: Dilatation is noted in 60% of the male rats in the 300ug/kg group and 80% of male rats in the 1000 ug/kg group, 30% of females in the 300 ug group and 30% in the 1000 ug group. Hyperkeratosis was observed in 20% of male rats in the 300 ug group and 100 % of male rats in the 1000 ug/kg group, 100% of female rats in the 1000 ug group. Hypertrophy is reported for 60% of male rats in the 300 ug group and 100% in the 1000 ug group, 30% of female rats in the 300 ug group and 100% in the 1000 ug group. These findings are generally rated as moderate to severe.

Recovery: Microscopic examination of tissues from rats from the recovery groups (1, 4 and 5) indicates that the effect of study drug on the GI system epithelial tissues fully recovered. No findings of goblet cell hyperplasia or hyperkeratosis were noted. Hyperplasia and hyperkeratosis of the tongue was not observed on day 57.
The following findings persisted through the 28-day recovery period:

- Thyroid follicular hypertrophy and increased number of follicles and fibrosis. These findings persisted at approximately the same incidence rate as at day 29.
- Kidney glomerular hypertrophy, glomerulosclerosis, protein cast and mineralization. Urothelial hyperplasia was not reported at day 57. At day 57, mineralization of the kidney is reported for animals across all groups.
- At day 57, atrophy of the pancreas is noted in 40% of females in the 1000 ug/kg group.
- Extramedullary hematopoiesis is reported at a higher incidence than at day 29 but is reported for all groups. (75% to 100% of female rats across all groups showed this finding. For male rats at day 57, this finding is reported for 40% of control rats, 20% for the 300 ug/kg group and 80% of 1000 ug/kg group)

Toxicokinetics:
See the table below for TK sampling schedule. Table provided by the sponsor.

Time post-dose	Subset[a]	No. Animals for PK Serum Sampling		
		Main Study Groups[b]	Recovery Groups[c]	Total Sampled
30 min	A	2♂/2♀	1♂/1♀	3♂/3♀
2 hr	B	2♂/2♀	1♂/1♀	3♂/3♀
4 hr	C	2♂/2♀	1♂/1♀	3♂/3♀
8 hr	D	2♂/2♀	1♂/1♀	3♂/3♀
24 hr	E	2♂/2♀	1♂/1♀	3♂/3♀

[a]Five different subsets of animals in each dose group, including the controls, were sampled as above. The same animals in each subset were sampled at the same time points on each of the sampling days.
[b]5/sex/group started treatment on 03/23/95 (Day 1) and the remaining 5/sex/group started treatment on 03/24/95 (Day 1 for this subgroup).
[c]Started treatment on 03/23/95 (Day 1).

Other: Anti-drug antibodies were detected in all groups except control. No gender specific differences are noted in this response to the study drug.

Study title: 28-day repeated IV dose toxicity study of rHuKGF in male and female rats

Key study findings:
The purpose of this study was to evaluate potential toxic effects of palifermin when administered IV on a daily basis for 28 days to male and female rats. In addition, this study included a 28-day recovery period for assessment of the recovery potential for any identified toxic effects.

Adverse clinical signs (ruffled and discolored fur) were noted only in male rats in the 1000 ug/kg/day group; no treatment-related deaths occurred at any dose level. No ophthalmic effects occurred as a result of treatment with palifermin. The male rats receiving palifermin maintained body weights comparable to those of the control rats during the 28-day treatment period, while the female rats receiving palifermin had overall weight gains that were 14-24% higher than those of the control rats and therefore had a higher mean body weight at the end of the treatment period. Mean body weights for female rats on Day 29 were 4-8% higher than those of the controls; these differences were statistically significant for the 100, 300, and 1000 ug/kg/day groups. During recovery, male rats that had received palifermin had overall body weight gains that were 25-56% lower than those of the controls rats and female rats had overall body weight gains that were 20-53% lower than controls. At the end of the recovery period the mean body weights for male rats that had been treated with palifermin were 8-20% lower (statistically significant for the 1000 ug/kg/day group) and those for female rats were comparable to that of the controls. Food consumption in male and female rats followed the same pattern relative to gender as did body weights during the treatment and recovery periods. This suggests that the pattern of weight changes was related to altered food consumption.

A transient, mild, anemia (decreased red blood cell counts and hemoglobin and hematocrit levels) was observed in male and female rats in groups 5 and 6, and increased reticulocyte and platelet counts were observed in male and female rats in the 100, 300, and 1000 ug/kg/day groups on Day 15; these parameters had returned to normal or near normal levels by Day 29 or Day 57.

Administration of palifermin produced marked liver enlargement in male and female rats: absolute and relative liver weights were significantly increased on Day 29 in both males and females receiving doses of 30, 100, 300, and 1000 ug/kg/day. Absolute liver weights were 19-31% higher in male rats and 20-73% higher in female rats relative to control rats (statistically significant difference for all groups of males and all but the 30 ug/kg/day group of females). Liver-to-body weight ratios were 17-34% higher in male rats than those of the controls and 14-57% higher in female rats. These differences were statistically significant for all groups of rats that received palifermin. The increase in liver size could account for the significant increases in total protein, globulin, albumin, calcium, cholesterol, and triglycerides that occurred at all dose levels. This effect appeared to be reversible, since increased liver size and alterations in serum chemistries were not present at the end of the recovery period. No other treatment-related effects on clinical chemistry parameters were observed. An increased incidence of centrilobular apoptosis was present in the liver of male and female rats in the 1000 ug/kg/day dose group on Day 29, and a minimal degree of increased centrilobular apoptosis was still present in the liver of male and female rats in this group on Day 57.

The number and size of thyroid follicles were increased on Day 29 in male and female rats given either 300 or 1000 ug/kg/day. In most groups, this lesion had not reversed by the end of the recovery period on Day 57, when these changes were present at an even greater incidence in male rats in both groups and in female rats in the 1000 ug/kg/day group.

Hyperplastic/hypertrophic changes were present in the stomach, duodenum, jejunum, ileum, cecum, colon, and rectum of both male and female rats in the 100 (jejunum and

ileum only), 300, and 1000 ug/kg/day dose groups at the end of the 28 day treatment period (Day 29), but these intestinal lesions had resolved after the 28 day recovery period (Day 57). Nonglandular hypertrophy and hyperkeratosis of the stomach were still present, although with a reduced incidence, on Day 57 in male rats given 1000 ug/kg/day. Urinary bladder epithelial hyperplasia was present on Day 29 in male rats given 300 or 1000 ug/kg/day and female rats given 100, 300, or 1000 ug/kg/day. At the end of the recovery period on Day 57, this change was present in only one female rat given 1000 ug/kg/day.

Significant decreases in the size of the thymus observed grossly on Day 29 in male and female rats given doses of 300 and 1000 ug/kg/day were reflected by thymus weights, which were 61-71% lower in males and 27-38% lower in females relative to control. These decreases were primarily due to thymus gland lymphoid depletion and were more prominent in male rats given 300 and 1000 ug/kg/day than in female rats receiving the same dose levels. The lymphoid depletion appeared to be reversible. On Day 57, it occurred in only one male rat in each of these dose groups and was absent in female rats in these same groups. In addition, thymus weights on Day 57 were higher than those of control rats in all groups of female rats receiving palifermin (statistically significant only for the 1000 ug/kg dose group) and were comparable to the controls for male rats in all groups.

Kidney weights in female rats at all dose levels were 10-27% relative to controls on Day 29 but occurred to a lesser degree on Day 57 and only in female rats in the 300 and 1000 ug/kg/day dose groups (15-20% higher than controls). The lower incidence and severity at Day 57 suggests that the effect was reversible. Organ weights for the lungs and ovaries of female rats in the 1000 ug/kg/day dose group were also increased on Day 29 but not on Day 57, findings indicating that the effect was reversible. However, no correlating microscopic lesions were found for the kidneys, lungs, and ovaries of these female rats, and the significance of these effects is unknown.

No histologic changes were noted at doses of 30 ug/kg/day. At 100 ug/kg/day and above, reversible histologic changes such as hyperplastic/hypertrophic changes of the gastrointestinal tract and urinary bladder were observed. These changes are consistent with the expected pharmacologic action of the test article. Doses of 300 ug/kg/day and above were associated with hyperplasia of the thyroid gland and reversible lymphoid depletion of the thymus gland. Hyperplasia of the thyroid had reversed by Day 57 at 300 ug/kg/day, but not at 1000 ug/kg/day. Based on the results of this study, the no-effect level for microscopic lesions in male and female rats appears to be less than 100 ug/kg/day of rHuKGF administered once daily for 28 consecutive days. The no adverse effect level based on reversible liver enlargement and associated effects on clinical chemistry parameters was less than 30 ug/kg/day palifermin.

Study no.: — #M016-95; Amgen #T-95-KGF-002
Volume #, and page #:
Conducting laboratory and location: [redacted]

Date of study initiation: 2/28/95
GLP compliance: Yes
QA report: yes (X) no ( )

Drug, lot #, and % purity: rHuKGF lot# 01195A5A
Diluent lot # 02095B5
Placebo lot # 2085B5

Methods

Doses: 0, 30, 100, 300 or 1000 ug/kg/day
Species/strain: Sprague Dawley rats
Number/sex/group or time point (main study): 15/sex/group (5 groups)
Route, formulation, volume, and infusion rate: Administered via IV infusion through the lateral tail vein in a volume of 1 ml/kg
Satellite groups used for toxicokinetics or recovery:
Age: 6-7 weeks
Weight (nonrodents only): males: 201-243g, females: 142-180 g
Unique study design or methodology (if any):
The study design is illustrated by the table below, provided by the sponsor:

Group	Dose (µg/kg/day)	Total No. Animals	No. Animals Sacrificed	
			Day 29[1]	Day 57[2]
1 (Vehicle)	0	15♂/15♀	10♂/10♀	5♂/5♀
2 (Low dose)	30	15♂/15♀	10♂/10♀	5♂/5♀
3 (Lower mid dose)	100	15♂/15♀	10♂/10♀	5♂/5♀
4 (Upper mid dose)	300	15♂/15♀	10♂/10♀	5♂/5♀
5 (High dose)	1000	15♂/15♀	10♂/10♀	5♂/5♀

[1]5/sex/group started treatment on 02/28/95 (Day 1) and the remaining 5/sex/group started treatment on 03/01/95 (Day 1 for this subgroup).
[2]Started treatment on 02/28/95 (Day 1)

Observation times and results

Mortality: Monitored twice daily on weekdays, once daily on weekends and holidays

- No treatment-related deaths are reported. One female rat in the 1000 ug group (group 5) was euthanized on day 28 due to a broken leg. It is believed that the injury occurred during restraint of the animals for drug administration.

Clinical signs: Cageside clinical observations made once per day.

- No test article-related effects on clinical signs are reported for any group except male rats in group 5 (1000 ug/kg). These animals (100%) began showing ruffled and discolored fur after SD 16. This finding persisted for approximately one week into the recovery period (no longer present by SD37).

Body weights: Recorded pre-dosing on days 1, 8, 15, 22, 29, 36, 43, 50, 57

- All rats gained weight during the course of the study. However, treatment related effects on body weight gain are reported: During the 28-day treatment period, male rat weight gain was comparable to control but slowed during the recovery to less than control. The reduction in weight gain during recovery was statistically significant for group 4 and 5 males, resulting in reduced overall weight gain relative to control. Female rats showed a dose-related increase in body weight and weight gain during the treatment phase. On day 29, mean body weight for females in groups 2, 3, 4 and 5 were significantly greater than control. During the recovery period, the weight gain slowed to less than control rate resulting in overall body weight and weight gain comparable to control

Food consumption: Recorded weekly for the first week and then 3X/week thereafter.

- The increase in body weight gain for female rats in groups 2, 3, 4, and 5 is associated with increased food in take during the treatment phase and was comparable to control during the recovery phase. For male rats, food consumption during the treatment phase was comparable to control and dropped to less than control during recovery.

Ophthalmoscopy: Performed pre-study, then on days 28 or 29, 56 or 57 (pre-necropsy)

- No treatment related effects are reported.

EKG: No done

Hematology: Blood samples collected for clinical pathology on days 15, 29, 57

- Reductions in RBC levels were observed for both males and females during the course of the study. On day 15, male rats showed a small dose-related trend toward reduced RBC levels that returned to control levels by day 29. On day 15, female rats showed a significant reduction in RBC levels in groups 4 and 5 relative to control (7% and 8% for groups 4 and 5, respectively). No differences in RBC levels were observed on day 29.
- Dose related deceases in HGB and HCT were observed for male rats in groups 4 and 5 on day 15. For HGB decreases of 7% for both groups 4 and 5 males are reported. For HCT decreases of 7% and 5% are reported for group 4 and 5, respectively. These parameters remained slightly reduced at day 29 and were not observed for day 57. For female rats, slightly greater effects were observed. On day 15, HGB was reduced by 8% and 11% for groups 4 and 5, respectively. HCT was reduced by 8% and 10%, for groups 4 and 5, respectively. Values remained slightly reduced on day 29 and both HGB and HCT were comparable to control by day 57.
- RET values were increased in male and female rats on day 15 but were comparable to controls by day 29. (Day 15 males increased 43%, 46% and 43% for groups 3, 4, and 5, respectively. Day 15 female values increased 50%, 50% and 79% for groups 3, 4 and 5, respectively.) This study drug effect appears to be more severe in female rats than males at equivalent doses.

- PLC values increased for both male and female rats in groups 3, 4 and 5 at day 15 and peaked at day 29. This effect was slightly less marked in female rats. For male rats, day 15 PLC values were increased 22% and 26% for groups 4 and 5, respectively. On day 29, values were elevated by 15%, 23% and 44% for groups 3, 4, and 5, respectively. For females, PLC was elevated by 15 in group 5. On day 29, PLC values were elevated by 14% and 18% in groups 4 and 5, respectively. These values for both males and female rats were comparable to control by day 57.
- Sporadic changes in ANS and AMO were noted in male rats during the study. The ANS values were elevated in a dose dependent manner across group, reaching statistical significance for group 5 males on day 29. This effect could be test article related but was not observed in female rats in the same group. AMO values were elevated for group 4 male rats at day 29 but for females or males in any other group.

Clinical chemistry: See above for sample collection.

- Dose-dependent increases in total protein are reported for both male and female rats on day 15, with respect to control. These values remained elevated on day 29 but were comparable to control by day 57. For male rats on day 15: increases of 14%, 20%, 28% and 33% for groups 2, 3, 4 and 5, respectively. For female rats on day 15: increased of 12%, 24%, 27% and 36% for groups 2, 3, 4 and 5, respectively.
- Significant, dose-related increases in triglycerides are ported for both male and female rats in groups 3, 4 and 5 at day 15, relative to control and baseline values. These effects persisted through day 29 and were absent on day 57. Males, day 15, relative to control: up 58%, 108% and 138% for groups 3, 4 and 5, respectively. Females, day 15, relative to control: up 52% (NS), 93% and 153%, for groups 3, 4 and 5, respectively.
- Significant increases in cholesterol are reported for both males and females in day 15, relative to control and baseline values. The effect persisted through day 29 was not observed on day 57. For male rats, day 15, relative to control: up 50%, 72%, 92% and 114% for groups 2, 3, 4 and 5, respectively. For female rats, day 15, relative to control: up 18%, 37%, 47% for groups 3, 4 and 5, respectively.
- Significant increases in albumin and globulin are reported for both males and females by day 15, relative to control and baseline values. These values remained elevated on day 29 and returned to control levels by day 57. For male rats, on day 15, relative to control: up 6%, 9%, 15% and 18% and globulin up 23%, 32%, 42% and 48% for groups 2, 3, 4 and 5, respectively. For female rats, day 15, relative to control: globulin up 16%, 34%, 41% and 56% and albumin up 9%, 14%, 14% and 17% for groups 2, 3, 4 and 5, respectively. Concomitant deceased in A/G ratio id also reported for these groups.
- Significant changes in electrolytes are reported for both males and females, including calcium, phosphate, chloride, potassium, and sodium.
  Calcium levels were increased (6%-10%) in all treated dose groups on Days 15 and 29 but not on Day 57 (except 300 ug/kg/day dose group). Slight decreases were observed in chloride levels (decreased 3% to 8% for males in groups 3, 4

and 5) on days 15 and 29. For female rats, chloride levels were decreased 2% to 9% for female rats in all treated dose groups on days 15, 29, and 57. Slight decreases in phosphorus levels (decreased 7% to 13%) were observed in groups 4 and 5 on day 15 in male rats but not in female rats. On Day 57, PHO levels were elevated 2 1% and 24% for groups 4 and 5, respectively, in males and elevated 37% and 52%for groups 4 and 5, respectively, in females. Slight increases in potassium (increased 11% to 13% for groups 3, 4 and 5) were observed on Day 29 in female rats but not in male rats. POT remained elevated (increased 15% for group 5) in female rats on Day 57. These changes are generally small and within the historical ranges for this species so biological significance is not clear. However, the electrolyte changes correlate with histological findings in the kidneys of treated rats. Therefore, a test article relationship is possible.

- Statistically significant decreases in liver enzymes (ALP, ALT, AST) are reported for both male and female rats on days 15 and 29. For the higher dose groups, these effects were apparent only on day 29. Values were comparable to control on day 57. The sponsor states that decreased levels of these enzymes is not biologically significant. However, histopathology shows dose dependent increases in centrolobular apoptosis in the liver.
- Statistically significant increases on creatinine are reported for both males and females. On day 15, CRE levels were in male rats for groups 3 and 4. On day 29 these values were increased for male rats in groups 3, 4 and 5. The values returned to control levels on day 57. Although small, these findings appear to be dose-dependent. For female rats, no changes are reported except on day 57 for group 4. Although the changes are not consistent across genders, there may be a test article effect especially in light of the gross and microscopic kidney changes reported. See below.
- BUN values were decreased for females on day 57 in groups 4 and 5.

Urinalysis: Not done.

Gross pathology: 10/sex/group sacrificed on day 29, 5/sex/group sacrificed on day 57.

Organ weights (specify organs weighed if not in histopath table): Organs from 10/sex/group taken on day 29, 5/sex/group on day 57.

- Significantly increased liver weights are reported for all groups on day 29 with a dose dependent relationship (up approximately 30% for group 5 males and females)
- Reduced thymus weight on day 29 for groups 4 and 5 (down 70% for group 5 males). For females, group 4 and 5 thymus weights were reduced approximately 30% on day 29. thymus weights for male rats were not different from control on day 57. Increased thymus weight is reported for female rats on day 57 with a dose dependent relationship (significant for group 5).
- Lung weight was increased in group 5 females on day 29. This finding was not reported for male rats. Lung weights for group 5 females were not different from control on day 57.

- Kidney weight was increased in female rats from groups 3, 4 and 5 on day 29 (approximately 27% for group 5) but was not reported for male rats. Kidney weight for group 4 and 5 females was also reported on day 57, although the difference (approximately 20%) from control was smaller than at day 29.
- Ovary weight was decreased for group 5 females on day 29 but no differences are reported for day 57.
-

Histopathology: Adequate Battery: yes ( ), no ( )—explain
Peer review: ( ), no ( )

- Adrenal glands (pair)
- Aorta
- Bone marrow smear (femur)
- Bone and marrow, contralateral femur with
- femorotibial joint
- Buccal cavity
- Brain (fore-, mid-, and hindbrain)
- Cecum
- Cervix
- Colon
- Duodenum
- Esophagus
- Eyes, with optic nerve
- Cross lesions (including tissue masses and abnormal regional lymph nodes)
- Heart
- Ileum
- Injection site (tail)
- jejunum
- Kidneys
- Larynx (pharynx examined at necropsy)
- Liver
- Lungs with bronchi
- Lymph nodes (mandibular and mesenteric)
- Mammary gland (to include nipple and surrounding tissue)
- Ovaries and oviduct
- Pancreas
- Pituitary gland
- Prostate, Rectum, Salivary gland
- Sciatic nerve
- Seminal vesicle
- Skeletal muscle
- skin (abdominal; taken with mammary)
- Spinal cord,

- Spleen, Stomach
- Testes
- Thymus,
- Thyroid and,
- Tongue,
- Trachea,
- Urinary bladder,
- Uterus,
- Vagina

Histopathology Results: The following dose-related effects are reported:

- Goblet cell hyperplasia of the GI tract including cecum, colon, duodenum, ileum, jejunum, rectum. 100% of rats of both sexes were affected for group 5, up to 100% of group 4 rats of both sexes for group 4, and up to 70% of group 3. These findings were not observed on day 57.
- Stomach: Dilatation is reported for 100% of male and female rats in group 5, up to 89% of rats of both sexes from group 4. Hyperkeratosis and hypertrophy are reported for 100% of both male and female rats in group 5 and up to 100% of both sexes from group 4. By day 57, these findings were not present for female rats of any group but persisted in 40% of male rats from group 5.
- Injection site reactions are reported for male rats in the higher dose groups but not control. This finding is not reported for female rats in any dose group. Biological significance is not clear.
- Lymphoid depletion is reported for the thymus gland of 80% of male rats in group 5 and 67% from group 4, day 29. For female rats the finding is less pronounced, affecting 105 in each of groups 4 and 5. These findings are not reported for day 57.
- Increased follicle size and number in the thyroid is reported for males and females in groups 4 and 5 on day 29. For males the increase was seen in 90% of group 5 and 44% for group 4. For females, 40% of group 5 and 10% of group 4. These findings were still apparent on day 57 and had increased to 60% of female rats for group 5.
- Urinary bladder hyperplasia is reported for 100% of both males and females from group 5, and up to 90% from group 4 on day 29. On day 57, this finding is not reported for male rats and was presence for 25% of females in group 5.
- No effect of treatment on ovary histology is reported.
- Dilatation of the uterus is reported on day 29 for all treated groups (10%, 33%, 10% and 20% for groups 2, 3, 4, and 5, respectively). This effect is reported at similar rates for all groups on day 57, including control. Biological significance is unknown.

Toxicokinetics:

Samples for TK were taken according to the schedule illustrated below (table provided by the sponsor)

Time post-dose	Subset[1]	No. Animals for PK Serum Sampling		
		Main Study Groups[2]	Recovery Groups[3]	Total Sampled
5 minutes	A	2♂/2♀	1♂/1♀	3♂/3♀
1 hour	B	2♂/2♀	1♂/1♀	3♂/3♀
4 hours	C	2♂/2♀	1♂/1♀	3♂/3♀
8 hours	D	2♂/2♀	1♂/1♀	3♂/3♀
24 hours	E	2♂/2♀	1♂/1♀	3♂/3♀

[1]Five different subsets of animals in each dose group, including the controls, were sampled as above. The same animals in each subset were sampled at the same timepoints on each of the sampling days.
[2]5/sex/group started treatment on 02/28/95 (Day 1) and the remaining 5/sex/group started treatment on 03/01/95 (Day 1 for this subgroup).
[3]Started treatment on 02/28/95 (Day 1)

Anti-drug antibodies were detected only in the 300 ug/kg group at approximately 30 days into the study. No other TK data are provided.

Study title: A 4-week repeated dose toxicity study of recombinant-human keratinocyte growth factor (r-HuKGFd23) administered subcutaneously to rhesus monkeys followed by a 4-week recovery period

Key study findings:
The purpose of this study was to evaluate the safety of palifermin when administered subcutaneously to rhesus monkeys at dose levels of 1, 10, 30, 100 and 300 ug/kg/day for a period of 4 weeks, and to assess the reversibility of any toxic changes during a 4-week recovery period. No deaths were noted during the dosing or the recovery periods. No test article-related abnormalities were noted in clinical signs, food consumption, body weight, ophthalmology, electrocardiogram, urinalysis, hematology or serum biochemistry.

Numerous gross and microscopic fingings that appeared to be dose dependent were reported. In the gross necropsy examination, thickening of the oral mucosa was present in the 100 ug/kg group (males) and the 300 ug/kg group (both sexes), and in the 300 ug/kg group, thickening (1 female) or villous thickening (2 males, 1 female) of the esophageal mucosa was present.
Microscopic changes in the 300 ug/kg group included acanthosis in the skin (scalp of both sexes, skin overlying the mammary gland of 2 males and 2 females, injection site of 2 males and 1 female, gluteal of 2 males) and the mucosa (esophagus and buccal mucosa of both sexes, tongue of all males and 2 females, anus of 1 female). Also in the 300 ug/kg group, vesicle formation (all males and 1 female) and eosinophil infiltration (all males and 2 females) were noted in the esophagus, and hyperkeratosis was also noted in the scalp (2 males). Involution of the thymus was observed in both sexes of the 300

ug/kg group, rated moderate or marked, as well as a high absolute and relative weight of the submandibular gland. In the 100 ug/kg group, acanthosis was present in the mucosa (esophagus of 1 male and 1 female, tongue of 1 male, buccal mucosa of 1 male). and the skin overlying the mammary gland (2 males) and the injection site (1 male). The findings of the 100 ug/kg group were of lower incidence and severity than those of the 300 ug/kg group.

All test article-related findings present at the end of the dosing period were absent in the recovery group.

Toxicokinetic evaluation confirmed exposure to the study drug based on detectable serum concentrations and/or anit-drug antibody formation. The study drug was rapidly absorbed into the systemic circulation, with peak concentrations appearing at 1 hour. No gender-difference in drug disposition was observed. Repeat dosing resulted in an increase in serum concentrations observed at all dose-groups beyond Day 7 of the study. The observation was consistent with the development of circulating antibodies, which could decrease the systemic clearance of palifermin.

From these results and under these conditions, the non-effect dose level of palifermin was considered to be 30 ug/kg. The histologic changes present were consistent with the pharmacological action of the test article.

Study no.: 39-26
Volume #, and page #:
Conducting laboratory and location:
Date of study initiation: 4/4/1995
GLP compliance: YES
QA report: yes (X) no ( ) palifermin lot # 01195A5A, placebo lot # 02085B5
Drug, lot #, and % purity: 01195A5A.

Methods

Doses: 0, 1, 10, 30, 100, 300 ug/kg/day, once daily for 4 week (total 28 doses)

Species/strain: rhesus monkey

Number/sex/group or time point (main study): See table below, 18 animals of each sex were used for the main study, 8 animals of each sex were used for the recovery phase of the study.

Route, formulation, volume, and infusion rate: subcutaneous

Age: males: 4-8 years

Weight: males: 5-9 kg, females: 3-7 kg

Unique study design or methodology (if any):

Animals will be randomized into groups as follows:

Group Number	Dose Level (µg/kg/day)	Dose Conc. (mg/ml)	Dose Volume (ml/kg/day)	Number of Animals** Male (animal number)	Female (animal number)
1	0 *	0	0.06	3+2 (1 ~ 5)	3+2 (6 ~ 10)
2	1	0.1	0.01	3 (11 ~ 13)	3 (14 ~ 16)
3	10	1	0.01	3 (17 ~ 19)	3 (20 ~ 22)
4	30	1	0.03	3+2 (23 ~ 27)	3+2 (28 ~ 32)
5	100	5	0.02	3+2 (33 ~ 37)	3+2 (38 ~ 42)
6	300	5	0.06	3+2 (43 ~ 47)	3+2 (48 ~ 52)

* The excipient control will be administered in the same manner as the test article.

** Recovery Animals: Animal nos. 4, 5, 9, 10, 26, 27, 31, 32, 36, 37, 41, 42, 46, 47, 51, 52

Results

Mortality: All animals survived to scheduled necropsy.

Clinical signs: Cageside observations were made at least three times daily during the dosing period. During the recovery period observations were made once daily.

- No test article related clinical signs are reported.

Body weights: Data collected once prior to study initiation, then once per week thereafter with final weight taken on necropsy day.

Food consumption: Data collected daily during the dosing period and weekly thereafter.

- No effect of test article administration on food consumption are reported.

Ophthalmoscopy: Exam performed once prior to study initiation, wk 4 of the dosing period and week 4 of the recovery period.

- No test article related effects are reported.

EKG: Exam performed on all animals once prior to study initiation, week 4 of the dosing period and week 4 of the recovery period.

- No effects of test article on EKG results are reported.

Hematology: Testing battery performed prior to dosing on days 0, 6, 13 and 27 during the dosing period and days 6 and 27 of the recovery period.

- erythrocyte count (RBC : ⊏] ⊇ detection method)
- leukocyte count (WBC : ⊏] detection method) isolated changes in white cell populations are reported during the dosing period by these findings are sporadic and isolated and most likely not related to test article administration.
- Lymphocyte counts: reduced in males in group on day 6 relative to control

- Monocyte counts: Ratios increased for female rats in groups 2, 3, 5 and 6 on day 27.
- APTT
- platelet count (electric resistance detection method): PLC values were elevated during the dosing period relative to control for females in the 3 and 4. This effect is inconsistent and does not appear to be dose related.
- hematocrit value ([redacted] detection method)
- hemoglobin concentration ([redacted] method)
- mean corpuscular volume (MCV)
- mean corpuscular hemoglobin (MCH)
- mean corpuscular hemoglobin concentration (MCHC)
- reticulocytes: Reduced by 60% in groups 5 and 6 relative to control.

The findings noted above as statistically significant appeared to be transitory and somewhat sporadic. In addition, they fell for the most part within historical normal range for this species. Biological significance is not clear. No effects were noted at the end of the recovery period.

Clinical chemistry: Analyses performed on the same schedule as hematology analyses (see above).

- Aspartate aminouansferase (AST: [redacted] method)
- Alanine aminotransferase (ALT: [redacted] method)
- Alkaline phosphatase (ALP: [redacted] method)
- Lactate dehydrogenase (LDH: [redacted] assay)
- γ-Glutamyl transpeptidase (γ-GTP: [redacted] method)
- Total bilirubin ([redacted] method)
- Total protein ([redacted] method)
- Albumin ([redacted] method)
- Total cholesterol ([redacted] method)
- Triglyceride ([redacted] method)
- Glucose (GlcK [redacted] method)
- Blood urea nitrogen (BUN: [redacted] method)
- Creatinine (Jaffe method)
- Uric acid (Uricase - [redacted] method)
- Inorganic phosphorus ([redacted] direct method)
- Calcium (Ca2+ : [redacted] method)
- Sodium (Na+ [redacted] method)
- Potassium (K+ : [redacted] method)
- Chloride (Cl- : [redacted] method)

Although some statistically significant changes were recorded during the dosing phase of the study, they did not appear to be related to the test article administration. These findings appeared to be transient and inconsistent. No statistically significant test article related changes are reported for serum chemistry parameters.

Urinalysis: Exam performed once prior to study initiation, week 4 of the dosing period and week 4 of the recovery period. The following analyses will be performed: (Tables supplied by the sponsor)

- No dose dependent effects on urinalysis results are reported. (a small decrease in specific gravity was observed for female rats in group 4 but this finding does not appear to be dose dependent as it did not occur in higher dose groups or in the males in group 4.)

Parameter	Method	Apparatus
Fresh Urine		
Color	Visual	
pH	Test paper	
Glucose	Test paper	
Ketone body	Test paper	
Bilirubin	Test paper	
Urine occult blood	Test paper	
Urobilinogen	Test paper	
Urine sediments	Microscopic examination of urine sediments stained with Sternheimer-Malbin stain after centrifugation (— r.p.m., 5 minutes).	

18- hour excreted urine analyses will be performed: urine volume, specific gravity, protein, creatinine, sodium, potassium and chloride.

Gross pathology: Animals were euthanized on the day after the last dose of the last day of the recovery period. Organs and tissues were examined at necropsy for gross lesions and deformities.

- In animals sacrificed on day 29, an apparent test article related thickening of mucosa in the oral cavity of 2 of 18 males in group 5 and 100% of all males and females in group 6 was observed. (Rated as slight to moderate)
- Villous thickening of esophageal mucosa was noted for two males and one female in group 6. Rated as slight to moderate.
- Thickening of mucosa of the esophagus was noted in one female in group 6. Rated as slight.

Organ weights (specify organs weighed if not in histopath table):
The following organs were removed and weighed:

- heart	- pituitary	- brain (with cerebellum and brain stem)
- pancreas	- liver	- thyroids (with parathyroid)
- thymus	- spleen	- submandibular glands
- epididymides	- adrenals	- lungs (with bronchus)
- ovaries	- uterus	- kidneys
- prostate	- seminal vesicles	- testes

Day 29:

- Increased submandibular gland weight for for males and females in group 6. (Relative to control: 198% for males and 109% for females.)
- High absolute pituitary weights were noted for females in group 6.
- Low liver weights relative to control were noted for males in groups 2 and 3.
- Low lung weight relative to control were noted for females in group 6.

No differences in organ weight were observed at the end of the recovery period.

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes ( ), no ( )

The following tissues were retained and examined microscopically:
heart, spleen, liver, lungs, trachea, esophagus, stomach (fundus, pylorus), kidneys, gall bladder, uterus, ovaries, testes, epididymides, prostate, thyroids, adrenals, cerebrum, brain stem, sciatic nerve, lacrimal gland, buccal mucosa, injection sites, thoracic aorta, thymus, submandibular lymph node, bronchus, tongue, pancreas, small intestine (duodenum, jejunum, ileum), large intestine (cecum, colon, rectum), anus, vagina, urinary bladder, penis (epithelial lining of ureter), seminal vesicles, pituitary, parathyroid (if possible), quadriceps muscle of thigh, cerebellum, lumbar spinal cord, skin (gluteal, scalp), eye (with optic nerve), submandibular gland, mesenteric lymph node, bones and bone marrow (femur: bone marrow was observed as specimens without decalcifying after fixation, and bone was observed as decalcified specimens), mammary gland (including a cross section of the nipple and underlying breast, and two random samples of breast parenchyma other than that near the nipple)

- Acanthosis: Observed in group 6 for all animals in the esophagus, scalp and buccal mucosa, all males and 2 females in the tongue, in the skin of the mammary gland in 2 males and 2 females, in the skin at the injection site for 2 males and 1 female, in the gluteal skin for 2 males and 1 female, at the skin of the anus for one female. The severity of these findings ranges from slight to marked.
- Involution of the thymus gland was noted for all animals in group 6 and rated as moderate to marked.
- Slight hyperkeratosis was observed in the scalp for 2 males from group 6.
- Esophageal epithelium showed slight eosinophil infiltration into the lamina propria in all males and 1 female of group 6.
- Acanthosis was observed at a lower rate and severity in group 5 in similar regions as described for group 6.

- Other histological findings were considered to be not dose related or were observation commonly seen in this species so could not be attributed to test article administration.

Recovery animal histopathology: No test article related changes were observed at the end of the recovery period.

Toxicokinetics: Blood samples for TK were collected from 3 animals/sex/group. Samples were taken once immediately prior to the initiation of dosing; at 0, 1, 2, 4, 8 and 24 hours after administration on Days 0 and 27; 1 and 24 hours after administration on Days 6 and 18; and on Days 6 and 13 of the recovery period.
Average serum drug concentrations were below the level of detection for groups 1, 2 and 3. Peak and overall exposure between 4 and 5 were dose proportional. Lower doses could not be evaluated for proportionality due to the limit of quantitation.

TK results are illustrated in the table below, supplied by the sponsor:

Table 9-2 Toxicokinetics

Serum concentrations and pharmacokinetics parameters (mean±SD) of r-HuKGF in male and female Rhesus monkeys following subcutaneous administration at 300 µg/kg on Day 0 of study

Time post-dose (hours)	Serum concentrations of r-HuKGF (ng/mL)			
	Male + Female (n=6)	Male (n=3)	Female (n=3)	
Pre-dose	0.00±0.00	0.00±0.00	0.00±0.00	
1	28.7±11.0	23.8±12.8	33.6±8.18	
2	14.9±4.96	11.4±2.72	18.5±3.98	
4	12.3±4.45	9.99±3.84	14.6±4.31	
8	8.55±2.68	7.85±3.06	9.25±2.66	
24	3.77±1.43	3.33±1.83	4.21±1.08	
Time post-dose (hours)	Pharmacokinetic parameters of r-HuKGF			ANOVA (Male vs. Female)
t_{max} (h)	1.00±0.00	1.00±0.00	1.00±0.00	NS[2]
C_{max} (ng/mL)	28.7±11.0	23.8±12.8	33.6±8.18	NS
AUC[1] (ng•h/mL)	204±50.6	176±43.5	231±46.8	NS

[1] AUC was calculated by the trapezoidal method from 0 to 24 hours post-dose
[2] NS : No significant difference ($p<0.05$)

Other:
Detection of anti-drug antibody levels:

Blood samples for antibody titers were collected from all the animals once prior to the initiation of dosing, 24 hours after administration on Days 0, 13 and 27, and on Day 27 of the recovery period. Anti-drug antibodies were detected as early as SD13 in 100% of animals in group6.

Study title: A preliminary 1-week repeated dose toxicity study of recombinant human keratinocyte growth factor (r-HuKGFd23) administered subcutaneously to cynomolgus monkeys

Key study findings:
This study was intended as a preliminary study to evaluate the toxicity of daily subcutaneous administration of palifermin to cynomolgus monkeys for one wee. No control group was included and no recovery data was collected. The study was not performed under GLP conditions. Therefore, only a brief review of this study was performed.

During the administration period, none of the animals died in any group. In the 30 and 300 ug/kg treated animals, no abnormalities were observed in the clinical signs; however, in the 1000 ug/kg animal flushing (reddening skin) was observed over the face and lower belly, including the scrotum. No abnormalities were noted at any dose level, in food consumption, body weight, urinalysis, hematology or serum biochemistry.

No abnormalities were observed in the gross pathology examination or organ weights of any palifermin treated animals.
In microscopic evaluation, acanthosis in the scalp skin and buccal mucosa was observed in the 300 and 1000 ug/kg treated animals, and hyperkeratosis in the scalp skin was also observed of the 1000 ug/kg treated animals.
The NOAEL was considered to be 30 ug/kg under the conditions of this study.

Study no.: 39-26-10
Volume #, and page #:
Conducting laboratory and location:
Date of study initiation: 2/15/1995
GLP compliance: NO
QA report: yes ( ) no ( )
Drug, lot #, and % purity: palifermin lot #01195A5A

Methods

Doses: 30, 300 or 1000 ug/kg
Species/strain: male cynomolgus monkeys
Number/sex/group or time point (main study): 3 males used total
Route, formulation, volume, and infusion rate:
Satellite groups used for toxicokinetics or recovery: NA
Age: 3-4 years
Weight: 2-4 kg
Sampling times:
Unique study design or methodology (if any):

Observations and times and Results

Mortality: All animals survived to scheduled sacrifice.

Clinical signs: No test article-related effects are reported except flushing of the skin over the face, and lower belly for the one animals receiving 1000 ug/kg.

Body weights: No effects on body weight are reported.

Food consumption: No effects on food consumption are reported.

Ophthalmoscopy: Not Done.

EKG: Not Done.

Hematology: No treatment related effects are reported.

Clinical chemistry: No treatment related effects are reported.

Urinalysis: No treatment related effects are reported.

Gross pathology: No treatment related effects are reported.

Organ weights (specify organs weighed if not in histopath table): No treatment related effects are reported.
Organs weighed: heart, lung, kidney, liver and spleen
Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes ( ), no (X)
The only test article related finding reported is acanthosis of the skin (scalp) and buccal mucosa seen in animals treated with either 300 ug/kg or 1000ug/kg. Other findings are commonly observed in this species so could not be attributed to test article administration.

Toxicokinetics: Not Done.

Other: NA

Study title: A 4-week repeated dose toxicity study of recombinant-human keratinocyte growth factor (r-HuKGFd23) administered intravenously to rhesus monkeys followed by a 4-week recovery period.

Key study findings:
The purpose of this study was to evaluate the potential toxic effects of IV administration of palifermin to rhesus monkeys. Six groups of monkeys received daily iv infusions of palifermin in doses of 0, 1, 10, 30, 100 and 300 ug/kg. Two animals from the control, 30,

100 and 300 ug /kg groups survived 4 additional weeks after the final dose to assess potential for recovery of any identified toxicities.
One male animal from the high dose group was euthanized moribund early in the dosing phase of the study (SD11) after showing negative clinical signs (low food consumption, sedation, hypothermia, prone position advancing to coma) after SD6. Examination of that animal indicated that study drug related changes in the GI system caused reduced food consumption followed by deteriorating body condition. This was the only death on study.
Hematology parameters revealed several study drug related effects. Animals in groups 5 and 6 showed a mild decrease in the RBC count, HCT and HGB values. The HCT and HGB values in the 30 ug/kg group were also reduced. Reticulocye counts were not affected.

The serum biochemistry examination revealed low values in the total cholesterol, Ca+ concentration, total protein and albumin in the 300 ug/kg group. In the 100 and 300 ug/kg groups, a high alpha-l globulin level, or a tendency, and low inorganic phosphate levels were also noted. These changes were rated as mild and not accompanied by changes of the serum biochemical or pathological parameters indicating functional disturbances of liver or kidneys. In the determination of serum amylase and lipase, mean serum amylase levels were elevated, consistent with increased submandibular gland weights and histological changes, and considered to be a result of the pharmacological action of the study drug.

In the gross necropsy examination, slight or moderate thickening or villous thickening of the buccal mucosa in the oral cavity, tongue or esophagus was present in the 30,100 and 300 ug/kg groups, and slight or moderate squama (scale) of the skin was also present in the 300 ug/kg group. High absolute and relative weights of the submandibular gland were observed in the 100 and 300 ug/kg groups. In the 300 ug/kg group, this was accompanied by very slight hypertrophy of the acinar cells in the gland. A pool of yellow transparent fluid (approximately 8 - 30 ml) was found in the thoracic and/or abdominal cavity in the 100 and 300 ug/kg groups.

The microscopic analysis revealed the following findings that were thought to be related to the pharmacological action of the test article: very slight to marked acanthosis in the mucosa of the tongue, buccal mucosa, esophagus, scalp skin and the injection site skin of the 30, 100 and 300 ug/kg groups and in the skin overlying the mammary gland and the gluteal skin of the 300 ug/kg group; very slight vesicle formation in the mucosal epithelia in the tongue of the 300 ug/kg group and in the esophagus of the 100 and 300 ug/kg groups; very slight or slight hyperkeratosis in the buccal mucosa, the skin overlying the mammary gland, the gluteal and scalp skin of the 300 ug/kg group, and the skin of the injection site of the 30, 100 and 300 ug/kg groups; very slight to moderate hyperplasia of the mucous cells in the stomach of the 30, 100 and 300 ug/kg groups; and very slight to moderate goblet cell hyperplasia in the duodenum, ileum, cecum, colon and rectum of the 30,100 and 300 ug/kg groups and in the jejunum of the 100 and 300 ,ug/kg groups. Marked involution of the thymus was present in the 10, 30, 100 and 300 ug/kg groups, and it was accompanied by low absolute and relative thymus weights in the females of these groups.

No abnormalities related with the test article treatment were noted in the 1 ug/kg group during the dosing period.

At the end of the recovery period, villous thickening of the tongue mucosa (slight), hyperkeratosis of the tongue (slight) and goblet cell hyperplasia (very slight) in the duodenum were noted histologically in 1 female out of 5 animals of the 300 ug/kg group. All of the other findings noted during the dosing period were absent at the end of the recovery period, suggesting reversibility.

The toxicokinetic analysis showed that serum concentration time profiles of palifermin displayed an initial rapid decline followed by a "hump" or plateau between 1 and 3 hours post-dose. The incidence of anti-drug antibodies increased with dose and dosing duration. The presence of anti-drug antibodies appeared to increase palifermin serum concentration. The pharmacokinetics appeared dose-independent throughout the dosing period. However, interpretation after Day 13 was confounded by the presence of antibodies. Terminal half-life was approximately 3.5 hours on Days 0 and 27. There was no consistent difference between the serum concentration-time profiles for male and female monkeys.

The incidence of anti-drug antibodies appeared to increase with increasing dose from 10 ug/kg to 300 ug/kg palifermin. The apparent antibody response was not associated with any observable adverse events throughout the study.
From the results under these conditions, the no observed adverse effect level (NOAEL) of palifermin was considered to be 1 ug/kg, since the observations were similar to the control in this study.

Study no.: — 39-27 (Amgen study number T-95-KGF-005)
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: 3/22/1995
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Palifermin lot no. 01195A5A, placebo lot no. 02085B5

Methods

Doses: 0, 1, 10, 30, 100 or 300 ug/kg
Species/strain: Rhesus monkeys
Number/sex/group or time point (main study): 3/sex/group for the dosing phase, 2/sex/group for groups 1, 4, 5 and 6 for the recovery phase of the study.
Route, formulation, volume, and infusion rate: IV infusion daily for 4 weeks
Satellite groups used for toxicokinetics or recovery: Yes. See below.
Age: 4-8 years
Weight: males: 5-9 kg, females: 3-7 kg
Unique study design or methodology (if any):
Study design is illustrated in the table below, supplied by the sponsor:

Animals will be randomized into groups as follows:

Group Number	Dose Level (µg/kg/day)	Dose Conc (mg/ml)	Dose Volume (ml/kg/day)	Number of Animals** Male (animal number)	Female (animal number)
1	0 *	0	0.06	3+2 (1 – 5)	3+2 (6 – 10)
2	1	0.1	0.01	3 (11 – 13)	3 (14 – 16)
3	10	1	0.01	3 (17 – 19)	3 (20 – 22)
4	30	1	0.03	3+2 (23 – 27)	3+2 (28 – 32)
5	100	5	0.02	3+2 (33 – 37)	3+2 (38 – 42)
6	300	5	0.06	3+2 (43 – 47)	3+2 (48 – 52)

* The excipient control will be administered in the same manner as the test article

** Recovery Animals: Animal nos 4, 5, 9, 10, 26, 27, 31, 32, 36, 37, 41, 42, 46, 47, 51, 52

Observations and times Results

Mortality: One male animal in 6 was euthanized moribund on day 11 if the study. The effects were first noted on day 6 and included sedation, hypothermia, prone position and advanced to coma.

The serum biochemistry examination of this animal at sacrifice revealed high BUN, creatinine and uric acid levels, high inorganic phosphate, sodium and Cl concentrations and a low glucose level.

The pathology examination showed, acanthosis present in the tongue (marked), esophagus (marked), buccal mucosa (very slight) and skin (scalp, slight); and slight or moderate goblet cell hyperplasia was present in the small and large intestines (duodenum, ileum, cecum, colon and rectum). It was concluded that, the pathological changes in the digestive system caused the decrease of food consumption, which was followed by a gradually deteriorating body condition. Other findings noted in the thymus (severe) and adrenals (slight) were thought to be secondary changes relating to the deteriorating body condition. The slight dilatation of the stomach and moderate atrophy of chief cells in the stomach were also noted. Slight hypocellularity of the sternal bone marrow was also noted in this animal.

Clinical signs: Performed at least 3 times per day during the dosing period: prior to dosing, immediately after and 2-3 hours post-dosing.

- Surviving animals in the high dose group (grp 6) showed swollen lips of varying degrees and scaling of the skin (4 males and 2 of 5 females). Skin scaling was reported for 2 males in group 6 recovery group but resolved by the end of the recovery phase.
- Increased salivation (slight to severe) was noted for 2 of 4 males. Resolved by the end of the recovery phase.
- For the 100ug/kg group, 1 male of 5 showed a swollen lip (slight) during the dosing period.

- No clinical signs related to the study drug are reported for groups 1-4.

Body weights: Data collected once prior to study initiation, once per week thereafter and prior to necropsy.

- Slight decreases in mean body weight are reported for males and females in group 6 during weeks 2 and 3 of the dosing phase. At week 4, the body weight reduction relative to prestudy weight for males and females in group 6 were 1.056 kg and 0.416 kg, respectively. No test article effects were noted at the end of the recovery phase.
- No test article related effects are reported for groups 1-5.

Food consumption: Calculated daily.

- Food consumption was severely reduced on day 5 and later for the animal that was sacrificed moribund on day 11.
- Decreases in food consumption rated moderate to severe were noted for all group 6 animals during the middle of the dosing phase of the study (Days 6-20 for males, days 8-11 for females). No effects were noted for any other groups.
- No effects related to the test article were noted during the recovery phase of the study.

Ophthalmoscopy: Data collected once prior to study initiation, and once at week 4 of the dosing phase. If any abnormalities were noted, examination was performed at the end of the recovery phase.

- No test article effects were noted during the dosing phase of the study so this test was not performed during the recovery phase.

EKG: Data collected once prior to study initiation, once at week 4 of the dosing phase. If any findings were noted, the exam was to be repeated at the end of the recovery period.

- No test article related effects were noted during the dosing phase so this test was not performed during the recovery phase.

Hematology: Data collected prior to study initiation and on day 0, 6, 13 and 27 of the dosing phase, days 6 and 27 of the recovery phase.

- erythrocyte count (RBC : [redacted] detection method)
- leukocyte count (WBC : [redacted] detection method)
- platelet count (PLC, [redacted] detection method)
- hematocrit value (HCT, [redacted] detection method)
- hemoglobin concentration (HGB, [redacted] method)
- mean corpuscular volume (MCV)
- mean corpuscular hemoglobin (MCH)
- mean corpuscular hemoglobin concentration (MCHC)
- Reticulocyte count and differential leukocyte count
- Coagulation

Clinical chemistry: Data collected at the same time points as above for hematology.

- Aspartate aminotransferase (AST: [ ] method)
- Alanine aminotransferase (ALT: [ ] method)
- Alkaline phosphatase (ALP: [ ] method)
- Lactate dehydrogenase (LDH: [ ] method)
- γ-Glutamyl transpeptidase (γ -GTP: [ ] method)
- Total bilirubin [ ] method)
- Total protein ([ ] method)
- Albumin ([method)
- Total cholesterol ([ ] method)
- Triglyceride ([ ] method)
- Glucose (GlcK . [ ] method)
- Blood urea nitrogen (BUN: [ ] method)
- Creatinine (— method)
- Uric acid ([ ] method)
- Inorganic phosphorus ([ ] direct method)
- Calcium (Ca : — method)
- Sodium (Na : — method)
- Potassium (K : — method)
- Chloride (Cl : [ ] method)
- Protein fractions, including A/G ratio

Urinalysis: Data collected once prior to study initiation, once at week4 of the dosing phase and at week 4 of the recovery phase. The urine was collected 2 hours prior to dosing, using a metabolic cage, and used for color, sedimentation rate, pH, glucose, ketone body, bilirubin, occult blood and urobilinogen analyses. 18 hour samples were measured for volume and specific gravity, sodium, chloride, and creatinine, determination.

- Increased volume was noted for all treatment groups with respect to prestudy levels and control. Volume returned to prestudy levels by the end of recovery period. Increases ranged 30%-40% but did not have a clear does relationship among groups 2-6. Increased volume in female animals from groups 2-6 is also observed. However, female control animals also showed an increase in volume during the dosing period. This increase may be due to increase fluid from the drug infusions.
- Creatinine levels were reduced for groups 5 and 6 at the end of the dosing phase. Values were higher but still reduced compared to pre-study and control levels at the end of the recovery phase.
- Sodium was reduced in group 5 and 6 male animals with respect to prestudy levels and control values.
- Chloride levels were reduced at the end of the dosing phase (relative to pre-study values) for group 6 males but returned to normal limits by the end of the recovery phase. Large interanimal variability is noted for these values. Biological significance is not clear.

- Slightly increased mean protein level is reported for group 6 females during the dosing phase but returned to baseline by the end of the recovery phase.

Gross pathology:

- The following gross findings are reported for the animals euthanized early in a moribund condition: thickening of the buccal mucosa, villous thickening of the tongue mucosa, thickening of esophageal mucosa, emaciation (not eating) small spleen size, thymic atrophy, empty stomach and small intestine, bilateral soft white testes, bilateral enlargement of adrenal glands.

The following findings are reported for animals euthanized after the 4-week dosing phase:

- Emaciation was noted for 2 females in group 6.
- Pool of pale yellow fluid found in the thoracic cavity of one male in group6
- Pool of yellow fluid in the abdominal cavities of 1 male in group 5, 1 male and 2 females in group 6.
- Thickening of the buccal mucosa of all males and females in groups 5 and 6 and 1 male in group 4.
- Thickening of the tongue mucosa in 1 male and 2 females of group 5, 1 male and 3 females of group 6.
- Thickening of esophageal mucosa of 1 male from group 4, 3 males and 2 females of group 5 and 2 males and 3 females from group 6.
- Scaling of the skin was noted in 3 females in group 6.

By the end of the recovery period only villous thickening of the tongue mucosa was noted, suggesting recovery potential.

Organ weights (specify organs weighed if not in histopath table):
The following organs were removed at necropsy and weighed:

Heart, pancreas, thymus, epidiymides, ovaries, pituitary, liver, spleen, adrenals, uterus, brain with brainstem and cerebellum, thyroid with parathyroid, submandibular glands, lungs with bronchus, kidneys, prostate, testes, seminal vesicles.

- Increased organ weight for the submandibular gland is reported for males and females in groups 5 and 6. Not reported at the end of the recovery period.
- Reduced thymus weight is reported for female animals in groups 2-6. (Values reduced up to 83% for males and females in group 6. Definitely test article related. No group differences noted at the end of the recovery period.
- Increased absolute spleen weight noted for one female in group 6.
- Increased absolute thyroid weight for 1 female in group 6.
- No differences were noted for the animals in group 2.
- No differences in any organ weight was noted for animals sacrifieced at the end of the recovery phase.

Histopathology: Adequate Battery: yes (X), no ( )—explain

Peer review: yes ( ), no ( )

A full panel of tissues was retained and prepared for histological examination for dosing phase animals on day 29 and for recovery phase animals on day 57.

At the end of the dosing period the following observations were noted:

- Tongue: acanthosis was noted in the tongue of 2 males and 2 females in group 4, and all males and females in groups 5 and 6. The severity of this finding was very slight for group 4, slight for group 5, very slight to marked for group 6.
- Very slight vesicle formation was present in the mucosal epithelia of the tongue of 1 male and 1 female in group 6.
- Buccal Mucosa: Acanthosis was present in the buccal mucosa of 2 males and all females in group 4, and all males and females in groups 5 and 6. The severity of this finding was very slight for group 4, very slight or slight for group 5, and very slight to marked for group 6.
- Very slight hyperkeratosis of the buccal mucosa was present in 2 females in group 6.
- Esophagus: Acanthosis was present in the tongue of 2 males and 2 females in group 4, and all males and females in groups 5 and 6. The severity of this finding was reported as very slight or slight for the group 4, slight or moderate for group 5, and moderate or marked for group 6. Vesicle formation rated as very slight was observed in the mucosal epithelia of all females in group 5 and all males and females in group 6.
- Stomach: Hyperplasia of the mucous cells was present in the stomach of 1 male and 1 female in group 4, and all males and females in the groups 5 and 6. The severity of this finding was rated very slight for group 4, slight for group 5 and slight or moderate for group 6.
- Small Intestine: Goblet cell hyperplasia was present in the following regions: the duodenum of 2 females in group 4, and all males and females in groups 5 and 6; the jejunum of 1 and 2 females in group 5 and 1 female in the group 6; and the ileum of all males and females in the groups 4, 5 and 6. The severity of these findings was rated as very slight for the group 4, very slight or slight for group 5 and very slight, slight or moderate for group 6.
- Large Intestine: Goblet cell hyperplasia was present in the following regions: the cecum of 2 males and 1 female in the group 4, and all males and females in groups 5 and 6; the colon of 1 male and 1 female in group 4, and all males and females in groups 5 and 6; the rectum of all males and females in groups 4, 5 and 6. The severity of this finding was rated as very slight or slight for group 4, slight for group 5 and slight or moderate for group 6.
- Mammary Gland (nipple): Very slight acanthosis was present in the skin at the nipple of 1 female in group 6. Very slight hyperkeratosis was present in the skin at the nipple of all males in group 6.
- Mammary Gland (breast parenchyma): Very slight or slight acanthosis was present in the skin overlying the mammary gland of 2 females in group 6.
- Skin (gluteal): Acanthosis rated as very slight or slight was present in 1 female in the group 4 and 2 females in group 6. Hyperkeratosis rated as very slight or slight was present in all males and 1 female in group 6.

- Skin (scalp): Acanthosis was present in 1 female in group 4, 1 male and 2 females in the group 5 and all males and females in group 6. The severity of this finding was rated as very slight for groups 4 and 5 and slight for group 6. Very slight or slight hyperkeratosis was present in all males and 2 females in group 6.
- Injection Site: Acanthosis was present in the skin of the injection site of 1 female in group 4, 1 male and 1 female in group 5 and all females in group 6. The severity of the acanthosis was rated as very slight for the group 4, very slight or slight for group 5 and slight or moderate for group 6. Hyperkeratosis was also observed in the skin of the injection site of 1 male in group 4, 1 male in group 5 and all males and females of group 6. The severity of the hyperkeratosis was rated as very slight for group 5 and very slight or slight for group 6. Hemorrhage, inflammatory cell infiltration or mononuclear cell infiltration in the subcutaneous tissue, fibrosis in the vascular wall and crust formation were present at the injection site. Severity and incidences of these findings appeared to be similar among the groups, and no irritation related to the test article was observed.
- Thymus: Marked involution of the thymus was present in 2 males and 1 female in group 3, 1 male and 2 females in group 4 and all males and females in groups 5 and 6. The remaining animals, 1 male and 2 females in group 3 and 1 male and 1 female in group 4, showed slight or moderate involution of the thymus. Very slight to moderate involution of the thymus was also present in all males and 1 female of the control group, 2 males and 1 female group 2. However, the very slight to moderate involution noted in the 1, 10 and 30 ug/kg groups was thought to be a spontaneous change since it was noted in the control group and is commonly observed in the Macaca monkeys of the testing facility.
- Submandibular Gland: Very slight hypertrophy of the acinar cells was present in the submandibular gland in all males and 2 females from group 6.
- Sternal Bone Marrow: Very slight hypocellularity was present in 1 male from group 6.

Other histological findings in the test article treatment groups were present in the heart, thoracic aorta, spleen, thymus, femoral bone marrow, sternal bone marrow, mesenteric and submandibular lymph node, lungs, pancreas, liver, kidneys, urinary bladder, testes, epididymides, seminal vesicles, prostate, ovaries, uterus, pituitary, adrenals, thyroids, parathyroid, eye ball (with optic nerve) and quadriceps muscle of the thigh. These changes either did not occur dose dependently, or were changes that are commonly observed in Macaca monkeys of the testing facility; and therefore, were thought to be spontaneous.

Histology of recovery animals:

The following test article-related changes were present.

- Very slight goblet cell hyperplasia in the duodenum of 1 female in group 6.
- Marked involution of the thymus of 1 male and 1 female of the group 5 and 1 female from group6;
- marked acanthosis of the tongue in 1 female from group 6.
- Slight hypocellularity in the sternal bone marrow was present in 1 female from group 6.

Toxicokinetics:

Blood samples for toxicokinetic examinations were taken from three animals per sex in each group once immediately prior to the initiation of dosing; 0, 5 min., and 0.5, 2, 4, 8 and 24 hours after administration on Days 0 and 27 of the dosing period; and 0.5 and 24 hours on Days 6 and 13 of the dosing period.

Other:
Anti-drug antibody levels: Blood samples for antibody titers were collected from all the animals once prior to the initiation of dosing, 24 hours after administration on Days 0, 13 and 27, and on Day 27 of the recovery period.

Amylase and lipase levels: amylase and lipase levels were measured on 3 animals per sex from each group from serum samples remaining after non-GLP toxicokinetic analysis on Days 0, 1, 6, 13, 27 of the dosing period, and on Days 6 and 27 of the recovery period.

- Significant increases in log mean serum amylase values were noted for monkeys from groups 4, 5, and 6 during the treatment period. Values were comparable to control values by the end of the recovery phase. This finding is correlated with increased submandibular gland weight for group 5 and 6 and hypertrophy (rated slight) of the acinar cells of that gland in group 6 animals.
- No test article related effect was noted for serum lipase levels.

Study title: A preliminary 1-week repeated dose toxicity study of recombinant human keratinocyte growth factor (r-HuKGFd23) administered intravenously to cynomolgous monkeys

Key study findings:
The purpose of this study was to evaluate the safety of palifermin when administered intravenously to 3 male cynomolgus monkeys at dose levels of 30, 300 and 1000 ug/kg/day for a period of 7 consecutive days.
All animals survived to scheduled termination. No abnormalities caused by palifermin were observed in the food consumption, body weight or hematology examinations.
At doses of 300 and 1000 ug/kg, flushing (reddening skin) was observed in the face and lower belly including the scrotum, and later a slight swelling and abnormal scaling were observed in these areas. In the urinalysis examination, at 300 ug/kg, a high creatinine level was noted. In the serum biochemistry examination, at 300 and 1000 ug/kg triglyceride levels were observed.
Gross pathology examinations revealed abnormal scaling in the facial and scrotal skin of the animals at 300 and 1000 ug/kg doses. A high relative liver weight was noted at 300ug/kg.
The microscopic examination revealed acanthosis and hyperkeratosis in the skin (scalp, face and scrotum) at 300 and 1000 ug/kg. Acanthosis and vacuolation of the prickle cells were observed in the buccal mucosa at all dose levels. Vacuolation of the prickle cells in the scrotum and mononuclear cell infiltration in dermis of the scalp skin and basal layer of the buccal mucosa were also observed at 1000 ug/kg. Perivascular eosinophil and/or lymphocyte infiltration in the facial subcutis was observed in the 300 and 1000 ug/kg

treated animal. Vacuolation of the hepatocytes was observed at 300 and 1000 ug/kg. The severity of this lesion was marked at 300 ug/kg and slight at 1000 ug/kg.
From these results, the non toxic dose level was considered to be less than 30 ug/kg, under the conditions of this study.
The animals receiving the 300 ug dose consistently showed increased toxicity relative to the high dose animal. No dose verification of dose was included as this study was not conducted under GLP conditions. Because the relationship to dose was consistent for urinalysis, histopathology, and organ weights, there is some reason to question the magnitude of exposure of these animals to the test article relative to one another.

Study no.: — 39-27-10
Volume #, and page #:
Conducting laboratory and location:
Date of study initiation: 2/15/1995
GLP compliance: NO
QA report: yes ( ) no (X)
Drug, lot #, and % purity: Palifermin lot# 01195A5

Methods

Doses: 30, 300 or 1000 ug/kg (no placebo control)
Species/strain: cynomolgus monkeys
Number/sex/group or time point (main study): 3 male monkeys, 1/group
Route, formulation, volume, and infusion rate: IV bolus infusion once daily for 7 days
Satellite groups used for toxicokinetics or recovery: NO
Age: 3-8 years
Weight: 2-4 kg
Unique study design or methodology (if any): This study was designed as a preliminary study and included no control animals. The design is illustrated in the table, below, supplied by the sponsor.

Group Number	Test Article	Dose Level (µg/kg/day)	Dose Volume (ml/kg/day)	Number of Animals** Male (animal number)
1	r-HuKGFd23	30	0.006	1 (1)
2	r-HuKGFd23	300	0.06	1 (2)
3	r-HuKGFd23	1000	0.2	1 (3)

Observations and times and Results

Mortality: All three animals survived to scheduled necropsy.

Clinical signs: AT least 3 times daily at cageside during the dosing period.

- No abnormal clinical findings were reported for group 1 (30 ug/kg)
- Flushing of face and lower belly including scrotum for groups 2 and 3 (300 and 1000 ug/kg) after SD3.
- After SD 5, 300 and 1000 ug/kg animals showed scaling and slight swelling of face and scrotum.

Body weights: Recorded prior to the study initiation, SD 1, 2, 4 and 6, then prior to necropsy.

- No abnormal findings related to body weight were reported.

Food consumption: Data recorded daily.

- No abnormal findings relating to food consumption were reported.

Ophthalmoscopy: Not Done.

EKG: Not Done.

Hematology: Data collected once pre-study, and on SD6
Standard battery will be performed.

- For the 1000 ug animal, a small reduction in HGB, HCT and RBC levels was reported. Because the RET level was not affected, the sponsor concluded that this finding was not of toxicological significance. However, repeat-dose studies in the rat showed consistent reduction in RBC, HCT and HGB. In those studies the RET levels did increase but this finding did not appear to be seen consistently in relationship to reduced hemoglobin measures. Therefore, the reduced hemoglobin measures may reflect a test article-related toxicity. With no placebo animals for comparison, and such limited animal numbers biological or toxicological significance is difficult to determine.
- A 50% reduction in absolute lymphocytes counts and small reduction in % lymphocytes for the 300 and 1000ug doses. The 300 ug dose animals showed a slightly larger magnitude of change.

Clinical chemistry: Data collected as for hematology.
Standard battery will be performed

- No abnormal findings at 30 ug.
- For the higher doses, increased in triglycerides was noted.
- The sponsor states that no other findings were observed. However, the data appear to show a 50% drop in total cholesterol relative to baseline, as well as reductions in AST, ALT, ALP and LDH. Reduced liver enzyme parameters are also reported in the rat repeat-dose studies.

Urinalysis: Data collected prior to study initiation, and on SD6. Collection of fresh urine occurred 2 hours prior to dosing using a metabolic cage. Samples will be analyzed for pH, glucose, ketone bodies, bilirubin, occult blood, urobilinogen, color and sediment. 18-sample will be analyzed for volume, specific gravity, creatinine and electrolytes including Na, K and Cl.

- No apparent treatment related effects were recorded for any animal
- Elevated creatinine was noted for the 300 ug animal. This finding was not seen in the higher dose monkey, so was considered not to be test article related. However, kidney findings were reported for the repeat-dose rat studies, thus, kidney findings should be watched for effects in monkeys.

Gross pathology:
Limited necropsy on all animals performed on the day after the final dose.

- For the higher two doses abnormal scaling of the skin on face and scrotum was noted.
- No abnormal findings are reported for the 30 ug dose.

Organ weights (specify organs weighed if not in histopath table):
Limited panel of organ removed and weighed: heart, lung (with bronchus), kidneys (R&L), liver, spleen. Data expressed as % body eight at necropsy.

- Increased liver weight was noted at the 300 ug dose.
- No other findings are reported.

Histopathology: Adequate Battery: yes ( ), no ( )—explain
Peer review: yes ( ), no ( )
40+ tissues harvested and stored. Histological examination was performed on heart spleen, lungs, liver, kidneys, scalp skin, and buccal mucosa.

- Acanthosis, hyperkeratosis in skin of scalp, face and scrotum (300 and 1000 ug groups)
- Vacuolization of the prickle cells of the scrotum (1000 ug group)
- Mononuclear cell infiltration of the dermis in scalp and buccal mucosa at the 1000 ug dose.
- Perivascular eosinophil and/or lymphocyte infiltration of deep tissue layers of facial skin and scalp at 300 and 1000 ug dose. (rated slight or very slight)
- Vacuolization of hepatocytes at 300 and 1000 ug doses, rated marked and very slight, respectively.
- Kidney effects at the 300 ug dose: slight degeneration or tubule necrosis in papilla, crystalline-like substance in giant cells in the renal cortex.

Toxicokinetics: Not Done.

Other:

Study title: A 3- day toxicity study of recombinant-human keratinocyte growth factor (rHuKGF) via intravenous administration in rhesus monkeys

Key study findings:

The purpose of this study was to evaluate the toxicity of palifermin when administered intravenously to rhesus monkeys at dose levels of 500, 1000, 2000 and 4000 ug/kg/day for 3 consecutive days. In this study, 2 males and 1 female were used in the control,

1000 and 4000 ug/kg groups, and 1 male and 2 females were used in the 500 and 2000 ug/kg groups.
The following changes, thought to be related to the pharmacological effect of the test article, were observed in all test article treated groups: reddening of the facial skin in the clinical signs examination on Day 2, and in the gross pathological examination at necropsy on Day 3; very slight or slight acanthosis (with hyperkeratosis) in the nipple, injection site, lip (upper), buccal mucosa and tongue, very slight acanthosis in the skin (mammary gland) and esophagus, and very slight goblet cell hyperplasia in the small and large intestines (ileum, cecum, colon and rectum) were noted in the histopathological examination.
Test article related findings were revealed for urinalysis parameters that correlate with increased organ weight for the urinary bladder as well as microscopic abnormalities.
Test article related effects on food consumption in the two high dose groups were accompanied by low glucose values in groups 3, 4 and 5. Decrease in urine volume noted for all groups on day 2 may have been related to reduced food intake. Test article-related changes were observed in the nipple (mammary gland), skin (mammary gland), injection site, lip (upper), buccal mucosa, tongue, esophagus, small intestine (ileum), large intestine (cecum, colon, rectum), submandibular gland and urinary bladder.

Study no.: — -39-35/Amgen study # 970159
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: 12/22/1997
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: palifermin lot# 1107096D7
Placebo lot# 02D07226G6

Methods

Doses: 0, 500, 1000, 2000 and 4000 ug/kg/day (doses chosen as multiples of the expected human clinical dose, at that time was 100 ug/kg)

Species/strain: rhesus monkeys

Number/sex/group or time point (main study): Group 1: control, 2 males and 1 female, group 2: 500 ug, 1 male and 2 females, group 3: 1000 ug, 2 males and 1 female, group 4: 2000 ug, 1 male and 2 females, group 5: 4000 ug, 2 males and 1 female

Route, formulation, volume, and infusion rate: IV bolus Injection at rate of 2 ml/minute in a volume of 0.8 ml/kg

Satellite groups used for toxicokinetics or recovery:

Age: males 7-9 years, females 5-7 years

Weight: males: 7.54 – 9.31 kg; females: 3.83- 5.88 kg

Unique study design or methodology (if any):

Observations and times Results

Mortality:

Clinical signs: twice daily during dosing

- Reddening of the face on day 2 in all test article-treated groups. One female in group 2 (500 ug) did not show this finding.

Body weights: Data collected once prior to study initiation, once of SD 2 and prior to necropsy on SD3.

Food consumption: Recorded daily

- Reduced food intake in food consumption was noted in 2 males and 1 female in the 1000 ug group and 1 male in the 4000 ug group.

Ophthalmoscopy: Not Done

EKG: Not Done.

Hematology: Samples were collected once prior to study initiation, once prior to dosing on SD1 and once prior to necropsy on SD3. A standard hematology testing battery was performed including coagulation parameter.

- SD 1: low WBC for the 500 ug group,
- SD 3: high WBC count and elevated seg.neutrophils in the 1000 ug group.

These findings were not observed in the 2000 or 4000 ug group, thus biological significance is not clear.

- Reduced hemoglobin measures are reported for all groups on day 1 and 3. The sponsor attributes these findings to the effects of blood draws. However, similar findings are noted in other repeat dose studies so biological significance can not be discounted.

Clinical chemistry: Samples taken under the same schedule as for hematology. A standard testing battery was performed. In addition, serum amylase, serum lipase and C-reactive protein were assessed.

- low total cholesterol valuc in the 2000 and 4000 ug/kg groups on Day 3, and a trend towards a decreased total cholesterol level in the 500 and 1000 ug/kg groups
- low glucose level in the 1000 ug/kg group on Day 3; and a low BUN in the 1000, 2000 and 4000 ug/kg groups on Day 1. Low glucose values were also noted in 1 animal in each of the 2000 and 4000 ug/kg groups on Day 3.
- No test article related effects on serum amylase are reported.
- No test article related effects on serum lipase are reported.
- No test article related effects on C-reactive protein are reported.

Urinalysis: Data collected prior to study initiation and once on SD2
Fresh urine was collected for 2 hours. pH, glucose, ketone body, bilirubin, urine occult blood and urobilinogen and protein content were determined. Urine color was examined visually. The urine sediment was examined microscopically.
The urine samples excreted over 16 hours were measured for urine volume, and specific gravity was measured. Electrolyte concentration was analyzed including NA, K. Cl. The

total excretion volume of the urinary electrolytes was calculated from urine volume and electrolyte concentration.

- SD 2: reduced urine volume in all group 2 animals (500 ug).
- SD 2: Reduced Na excretion in all but one female in group 2.
- SD 2: reduced total potassium in all animals in group 2.
- SD 2: Reduced total Na excretion in 2 males and 1 female of group 3 (1000 ug), 1 male and 2 females in group 4 (2000 ug), 1 male and 1 female in group 5 (4000 ug)
- SD 2: reduced Na in 1 male and one female in group2, 2 males and 1 female in group 3, 1 male and 1 female in group 4, and one male in group 5.
- SD 2: Reduced Cl concentration in 1 male and 1 female in group 3, 1 female in group 4 and 1 male in group 5.
- SD 2: Increased specific gravity in 1 male and 1 female in group 5.
- SD 2: increased protein concentration in 1 male and 1 female in group 4 and 1 female in group 5.

Correlate these findings with microscopic findings under the gross pathology and histopathological results.

Gross pathology: Animals euthanized one day after the final dose.

- Reddening of the facial skin was observed in all test article treated animals except 1 female in group 2.
- Reddening of the mucosa in the urinary bladder was observed in 1 male and 1 female from group 4, and 1 male from group 5.
- Moderate induration in the right posterior lobe of the lungs was observed in 1 male from group 5.

Organ weights (specify organs weighed if not in histopath table):
Liver, kidneys, urinary bladder, adrenals, thyroids (including parathyroids), submandibular glands, testes, ovaries, uterus, pituitary, thymus, heart, lungs (including bronchi), spleen, pancreas, epididymides, seminal vesicle, prostate

- High absolute and relative urinary bladder weights were noted in 1 male of the 1000 ug/kg group, and 1 male and 1 female each of the 2000 and 4000 ug/kg groups.

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes ( ), no ( )
40+ tissues saved. 30+ tissues examined histologically.

- Nipple (mammary gland): Very slight or slight acanthosis was present in all test article treated animals except 1 female of the 2000 ug/kg group. Very slight hyperkeratosis was present in all test article treated animals except 2 females from each of the 500 and 2000 ug/kg groups.
- Skin (mammary gland): Very slight acanthosis was present in all test article treated animals except 2 females from each of the 500 and 2000 ug/kg groups.
- Injection site: Very slight or slight acanthosis was present in all test article treated animals. Very slight hyperkeratosis was present in 1 male of the 1000 ug/kg

group. Hemorrhage, mononuclear cell or inflammatory cell infiltration in the subcutaneous tissue was present at the injection site of some animals. The severity and incidence of these findings appear to be similar among the groups, and no irritation specific to the test article was observed.

- Lip (upper): Very slight or slight acanthosis was present in the skin of all test article treated animals except 1 male from the 1000 ug/kg group. Very slight hyperkeratosis was present in the skin of 2 females from each of the 1000 and 2000 ug/kg groups, and 2 males of the 4000 ug/kg group. Very slight acanthosis was present in the mucosa of all test article treated animals. Very slight or slight hyperkeratosis was present in the mucosa of all test article treated animals except 1 female from the 1000 ug/kg group.
- Buccal mucosa: Very slight acanthosis was present in all test article treated animals. Very slight hyperkeratosis was present in all test article treated animals except 1 female from the 2000 ug/kg group.
- Tongue: Very slight or slight acanthosis and very slight hyperkeratosis were present in all test article treated animals.
- Esophagus: Very slight acanthosis was present in all test article treated animals except 2 females from each of the 500 and 1000 pg/kg groups.
- Small intestine (ileum): Very slight hyperplasia of goblet cells was present in all test article treated animals.
- Large intestine (cecum, colon, rectum): Very slight hyperplasia of goblet cells was present in the following tissues: the cecum of all test article treated animals except 1female of the 2000 ug/kg group; the colon of all test article treated animals except 1 female from the 1000 ug/kg group; and the rectum of all test article treated animals except 1 male from the 2000 pg/kg group.
- Submandibular gland: Very slight decrease in the number of mucous cells was observed in 2 males and 1 female from the 4000 ug/kg group.
- Pancreas: Very slight decrease in the zymogen granules in the acinar cells was observed in 1 female from the 500 ug/kg group, 1 male and 1 female each from the 1000 and 2000 ug/kg groups.
- Urinary bladder: Very slight or slight edema in the submucosa was present in 1 from the 500 ug/kg group, 2 males and 1 female from the 1000 ug/kg group, 1 male and 1 female of the 2000 ug/kg group, and 2 males and 1 female from the 4000 ug/kg group. Very slight or slight hemorrhage in the submucosa was present in 1 male the 500 ug/kg group, 2 males from the 1000 ug/kg group, 1 male and 1 female each from the 2000 and 4000 ug/kg groups. Very slight or slight hemorrhage in the mucosal epithelium was present in 3 males from each of the 500, 1000 and 4000 ug/kg groups. These findings were not observed in the control animals, thus, are most likely test article related.
- Prostate: Very slight interstitial mono nuclear infiltration in 1 male from the 2000 and 4000 ug groups each.
- Other histological findings in the test article treatment groups were present in the liver, kidneys, testes, ovaries, pituitary, thyroids, parathyroid, adrenals, submandibular gland, pancreas and lung. These changes either did not occur dose

dependently, or were changes that are commonly observed in rhesus monkeys of this facility. Thus, biological significance is difficult to determine.

Toxicokinetics:

Blood samples for toxicokinetics were taken from all animals once prior to study initiation; once at 5 and 30 minutes, and once at 1, 2, 4, 8 and 24 hours after dosing on Days 0 and 2 of the dosing period.

- Control animals showed no evidence of exposure based on serum concentrations.
- Intravenous administration of palifermin resulted in serum concentration-time profiles which "plateau" between 1 and 3 hours post-dose.

The "plateau" became less pronounced dose as the dose increased, but did not alter over time. Serum concentrations on Day 2 were generally lower for every animal than on Day 0. Consequently, clearance and volume of distribution increased over time; terminal half-life was time-independent. There were no consistent changes with dose for any of the parameters indicating dose-linearity. However, on Day 2, serum concentrations for males had a tendency to be higher than in females (up to 4-fold greater at 5 minutes), resulting in increased C_0 (up to 5.01-fold) and $AUC_{(0-w)}$ (up to 2.42-fold) for males when compared to females.

Dosing Scheme: Daily IV bolus for 3 days
Formulation: Lyophilized Assay: ELISA (LOD: —— ng/mL)

Group:	2		3		4		5	
Dose (ug/kg):	500		1000		2000		4000	
Day 0 Results								
Parameter (units):	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C_0 (ng/mL)	2210	564	5610	3860	6970	4060	12400	3440
V_{ss} (L/kg)	2.29	0.411	1.79	0.385	2.72	1.21	1.90	0.364
$t_{1/2,\lambda}$ (hr)	3.90	0.258	4.16	0.448	4.27	0.357	4.03	0.401
$AUC_{(0-\infty)}$ (ng hr/mL)	904	155	2170	411	3780	1960	8050	2150
$nAUC_{(0-\infty)}$ (ng hr/mL)/(ug/kg)	1.81	0.310	2.17	0.411	1.89	0.982	2.01	0.538
Cl (L/hr/kg)	0.565	0.107	0.471	0.0802	0.620	0.271	0.525	0.159

Other:
Measurement of hepatic cytochrome P-450

- No test article related effects are reported.

Study title: An intermittent (11-day) intravenous administration toxicity study of recombinant-human KGF (rHuKGF) in rhesus monkeys

Key study findings:

The purpose of this study is to evaluate the toxicity of r-HuKGFd23 when administered to rhesus monkeys for two cycles of 3 consecutive dosing, days with an interval of 5 non-dosing days. This regimen is similar to the expected clinical regimen.

Reddening of the facial skin was observed in all animals that received the test article by SD2.
Test-article related changes were observed in the nipple (mammary gland), skin (mammary gland), injection site, lip (upper), palm, vagina, buccal mucosa, tongue, esophagus, small intestine (ileum), large intestine (cecum, colon, rectum), pancreas and urinary bladder.
Under these conditions, the no observable adverse effect level (NOAEL) for palifermin was considered to be less than 500 ug/kg, since edema and hemorrhage in the submucosa of the urinary bladder were present at this dose level in this study. No recovery group was included in this study.

Study no.: — 39-36/ Amgen # 970160
Volume #, and page #:
Conducting laboratory and location: [ ]
Date of study initiation: 12/22/1997
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: palifermin lot# 1107096D7
Placebo lot #02D07226G6

Methods

Doses: 0, 500 ug/kg/day (group 2), 1000 ug/kg/day, (grp 3), 2000 ug/kg/day (grp 4), 4000 ug/kg/day (grp 5) These doses were chosen as multiples of the expected human clinical dose.

Species/strain: rhesus monkeys (7 males and 8 females) 3/group

Number/sex/group or time point (main study):

Groups 1, 3, and 5: 1 male and 2 females;

Groups 2 and 4: 2 males and 1 female

Route, formulation, volume, and infusion rate: IV bolus infusion in a volume of 0.8 ml/kg and a rate of 2 ml/minute.

Satellite groups used for toxicokinetics or recovery:

Age: males: 7-10 yr, females: 5-8 years

Weight: males: 7.79 – 9.97 kg; females: 3.61-5.52 kg

Unique study design or methodology (if any):

The study design is illustrated in the table, below, supplied by the sponsor:

STUDY DESIGN

Group Number	Test Article	Concentration (mg/mL)	Dose Level (µg/kg/day)	Number of Animals** Male	Female
1	KGF Placebo*	0	0	1 or 2	1 or 2
2	r-HuKGFd23	1.25	500	1 or 2	1 or 2
3	r-HuKGFd23	2.5	1000	1 or 2	1 or 2
4	r-HuKGFd23	5	2000	1 or 2	1 or 2

* The control group will receive the control in the same manner as the test article.
** There will be at least one male and one female per group.

The study design calls for daily injections on study days 0, 1, and 2 followed by a 5-day drug-free interval, then daily injections on days 8, 9, and 10. Necropsy was performed on all animals on day 11.

Observations and times Results

Mortality: All animals survived to scheduled necropsy.

Clinical signs: Data collected twice daily

- SD2: reddening of the facial skin was observed in all test article treated animals except 1 female from each of the 500 and 1000 ug/kg groups.
- During the 5-day drug-free interval: Reddening of the facial skin persisted as follows:
  500 ug/kg group: 2 males and 1 female on SD3
  1000 l ug/kg group: 1 female on Day 3, 1 male on Day 3 and 4, and 1 female from Day 3 through Day 5;
  2000 ug/kg group: 1 female on Day 3 and 4, 1 male from Day 3 through Day 5, and 1 male from Day 3 through Day 6
  4000 ug/kg group: 1 male and 2 females from Day 3 through day 6
- No abnormalities were noted on SD 7.
- During the second dosing sequence:
  1000 ug/kg group: reddening of the facial skin in 1 male on day 9 and day 10, and one female on day 10
  2000 ug/kg group: reddening and scaling of the facial skin in 2 males on day 9 and day 10, and 1 female on day 10
  4000 ug/kg group: reddening of the facial skin in one female on day 9 and 10; reddening and scaling of the facial skin in 1 male and 1 female on days 9 and 10

These are expected, dose-related effects of the test article and have been observed in other non-clinical studies. They show increased incidence and severity with increasing dose and dosing duration.

No test article related effects were noted for the 500 ug/kg group during the second dosing sequence.

Body weights: Data collected once prior to study initiation, SD 3, 7, 10 and prior to necropsy.
During the first dosing sequence:
On Day 3, no test article-related changes were noted in any group.
On Day 7, a slight decrease in body weight was noted in 1 male from each of the 1000 and 2000 ug/kg groups, respectively, as compared to the pre-dosing values.

During the second dosing sequence:
No test article related effects were noted in any group on SD 10 relative to SD 7.

Food consumption: Calculated daily.
During the first dosing sequence:
On Day 2, a slight decrease (almost less than half) in food consumption relative to the control group was noted in 1 male and 1 female of the 1000 ug/kg group, 2 males of the 2000 ug/kg group, and 1 male of the 4000 ug/kg group.

During the 5-day drug-free interval:
On Day 3, 1 male of the 1000 ug/kg group, 2 males of the 2000 ug/kg group, and 1 male of the 4000 ug/kg group noted loss of appetite. A slight decrease in appetite was also noted in 1 male of the 500 ug/kg group, 1 female each of the 1000 and 2000 ug/kg groups, respectively, and 2 females of the 4000 ug/kg group. From Day 6, normal food intake was observed for all animals.
These data reflect a dose dependent increase in severity and incidence of food intake reduction that persisted into the drug-free interval. The effects had resolved by SD 6 and not subsequent effects were noted during the second dosing sequence.

No test article related effects were noted during the second dosing sequence.

Ophthalmoscopy: Not Done.

EKG: Not Done.

Hematology: Blood samples taken prior to dosing on SD 1 and 9, SD 3 and 7, and SD11

- erythrocyte count ([redacted])
- leukocyte count ([redacted])
- blood platelet count ([redacted])
- hematocrit value ([redacted] method detection)
- hemoglobin concentration [redacted] method)
- mean corpuscular volume (calculation)
- mean corpuscular hemoglobin (calculation)
- mean corpuscular hemoglobin concentration (calculation)
- Reticulocyte count and
- differential leukocyte count
- coagulation parameters

No treatment related effects were noted on SD1 for any group.

Reduced lymphocyte ratio (%) in the 1000 and 2000 ug/kg groups was noted on Day 3. but was not found in the 4000 pg/kg group.
No test article related effects were observed in any group on SD 9 or 10.

Sporadic reductions in hemoglobin parameters (RBC, HCT, HGB) were noted during the study. These findings may have been related to blood draws. There was a concomitant increase in RET counts which supports this conclusion.

Clinical chemistry: Samples taken under the sample schedule as for hematology.

- aspartate aminotransferase
- alanine aminotransferase
- alkaline phosphatase
- lactate dehydrogenase
- creatine phosphokinase
- total bilirubin
- total protein
- albumin
- total cholesterol
- triglyceride
- phospholipid
- glucose
- blood urea nitrogen
- creatinine
- inorganic phosphorus
- calcium
- sodium
- potassium
- chloride
- Protein fractions, including A/G ratio

No test article related effects were reported for SD1.
During the 5-day drug-free interval:

- Relative to control: a high alkaline phosphatase value in the 2000 and 4000 ug/kg groups on Day 3; a low total cholesterol value in all test article treated groups on Day 3 and in the 2000 ug/kg group on Day 7.

Low glucose values were noted in 1 female of the 1000 ug/kg group and 1male of the 2000 ug/kg group on Day 3.

During the second dosing period:
The following statistically significant change was noted in comparison to the control group: a low total cholesterol value in the 1000 and 2000 ug/kg groups on Day 9 and in all test -article treated groups on Day 11; a low gamma globulin level in all test article treated groups on Day 11.

Statistical significance in comparison to the control was also noted in the following values: a low phospholipid value in the 1000 and 2000 ug/kg groups on Day 11; a high alpha-l globulin level in the 500 and 2000 ug/kg groups on Day 3, and in the 500, 1000 and 2000 ug/kg groups on Day 11; a high alpha-2 globulin level in the 1000 and 4000 ug/kg groups on Day 11. However, these changes were not dependent on the dose levels. Therefore, the changes were thought to be incidental. An increase in the AST, LDH and CPK levels was noted in every group including the control group on Days 1 and/or 3. These increases were concluded to have been caused by blood sampling.

Serum amylase:
No test article related effects noted. Elevations relative to base line were noted in the 500 ug/kg group and the 2000 ug/kg group were noted. However no such effects were noted for the 1000 or 4000 ug/kg groups so this does not appear to be dose related. Statistical significance in comparison to the control group was also noted in the following values: a high total amylase value in the 500 ug/kg group on Days 2, 8, 9, 10 and 11, and in the 2000 ug/kg group on Days 8 and 9; a high pancreatic amylase value in the 500 ug/kg group on Day 10; a high salivary amylase value in the 500 ug/kg group on Days 0, l and 9, and in the 2000 ug/kg group on Days 8 and 9. However, similar changes were not found in the 4000 ug/kg group in this study. Therefore, the above findings were concluded to be incidental.

Serum lipase
No test article related effects noted
C-reactive protein
No test article related effects noted. All group had apparent increases relative to baseline for the respective group. None specific to dose.

Urinalysis: Samples taken prior to study initiation, SD2, 7, 10.

- Fresh urine was collected for 2 hours. Samples were assessed for: pH, glucose, ketone body, bilirubin, urine occult blood, urobilinogen and urine color. The urine sediment was examined microscopically. Protein content was assessed.
- The urine samples excreted over 16 hours were measured for urine volume and specific gravity. The sodium, potassium and chloride levels were examined. The total excretion volume of the urinary electrolytes was calculated from urine volume and electrolyte concentration.

During the first dosing sequence:
Relative to the control values and baseline values, the following changes were noted on SD2:

- a slight decrease in urine volume in 1 male of the 2000 ug/kg group, and 2 females from the 4000 ug/kg group;
- a slight decrease in Na concentration in 2 males of the 2000 ug/kg group;
- a slight decrease in Cl concentration in 1 male each from the 2000 and 4000 ug/kg groups;

- a slight increase in protein concentration in 1 male and 1 female from the 4000 ug/kg group;
- a slight decrease in total Na+ excretion in 1 male from the 2000 ug/kg group, and 2 females from the 4000 ug/kg group;
- a slight decrease in the total Cl excretion value in 1 male from the 2000 ug/kg group, and 1 male and 1 female from the 4000 ug/kg group.

No effects were noted on SD7
During the second dosing sequence:
Relative to control and baseline data, the following effects were noted on Day 10:

- a slight decrease in urine volume in 1 male from the 2000 ug/kg group
- a slight decrease in Nat and Cl- concentration and total Na+ and Cl- excretion levels in 1 male from the 2000 ug/kg group;
- a slight increase in protein concentration in 1 male from the 500 ug/kg group, and 1 female from the 4000 ug/kg group

Gross pathology: Necropsy was performed on all animals on SD11 (one day after the final dose).
4000 ug/kg group: scale on the facial skin in 1 male and 1 female, scale on the skin of the hands in 1 male, and slight thickening of the mucosa in the urinary bladder and enlargement of the vagina in 2 females were observed.
2000 ug/kg group: reddening of the facial skin in 2 males, scale on the facial skin in 2 males and 1 female, slight thickening and reddening of the mucosa in the urinary bladder in 2 males were observed.
1000 ug/kg group: reddening of the facial skin in 1 female, and scale on the skin of the hands in 1 male were observed.

Organ weights (specify organs weighed if not in histopath table):

- liver - kidneys
- urinary bladder - adrenals
- thyroids (including parathyroids) - submandibular glands
- testes - ovaries
- uterus - pituitary
- thymus - heart
- lungs (including bronchi) - spleen
- pancreas - epididymides
- seminal vesicle - prostate

High absolute and relative urinary bladder weights were noted in 1 male in the 500 ug/kg group, 1 female of the 1000 ug/kg group, 2 males of the 2000 ug/kg group, and 2 females of the 4000 ug/kg group. These findings are consistent with those of other monkey studies and are accompanied by test article related effects on urinalysis results.

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes ( ), no ( )

- tongue - buccal mucosa
- esophagus - stomach (fundus, pylorus)

- duodenum - jejunum
- ileum - cecum
- colon - rectum
- liver - kidneys
- urinary bladder - testes
- epididymides - seminal vesicle
- prostate - ovaries
- uterus - vagina
- pituitary - thyroids
- parathyroid (as much as possible) – adrenals
- skin (mammary gland) - mammary gland
- submandibular gland - injection site
- thymus

Nipple (mammary gland): Very slight or slight acanthosis was present in all palifermin treated animals except 1 female of the 500 ug/kg group. Very slight or slight hyperkeratosis was present in 1 male of the 500 ug/kg group, 1 female of the 1000 ug/kg group, 1 male and 1 female of the ug/kg 2000 group, and 1 male and 2 females of the 4000 ug/kg group.

Skin (mammary gland): Very slight or slight acanthosis was present in all palifermin treated animals except 1 female of the 500 ug/kg group. Very slight hyperkeratosis was present in 1 male of the 2000 ug/kg group, and 1 male and 2 females of the 4000 ug/kg group. Very slight hyperplasia of the sebaceous gland was present in all test article treated animals except 2 males of the 500 ug/kg group.

Injection site: Very slight or slight acanthosis was present in all palifermin treated animals. Very slight hyperkeratosis was present in 1 male and 1 female of the 1000 ug/kg group, and all males and females of the 2000 and 4000 ug/kg groups. Very slight hyperplasia of the sebaceous gland was present in all test article treated animals except 1 male of the 500 ug/kg group. Hemorrhage, inflammatory cell infiltration or fibrosis in the subcutaneous tissue was present at the injection site, as compared to the control group.

Lip (upper): Very slight, slight or moderate acanthosis was present in the skin of all palifermin treated animals. Very slight or slight hyperkeratosis was present in the skin of all palifermin treated animals except 1 male and 1 female of the 500 ug/kg group. Very slight hyperplasia of the sebaceous gland was observed in the skin of all palifermin treated animals. Very slight or slight acanthosis was present in the mucosa of all test article treated animals. Very slight or slight hyperkeratosis was present in the mucosa of all test article treated animals.

Palm: Very slight, slight or moderate acanthosis was present in the skin of all test article treated animals. Very slight or slight hyperkeratosis was present in the skin of all test article treated animals.

Vagina: Slight acanthosis was present in all test article treated female animals except 1 female of the 500 ug/kg group.

Buccal mucosa: Very slight acanthosis was present in all palifermin treated animals. Very slight or slight hyperkeratosis was present in all palifermin treated animals.

Tongue: Very slight acanthosis was present in all test article treated animals.
Very slight or slight hyperkeratosis was present in all test article treated animals except 1 male of the 500 ug/kg group.

Esophagus: Very slight acanthosis was present in all palifermin treated animals.
Very slight or slight hyperkeratosis was present in 1 male and 1 female from each of the 500 and 2000 ug/kg group, 1 female of the 1000 ug/kg group, and all male and females of the 4000 ug/kg group.

Small intestine (ileum): Very slight hyperplasia of the goblet cells was present in all test article treated animals.

- Large intestine (cecum, colon, rectum): Very slight hyperplasia of the goblet cells was present in the following tissues: the cecum of all test article treated animals except 1 male of the 4000 ug/kg group; the colon and the rectum of all test article treated animals.
- Pancreas: Very slight decrease in the zymogen granules in the acinar cells was observed in 2 females of the 1000 ug/kg group, 1 male and 1 female of the 2000 ug/kg group, and 1 female of the 4000 ug/kg group.
- Urinary bladder: Very slight or slight edema in the submucosa was present in 1 male and 1 female of the 500 ug/kg group, 2 females of the 1000 ug/kg group, 2 males and 1 female of the 2000 ug/kg group, and 1 male of the 4000 ug/kg group. Moderate edema in the submucosa was present in 2 females of the 4000 ug/kg group. Very slight hemorrhage in the submucosa was present in 1 male and 1 female of the 500 ug/kg group, 1 male of the 2000 ug/kg group, and 1 female of the 4000 ug/kg group.
- Hemorrhage, inflammatory cell infiltration or fibrosis in the subcutaneous tissue was present at the injection site, as compared to the control group. The severity and incidence of these findings were thought to be similar among the groups, and no irritation related to the test article was observed.

Other histopathological findings in the test article treatment groups were present in the liver, kidneys, testes, seminal vesicle, prostate, pituitary, thyroids, parathyroid, adrenals, stomach, duodenum, submandibular gland and pancreas.
These changes either did not appear to be dose dependent, or were changes that are commonly observed in rhesus monkeys seen historically at the testing factility; and therefore, were thought to be spontaneous.

Cytochrome P-450 assay: No effects related to palifermin administration were noted.

Toxicokinetics:
The toxicokinetics were dose-linear between doses of 500 and 4000 ug/kg, as assessed byAUC and t 1/2. However, upon multiple dosing, exposure decreased with time (60 to 78% by Day 10), distribution volume had a tendency to increase and terminal half-life remained constant. Male monkeys tended to have higher serum concentrations than female monkeys resulting in 1.01 - to 2.36-fold increase in AUC(o-∞). Intravenous administration of palifermin resulted in serum concentration-time profiles which

displayed plateau between 1 and 3 hours post-dose, with a terminal half-life of 3 to 4 hours. No antibodies were detected in any of the monkeys by Day 11 and none of the control animals appeared to have been dosed with study drug.

Study Design: In-Life GLP: Yes Bioanalytical GLP: Yes
Test Compounds: rHuKGF (Lot #1107096D7 and 2400F5), Placebo: (Lot # 02D07226G6), Reconstitution Solution (Lot # 02D07226G6A)
Dosing Scheme: IV bolus: Day 0, 1, 2, 6, 9 and 10
Formulation: Lyophilized Assay: ELISA (LOQ: — ng/mL)

Group:	2		3		4		5	
Dose (µg/kg):	500		1000		2000		4000	
Day 0 Results								
Parameter (units):	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C_0 (ng/mL)	2360	1840	6010	1560	9870	2980	12800	9530
V_{ss} (mL/kg)	2070	1190	1840	469	1860	395	2300	1110
$t_{1/2,z}$ (hr)	3.84	0.297	3.85	0.0815	4.04	0.151	3.95	0.816
$AUC_{0-\infty}$ (ng hr/mL)	1080	176	2050	302	4210	767	7880	2910
$nAUC_{0-\infty}$ (ng hr/mL)/(µg/kg)	2.16	0.351	2.05	0.302	2.10	0.383	1.97	0.729
CL (mL/hr/kg)	471	82.4	497	80.2	486	90.3	551	221

Samples taken prior to initiation of dosing, 5 and 30 minutes, 1, 2, 4, 8 and 14 hours after dosing on SD 0, 2 and 10. In addition, samples were taken 5 minutes post-dose on SD 8 and 9.

Other:
Anti-drug antibody detection: Samples taken prior to study initiation and on SD11. No anti-drug antibodies were detected in any animal on day11. It is not clear how valuable these data are sine the sensitivity of the detection assay may not be sufficiently sensitive and there may be a masking effect from test article still in circulation. However, with a half-life of 3-4 hours, sufficient drug should have been cleared in the 24 hours between the final dose and sampling for antibodies.

Study title: A 7-day intravenous and subcutaneous toxicity study of rHuKGF in rats

Key study findings:
Possible toxic and pharmacological effects of palifermin were evaluated in this 7-day study in rats. The test article was administered by intravenous injection to one group and by subcutaneous injection to another group once daily for seven consecutive days.
A dosage level of 1.0 mg/kg/day and a dose volume of 0.2 ml/kg were used for both groups. Each group consisted of three male and three female Sprague Dawley rats. For comparative purposes, a concurrent control group of identical design received the control article by intravenous injection on a comparable regimen at a volume of 0.2 ml/kg. The rats were observed for signs of overt toxicity, and effects on body weight, food consumption and clinical pathology parameters. Complete necropsies were performed on all animals, and the brain, liver and pancreas were weighed. The liver and pancreas from all animals were examined microscopically.
Survival, clinical condition and body weights were unaffected by treatment with rHuKGF.

Study no.: — 120072/amgen study # 100148
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: 4/14/1998
GLP compliance: YES
QA report: yes (X) no ( )
Drug, lot #, and % purity: palifermin lot # 24006M7A
Diluent lot# 02D07226G6A
Placebo lot # 02D07226G6

Methods

Doses: 1 mg/kg daily for 7 days by either SC or IV route
Species/strain: Sprague Dawley rat — CD@BR
Number/sex/group or time point (main study):
Route, formulation, volume, and infusion rate: IV and SC
Satellite groups used for toxicokinetics or recovery:
Age: 6-7 weeks of age
Weight:
Unique study design or methodology (if any):

Group Number	Treatment	Route	Dosage Level (mg/kg/day)	Dosage Volume (ml/kg)	Number of Animals Males	Females
1	rHuKGF Placebo	IV[a]	0	0.2	3	3
2	rHuKGF	IV	1.0	0.2	3	3
3	rHuKGF	SC[b]	1.0	0.2	3	3

[a] = Intravenous via tail vein
[b] = Subcutaneous in the scapular area

Observations and times Results

Mortality: Observations made twice daily. All animals survived to scheduled necropsy.

Clinical signs: Evaluation will be performed daily prior to dosing and 1-2 hours post-dosing. No remarkable clinical findings attributable to test article administration were noted.

All animals received a detailed physical examination weekly, beginning one week prior to dosing, on the day of randomization and just prior to the scheduled necropsy.

Body weights:

Individual body weights will be recorded weekly, beginning one week prior to test article administration, on Day 0, 2 and 6 and a fasted body weight just prior to the scheduled necropsy on Day 7.

- All groups gained weight through the study. No effects on body weight or body weight gain attributable to test article administration were noted. A trend toward lower rate of weight gain was possibly present for the group receiving test article by IV administration but this finding did not reach statistical significance. (Example: total weight gain for each group for males: 37, 21 and 32 grams for control, IV dosing and SC dosing groups, respectively.

Food consumption:
Individual food consumption will be determined weekly, beginning one week prior to test article administration and twice during the week of dosing.

- Slightly reduced food consumption was noted for the IV dosed group relative to controls and SC dosed group. These findings did not reach statistical significance.

Ophthalmoscopy: Not Done

EKG: Not Done

Hematology: Not Done.

Clinical chemistry:
Blood samples were collected during the pretest week, on Study Day 3 and on Study Day 7. The animals will be fasted overnight prior sample collection.
The following clinical pathology parameters were evaluated:

- Serum Amylase: No significant effects of the test article administration were noted for the male ainmals. However, there appears to be a trend toward increased amylase levels for both the treated groups with, perhaps, a greater magnitude for the IV dosed group over the course of the study, as would be expected. In female animals, there was a significant, dose related increase inserum amylase on both SD3 and SD7 for both treated groups.
- C-Reactive protein: Values for the treated groups were significantly higher than control values at baseline. Therefore, all values for test days were also greater than control. For each treated group, there appears to be an increase in levels on both SD3 and SD7 relative to the respective baseline values, but no difference between treatment groups.
- Glucagon: Hormone levels appeared to slightly higher for the treated groups relative to control but this finding did not achieve statistical significance. The magnitude of increase may be slightly greater for the IV dosed group.
- Insulin: hormone levels were significantly lower for the treated groups relative to control. This finding could have been related to food consumption. For females in the IV dosed group, higher insulin levels are noted. (the mean value for the group is 3X that of control, but not statistically significant.
- Serum Lipase: Significantly higher lipase levels detected for the IV dosed group on SD3 and SD7 relative to control and baseline value. Elevated serum lipase

was also noted for the SC dosed group on SD3 and SD7 relative to control and baseline. The magnitude of the increase for the SC group was greater than that of the IV dosed group. On SD7 the serum lipase value for the IV dosed group was 2.3 X greater than baseline value; the value on SD7 for the SC dosed group was 3.6 X the baseline for that group.

Urinalysis: Not Done.

Gross pathology:
A complete necropsy will be conducted on all animals.
Necropsy included examination of the external surface, all orifices and the cranial, thoracic, abdominal and pelvic cavities including viscera. At the time of necropsy, the following tissues and organs will be collected and placed in 10% neutral buffered formalin (except as noted):

- Adrenals (2)
- Aorta
- Bone with marrow
- Femur
- Bone marrow smear'
- Buccal mucosa
- Brain: forebrain, midbrain, hindbrain
- Eyes with optic nerve (2)
- Gastrointestinal tract: esophagus, stomach, duodenum, jejunum, ileum, cecum, Colon, Rectum
- Heart
- Kidneys (2)
- Harderian gland
- Lacrimal glands
- Liver (sections of two lobes)
- Lungs (including bronchi, fixed by inflation with fixative)
- Lymph node, mesenteric
- submandibular
- Mammary gland (females only)
- Ovaries with oviducts (2)
- Pancreas
- Peripheral nerve (sciatic)
- Pituitary
- Prostate
- Salivary glands [submaxillary (2)]
- Seminal vesicles (2)
- Skeletal muscle (vastis medialis)
- Skin
- Spinal cord: cervical, midthoracic, lumbar
- Spleen

- Testes with epididymides (2)
- Thymus
- Thyroids [with parathyroids
- if present (2)l
- Tongue
- Trachea
- Urinary bladder
- Uterus with vagina
- All gross lesions (when possible)
- Injection Site

Organ weights (specify organs weighed if not in histopath table):

The following organs from all animals at scheduled and unscheduled necropsies will be weighed:

- Brain
- Liver: slightly enlarged for both males and females, statistically significant for the female of both dose groups. Relative to bodyweight the increased liver weight was significant for both
- Pancreas

Organ to body weight and organ to brain weight ratios will be calculated. The organ weights at the unscheduled necropsies were excluded from statistical analyses.

Histopathology: Adequate Battery: yes ( ), no ( )—explain
Peer review: yes ( ), no ( )

Representative sections of the liver and pancreas from all animals will be processed, sectioned and stained with hematoxylin and eosin. Microscopic examination of these sections was conducted on all animals. Examination was planned to be extended to other organs or tissues if deemed necessary (by amendment).

- Increased liver weight is correlated with microscopic finding of ductal hyperplasia, rated minimal, for both treated groups relative to control. The finding appears to be of slightly greater magnitude for the IV dosed group.
- Ductal hyperplasia (interlobular and intrainsular), rated minimal to mild, is reported for the pancreas for both treated groups.

Toxicokinetics: Not Done.

6.6.6.4 Genetic toxicology

Genetic toxicology testing is not usually required for biological therapeutic products. Therefore, only a brief review of the studies submitted with this BLA will be included.

Study title: Mutagenicity test with recombinant human keratinocyte growth factor in Salmonella-escherichia coli/mammalian-microchromosome reverse mutation assay preincubation method with a confirmatory assay

Key findings:
The results of the Salmonella - Escherichia coli/Mammalian-Microchromosome Reverse Mutation Assay, Preincubation Method With a Confirmatory Assay, indicate that, under the conditions of this study, in both an initial and confirmatory assay, the test article, Recombinant Human Keratinocyte Growth Factor (rHuKGF, palifermin), did not cause a positive increase in the number of revertants per plate of any of the tester strains either in the presence or absence of microsomal enzymes prepared from AroclorTM-induced rat liver (S9).

Study no.: 17686-0-422R/Amgen study # 960041

Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: 6/27/1996
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity:

Study title: In vitro mammalian cytogenetic test

Key findings:
The test article, Recombinant-Human Keratinocyte Growth Factor (r-HuKGF), was tested in the chromosome aberration assay using Chinese hamster ovary cells. The assay was performed in two phases. The first phase, the initial chromosome aberration assay, was conducted to establish the dose range for testing and to evaluate the clastogenic potential of the test article. The second phase, the independent repeat chromosome aberration assay, was performed to confirm the test system response to the test article seen in the initial assay. Both phases were conducted in the absence and presence of metabolic activation.
Dose levels of 15, 50, 150 and 500 pg/ml were selected for microscopic analysis in both the non-activated and S9-activated studies. No statistically significant increases in chromosome aberrations, relative to the solvent control group, were observed in the non-activated test system or in the S9-activated test system at any dose level evaluated.
An independent repeat chromosome aberration assay was conducted in the absence and presence of an Aroclor-induced S9 metabolic activation system at dose levels of 63, 125, 250 and 500 pg/ml. There was no toxicity observed (at least 50% cell growth inhibition) at any of the dose levels tested in the non-activated or S9-activated studies regardless of cell harvest time. No statistically significant increases in structural chromosome aberrations were observed in either the non-activated or S9-activated studies, regardless of dose level or harvest time. No statistically significant increases in numerical chromosome aberrations were observed in either the non-activated or S9-activated studies at the 44 hour harvest time, regardless of dose level.

Based on the findings of this study, Recombinant-Human Keratinocyte Growth Factor (r-HuKGF) was concluded to be negative for the induction of structural and numerical chromosome aberrations in the in vitro mammalian cytogenetics test.

Study no.: G95BS11.336

Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: 10/31/1995
GLP compliance: Yes, also performed in compliance with EC guidelines
QA reports: yes (X) no ( )
Drug, lot #, and % purity: palifermin lot# 01195B5C and 05035M5 (with and without histidine,
Placebo lot # 04105D5, 11155L5

Study title: Salmonella/Escherichia coli plate incorporation mutagenicity assay with a confirmatory assay

Key findings:
The purpose of this study was to evaluate the mutagenic potential of the palifermin by measuring its ability to induce reverse mutations at selected loci of several strains of Salmonella typhimurium and one strain of E. coli in the presence and absence of S9 activation.
In the preliminary toxicity assay, the maximum dose tested for E. coli was 5000 ug r-HuKGF per plate. The maximum dose tested for Salmonella was 2500 ug r-HuKGF per plate. Neither precipitate nor appreciable toxicity was observed with both E. coli and Salmonella. Based on the findings of the toxicity assay, the maximum dose plated in the mutagenicity assay for both E. coli and Salmonella was 5000 ug r-HuKGF per plate.

Under the conditions of this study, test article Recombinant Human Keratinocyte Growth Factor (r-HuKGF) was concluded to be negative in the Salmonella/Escherichia coli Mutagenicity Assay with a Confirmatory Assay. Although a positive response was observed in a single experiment, it was not reproducible in two other independent experiments. Therefore, the non-reproducible response does not meet the regulatory requirement for reproducibility and is concluded to be negative.

Study no.: G95BS11.502001/Amgen study# T-95-KGF-010

Volume #, and page #:
Conducting laboratory and location: [redacted]

Date of study initiation: 11/15/1995
GLP compliance: YES
QA reports: yes (X) no ( )
Drug, lot #, and % purity:

Study title: CHO/HGPT mutation assay with confirmation

Key findings:
The purpose of this study was to evaluate the mutagenic potential of the test article based on quantitation of forward mutations at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus of Chinese hamster ovary (CHO) cells.

All criteria for a valid study were met as described in the protocol. The results of the CHO/HGPRT Mutation Assay indicate that, under the conditions of this study, Recombinant Human Keiatinocyte Growth Factor (r-HuKGF) did not cause a positive response in the non-activated and S9-activated systems and was concluded to be negative.

Study no.: G95BS11.782001/Amgen study # T-95-KGF-008

Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: 10/25/1995
GLP compliance:
QA reports: yes ( ) no ( )
Drug, lot #, and % purity: placebo lot#04105d5
Palifermin lot# code 95BS11

Study title: Micronucleus genetic assay in mice

Key findings:
The purpose of this study was to assess the clastogenic potential of a test article to increase the incidence of micronucleated polychromatic erythrocytes in bone marrow of male and female mice.
The test article, Recombinant Human Keratinocyte Growth Factor (r-HuKGF, palifermin), was tested in the mouse micronucleus assay. The assay was performed in two phases. The first phase, designed to set dose levels for the definitive study, consisted of a pilot assay. In the second phase, the micronucleus study, the potential of the test article to increase the incidence of micronucleated polychromatic erythrocytes in bone marrow of male and female mice was evaluated. In both phases of the study, test and control articles were administered in a constant volume of 20 ml/kg body weight by a single intraperitoneal injection. In the micronucleus assay, male and female mice were dosed with 25, 50 or 100 mg/kg body weight of Recombinant Human Keratinocyte Growth Factor (r-HuKGF). No mortality or clinical signs were observed in any male or female mice in the micronucleus study. Slight reductions (up to 27%) in the

ratio of polychromatic erythrocytes to total erythrocytes were observed in some of the test article-treated groups relative to the respective vehicle controls.
No significant increase in micronucleated polychromatic erythrocytes in test article-treated groups relative to the respective vehicle control group was observed in male or female mice at 24, 48 or 72 hours after dose administration. The results of the assay indicate that under the conditions described in this report, Recombinant Human Keratinocyte Growth Factor (r-HuKGF) did not induce a significant increase in micronucleated polychromatic erythrocytes in either male or female mice. Recombinant Human Keratinocyte Growth Factor (r-HuKGF) was concluded to be negative in the mouse micronucleus assay.

Study no.: G95BS11.122/Amgen study # T-95-KGF-006

Volume #, and page #:
Conducting laboratory and location:
Date of study initiation: 8/04/1995
GLP compliance:
QA reports: yes ( ) no ( )
Drug, lot #, and % purity: palifermin lot# 01195B5C
Placebo lot# 02095B5, 04105D5

	Number Mice Per Sex Dosed	Number Mice Per Sex Used For Bone Marrow Collection After Dose Administration 24 hr	48 hr	72 hr
Vehicle Control	15	5	5	5
Low test dose (25 mg/kg)	15	5	5	5
Mid test dose (50 mg/kg)	15	5	5	5
High test dose (100 mg/kg)	15	5	5	5
CP, 60 mg/kg	5	5		

2.6.6.5 Carcinogenicity

Study title: Screen of tumor cell lines for expression of KGFR mRNA

Study number: 00-188
Conducting laboratory and location: Amgen, Inc.
Date of study initiation: not stated, completion date is 9/29/2000
GLP compliance: No
QA report: yes ( ) no (X)
Drug, lot #, and % purity:

Summary:
As part of an investigation of the effect of rHuKGF on proliferation of human tumor cell lines (Amgen study no. 100928, see below), expression of KGF receptor (KGFR) in lysates of these same cell lines was examined using an RNase protection assay (RPA).

BALB/MK, a mouse keratinocyte cell line that was used as a positive control for KGF-induced cell proliferation, was also tested for KGFR expression.
Eleven cell lines tested were found to express the KGFR mRNA: NCI-322M, T-47D, SCC15, CaCo2, DLD-1, HCC-116, HT-29, KM-12, KM20L2 and Balb/MK.

Study title: The effects of r-HuKGF on the proliferation rates of 41 human tumor cell lines
Study number: Amgen number 100928, [redacted] number A068.1
Conducting laboratory and location: [redacted]
Date of study initiation: 6/1/2000
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: palifermin lot # 24010C9, placebo lot # 24-07D9

Key study findngs:
This study was undertaken to determine the effects of palifermin on a panel of human tumor cell lines consisting of 13 lung, 11 colon, 10 mammary, 3 leukemia, 3 prostate, and 1 tongue tumor type. One normal mouse keratinocyte cell line, BALBMK, was also included in the tested lines. Beginning on Day 0, the cell lines were cultured with the test article at various concentrations (0, 10, 100, 1,000, 10,000, 100,000 ng/mL) and the controls included placebo treated and untreated cells. On Days 2 and 5, cells were treated with 3H-thymidine and analyzed to measure 3H-thymidine incorporation.

The major finding of this study was that treatment at the highest level of palifermin (100,000 ng/mL) decreased the incorporation of tritiated thymidine, which indicates either fewer cells present or fewer cells synthesizing DNA and may indicate a cytotoxic effect. There is evidence that the test article-induced proliferation at one or more lower concentrations in cell lines DU 145 (prostate) , NCI-H82 (lung), NCI-H322M (lung), EKVX (lung), KM2OL2 (colon), DLD-1 (colon), HCC-2998 (colon), HT-29 (colon), T47D (mammary), [redacted]4 (mammary), and UISO-BCA-1 (mammary). This effect was not of great magnitude, representing a 30%-100% increase in tritiated thymidine incorporation.

Further studies were carried out (See study # 00-188, above) to determine whether mRNA fro KGFR is expressed in these cell lines. Of the cell lines studied in 100928, T47D, HT-29, KM20L2, DLD-1, EKVX, NCI-322M, HCC-2998 (low) were shown to express mRNA for KGFR under study #00-188.

Tumor cell lines used are illustrated in the following table, supplied by the sponsor:

Cell Line	Tumor Type	Histology
A-427	Lung	Carcinoma
A-549	Lung	Carcinoma
EKVX	Lung	Adenocarcinoma
HOP-62	Lung	Carcinoma
LX-1	Lung	Not Applicable
NCI-H23	Lung	Large Cell Adenocarcinoma
NCI-H249	Lung	Small Cell Carcinoma
NCI-H322M	Lung	Small Cell Carcinoma
NCI-H358M	Lung	Mixed Cell Carcinoma
NCI-H460	Lung	Large Cell Carcinoma
NCI-H522	Lung	Large Cell Adenocarcinoma
NCI-H69	Lung	Small Cell Carcinoma
NCI-H82	Lung	Small Cell Carcinoma
BT-474	Mammary	Ductal Carcinoma
BT-549	Mammary	Carcinoma
Hs 578T	Mammary	Ductal Carcinoma
MCF7	Mammary	Adenocarcinoma
MDA-MB-231	Mammary	Adenocarcinoma
MDA-MB-435	Mammary	Adenocarcinoma
MX-1	Mammary	Carcinoma
T-47D	Mammary	Ductal Carcinoma
UISO-BCA-1	Mammary	Adenocarcinoma
ZR-75-1	Mammary	Ductal Carcinoma
CCRF CEM	Leukemia	T-Cell Lymphoblastic
HL-60	Leukemia	Promyelocytic
K-562	Leukemia	Chronic Myelogenous
DU 145	Prostate	Carcinoma
LNCaP	Prostate	Adenocarcinoma
PC-3	Prostate	Carcinoma
SCC-15	Tongue	Squamous Cell Carcinoma
COLO 205	Colon	Adenocarcinoma
COLO 320DM	Colon	Adenocarcinoma
Caco-2	Colon	Adenocarcinoma
DLD-1	Colon	Adenocarcinoma
HCC-2998	Colon	Carcinoma
HCT 116	Colon	Carcinoma
HCT-15	Colon	Adenocarcinoma
HT-29	Colon	Adenocarcinoma
KM12	Colon	Carcinoma
KM20L2	Colon	Adenocarcinoma
SW-620	Colon	Adenocarcinoma

Mouse cell lines tested:

Cell Line	Tumor Type	Histology
BALB/MK	Normal	Keratinocyte

Study title: Effect of intermittent rHu KGF on growth of subcutaneous DU-145, HT-29, [ ], UISO-BCA-1, and EKVX xenografts in athymic mice

Key study findings:
This series of non-GLP preclinical studies was designed to evaluate the effects of palifermin on the growth of a variety of subcutaneously implanted human tumor xenografts in athymic nude mice. Various human cancer cell lines were implanted subcutaneously into male athymic nude mice. Groups of mice with each tumor type were treated with 0, 150, 500, 1500 or 4000 ug/kg, IV, of palifermin for a series of treatment cycles (three days treatment followed by 4 days off treatment). Tumor volumes were calculated for each dose group of each tumor type and vehicle control. The time to two tumor volume doublings was calculated for each dose group for each tumor type and compared to that of the relevant control group. The results showed growth of one tumor cell line to be significantly enhanced by palifermin treatment. However, choice of cell lines and other factors make use of the data as predictor of tumor promotion potential of palifermin in humans questionable.

Evaluation of tumor findings and adequacy of model: One tumor type [ ] showed a statistically significant growth enhancement in a dose dependent manner when exposed to increasing doses of palifermin. However, at least one additional experiment appeared to show a dose dependent increase in tumor growth (see results using [ ] below). In that case, the sponsor states that statistical analysis did not show significance. Some of the tumor types appeared to be inhibited by palifermin at the higher doses. The sponsor interprets this phenomenon as supporting the lack of test article related effect because a continued dose-related growth enhancement is not seen. This may be a pre-mature conclusion. No toxicokinetics were performed for this study so actual systemic levels or exposure could be determined. In addition, no positive control was run for any cell line, although each experiment verified drug activity using stomach size enhancement relative to control as a biomarker. It is not clear that all of the cell lines examined express functional KGFR, nor is there any way to determine receptor occupancy. Also, groups of mice were small (20 for control groups, 10 for each treatment group) and a large incidence of animal death was encountered in many groups, including controlgroups. Therefore, use of these data to predict potential for tumor promotion in humans is questionable.

Study no.: 100930 AMG-4, AMG-5, AMG-6, AMG-7, AMG-8, AMG-10
Volume #, and page #:
Conducting laboratory and location: [ ]
Date of study initiation:
GLP compliance:

QA report: yes ( ) no ( )
Drug, lot #, and % purity:
CAC concurrence:

Methods

Doses: 0, 150, 500, 1500, or 4000 ug/kg
Basis of dose selection (MTD, MFD, AUC etc.):
Species/strain: male athymic nude mice
Number/sex/group (main study): Control group: 20 mice, all dose groups: 10/group
Route, formulation, volume: IV via tail vein, 0.05 ml/10 gm body weight
Frequency of dosing: Regimen consisted of 3 daily IV injections followed by 4 days with no treatment, dosing cycle repeated until 2 volume doublings were achieved.
Satellite groups used for toxicokinetics or special groups: NA
Age: 6 weeks
Study design: Mice were implanted subcutaneously with tumor fragments grown from each cell line. For each tumor type, the implanted tumor fragments were allowed to grow to a pre-specified tumor size prior to initiation of treatment. Treatment cycles as described above, were carried out for varying numbers of cycles depending tumor volume doubling time. One to three volume doublings are commonly used I this type of study for detection of tumor growth delay.
The tumor volume doubling time of the various groups was determined as the median doubling time of the individual tumors within the group, and this was used to calculate a "T - C" value. The T-C value is calculated as the difference in doubling time between the control group and each treatment group. Results are expressed in number of days of difference in time to a pre-specified number of volume doublings between each dose group and control for tumor type.

Observation times and Results
Mortality: Observed daily. Multiple animal deaths were noted for all dose groups for each experiment, including control groups.
Clinical signs: Observations recorded daily

Body weights: recorded twice weekly, tumors measured twice weekly during each treatment cycle.
No toxicokinetics was performed for these studies.
Gross pathology: Limited necropsy was performed at the end of each study. As a biomarker of exposure to palifermin, the stomach was removed and weighed. Individual mice were euthanized when tumor weights exceeded 4000 mg.

Tumor growth results:

- EKVX tumors: T-C values of -4.0, 2.7, -1.8 and 13 days were reported for the 150, 500, 1500 and 4,000 ug dose groups, respectively. These results indicated that, at the higher concentration, palifermin may have had an inhibitory effect on growth of this tumor type. Expected dose related increase in stomach weight was observed, confirming biological activity of the test article.

- UISO-BCA-1 tumors: T-C values of -1.9, -4.4, 0.7 and 2.6 days were reported for for the 150, 500, 1500 and 4000 ug groups, respectively. Statistical analysis indicated that palifermin treatment had no significant effect on tumor growth. Stomach weights showed the expected dose-dependent increase confirming exposure to, and biological activity of the test article.
- [ ] tumors: T-C values of -2.4, -4.6, -4.0 and -5.0 days were reported for the 150, 500, 1500 and 400ug dose groups, respectively. The sponsor reported that statistical analysis indicated that palifermin treatment had no effect on tumor growth. Stomach weights increased in a dose dependent pattern, as expected.
- [ ] tumors: T-C values of -1.9, -2.2, -2.9 and -3.0 were reported for the 150, 500, 1500 and 4000 ug dose groups, respectively. Statistical analysis indicated that palifermin exposure had a significant tumor promoting effect for this tumor type.
- HT-29 tumors: T-C values of -3.6, -7.8, 3.1 and 2.6 days were reported for the 150, 500, 1500 and 4000 ug dose groups, respectively. Statistical analysis indicated that there was no effect of palifermin treatment on tumor growth. However, the increase in growth for the lower doses is seen with other such cell-line originated tumors. The sponsor concludes that, because the increase in growth was not continued in a dose dependent manner at the higher doses, that the increase in growth at the 500 ug dose level was not test article related. This conclusion may be premature. Receptor occupancy was not evaluated so a saturation level could have been reached at the high doses.
- DU-145 tumor: T-C values of -0.9, -1.8, 0.6 and -0.3 days were reported for the 150, 500, 1500 and 4000 ug dose groups, respectively. Statistical analysis suggests that palifermin had no effect on tumor growth rate. This tumor cell line did not test positive for KGFR under 00-188.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: Intravenous fertility and general reproductive toxicity study of recombinant human keratinocyte growth factor (r-HuKGF) in rats

Key study findings:
The purpose of this study was to test for toxic effects/disturbances resulting from palifermin treatment of Srprague Dawley male and female rats before cohabitation, through mating and implantation. This study was designed to address ICH Harmonised Tripartite Guideline stages A and B of the reproductive process to detect effects on the estrous cycle, tubal transport, implantation, and development of pre-implantation stages of the embryos of female rats and permit detection of functional effects (e.g., effects on libido or epididymal sperm maturation) that may not be recognized in histological examinations of male rat reproductive organs.
A dose of 100 ug/kg/day of palifermin did not affect mating or fertility. However, effects on body weights in male and female rats, clinical observations, increased food consumption and increased epididymides weights were seen in all dose groups. There fore, a NOAEL could not be identified. Higher doses produced clinical signs and

affected body weights, food consumption, organ weights in at least one sex. Adverse effects on male and female reproductive performance and the number and viability of the offspring occurred at the 300 and 1000 ug/kg/day doses.
Dose-dependent histopathological alterations were observed in the reproductive organs of the high dose group male rats. These included hyperplasia of the tubular epithelium of the epididymides in all rats in this group, and necrotic germ cells (10/25) and/or hypospermia within the epididymal tubules (3/5, two of which were the moribund sacrifices). Signs of reduced secretory activity were observed in the seminal vesicles (21/24) and prostate (17/24) of nearly all the high dosage group rats, and four rats in this group had muitifocal or diffuse testicular degeneration, one of which was moribund sacrificed.
The fertility index (number of pregnancies/number of rats that mated: 48%) and the number of pregnant rats/number of rats in cohabitation (44%) were significantly reduced in the 1000 ug/kg/day dosage group, relative control (100%).

The 300 and 1000 ug/kg/day dosage groups had significant reductions (ranging from approximately 16% to 27%; in cauda epididymal sperm counts and concentrations, relative to control. Sperm motility was unaffected by dosages of the test article as high as 1000 ug/kg/day.

Estrous cycling: the average number of days in cohabitation before mating and mating incidences were unaffected by dosages of the test article as high as 1000 ug/kg/day.

The number of pregnant rats per rats in cohabitation were significantly reduced in the 1000 ug/kg/day dose group, relative to control. The 1000 ug/kg/day dose group also had significant reductions in the litter averages for corpora lutea and implantations.

The 300 and 1000 ug/kg/day dosages of the test article were associated with increases in embryo deaths evident as significant increases in the litter averages for nonviable embryos and percent nonviable embryos, significant reductions in the litter averages for viable embryos and litter sizes (the sum of viable and nonviable embryos) and increases in the number of dams with nonviable embryos.

Study no.: 19901-012/Amgen number T-095-KGF
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: 4/23/1996
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: palifermin lot #05035E5A
Placebo lot #11155L5

Methods

Doses: 0, 100, 300 or 1000 ug/kg/day

Species/strain: Sprague/Dawley rats: male 89days old, female: 66 days old
Number/sex/group: 25/sex/group
Route, formulation, volume, and infusion rate: IV injection in a volume of 1ml/kg
Satellite groups used for toxicokinetics:
Study design:
The test article, palifermin, or control article, r-HuKGF Placebo, was administered via intravenous injection once daily to these male and female rats 28 and 15 days, respectively, before a 21-day cohabitation period. Daily injection continued through cohabitation until the day before scheduled sacrifice (male rats) or day 7 of presumed gestation.

Dosage Group	Dosage (µg/kg/day)	Number of rats per sex	Concentration (mg/mL)	Assigned Numbers	
				Male Rats	Female Rats
I	0[ControlArticle(Placebo)]	25	. 0	801 - 825	901 - 925
II	100	25	0.1	826 - 850	926 - 950
III	300	25	0.3	851 - 875	951 - 975
IV	1000	25	1.0	876 - 900	976 - 1000

Parameters and endpoints evaluated:
The rats were observed for viability at least twice each day of the study. The rats were also examined for clinical observations of effects of the test article, abortions, and premature deliveries before and once daily after dosing. These observations were also conducted daily during the postdosing period for female rats.
Body weights were recorded daily during the dosing period and postdosing period (female rats) and at sacrifice. Food consumption values were recorded weekly to cohabitation (all rats), weekly until sacrifice (male rats) and on DGs 0, 7, 8 and 13 (female rats). Estrous cycling was evaluated by examination of vaginal cytology for 14 days before the initiation of dosage and then for another 14 days, beginning with the first day after injection. Evaluation of the vaginal cytology continued during the cohabitation period until spermatozoa were observed in a smear of the vaginal contents or a copulatory plug was observed in situ (DG 0).
Male rats were euthanized on the day after cohabitation ended. Gross necropsy was performed. The right testis, left testis, left epididymis (whole and cauda), right epididymis, seminal vesicles (with and without fluid), prostate, kidneys (paired weights) and liver were excised, weighed and appropriate sections were retained. Cauda epididymal sperm count, viability and motility were evaluated, and the testes, epididymides, prostate and seminal vesicles of each male rat were examined microscopically.

All surviving female rats were sacrificed on DG13, and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The liver and kidneys (paired) of each rat were excised and weighed. The kidneys, liver and ovaries of each rat were retained. The number of corpora lutea in each ovary was recorded. The uterus of each rat was excised and examined for pregnancy, number and distribution of implantations and viable and nonviable embryos. Placentae were evaluated for abnormalities in size, color or shape.

Results

Mortality:

- 2 male rats from the 1000 ug group were euthanized moribund on SD 43. One additional male rat from the 1000 ug group was found dead on SD 46.
- 2 female rats from the 300 ug/kg group were euthanized moribund or found dead.

Clinical signs:

- Dose-dependent increases in the numbers of male rats with adverse clinical observations occurred in all groups administered the test article, as compared with the control group values. The onset of effects was time- and dose-dependent, generally first observed after at least one week of dosage, and included ungroomed coat and chromorhinorrhea (100, 300 and 1000 ug/kg/day dose groups); red perinasal substance, red swollen right ear (generally the location of the eartag), and a mass at the ear-tag (300 and 1000 ug/kg/day dose groups); swollen red scaly nose, swollen forepaws and hindpaws, excess salivation, swollen ocular membranes, moist discolored fur, labored breathing, rales, soft or liquid feces, chromodactyorrhea, multiple red areas on the neck, axilla, chest and/or inguinal area, skin lesions on the forelimb or hindpaw, swollen red and/or scaly scrotum and prepuce, a mass on the head or tail, coldness to touch, and a red perioral substance, lacrimation and coldness to touch (1000 ug/kg/day dose group).

The 1000 ug/kg/day dose of the test article caused significant increases in the number of female rats with chromorhinorrhea and ungroomed coat during the precohabitation period. During gestation, chromorhinorrhea occurred in increased or significantly increased numbers of female rats in the 300 and 1000 ug/kg/day dose groups. Significantly increased numbers of 1000 ug/kg/day dose group female rats also had ungroomed coat, swollen, red ear and/or a mass at the eat-tag.

Body weight:

For the male rats: the 100 and 300 ug/kg/day dose groups had significant increases in body weight gains on SD1 to 8; the 1000 ug/kg/day dose group had significantly reduced weight gain during this interval, relative to Control. After the first week of treatment, the 100 and 300 ug/kg/day dose groups generally had body weight gains similar to or slightly higher than the control group values, whereas the 1000 ug/kg/day dose group had larger reductions in body weight gains. Body weight gains for the precohabitation period (calculated as SD1 to 28) were significantly increased (16%) in the 100 ug/kg/day dose group, tended to be increased (10%) in the 300 ug/kg/day dose group, and were significantly reduced (66%) in the 1000 ug/kg/day dose group. Body weight gain for the entire dosing period was increased 9% in the 100 ug/kg/day dose group, 3% for the 300 ug/kg/day dose group and was significantly reduced (89%) in the 1000 ug/kg/day dose group. Average body weights were significantly reduced in the 1000 ug/kg/day dose group beginning on day 8 of the study. Average body weights in the 100 and 300 ug/kg/day dose groups were comparable to the control group values.

Female rats in the 100, 300 and 1000 ug/kg/day dose groups had significant increases in body weight gains on SD1 to 8, the first week of treatment. The 1000 ug/kg/day dose

group tended to have reduced body weight gain on SD8 to 15. Reflecting these effects of the test article, body weights were significantly increased in the 300 and 1000 ug/kg/day dose groups on DS 8 and in the 300 ug/kg/day dosage group on DS 15, and body weight gains for the entire precohabitation period were increased (ranging from approximately 80% to 140%) in all groups that received the test article, although the value was significant only for the 300 ug/kg/day dose group.

As the result of increased weight gains in female rats in all groups administered the test article during the precohabitation period, maternal body weights were significantly increased on day of gestation (DG) 0 in the 100, 300 and 1000 ug/kg/day dose groups, as compared with the control group value. Although body weights in the test article-administered groups continued to be higher than in the control group throughout the gestation period, and were generally significantly increased in the 300 and 1000 ug/kg/day dose groups, significant reductions in body weight gains in the 100, 300 and 1000 ug/kg/day dose groups on DGs 8 to 13 resulted in only small differences in maternal body weights after DG 10. The reductions in maternal body weight gains in the 300 and 100 ug/kg/day dose groups were associated with increased resorption of the conceptuses in these dosage groups.

Food consumption:
Absolute (g/day) and relative (g/kg/day) feed consumption values were significantly reduced in the 1000 ug/kg/day dose group on days 1 to 8 of the study. Though the differences between the control and high dose groups in absolute feed consumption diminished in the subsequent weeks, these values were still significantly reduced in the high dose group in the cumulative intervals of SD1 to 28 and SD 1 to 56. In contrast, these values were generally significantly increased in the 100 and 300 ug/kg/day dose groups, which reflected the increases in body weight gains in these groups.

Absolute (g/day) and relative (g/kg/day) feed consumption values were significantly reduced in the 1000 ug/kg/day dosage group on SD 1 to 8, but tended to be slightly increased, as compared with the control group values, on SD8 to 15. As a result, only the relative feed consumption value for the 1000 ug/kg/day dose group was significantly reduced for the entire precohabitation period. A significant reduction in the relative feed consumption value for the 100 ug/kg/day dose group on SD1 to 15 was considered unrelated to the test article because the value was not dose-dependent.

Necropsy:
Significantly increased numbers of 300 and 1000 ug/kg/day dose group male rats had a small thymus, and this observation also occurred in two 100 ug/kg/day dose group rats. The 300 and 1000 ug/kg/day dose group rats also had significantly increased incidences of a thickened stomach wall with a white substance present on the fundic and/or cardiac regions. Significant numbers of the 1000 ug/kg/day dose male rats had a small prostate and/or flaccid seminal vesicles.

Organ weights:

The 100, 300 and 1000 ug/kg/day dosage groups had significant, dose-dependent increases (ranging from approximately 10% to 50% of the control) in ratios of organ weight to terminal body weight in the left and right epididymides, liver and paired kidneys. The absolute weights of these organs were also significantly increased in the 100 and 300 ug/kg/day dose groups. Absolute and relative weights of the seminal vesicles with fluid were significantly reduced in the 300 (approximately 10%) and 1000 (approximately 55% for the absolute value and 40% for the relative value) ug/kg/day dose groups. Changes in the weights of seminal vesicles without fluid were smaller in magnitude, and only the value for the absolute weight in the high dose group was statistically significant. Prostate weights, both absolute and relative, were significantly reduced (approximately 40% and 20%, respectively) in the high dose group. The absolute weights of both the left and right testis were significantly reduced in the 1000 ug/kg/day dose group. However, the ratios of the weight of either testis to terminal body weight were significantly increased.

Histopathology:
Dose-dependent histopathological alterations were observed in the reproductive organs of the high dose group male rats. These included hyperplasia of the tubular epithelium of the epididymides in all rats in this group, and necrotic germ cells (10/25) and/or hypospermia within the epididymal tubules (3/5, two of which were the moribund sacrifices). Signs of reduced secretory activity were observed in the seminal vesicles (21/24) and prostate (17/24) of nearly all the high dosage group rats, and four rats in this group had muitifocal or diffuse testicular degeneration, one of which was moribund sacrificed.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

- The fertility index (number of pregnancies/number of rats that mated: 48%) and the number of pregnant rats/number of rats in cohabitation (44%) were significantly reduced in the 1000 ug/kg/day dosage group, relative control (100%).

- The 300 and 1000 ug/kg/day dosage groups had significant reductions (ranging from approximately 16% to 27%; in cauda epididymal sperm counts and concentrations, relative to control. Sperm motility was unaffected by dosages of the test article as high as 1000 ug/kg/day.

- Estrous cycling: the average number of days in cohabitation before mating and mating incidences were unaffected by dosages of the test article as high as 1000 ug/kg/day.

The number of pregnant rats per rats in cohabitation were significantly reduced in the 1000 ug/kg/day dose group, relative to control. The 1000 ug/kg/day dose group also had significant reductions in the litter averages for corpora lutea and implantations.

The 300 and 1000 ug/kg/day dose of the test article were associated with increases in embryo deaths evident as significant increases in the litter averages for nonviable

embryos and percent nonviable embryos, significant reductions in the litter averages for viable embryos and litter sizes (the sum of viable and nonviable embryos) and increases in the number of dams with nonviable embryos.

Embryofetal development

Study title: An intravenous rabbit developmental toxicity study with rHuKGF

Key study findings:
This study was designed to determine the maternal and developmental toxicity, including the teratogenic potential, of rHuKGF in New Zealand White rabbits. The study consisted of 3 treatment groups and 1 placebo control group (22 time-mated female rabbits/group). Animals were treated from day 6 to day 18 of gestation. Observations of does included clinical signs, gestation body weights, and food consumption. Animals were euthanatized on gestation day 29 (GD29) and subjected to a necropsy. Gravid uterine weights were recorded. The total number of corpora lutea, uterine implantations, early and late resorptions, viable and nonviable fetuses, and the sex and individual body weights of fetuses were recorded. All fetuses were examined for external, visceral (microdissection), and skeletal malformations and developmental variations. Approximately one-half of the fetuses in each litter were decapitated, and the heads were fixed and head sections were evaluated under a dissecting microscope.
The TK component was comprised of 3 treatment groups (same dose levels as in the main study) and 1 placebo control group (3 timed pregnant female rabbits/group). Blood was collected from the TK animals on GD6 and GD18 at 8 time points: predose, 5 minutes, 30 minutes, and 1, 4, 6, 8, and 24 hours postdosing. Following the last blood collection on GD19, the TK animals were euthanized. Pregnancy status and the total number of corpora lutea and uterine implantations (distinguished as viable fetuses and early resorptions) were recorded, and the carcasses were discarded. Drug concentrations were measured using a validated enzyme-linked immunoabsorbent assay (ELISA) with a lower limit of quantification of [redacted] ng/mL. Toxicokinetic parameters were estimated using noncompartmental analysis.

All treated animals in the developmental study and TK component survived to scheduled euthanasia. One control animal in the developmental study died during the study (GD15). Pregnancy rates in the developmental study were 90.9%, 100%, 95.5%, and 90.9% in the control, 5, 60, and 150 ug/kg/day groups, respectively. One female in each of the 60 and 150 ug/kg/day groups aborted. Four animals in the 150 ug/kg/day group that was euthanatized on GD29 did not have grossly visible uterine implants (fetuses or resorptions) and were confirmed pregnant solely on the basis of stained foci in utero. There were 19, 22, 20, and 15 litters with viable fetuses for evaluation on GD29 in the control, 5, 60, and150 ug/kg/day groups, respectively. In the TK study segment, pregnancy rates were 100% in all groups.

No effect of treatment at the 5 ug/kg/day dose level was evident from clinical examinations, gestational parameters (body weight, body weight gain, and food consumption), or macroscopic findings.

Clinical findings seen at the 60 and/or 150 ug/kg/day dose levels and considered related to treatment included ocular discharge (clear), swelling around eye(s), swollen lips, swollen ear(s), white material around eye (s), ears discolored red, and skin discolored about eye(s). More animals at these dose levels showed reduced fecal output (feces few/absent) and soft stool. Animals at the 60 and 150 ug/kg/day dose levels also gained less weight and were eating less during the treatment period. No effect of treatment at the 5 and 60 ug/kg/day dose levels was evident from uterine implantation data and fetal sex distribution. Fetal body weights, distinguished by sex and for both sexes combined, in these groups were slightly lower than control. At the 150 ug/kg/day dose level, there was an increase in postimplantation loss and a corresponding decrease in litter size (viable fetuses/doe). No effect of treatment was evident from fetal sex ratios, but fetal body weights were about 15% lower than controls. Dose-related increases on the incidence of some common developmental variations (e.g., small gallbladder, bent hyoid arch) were observed. The sponsor did not consider the abnormalities top be toxicologically meaningful because they were seen at relatively low incidence and are common malformations in this species. However, they appeared to be dose dependent and a dose related effect of palifermin cannot be ignored in this basis. In this intravenous rabbit developmental toxicity study with rHuKGF, the No-Observable-Adverse-Effect-Level (NOAEL) for maternal toxicity was 5 ug/kg/day. This was based on clinical signs, lower maternal body weight gain, and reduced food consumption during the treatment period at dose levels of 60 ug/kg/day and above. The NOAEL for developmental toxicity was considered 60 ug/kg/day based on increased postimplantation loss, smaller litter size, and lower fetal body weights at the 150 ug/kg/day dose level, which was the highest dose level evaluated.

Study no.: Amgen #529-048, — ' # 101728
Volume #, and page #:
Conducting laboratory and location: [redacted]

Date of study initiation: 7/16/01
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: rHuKGF lot # 24010J0, vehicle lot # A0104270000

Methods

Doses: 0, 5, 60 and 150 ug/kg/day

Species/strain: New Zealand White rabbits, 5-8 months

Number/sex/group: 3 treatment groups and 1 placebo group, 22 time-mated females per group

Route, formulation, volume, and infusion rate: IV injection via ear vein, single dose per day in a volume of 0.3 ml/kg

Satellite groups used for toxicokinetics: See Table, below.

Study design: Study design is illustrated in the table provided by the sponsor:

4. STUDY DESIGN

TREATMENT GROUP	rHuKGF DOSE LEVEL µg/kg	NOMINAL (TARGET) CONCENTRATION µg/ml	INITIAL (N) DOSED DAILY (DAY 6-DAY 18) F	CESAREAN SECTION/ NECROPSY (DAY 29) F	MICROSCOPIC PATHOLOGY F
1	0	0	22	22	A.R.
2	5	16.7	22	22	A.R.
3	60	200	22	22	A.R.
4	150	500	22	22	A.R.
5	0	0	3	-[a]	-
6	5	16.7	3	-[a]	-
7	60	200	3	-[a]	-
8	150	500	3	-[a]	-

A.R. = As Required: Study Director decision in consultation with the Sponsor.

[a] – TK animals will be euthanized and pregnancy status determined after the last blood collection on Gestation Day 19.

Parameters and endpoints evaluated:

- Cageside and detailed observations made on all rabbits twice daily. On days 6-29, each main study rabbit given a detailed physical exam.
- Body weights: Weights were recorded on gestation days 0, 6, 9, 12, 15, 19, 21, 25 and 29. TK animals will be weighed on the same dates but will not be analyzed as will those from the main study. TK animal weights will be used for adjustment of dose volumes to the most recent body weight.
- Food consumption: Recorded daily for main study animals only.
- Mortality/moribundity: Daily.
- Abortion/premature delivery: Females showing signs of abortion earlier than 24 hour prior to scheduled necropsy will be subjected to necropsy and uterine examinations. Females showing signs of premature delivery within 24 hours of scheduled sacrifice will be euthanized and necropsied.

Results

Mortality: All treated does survived to scheduled sacrifice. One control animal died suddenly due to unknown causes.

Clinical signs:

- A dose dependent increase in observed swelling around the eyes of the does in the high dose group accompanied by clear or white ocular discharge. 230 observations in 20 animals for the left eye and 229 observations in 20 animals for the right eye in the high dose group. For the 60 ug/kg group, the same observations were noted 69 times in 11 left eyes and 74 observations for the right eye in 12 animals. No such observations were reported for either the low dose or control groups. A similar pattern is reported for the eye discharge with no observations in the low or control groups, 22-26 observations in 7 animals for the

mid dose group and 75 observations for 15 animals in the high dose groups for each eye.

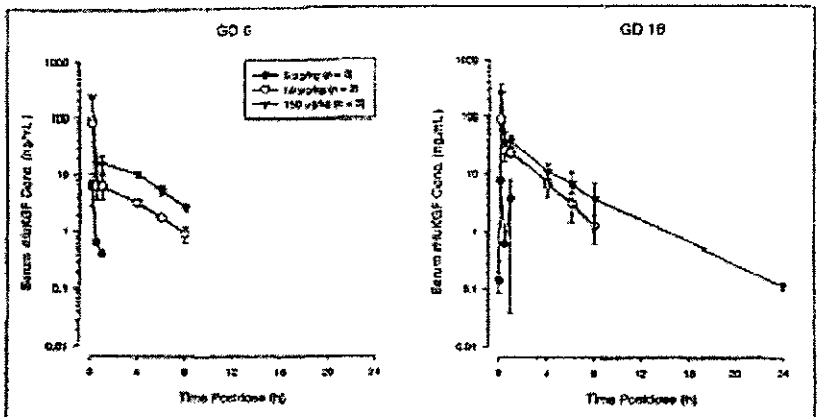
- Lip swelling is reported showing a similar dose dependent relationship. No observations reported for the control and low dose group, 19 observations in 3 animals in the mid dose group and 83 observations in 15 animals in the high dose group.
- Incidence of soft feces occurred in all dose groups including control but showed a dose related in crease in incidence
- A dose related increase in pelage and skin findings was noted: hair absent, hair discolored. Such findings are consistent with previously reported findings in rat toxicity studies.

Body weight: All groups of animals are reported to gain weight over time during the study. However, animals in the high dose group gained less rapidly than controls and mean group weight was significantly less that control at all time points after day 12. This appears to be study drug administration related and may correlate with the swollen lip observation noted above. Calculation of body weight change values for each interval shows a trend toward reduced weight gain in the treated animals with increasing frequency over the dose groups (reported differences in the calculated values most often noted for the high dose group.)

Food consumption: The dose dependent reduction in weight gain is correlated with a dose dependent reduction in food consumption over the course of the study. It is clear that the treated animals ate less than controls with severity increasing with the dose of the study drug.

Toxicokinetics:

Blood will be collected from the TK rabbits (Groups 5-8) at 8 time points on each of 2 days: predose, 5 minutes, 30 minutes, 1 hour, 4 hours, 6 hours, 8 hours, and 24 hours postdose, following the first dose on Gestation Day 6 and the last dose on Gestation Day 18. When thelast sample is collected on Gestation Day 19, the TK animals will be euthanized. The abdominal cavity was opened and the pregnancy status determined and number of implants distinguished as viable and nonviable fetuses and resorption recorded. The animal's carcass was then discarded with no further evaluations.
Results are summarized in the table below, provided by the sponsor:



Mean (SD) rHuKGF TK parameter values on GD 6 and GD 18 are depicted below.

Parameter	5 µg/kg (n = 3)*	60 µg/kg (n = 3)	150 µg/kg (n = 3)
	Gestation Day 6		
C_0 (ng/mL)	11.7 (NA)	142 (13.0)	332 (14.5)
AUC_{0-t} (ng·h/mL)	2.15 (NA)	45.9 (4.39)	128 (14.3)
	Gestation Day 18		
C_0 (ng/mL)	12.9 (0.920)	120 (49.3)	399 (243)
AUC_{0-t} (ng·h/mL)	2.58 (0.600)	99.7 (17.1)	202 (20.8)
AR	1.40 (NA)	2.16 (0.176)	1.58 (0.304)

*n = 2 on GD 6; therefore, no standard deviation is reported.
C_0 = Estimated concentration at time 0
AUC_{0-t} = Area under the serum concentration-time curve from time 0 to t (t corresponds to the time of the last quantifiable concentration)
AR = Accumulation ratio = $AUC_{0-t\ GD\ 18}/AUC_{0-t\ GD\ 6}$
Except at t = 0, concentrations below the limit of quantification [ ] ng/mL) were set to 0.000 for TK analysis.
NA = Not applicable
All numbers are reported to 3 significant figures.

Conclusion: One animal in the control group had measurable concentrations on GD 6 and 18; it is unclear if this observation is because of rHuKGF dosing or some other reason (e.g., sample contamination). All animals dosed with rHuKGF had measurable concentrations of rHuKGF as assessed on GDs 6 and 18. The increase in C_0 was approximately dose proportional in the dose range of 5 µg/kg to 150 µg/kg on both GD 6 and 18. A greater than dose proportional increase in AUC_{0-t} was observed between 5 µg/kg and 60 µg/kg doses, but AUC_{0-t} increased approximately dose proportionally between the doses of 60 to 150 µg/kg on GDs 6 and 18. The accumulation ratios were 1.40, 2.16, and 1.58 for the 5-, 60-, and 150-µg/kg dose groups, respectively.

The sponsor suggests that the accumulation observed after multiple doses may be due to formation of anti-drug antibodies. Such antibodies were detected after GD 18 in a previous study in which animals were dosed daily with doses ranging from 15 ug/kg to 500 ug/kg.

Necropsy:

Does:

- No differences among groups were noted in number of animals pregnant.
- A slightly higher rate of abortion was reported for the high and mid dose groups (1 for each high and mid dose groups, 0 for the low and control groups) accompanied by a lower number of does with viable fetuses in the high dose group. (15 for group 4, 20 for group 3, 22 for group 2 and 19 for group 1)
- A dose dependent swelling around the eyes, lip and ears was noted in treated groups compared to controls. Eye redness and swelling was accompanied by discharge in the high dose group.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

- No differences in corpora lutea, implantation sites, fetal sex ratio, or preimplantation loss were noted among groups.
- A small reduction compared to control was noted for the number of viable fetuses in the high dose group.
- Post implantation loss showed a dose dependent increase across the treated groups reaching statistical significance for the high dose group (3.13% for control, 39.75 % for the high dose group).
- The number of total resorptions was significantly higher in the high dose group and the trend toward increased resorptions is noted in a dose dependent manner across all treated groups. The increase in resorptions appears to be accounted for by a significant increase in early resorption for the high dose group plus a trend for increase in late absorptions in the high dose group.
- Gravid uterine weight was significantly lower in the mid and high dose groups compared to control but, when adjusted to body weight the difference was lost. However, when adjusted to weight change from day 0 a significant reduction is noted across treated groups in a dose dependent manner.
- Fetal weight showed a significant reduction across treatment groups in a dose dependent manner.
- A slight increase in abnormal flexure of the forelimbs was noted for the mid and high dose groups (one fetus for each dose group, 0 for the low and control groups)
- A slight increase in abnormal head shape is reported for groups 3 and 4, one fetus for group 4 and 2 fetuses from one litter for group 3, 0 for the low dose group and control.
- One fetus in the high dose group showed abnormal hind limb flexure with 0 for each of the other dose groups.
- Gall bladder abnormalities showed an apparent dose dependent increase across groups: absent gall bladder noted in one fetus in group 3 and 2 fetuses in group 4, small gall bladder also showed an increase across groups in a dose dependent manner (number of affected fetuses was 12, 3, 7 and 8 for control, low, mid and high dose, respectively.) The biological significance of these findings is not clear.
- An increased number of fetuses from group 3 showed lateral ventricle and third ventricle hydrocephaly. This finding was not noted in the high dose group. The biological significance of this finding is not known. This group did receive an erroneously large dose (85ug) at one of the dosing timepoints but this amount was still substantially lower than the high dose.
- Eye lens was absent in 2 fetuses in the high dose group with 0 noted for the other dose groups.
- A small, dose dependent increase in skull malformations and hyoid bone malformations is noted. The sponsor does not consider these findings to be of significance so does not include them as evidence of toxicity.
- Comparison of total malformations among groups shows no significant difference between groups. However, the total number of "variations" shows a dose

dependent increase across treated groups. The definitions of "malformations and "variations" are key to this interpretation.

Study title: A dose range finding study of the effects of rHuKGF administered intravenously on embryo/fetal development and toxicokinetics in rabbits.

Key study findings:
The objective of the study was to determine dosage levels to be evaluated in a definitive embryo/fetal development study in rabbits. This study was not performed under GLP conditions.
The selected route of administration was intravenous (via marginal ear vein) since this is the intended route of clinical administration for the human. The New Zealand White rabbit, was selected as appropriate based on the availability of historical control data and susceptibility to known developmental toxicants. The test article was administered to four groups of five artificially inseminated New Zealand White rabbits once daily from gestation days 6 through 18. Doses of palifermin administered were 15, 50, 150 and 500 ug/kg/day administered in volumes of 0.3, 0.1, 0.3 and 0.1 ml/kg, respectively. A concurrent control group composed of five artificially inseminated females received the vehicle on a comparable regimen at 0.3 ml/kg. The route of administration was intravenous (via the marginal ear vein). Clinical observations, body weights and food consumption were recorded. On gestation day 29, a laparohysterectomy was performed on all surviving animals. The uteri and ovaries were examined and the numbers of fetuses, early and late resorptions, total implantations and corpora lutea were recorded. Mean gravid uterine weights and net body weight changes were calculated for each group. Fetuses were weighed and examined for external malformations and variations. Blood samples were obtained from all animals during the pretest period and at selected intervals for analysis of antibody response and palifermin concentration in the maternal plasma. An additional dose group containing five artificially inseminated rabbits was selected for the satellite pharmacokinetic phase. The dose for this group was 500 ug/kg/day administered in volume of 0.1 ml/kg. Maternal and fetal blood samples and amniotic fluid samples were drawn from one animal each at selected intervals on gestation day 18 (the only day of dosing for this group) and 19 for analysis of study drug concentration.

In the 500 ug/kg/day group, one female each died on gestation days 20 and 22; these mortalities were assumed to be treatment-related. Clinical observations noted for the two females that died included rocking, lurching or swaying while ambulating, brown matting on the hindlimbs and forelimbs and/or red mucoid feces and were observed on gestation days 20 or 21. No remarkable internal findings were observed at necropsy and the cause of death for both females was not determined.

One female in the 15 ug/kg/day group aborted on gestation day 21. Spontaneous abortions are not uncommon in this species and strain. No abortions were observed in the lower dose groups. Therefore, the abortion in the 15 ug/kg/day group was not considered to be related to test article administration. All other females survived to the scheduled necropsy on gestation day 29. Treatment-related clinical observations included hypoactivity, labored respiration, lacrimation, salivation, various ocular findings (white ocular discharge, everted, reddened, and/or swollen eyelids, conjunctiva, and/or

nictitating membrane), clear nasal discharge, various excreta-related findings (soft stool, small feces and decreased defecation), clear matting around the nose, mouth and forelimbs, hair loss around the eyes, reddened and swollen ears, brown material and/or red staining on the tail and reddened and/or swollen mouth area. Mean body weight losses or reduced mean body weight gains and reductions in food consumption occurred in the 150 and 500 ug/kg/day groups throughout the treatment period. Body weight data and food consumption in the 15 and 50 ug/kg/day groups were unaffected by treatment with the test article. Both surviving gravid females in the 500 ug/kg/day group had entirely resorbed litters (all early resorptions). An increase in postimplantation loss and a corresponding decrease in viable litter size were also noted in the 150 ug/kg/day group. Intrauterine growth and survival in the 15 and 50 ug/kg/day groups were unaffected by treatment with the test article. The external malformations and developmental variations observed in the treated groups were considered to be spontaneous in origin and not related to test article administration.

Deficiencies in sampling time and dosing precluded complete pharmacokinetic analysis. An initial estimate of a terminal half-life of rHuKGF in pregnant rabbits of 3.27 hours was obtained. No evidence of transplacental transfer was observed, based on rHuKGF levels being below the limit of detection of the assay in amniotic fluid and fetal serum. Intravenous administration of doses ranging from 15 to 500 ug/kg resulted in detectable antibody formation as early as gestation day 12, with most rabbits positive by gestation day 18. The incidence of antibody formation was similar across dose groups. The control group was sero-negative. Since pharmacokinetic satellite animals were only dosed once, no correlation between antibody results and pharmacokinetic analysis could be drawn.

In conclusion, maternal toxicity was manifested by mortalities at a dose level of 500 ug/kg/day, inhibition of body weight gain and food consumption at dose levels of 150 and 500 ug/kg/day and by changes in the clinical condition of the animals at all treated dose levels. Developmental toxicity was exhibited by an increase in prenatal mortality (early resorptions) in the 150 and 500 ug/kg/day group.

In the presence of lethality and/or deficits in body weight gain and food intake in the females, maternal toxicity may possibly have contributed to the observed developmental toxicity, but it is not conclusive within the scope of this study. No developmental toxicity was noted at dose levels of 15 and 50 ug/kg/day. Based upon the results of the study, a dose of 150 ug/kg would be selected as the high dose in a definitive developmental toxicity study of rHuKGF in rabbits.

Study no.: — ·120063/ Amgen study # 970021
Volume #, and page #:
Conducting laboratory and location: t 1
Date of study initiation: 4/30/1997
GLP compliance: NO
QA reports: yes ( ) no ( )
Drug, lot #, and % purity: Palifermin lot #1101226A6B
Placebo lot # 02D07226G6

Methods

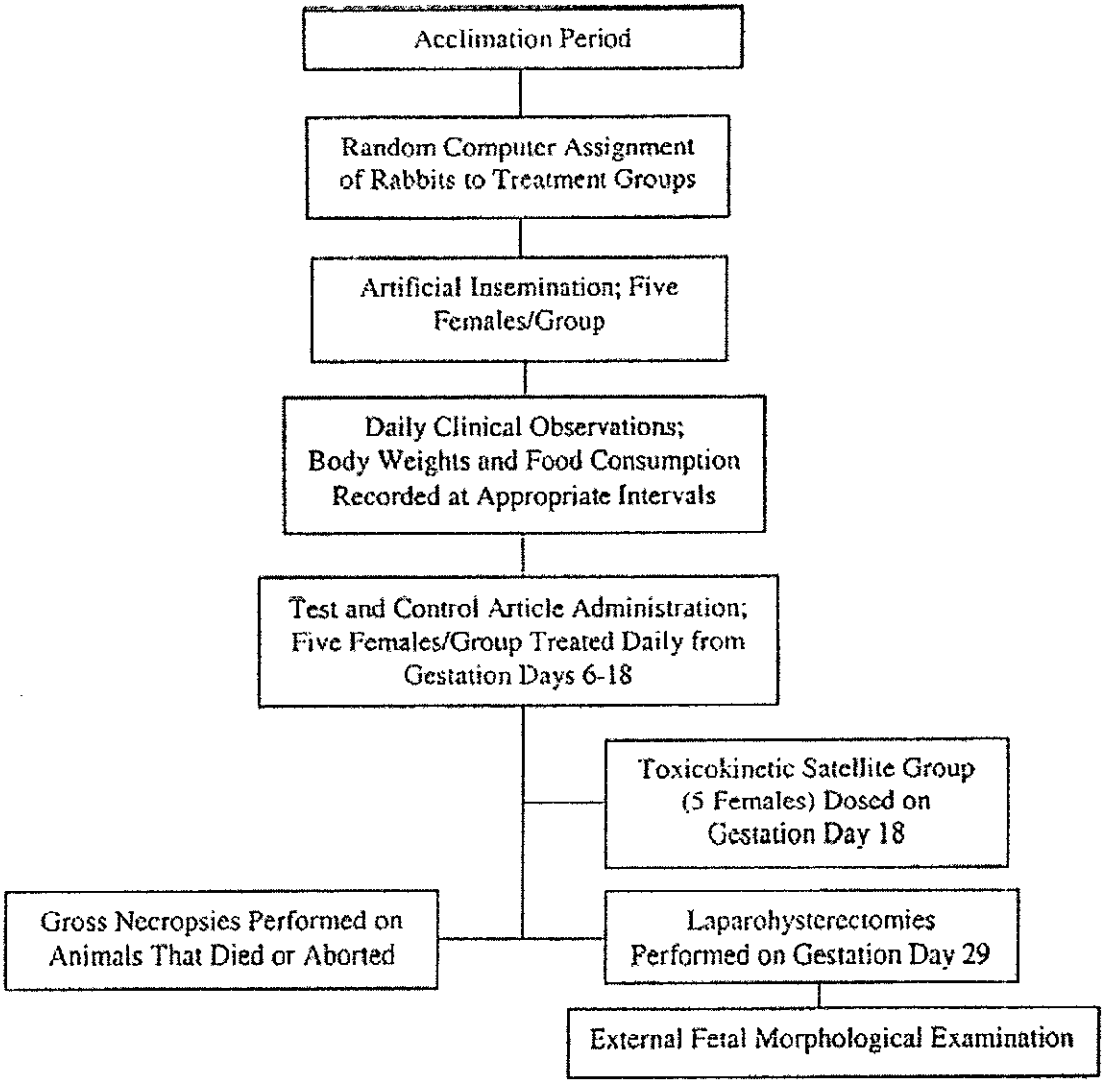
Doses: 0, 15, 50, 150 and 500 ug/kg/day

Species/strain: New Zealand white rabbits
Number/sex/group: 5 presumed pregnant females/group
Route, formulation, volume, and infusion rate: IV injection via the marginal ear vein as a single daily dose on GD 6 through 18. (one female, #23394, received two doses of 50 ug on GD18.

Satellite groups used for toxicokinetics:
Study design:

Group Number	Test Substance	Dosage Level (µg/kg/day)	Dosage Concentration (µg/ml)	Dosage Volume (ml/kg)	Number of Females
1	Vehicle Control[a]	0	0	0.3	5
2	rHuKGF	15	50	0.3	5
3	rHuKGF	50	500	0.1	5
4	rHuKGF	150	500	0.3	5
5	rHuKGF	500	5000	0.1	5
6[b]	rHuKGF	500	5000	0.1	5

[a] = rHuKGF placebo
[b] = Satellite pharmacokinetic group dosed on gestation day 18 only



Results

Mortality: Two deaths of pregnant dams in the 500 ug dose group occurred during the study: one on GD20 and one on GD22. One female in the 15 ug dose group aborted on GD21.

Clinical signs: Clinical signs of the dams assessed twice daily.

- All animals in the 15, 50, 150 and 500 ug/kg/day groups had a clear nasal discharge.
- Findings for all treated dose levels included clear matting around the nose, reddened and swollen conjunctiva and decreased defecation.

- Clinical signs noted at dose levels of 50, 150 and 500 ug/kg/day consisted of various ocular findings (lacrimation, swollen eyelids, reddened and swollen nictitating membranes), reddened and swollen ears and small feces.
- The 150 and 500 ug/kg/day group animals also had clear matting on the forelimbs, brown matting on the tail, everted eyelids and reddened and swollen mouths.
- Clinical signs noted only in the 500 ug/kg/day group included hypoactivity, red staining on the tail, labored respiration and salivation.

Body weight: Maternal body weights collected on GD 0, 6-19, 24, 29. Gravid uterine weight and net body weight change were calculated.

- For the 500 ug dose group, mean body weight losses were noted throughout the treatment period. During the post-treatment period (gestation days 19-29), mean body weight gain for the two surviving gravid females in the 500 ug/kg/day group was greatly increased relative to control. Statistically significant decreases in mean body weights were noted in the 500 ug/kg/day group for gestation days 17-19. A greatly reduced mean gravid uterine weight was observed in this group when compared to the control group value; both surviving gravid females in this group had entirely resorbed litters. Mean net body weight and net body weight gain in the 500 ug/kg/day group were comparable to the control group values.
- In the 150 ug/kg/day group, mean body weight losses or reduced mean body weight gain were noted during gestation days 6-9, 9-12, 12-19 and 6-19 relative to control. During the post-treatment period (gestation days 19-29), mean body weight gain in this group was greatly increased relative to control. Gravid uterine weight in the 150 ug/kg/day group was slightly reduced when compared to the control group value; the difference was not statistically significant. Mean body weights, net body weight and net body weight gain in the 150 ug/kg/day group were comparable to the control group values.
- No test article effects were noted for the 15 and 50 ug/kg dose groups.

Food consumption: Recorded daily from GD0 through GD 29

- For the 500 ug group, food consumption was reduced during the treatment period. During the post-treatment period (gestation days 19-29), food consumption in the two surviving gravid females in this group was comparable to that in the control group.
- In the 150 ug/kg/day group, food consumption was slightly reduced during gestation days 6-9 and 9-12; the differences from the control group during gestation days 9-12 were statistically significant. During the remainder of the treatment period (gestation days 12-19), food consumption in this group continued to be reduced when compared to that in the control group; the differences were statistically significant. When the overall treatment period (gestation days 6-19) was evaluated, statistically significant decreases in food consumption were noted in this group when relative to control. During gestation days 19-29, food consumption in the 150 ug/kg/day group was slightly greater relative to control group; the differences were statistically significant.

- No effects of the test article were noted for the 15 and 50 ug/kg groups.

Toxicokinetics:
Blood samples (approximately 1 ml) were to be collected from all animals (Groups 1-5) during the pretest period, at 5 minutes, 30 minutes, 1, 6 and 24 hours following dosing on gestation days 6, 12 and 18.

- Due to sampling time and dosing errors only the data from group 6 (500ug) were suitable for TK analysis. All animals in the group had quantifiable levels of palifermin on GD 18. Terminal half-life in pregnant rabbits was estimated at 3.27 hours. No evidence of transplacental transfer of test article since no detectable levels was found in the fetal serum and amniotic fluid.
- Intravenous administration of doses ranging from 0 to 500 ug/kg of rHuKGF to pregnant rabbits (GD 6 to 18) resulted in detectable antibody formation as early as gestation day 12 (after six doses). Most rabbits were positive for antibodies by GD 18. The incidence of antibody formation was similar across rHuKGF dose groups. The animals in the control group appeared to be sero-negative throughout the course of the study.

Necropsy:
In the 500 ug/kg/day group, two females died on gestation days 20 and 22, respectively. At necropsy, one had green fluid contents in the stomach, the other was macroscopically normal. Both of the females that died had entirely resorbed litters (all early resorptions). In addition, one female in the 15 ug/kg/day group aborted one early resorption on gestation day 21. This female had an accessory spleen and dark red lungs (all lobes) at necropsy.
At the scheduled necropsy on gestation day 29, no test article-related internal findings were noted. One female each in the control, 15 and 50 ug/kg/day groups had accessory spleens. Two control group females and one 500 ug/kg/day group female had cystic oviducts. All other females were macroscopically normal.

Fertility parameters and fetal malformationsc(mating/fertility index, corpora lutea, preimplantation loss, etc.):

- Increase in early resorptions. 500 ug/kg dose group
- Postimplantation loss in the two surviving gravid females in the 500 ug/kg/day group was excessive (100% per litter) relative to control (3.8% per litter). Both gravid females in the 500 ug/kg/day group had entirely resorbed litters (all early resorptions). The mean numbers of corpora lutea and implantation sites in this group were comparable to the control group values.
- Postimplantation loss (all early resorptions) was also increased in the 150 ug/kg/day group (44.9% per litter) when compared to the control group value and the maximum value in the [ ] historical control data (25.3% per litter). The difference in early resorptions from the concurrent control group was statistically significant. This increase was primarily due to one female with an entirely resorbed litter. A corresponding decrease in viable litter size was noted in the 150 ug/kg/day group (55.1% per litter) when relative to control group value (96.2%

per litter). The difference was not statistically significant. Mean fetal body weight and the mean numbers of corpora lutea and implantation sites in the 150 ug/kg/day group were comparable to the control group values.

- No test article related effects were noted for the 15 or 50 ug/kg dose group.

Fetal morphological data

The numbers of fetuses (litters) available for morphological evaluation were 37(5), 19(3), 38(5), 14(2) and 0(0) in the control, 15, 50, 150 and 500 ug/kg/day groups, respectively. Malformations were observed in 0(0), l(l), 2(2), 0(0) and 0(0) fetuses (litters) in these same respective dose groups. In the 50 ugkg/day group, one fetus had microcephaly and exencephaly (without an open eyelid). One fetus in this same dose group had an umbilical herniation of the intestine, cleft palate and a narrow pectoral region. One fetus in the 15 ug/kg/day group had an omphalocele. This fetus also had the only external developmental variation observed in this study (disseminated subcutaneous hemorrhages). All of the observed malformations in this study (cleft palate, narrow pectoral region, microcephaly, umbilical hemiation of the intestine, exencephaly and omphalocele) have also been observed in the test facility historical control data. Since the malformations were previously observed in the historical control data and occurred in the 15 and 50 ug/kg/day groups only while none were observed in the 150 and 500 ug/kg/day groups, these findings were considered to be spontaneous in origin.

Study title: A study of the effects of rHuKGF administered intravenously on embryo/fetal development in rats

Key study findings:

The objective of the study was to determine the potential of the test article, rHuKGF, to induce developmental toxicity after maternal exposure during the critical period of organogenesis, to characterize maternal toxicity at the exposure levels tested, and to determine a NOAEL (no observed adverse effect level) for maternal toxicity and developmental toxicity.

The potential maternal toxicity and developmental toxicity of rHuKGF were evaluated. The test article was administered to three groups of 25 bred Sprague Dawley rats once daily from gestation days 6 through 17. Doses were 100, 300, and 1000 ug/kg/day administered at a dose volume of 0.2 ml/kg. A concurrent control group composed of 25 bred females received the vehicle on a comparable regimen at 0.2 ml/kg. The route of administration was intravenous (via a caudal tail vein). Clinical observations, body weights, and food consumption were recorded. Blood samples were obtained from a caudal vein from 18, 22, 25, and 25 females in the control, 100, 300, and 1000 ug/kg/day groups, respectively, on gestation days 0 or 6 for analysis of seroreactivity. On gestation day 20, a laparohysterectomy was performed on all animals. Blood was collected from the vena cava of all animals for evaluation of serum chemistry parameters. The liver from all animals was weighed and sections preserved. Selected specimens of mammary gland tissue were obtained and examined microscopically. The uteri and ovaries were examined and the numbers of fetuses, early and late resorptions, total implantations, and corpora lutea were recorded. Mean gravid uterine weights and net body weight changes were calculated for each group. Fetuses were weighed, sexed, and examined for external, soft tissue, and skeletal malformations and variations.

All animals survived to the scheduled necropsy on gestation day 20.
Treatment-related clinical findings were observed at a dose level of 1000 ug/kg/day and included swollen and firm mammary tissue in the pectoral and inguinal area. In all rHuKGF treatment groups, a mean body weight loss occurred on gestation day 6-7. Reduced mean body weight gains were noted in the 1000 ug/kg/day group during gestation days 12-18 and 18-20. Mean gravid uterine weight in the 1000 ug/kg/day group was reduced. Therefore, the reduced body weight gain late in gestation in the 1000 ug/kg/day group was attributed to the delayed fetal development, rather than maternal toxicity. Mean body weight gains in the rHuKGF treatment groups were either comparable to or greater than the control groups for all other test periods. Mean net body weight gains in all treatment groups were comparable to the control group value. Food consumption in all treatment groups was reduced on gestation day 6-7. In the 1000 ug/kg/day group, food consumption on gestation days 7-8 and 6-9 continued to decrease, and the overall treatment period(gestation days 6- 18) was reduced.

The incidence of seroreactivity for females in the control group was 7/25 (28%) on gestation day 20. This was not substantially different than the incidences of seroreactivity in the 100 and 300 ug/kg/day groups of 28% and 36%, respectively, and was higher than the 8% incidence in the 1000 ug/kg/day group. These findings suggest that daily rHuKGF treatment did not result in increased seroreactivity in this experiment.
Dose-related increased serum levels of protein, albumin and globulin were observed in the 100, 300, and 1000 ug/kg/day groups while a decrease in A/G ratio was noted in the 1000 ug/kg/day group. A decrease in cholesterol level was also noted in all treatment groups.

At necropsy, test article-related internal findings were noted in the 1000 ug/kg/day group and included enlarged mammary tissue (inguinal, urogenital, thoracic, and/or abdominal) and thickened stomachs. Microscopically, a dose-related increase in hyperplasia of the mammary gland was observed in the 100, 300, and 1000 ug/kg/day groups while mammary gland ductal hyperplasia was also noted in the 1000 ug/kg/day group. Dose-related increases in mean liver weights were noted for all treated groups. Although the serum chemistry, necropsy, and microscopic findings were considered to be test article-related, these effects would not be unexpected in view of the pharmacologic activity of the test article.

In the 1000 ug/kg/day group, mean fetal body weight was decreased and postimplantation loss was increased. Intrauterine growth and survival were unaffected by test article administration at dose levels of 100 and 300 ug/kg/day. Fetal malformations were noted for one fetus each in the control and 1000 ug/kg/day groups and were considered to be spontaneous in origin. Increased and/or decreased incidences of several skeletal variants occurred in the 1000 ug/kg/day group. These consisted of a decreased incidence ossified cervical centrum no. 1, and increased incidences of unossified stemebrae nos. 5 and/or 6, and unossified stemebrae nos. 1, 2, 3, and/or 4. These variations were attributed to the intrauterine growth retardation (reduced fetal body weights) observed in the 1000 ug/kg/day group.

Based on the results of this study, the NOAEL (no-observed-adverse-effect level) for maternal endpoints was considered to be less than 100 ug/kg/day and a dose level of 300 ug/kg/day was considered to be the NOAEL (no-observed-adverse-effect level)

for developmental toxicity of rHuKGF in rats.

Study no.: — 120065/ Amgen # 970132
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: 12/29/1997
GLP compliance: YES
QA reports: yes (X) no ( )
Drug, lot #, and % purity: palifermin lot #1107096D7
Placebo lot#02D07226G6

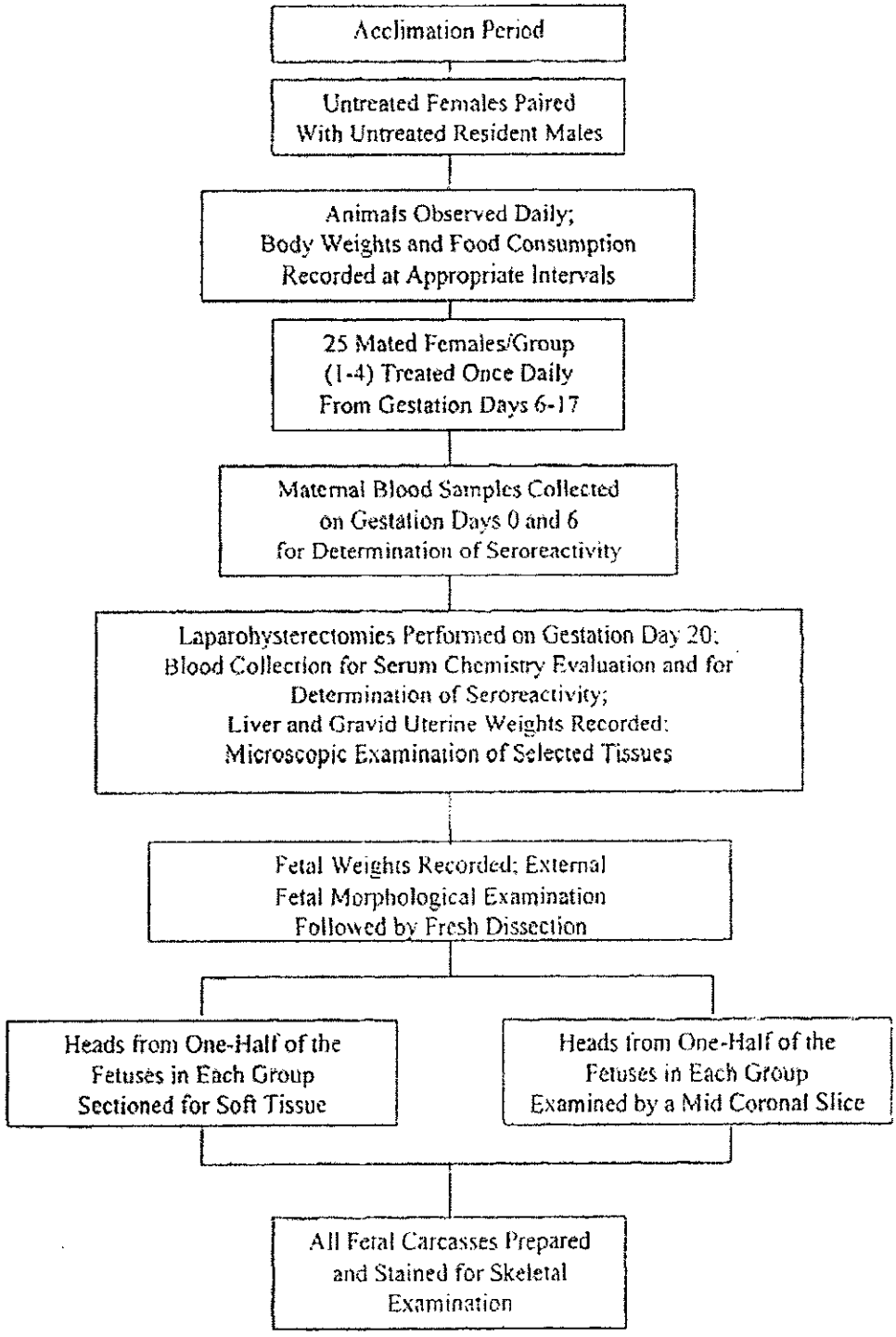
Methods

Doses: 0, 100, 300, 1000 ug/kg/day
Species/strain: female Sprague/Dawley rats, approximately 70 days old
Number/sex/group: 25
Route, formulation, volume, and infusion rate: IV admin in a volume of 0.2 ml/kg
Satellite groups used for toxicokinetics:
Study design:

Group Number	Test Article	Dosage Level (ug/kg/day)	Dosage Concentration (µg/ml)	Dosage Volume (ml/kg)	Number of Females
1	Vehicle Control[a]	0	0	0.2	25
2	rHuKGF	100	500	0.2	25
3	rHuKGF	300	1500	0.2	25
4	rHuKGF	1000	5000	0.2	25

[a] rHuKGF placebo

Appears This Way
On Original



Results

Mortality (dams): All animals survived to scheduled necropsy.

Clinical signs (dams): Observations were made twice daily. Detailed observations were made from GD0 to GD20 prior to dosing.

Body weight (dams): Data collected on SD0 and daily from GD6- GD20. Gravid uterine weight was collected and net body weight (the day 20 body weight exclusive of the weight of the uterus and contents) and net body weight change (the day 0-20 body weight change exclusive of the weight of the uterus and contents) were calculated and presented for each gravid female at the scheduled laparohysterectomy.

- All animals gained weight during the study. However, animals receiving 1000 ug/kg gained slower and showed significantly smaller weight gain over the course of the study relative to control and other treatment groups.
- Gravid uterine weights for the 1000 ug/kg group were significantly smaller than those of the other groups.

Food consumption (dams):
Individual maternal food consumption was recorded on GD0and GD6-GD20 (daily). Food intake was calculated as g/animal/day and g/kg/day for corresponding body weight change intervals.

- In general, a dose dependent reduction in food intake was noted relative to control. These findings reached statistical significance for the 1000 ug/kg group several days (but not every day) over the course of the study. These data are correlated with reduced over all body weight at the end of the study and reduction in weight gain for the 1000 ug/kg group.

Toxicokinetics:
Blood samples were obtained from 18, 22, 25, and 2.5 females in the control, 100, 300, and 1000 ug/kg/day groups, respectively, on gestation day 0 or 6 (prior to dosing) for determination of seroreactivity. Blood samples were also obtained from all females (25/group) on gestation day 20 for determination of seroreactivity.

Clinical pathology: Blood samples for clinical pathology evaluation were taken from the vena cava of all maternal animals, at the time of necropsy, on gestation day 20.
Albumin: A dose dependent increase in albumin relative to control was noted for all treatment groups (5.3 for 1the 1000 ug/kg group versus 4.0 for control)

- Total Protein: A dose dependent TPR was noted for all treated groups relative to control.
  - Globulin: A significant and dose dependent increase in globulin for the 300 and 1000 ug group was noted (3.1 for the 100 ug/kg group versus 1.9 for control).
  - A/G Ratio reduced for the 1000 ug/kg group relative to control
  - Total Cholesterol: total cholesterol was reduced for all treated groups relative to control

These findings are consistent with serum chemistry results from other rat studies.

Gross pathology:

Significantly increased liver weights were observed of the 300 and 1000 ug/kg groups. This finding is consistent with results from other rat toxicity studies.

Histopathology:

- Mammary gland: minimal glandular hyperplasia was observed for all treated animals. Increases in incidence and severity are noted with increasing dose.
- Mammary ductal hyperplasia is also noted for the 1000 ug/kg group only.

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.):
The number of corpora lutea on each ovary was recorded. The trimmed uterus was weighed and opened, and the number and location of all fetuses, early and late resorptions and the total number of implantation sites were recorded. The placentae were also examined. The individual uterine distribution was documented using the following procedure. All implantation sites, including resorptions, were numbered in consecutive order beginning with the left distal uterine horn, noting the position of the cervix, and continuing from the proximal to distal right uterine horn. The liver from each female was weighed. Sections of each liver were preserved in 10% neutral buffered formalin. In addition, a section of mammary gland (where enlargement was observed macroscopically) was obtained

- Increased early resorptions for the 1000 ug/kg group (40 versus 20 in ctrl.)
- Increased post-implantation loss: 20 for control, 42 for 100 ug group.
- Slightly fewer corpora lutea
- Slightly fewer implantation sites
- Fewer viable fetuses. (94.5% for control vs. 86.2% for high dose group.
- Significant reduction in fetal weight for both male and female fetuses.

Offspring (malformations, variations, etc.):

- A small increase of micropthalmia was reported for the 1000 ug/kg group (0 for control, and groups 2 and 3, 0.3 incidences /litter for the high dose group.
- A small increase in unossified sternebrae was noted for the high dose group. This finding was also observed in 22 of 327 fetuses in the control group versus 64 of 309 fetuses in the high dose group.

Study title: A dose range finding study of the effects of rHuKGF administered intravenously on embryo/fetal development and toxicokinetics in rats.

Key study findings:
This study was not conducted under GLP conditions. The objective of the study was to determine dosage levels of the test article to be evaluated in a definitive embryo-fetal development study in rats. The selected route of administration was intravenous (via a caudal vein) since this is the intended route of clinical administration for the human. The animal model, the Sprague/Dawley rat, was selected as an appropriate model based on the availability of historical control data and susceptibility to known developmental toxicants.

In the developmental toxicity phase, the test article was administered to four groups of six bred SD rats once daily from gestation days 6 through 19. Doses were 15, 50, 150 and 500 ug/kg/day administered at dose volumes of 0.3, 0.1, 0.3 and 0.1 ml/kg, respectively. A concurrent control group composed of six bred females received the vehicle on a comparable regimen at 0.3 ml/kg. The route of administration was intravenous (via a caudal vein). Clinical observations, body weights and food consumption were recorded. On gestation day 20, a laparohysterectomy was performed on all animals. The uteri and ovaries were examined and the numbers of fetuses, early and late resorptions, total implantations and corpora lutea were recorded. Mean gravid uterine weights and net body weight changes were calculated for each group. Fetuses were weighed, examined for external malformations and variations, and discarded. In the pharmacokinetic phase, one control group and four treated groups of identical design to those in the developmental toxicity phase were administered the control or test article on a comparable regimen. Blood samples were obtained from all animals (Groups 1-5) prior to dose administration on gestation day 6 and at selected intervals on gestation days 6, 12 and 19 for analysis of antibody response and rHuKGF concentration in the maternal serum. At the direction of the sponsor, the antibody samples were neither aliquoted nor analyzed due to high variability in serum volumes and color. An additional dose group (Group 6) containing six bred rats was selected for the satellite pharmacokinetic phase. The dose for this group was 500 ug/kg/day administered at a dose volume of 0.1 ml/kg on gestation day 19. Maternal and fetal blood samples were drawn from all surviving animals at selected intervals on gestation day 19 (Group 6) or from all animals on gestation day 20 (Groups 1-5) for analysis of rHuKGF concentration.

All animals survived to the scheduled laparohysterectomy on gestation day 19 or 20; no test article-related internal findings were observed in the developmental phase at any dose level. No clinical signs of toxicity were noted in the treated groups. Body weight and food consumption data in the 15, 50, 150 and 500 ug/kg/day groups were unaffected by test article administration. A reduced mean gravid uterine weight in the 500 ug/kg/day group was attributed to the reduced mean fetal body weight observed in this group.

The reduced mean fetal body weight in the 500 ug/kg/day group was the only effect on intrauterine growth and survival in the treated groups. The only fetal malformation observed was an umbilical hemiation of the intestine in one 150 ug/kg/day group fetus, which was not considered to be test article-related. No external developmental variations were noted in fetuses in this study. Because the fetal blood samples in the pharmacokinetic phase were destroyed prior to analysis, the pharmacokinetic phase of this study was repeated in another study. Under the conditions of this range-finding study, no maternal toxicity was observed at any dose level. Developmental toxicity was exhibited at a dose level of 500 ug/kg/day by reduced fetal body weights. No developmental toxicity was observed at dose levels of 15, 50, or 150 ug/kg/day. Based on the results of this study, doses of 100, 300, and 1000 ug/kg/day were selected for a definitive development toxicity study of rHuKGF in rats.

Study no.: — 120067
Volume #, and page #:
Conducting laboratory and location: [redacted]

Date of study initiation:
GLP compliance: NO
QA reports: yes ( ) no ( )
Drug, lot #, and % purity: Palifermin lot #1107096D7
Placebo lot # 02D07226G6

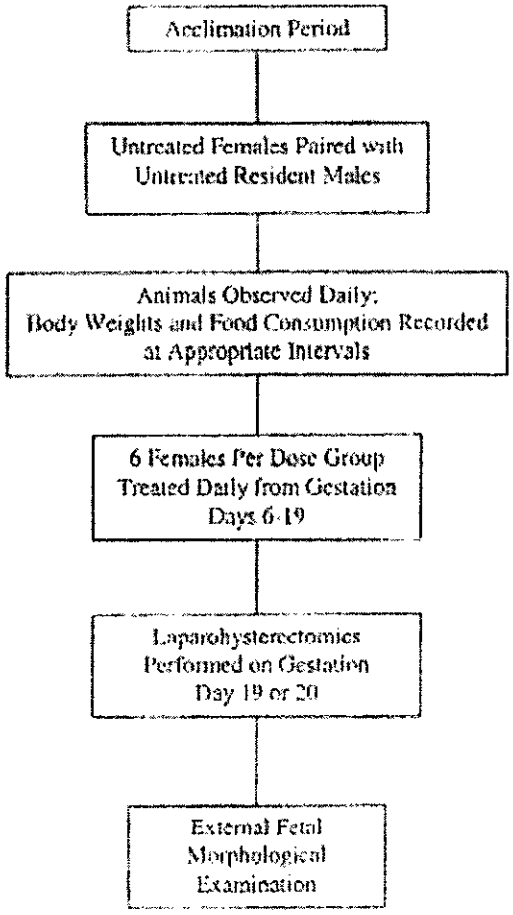
Methods

Doses: 0, 15, 50, 150, 500 ug/kg/day
Species/strain: Sprague-Dawley rats
Number/sex/group: 6
Route, formulation, volume, and infusion rate: administration by bolus IV injection as a single dose daily from GD6 to GD19
Satellite groups used for toxicokinetics: 6/group
Study design:

					Number of Females	
Group Number	Test Article	Dosage Level (ug/kg/day)	Dosage Concentration (ug/ml)	Dosage Volume (ml/kg)	Developmental Toxicology Phase	Pharmacokinetic Phase
1	Vehicle Control[a]	0	0	0.3	6	6
2	rHuKGF	15	50	0.3	6	6
3	rHuKGF	50	500	0.1	6	6
4	rHuKGF	150	500	0.3	6	6
5	rHuKGF	500	5000	0.1	6	6
6[b]	rHuKGF	500	5000	0.1	-	6

[a] = rHuKGF placebo
[b] = Satellite animals received the highest dose on gestation day 19 only.

Appears This Way
On Original



Results

Mortality (dams): All dams survived to scheduled necropsy.

Clinical signs (dams): Observations made twice daily. Observations were recorded prior to dosing throughout the treatment period.

- No effect of test article was noted for any group

Body weight (dams):
gestation days 0 and 6-20 (daily). A group mean body weight was calculated for each of these days. Mean body weight changes were calculated for each corresponding interval and also for gestation days 6-9, 9-12, 12-20, 6-20 and 0-20.
Gravid uterine weight was collected and net body weight (the day 20 body weight exclusive of the weight of the uterus and contents) and net body weight change (the day 0-20 body weight change exclusive of the weight of the uterus and contents) and net body

weight change (the day 0-20 body weight change exclusive of the weight of the uterus and contents) were calculated and presented for each gravid female at the scheduled laparohysterectomy.

Mean body weights and body weight gains in the 15, 50, 150 and 500 ug/kg/day groups were unaffected by test article administration throughout the study. The only statistically significant difference from the control group was an increased mean body weight gain in the 500 ug/kg/day group during gestation days 8-9. Mean net body weights, net body weight gains and gravid uterine weights in the treated groups were comparable to the control group values with the following exception. Mean gravid uterine weight in the 500 ug/kg/day group was slightly lower than the control group value, although the difference was not statistically significant.

Food consumption (dams):
Individual maternal food consumption was recorded on gestation days 0 and 6-20 (daily). Food intake was calculated as g/animal/day and g/kg/day for corresponding body weight change intervals.
No effects of test article were noted except for a significant decrease for the 500 ug group during the period from GD11-12.

Toxicokinetics: No data included in this report.

Gross pathology:
All females were euthanized by carbon dioxide inhalation on gestation day 19 or 20. For females in the developmental phase, the thoracic, abdominal, and pelvic cavities were opened by a ventral midline incision and the contents were examined. In all instances, the post mortem findings were correlated with the ante mortem clinical findings, and any abnormalities were recorded. The uterus and ovaries were excised. The number of corpora lutea on each ovary was recorded. The trimmed uterus was weighed and opened, and the number and location of all fetuses, early and late resorptions and the total number of implantation sites were recorded. The placentae were also examined. Findings were recorded as either developmental variations (alterations in anatomic structure that are considered to have no significant biological effect on animal health or body conformity, representing slight deviations from normal) or malformations (those structural anomalies that alter general body conformity, disrupt or interfere with body function or may be incompatible with life). Each fetus was weighed, sexed, euthanized by an intrathoracic injection of sodium pentobarbital and discarded, with the following exceptions. Fetuses with malformations and/or developmental variations were preserved in an appropriate fixative at the discretion of the study director. Crown-rump measurements were recorded for late resorptions, if present, and the tissues were discarded.

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.):
Mean fetal body weight in the 500 ug/kg/day group (2.9 grams) was reduced when compared to the control group value (3.5 grams) and to the minimum mean value in the

— historical control data (3.3 grams). The difference from the concurrent control group value was statistically significant. Mean fetal body weights in the 15, 50 and 150 ug/kg/day groups were comparable to the control group value. Other intrauterine parameters in the treated groups were unaffected by test article administration. Postimplantation losses, viable litter sizes, fetal sex ratios and the mean numbers of corpora lutea and implantation sites in the treated groups were similar to the control group values. None of the differences was statistically significant,

Offspring (malformations, variations, etc.):

The numbers of fetuses(litters) available for external morphological evaluation were 79(6), 70(5), 86(6), 76(6) and 78(6) in the control, 15, 50, 150 and 500 ug/kg/day groups, respectively. One fetus in the 150 ug/kg/day group had an umbilical herniation of the intestine. Since this malformation was not observed in the 500 ug/kg/day group and was observed similarly in the — historical control data, this finding was not considered to be test article-related. No other external malformations were observed. No external developmental variations were noted in fetuses in this study.

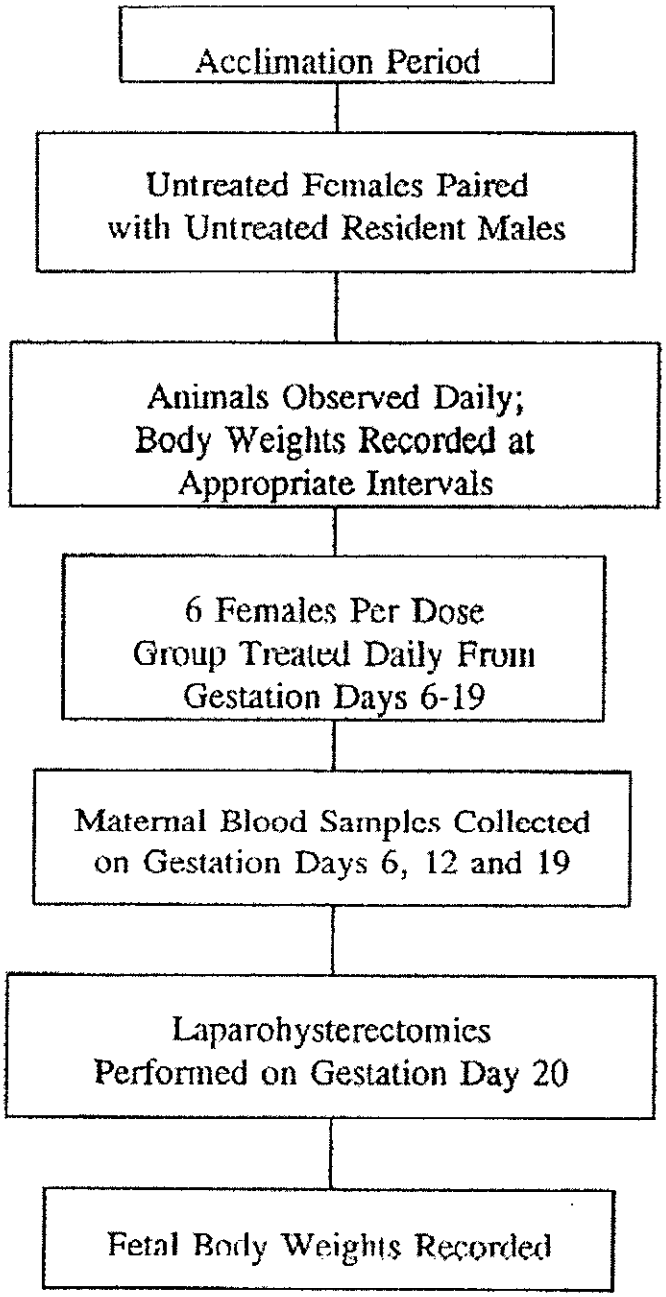
Study title: A study of the effects of rHuKGF administered intravenously in pregnant rats

Key study findings:
This study was not conducted under GLP conditions. The purpose of the study was 1) to determine whether the test article is transferred transplacentally after maternal exposure at various stages of organogenesis, 2) to provide information on the maternal blood serum levels and to compare to blood serum profiles from general toxicity studies performed with rHuKGF and 3) to evaluate for potential antibody response.

Study no.: — 120070/ Amgen study # 970138
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: 10/29/1997
GLP compliance: NO
QA reports: yes ( ) no (X)
Drug, lot #, and % purity: palifermin lot# 1107096D7
Placebo lot # 02D07226G6A

Methods

Doses: 0, 50, 100, 300 or 1000 ug/kg/day
Species/strain: Sprague-Dawley rats
Number/sex/group:
Route, formulation, volume, and infusion rate: IV in a volume of 0.1 or 0.2 ml/kg
Satellite groups used for toxicokinetics:
Study design:


Acclimation Period
Untreated Females Paired with Untreated Resident Males
Animals Observed Daily; Body Weights Recorded at Appropriate Intervals
6 Females Per Dose Group Treated Daily From Gestation Days 6-19
Maternal Blood Samples Collected on Gestation Days 6, 12 and 19
Laparohysterectomies Performed on Gestation Day 20
Fetal Body Weights Recorded

Group Number	Test Substance	Dosage Level (µg/kg/day)	Dosage Concentration (µg/ml)	Dosage Volume (ml/kg)	Number of Females
1	rHuKGF placebo	0	0	0.2	6
2	rHuKGF	50	500	0.1	6
3	rHuKGF	100	500	0.2	6
4	rHuKGF	300	1500	0.2	6
5	rHuKGF	1000	5000	0.2	6

Parameters and endpoints evaluated:

Results

Mortality (dams): All animals survived to scheduled necropsy

Clinical signs (dams):

Treatment-related clinical findings observed at the daily examinations included apparent mammary masses (classified as enlargements of the mammary tissue at necropsy) in the abdominal and thoracic areas of 3/6 and 6/6 females in the 300 and 1000 ug/kg/day groups, respectively. These findings were first observed on gestation days 15 and 13 in these same respective groups and continued to be observed through gestation day 20. Unkempt appearance was also noted for 5/6 females in the 1000 ug/kg/day group between gestation days 13 and 20. No other treatment-related clinical findings were observed at any dose level. A slightly increased incidence of red material around the nose was observed in the 1000 ug/kg/day group; however, the finding was observed sporadically throughout the treatment period and was generally limited to single occurrences for each animal.

Body weight (dams):
Dose-related increases in mean body weight gain were observed in the 300 and 1000 ug/kg/day groups during gestation days 6-9; the difference between the control and 1000 ug/kg/day group values was statistically significant. In all treated groups, mean body weight gains were increased for the remainder of the study [gestation days 9-12, 12-20 and 6-20 (overall treatment period)] and mean body weights were increased during the latter part of gestation relative to control; the increases in the 50, 100 and 1000 ug/kg/day groups were slight, while those in the 300 ug/kg/day group were often statistically significant. However, the values in the control group during these intervals were atypically low (due to one female that had only one viable fetus and did not gain as much weight as the females with normal-sized litters). In view of this and in the absence of a dose response, the slight increases for the 50, 100 and 1000 ug/kg/day groups during gestation days 9-12, 12-20 and 6-20 were not attributed to treatment. The significance of the increases in the 300 ug/kg/day group during these intervals is uncertain due to the

absence of similar increases in the high dose group. Further confounding interpretation of these data, slight mean body weight losses or lower-than-expected mean body weight gains were observed in all groups, including the control group, following each scheduled blood collection interval (gestation days 6-7, 12-13 and 19-20). Mean net body weight gains (the day 0-20 body weight change exclusive of the weight of the uterus and contents) in the 300 and 1000 ug/kg/day groups were significantly increased relative to the control group. Mean net body weight gains in the 50 and 100 ug/kg/day groups and mean net body weights and gravid uterine weights in all treated groups were unaffected by test article administration.

Toxicokinetics:
Analyses of pharmacokinetic data suggest that rHuKGF does not accumulate in pregnant rats and that there may be reduced exposure with daily administration. Analyses of fetal serum and amniotic fluid indicated negligible placental transfer of rHuKGF when administered at a single dose of 1000 ug/kg to pregnant rats.

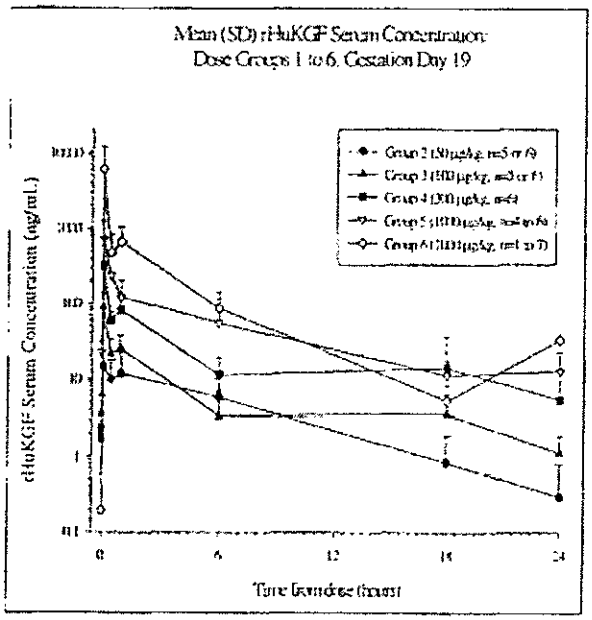
Dosing Scheme: Daily IV for 14 days (Gestation Days 6 through 19); Group 6: Single IV Dose on Gestation Day 19 Only.

Lot Number(s): 02D07226G6 (placebo), 1107096D7 (6 mg lyophilized powder per vial)

Formulation: Lyophilized

Group	2	3	4	5
Daily IV Dose (µg/kg)	50	100	300	1000
Study Day	19G*	19G	19G	19G
Parameter	Mean ± SD (n=6)	Mean ± SD (n=6)	Mean ± SD (n=6)	Mean ± SD (n=6)
$AUC_{(0\ min - 24\ hrs,\ Day\ 6G)}$	90.6 ± 61.9	101 ± 55.0	1070 ± 817	2660 ± 4590
$AUC_{(0\ min - 24\ hrs,\ Day\ 12G)}$	105 ± 176	77.2 ± 8.92	361 ± 261	1120 ± 831
$AUC_{(0\ min - 24\ hrs,\ Day\ 19G)}$	78.1 ± 78.1	118 ± 59.0	466 ± 326	1310 ± 1060
Accumulation Ratio, Day 19G	2.45 ± 3.90	1.35 ± 0.810	0.480 ± 0.140	0.997 ± 0.645

*G - Day of Gestation



Summary:

Upon multiple dose, intravenous injections of 50, 100, 300 or 1000 µg/kg rHuKGF to pregnant rats, all animals had quantifiable levels of rHuKGF on Days 6G, 12G and 19G. Animals in the control group showed no evidence of dosing. The variability of rHuKGF serum concentrations in each group on each sampling day was very high, resulting in highly variable AUC values. There was no consistent pattern in terms of accumulation, however, these data suggest that rHuKGF does not accumulate in pregnant rats and there may be reduced exposure with daily administration. The concentrations appeared to be lower than anticipated, based on non-pregnant rats. Analysis of fetal serum and amniotic fluid samples indicated negligible placental transfer of rHuKGF when administered intravenously at a single dose of 1000 µg/kg to pregnant rats.

Gross pathology:

At the scheduled necropsy on gestation day 20, enlarged mammary tissue (inguinal, urogenital, thoracic and/or abdominal areas) was observed in 4/6 and 6/6 females in the 300 and 1000 ug/kg/day groups, respectively. In these same respective groups, one and five females had thickened stomachs. Enlarged placentae (6-14 sites/dam) were noted for two, one and two females in the 100, 300 and 1000 ug/kg/day groups, respectively. The only other internal finding in the treated groups was an intussusception in the jejunum of one female in the 1000 ug/kg/day group. In the control group, one female had a thickened vagina (portion adjacent to the cervix) with dark red contents.

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.):

Offspring (malformations, variations, etc.):
Mean fetal body weight in the 1000 pg/kg/day group (3.3 grams) was reduced relative to control group value (4.2 grams) and was equivalent to the minimum mean value in the — historical control data (3.3 grams); the difference from the concurrent control group value was statistically significant at. Mean fetal body weights in the 50, 100 and 300 ug/kg/day groups were comparable to the control group value. Other intrauterine parameters in the treated groups were unaffected by test article administration. Postimplantation losses, viable litter sizes, fetal sex ratios and the mean numbers of corpora lutea and implantation sites in the treated groups were similar to the control group values. None of the differences were statistically significant.

Prenatal and postnatal development

Segment three studies were not submitted for this BLA

2.6.6.7 Local tolerance

Study title: Acute intravenous, subcutaneous, and intramuscular irritation of rHuKGF in rabbits

Key study findings:
The objective of this study is to determine the irritation potential of recombinant human keratinocyte growth factor (rHuKGF) following a single intravenous (IV), single subcutaneous (SC), and single intramuscular (IM) injection in rabbits. The study was conducted under required standards for the EU [ ]
The only clinical sign noted in rabbits treated with a total dose of 7,500 pg rHuKGF (2500 ug rHuKGF by each of three different injection routes) was a reddening of the eyelid 48 hr and up to seven days after treatment.
At 48 hr after intravenous injection with 2500 ug rHuKGF, the local irritability response was greater than that of the placebo-dosed group, but less than that of the 0.425% acetic acid- dose group. By 14 days after intravenous injection with rHuKGF, the local irritability response was comparable to that of the placebo-dosed group. At 48 hr and 14 days after intramuscular or subcutaneous injection with rHuKGF, the local irritability response for each of these routes of administration was comparable to that of the placebo-dosed group.

Study no.: — study # M079-95/Amgen study # T-95-KGF-014
Volume #, and page #:
Conducting laboratory and location: [ ]
Date of study initiation: 12/1/1995
GLP compliance: NO
QA reports: yes ( ) no ( )
Drug, lot #, and % purity: palifermin lot # 01195B5C
Placebo lot # 02095B5

Methods
Doses: 0 or 2500 ug
Animals: New Zealand white rabbits, young adult, 2-4 kg in weight, 3/sex/group
Study design:

The design of this study is illustrate in the table below, supplied by the sponsor:

Dose Levels and Number of Animals:

Group	Treatment	No. of Animals[a] Male	Female
1	Placebo	1	1
2	2,500 µg	3	3
3	0.425% & 1.7% Acetic Acid (Positive Control)	1	1

[a]Each rabbit is treated IV, SC, and IM.

Results:
All animals survived to scheduled necropsy. All animals gained weight during the study. Reduced food intake was noted for some animals, both male and female in the palifermin treated group on SD 3 (35-43% lower, relative to control).
Immediately after intravenous injection, the $\bar{x}$ for placebo and rHuKGF-treated animals was 0.33 (1/3 had a score of 1- slight- for erythema) and 0.40 (400 had a score of 1 for erythema), respectively. Edema was not observed in any of these animals, and erythema was not noted in any of the animals in these two groups at any of the subsequent timepoints.
Immediately after injection of 0.425% or 1.7% acetic acid intravenously, both rabbits had moderate erythema (score of 3) at both injection sites (0.425% and 1.7%). The response to the 0.425% injection thereafter, however, appeared to be more severe than that of the 1.7% injection in both rabbits. Erythema at the 1.7% injection site for both rabbits was reduced (score of 1) at 0.5 hr after injection until scheduled sacrifice at Day 3 (one rabbit) or until Day 6 (second rabbit), when the site appeared normal. Erythema at the 0.425% injection site increased in severity by 4 hr post-injection (score of 4; severe) in one rabbit; the erythema remained severe through Day 7 and decreased in severity through Day 11, and appeared normal on Day 12. Erythema at the 0.425% injection site of the second (female) rabbit decreased in severity by 0.5 hr post-injection (slight) and was well-defined through sacrifice on Day 3. Edema was not observed at any timepoint in these two animals. No erythema or edema could be discerned grossly upon visual inspection and palpation of the subcutaneous and intramuscular injection sites of any of the animals in any of the treatment groups at any timepoint after injection.

Histopathologic evaluation was performed on tissues from all injection sites for all rabbits from all dose groups at each sacrifice time and on the eyelid/conjunctival tissue from placebo control and rHuKGF-treated animals from each sacrifice time.

No microscopic changes were observed in the eyelid/conjunctival tissue of rabbits sacrificed 48 hr (Day 3) and 14 days (Day 15) after treatment with rHuKGF.
At 48 hr after intravenous and intramuscular injection with 2500 ug of rHuKGF, treatment-related alterations were characterized by minimal to mild hemorrhage, cellular infiltration of predominantly mononuclear cells, and/or edema ulceration of the skin was observed in one animal 48 hr after intravascular injection. At 14 days after injection, sites of injection of 2500 ug rHuKGF into the subcutis, muscle, or vein were comparable to placebo-injected sites and were essentially normal. The single intramuscular change present in one rabbit 14 days after injection with rHuKGF was composed predominantly of heterophils, and may indicate an inadvertent, focal site infection.

At 48 hr post-injection, the intravenous sites of 3/4 (75%) rabbits receiving rHuKGF had total local irritability grades greater than the placebo-dosed group, but less than the irritability response of the intravenous sites injected with the lower (0.425%) concentration of acetic acid. At 48 hr post-injection, 2/4 (50%) of the muscle sites receiving rHuKGF had local irritability grades greater than the 0.425% acetic acid-dosed group, but less than the irritability response of the 1.7% acetic acid-dosed group. At 14 days after injection with rHuKGF, only the intramuscular site of one rabbit had an irritability response greater than that of the placebo- treated group; however, this represented a cellular infiltration microscopically and was thought to be indicative of a minor local infection rather than treatment with rHuKGF. Subcutaneous sites injected with rHuKGF were comparable to those of the placebo-dosed group at 48 hr and 14 days after treatment.

2.6.6.8 Special toxicology studies

Study title: An antigenicity study of recombinant human keratinocyte growth factor using guinea pigs, mice and rats.

Key study findings:
The purpose of this study is to evaluate the antigenicity of palifermin using guinea pigs, mice and rats. To sensitize the guinea pigs, 100 and 1000 ug/kg of r-HuKGFd23 were administered intravenously five times weekly, and 1000 ug/kg of r-HuKGFd23 was also administered subcutaneously with Freund's complete adjuvant once weekly. To sensitize the mice, 100 and 1000 ug/kg of r- HuKGFd23 were administered intravenously five times weekly, and 1000 ug/kg of r- HuKGFd23 was administered intraperitoneally with 3% aluminium hydroxide gel once weekly. KGF Final Placebo was administered intravenously five times weekly as the excipient control, and ovalbumin with adjuvant was administered subcutaneously to guinea pigs and intraperitoneally to mice once weekly as the positive control. The administration period was scheduled for 3 weeks. However, due to the unexpected death of one guinea pig administered 1000 ug/kg of r-HuKGFd23 intravenously, the administration was terminated after the first administration

in the third week for all remaining animals in this group. All other groups received administrations as scheduled.

- All actively r-HuKGFd23 sensitized guinea pigs showed positive responses (grade 3 to 4) in the active systemic anaphylaxis response test after the intravenous challenge with 5000 ug/animal of r-HuKGFd23.
- All guinea pigs passively sensitized with sera from the actively r-HuKGFd23 sensitized guinea pigs showed positive responses (titer x16 to 264) in the 4-hour passive cutaneous anaphylaxis response test after the intravenous challenge with 5000 ug/animal of r-HuKGFd23.
- The rats passively sensitized with sera from mice actively sensitized with 100 and 1000 ug/kg of r-HuKGFd23 alone showed negative responses in the 48-hour passive subcutaneous anaphylaxis response test after the intravenous challenge with 5000 ug/animal of r-HuKGFd23. Only one rat passively sensitized with sera from mice sensitized with 1000 ug/kg of r-HuKGFd23 plus adjuvant showed weak positive responses (titer x 4).
- The animals sensitized with the excipient control showed negative responses in all tests. The animals sensitized with the positive control, showed positive responses in all tests.
- The results of this study indicate that palifermin could produce anaphylactic responses in guinea pigs and mice and weakly in rats.

Study no.: — -39-30/Amgen #960099
Volume #, and page #:
Conducting laboratory and location: [illegible]
Date of study initiation: 8/28/1996
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: palifermin lot # 1101226A6B
Placebo lot # 02D07226G6

Methods

Doses:
Animals: Male Hartley guinea pigs,
Male BALB/c mice
Male Sprague/Dawley rats.

Hemolytic potential:
Five study reports are included with this BLA that address the hemolytic potential of palifermin experimental animals (rat, rhesus monkey, human blood, rat blood).
The study numbers are: 100336, 100483, 100541, 101120

Key study findings:
The results of the assays indicate that under the conditions described in this report, r-HuKGF did not induce a hemolytic index greater than 5% using rat and human blood

substrate. It was concluded that the test articles were negative for inducing hemolysis in rat and human blood under the conditions of testing.
Therefore, based on the data from these studies, although the undiluted new formulation palifermin (— mg/ml) and undiluted new formulation palifermin placebo were hemolytic in rat blood, neither placebo (new formulation palifermin placebo and palifermin placebo) nor new formulation rHuKGF were hemolytic in human whole blood at the concentrations tested.

2.6.6.9 Discussion and Conclusions

The results of the toxicology studies showed that, at high doses, palifermin exposure may result in severe toxicities. Most of the effects can be attributed to exaggerations of the pharmacological activity of the molecule. In addition, most of the toxicities appear to be reversible. However, the pathological findings in the kidney, liver and thyroid in rats treated daily for 28 days with high doses of palifermin did not resolve completely, suggesting that irreversible damage may occur at high doses and long durations. The liver, kidney and thyroid findings were not observed in any monkey study. The toxicities found in monkeys receiving doses of up to 300 ug/kg/day for 28 days (IV or SC) appeared to be less severe than those observed in the rats at high doses. The findings observed in the monkeys at the highest doses and longest duration also appeared to be largely reversible. The treatment duration and dosing recommended for human use under the conditions proposed in this BLA are significantly less than those that produced the toxicities in animals. Furthermore, the toxicities observed in the animal studies can be monitored for in humans to avoid unreasonable risk

2.6.6.10 Tables and Figures

2.6.7 TOXICOLOGY TABULATED SUMMARY

Test Article: Recombinant Human Keratinocyte Growth Factor (rHuKGF)

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (µg/kg)	GLP Compliance	Testing Facility	Study No	Location Vol	Location Page
Single-dose Toxicity	Sprague-Dawley Rat	Intravenous	Acute	0, 100, 300, 1000, 5000, 10000, 30000	Yes		T-95-KGF-012 (M077-95)		
	Sprague-Dawley Rat	Subcutaneous	Acute	0, 100, 300, 1000, 5000, 10000, 30000	Yes		T-95-KGF-013 (M078-95)		
	Rhesus Monkey	Intravenous	Acute	10000, 50000	Yes		960098 (39-29)		
Repeat-dose Toxicity	Athymic NCr-nu Mouse	Intravenous	1 Day per Week for 6 Weeks	0, 10000, 15000, 25000, 50000	No		101623		
	Athymic NCr-nu Mouse	Intravenous	3 Days per Week for 6 Weeks	0, 5000 10000, 25000 50000	No		100929		
	Sprague-Dawley Rat	Intravenous Subcutaneous	7 Days	0, 1000	Yes		100148 (120072)		

For Single-Dose and Repeat-Dose Toxicity, the highest NOAEL is underlined

Test Article: Recombinant Human Keratinocyte Growth Factor (rHuKGF)

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (µg/kg)	GLP Compliance	Testing Facility	Study No.	Location Vol.	Location Page
Repeat-dose Toxicity (cont.)	Sprague-Dawley Rat	Intravenous	28 Days	0, 30, 100, 300, 1000	Yes		T-95-KGF-002 (M016-95)		
	Sprague-Dawley Rat	Subcutaneous	28 Days	0, 30, 100, 300, 1000	Yes		T-95-KGF-001 (M015-95)		
	Rhesus Monkey	Intravenous	3 Days	0, 500 1000, 2000, 4000	Yes		970159 (39 35)		
	Cynomolgus Monkey	Intravenous	7 Days	30 300, 1000	No		/9-27-10		
	Rhesus Monkey	Intravenous	11 Days	0, 500, 1000 2000, 4000	Yes		970160 (39-36)		
	Rhesus Monkey	Intravenous	28 Days	0, 1, 10, 30 100, 300	Yes		T-95-KGF-005 (39-27)		

For Single-Dose and Repeat-Dose Toxicity, the highest NOAEL is underlined

Test Article: Recombinant Human Keratinocyte Growth Factor (rHuKGF)

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (µg/kg or units shown)	GLP Compliance	Testing Facility	Study No	Location Vol.	Location Page
Repeat-dose Toxicity (cont.)	Cynomolgus Monkey	Subcutaneous	7 Days	30, 300, 1000	No		[redacted] 39-26-10		
	Rhesus Monkey	Subcutaneous	28 Days	0, 1, 10, 30, 100, 300	Yes		[redacted] 39-26		
Genotoxicity	Salmonella typhimurium Escherichia coli	Plate incorporation	-	100, 333, 1000 3333, 5000 µg/plate	Yes		T-95-KGF-010 (G95BS11.502001)		
	Salmonella typhimurium Escherichia coli	Plate incorporation	-	50, 100, 250, 500, 1000, 2500, 5000 µg/plate	Yes		960041 (17686-0-422R)		
	Chinese Hamster Ovary Cells	Media incorporation	-	100, 200, 300, 400, 500 µg/mL	Yes		T-95-KGF-008 G95BS11.782001)		

For Single-Dose and Repeat-Dose Toxicity, the highest NOAEL is underlined

Test Article: Recombinant Human Keratinocyte Growth Factor (rHuKGF)

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (µg/kg or units shown)	GLP Compliance	Testing Facility	Study No.	Location Vol.	Location Page
Genotoxicity (cont.)	Chinese Hamster Ovary Cells	Media Incorporation	-	63, 125, 250, 500 µg/mL	Yes		T-95-KGF-007 (G95BS11.336)		
	ICR Mouse	Intraperitoneal	Single Dose[a]	25000, 50000, 100000	Yes		T-95-KGF-006 (G95BS11.122)		
Reproductive and Developmental Toxicity	Sprague-Dawley Rat	Intravenous	b	0, 100, 300, 1000	Yes		T-095-KGF-015 (1901-012)		
	Sprague-Dawley Rat	Intravenous	14 Days (G6-G19)	0, 15, 50, 150, 500	No		970053 [redacted]-120067)		
	Sprague Dawley Rat	Intravenous	14 Days (G6-G19)	0, 50, 100, 300, 1000	No		970138 [redacted]-120070)		
	Sprague Dawley Rat	Intravenous	12 Days (G6-G17)	0, 100, 300, 1000	Yes		970132 [redacted]-120065)		

[a] Sampling times at 24, 48, or 72 hours after dosing
[b] Males were dosed for 28 days prior and through cohabitation, females were dosed for 15 days prior to cohabitation through gestation day 7.
G – gestation day

Test Article: Recombinant Human Keratinocyte Growth Factor (rHuKGF)

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (µg/kg or units shown)	GLP Compliance	Testing Facility	Study No.	Location Vol	Location Page
Reproductive and Developmental Toxicity (cont.)	New Zealand White Rabbit	Intravenous	13 Days (G6-G18)	0, 15, 50, 150, 500	No		970021 ([redacted]-120063)		
	New Zealand White Rabbit	Intravenous	13 Days (G6-G18)	0, 5, 60, 150	Yes		101728 (529-048)		
Local Tolerance	New Zealand White Rabbit	Intravenous, Subcutaneous, Intramuscular	1 Day	2500	Yes		T-95-KGF-014 (M079-95)		
Other Toxicity Studies - Antigenicity	Hartley Guinea Pigs, Mice, Rats	Intravenous Subcutaneous, Intraperitoneal	21 Days	100, 1000	Yes		960099 ([redacted] 39 30)		
Other Toxicity Studies - Other	Sprague-Dawley Rat, Rhesus Monkey, Human	In vitro	4 Hours	0.3, 1, 3, 10 µg/mL	Yes		100336 (G98AW70.100)		
	Sprague-Dawley Rat	In vitro	4 Hours	3000, 15000 µg/mL	No		100483 (AA12MB.100, [redacted])		

G = gestation day

Test Article: Recombinant Human Keratinocyte Growth Factor (rHuKGF)

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (µg/kg or units shown)	GLP Compliance	Testing Facility	Study No.	Location Vol	Location Page
Other Toxicity Studies - Other (continued)	Sprague-Dawley Rat, Human	In vitro	4 Hours	60x, 300x dilutions	No		100541 (AA14YM.100, [redacted])		
	Sprague Dawley Rat, Human	In vitro	45 Minutes	2, 5100 µg/mL	Yes		101120 7725XA31.002)		
	Human – Tumor Cell Lines	In vitro Incubation	5 Days	0, 10, 100, 1000, 10000, 100000 ng/mL	No	Amgen, Inc., Thousand Oaks, CA, USA	102101		
	Human – Tumor Cell Lines	In vitro Incubation	5 days	0, 10, 100, 1000, 10000, 100000 ng/mL	Yes		100928 (A068.1)		
	Human – Tumor Cell Lines	In vitro Incubation	-	-	No	Amgen, Inc., Thousand Oaks CA, USA	00-188		
	Athymic Nude Mice	Intravenous	5 Weeks	0, 150, 500, 1500, 4000	No		100930 AMG-4		

Test Article: Recombinant Human Keratinocyte Growth Factor (rHuKGF)

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (µg/kg)	GLP Compliance	Testing Facility	Study No.	Location Vol.	Location Page
Other Toxicity Studies - Other (continued)	Athymic Nude Mice	Intravenous	6 Weeks	0, 150, 500, 1500, 4000	No		100930 AMG-5		
	Athymic Nude Mice	Intravenous	6 Weeks	0, 150, 500, 1500 4000	No		100930 AMG-6		
	Athymic Nude Mice	Intravenous	6 Weeks	0, 150, 500, 1500, 4000	No		100930 AMG-7		
	Athymic Nude Mice	Intravenous	6 Weeks	0, 150, 500, 1500, 4000	No		100930 AMG-8		
	Athymic Nude Mice	Intravenous	6 Weeks	0, 150, 500, 1500, 4000	No		100930 AMG-10		
	Athymic Nude Mice	Intravenous	3 Weeks	150, 500, 1500, 4000	No	Amgen Inc., Thousand Oaks, CA, USA	R2002083		

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:
The results of the toxicology studies showed that, at high doses, palifermin exposure may result in severe toxicities. Most of the effects can be attributed to exaggerations of the pharmacological activity of the molecule. In addition, most of the toxicities appear to be reversible. However, the pathological findings in the kidney, liver and thyroid in rats treated daily for 28 days with high doses of palifermin did not resolve completely, suggesting that irreversible damage may occur at high doses and long durations. The liver, kidney and thyroid findings were not observed in any monkey study. The toxicities found in monkeys receiving doses of up to 300 ug/kg/day for 28 days (IV or SC) appeared to be less severe than those observed in the rats at high doses. The findings observed in the monkeys at the highest doses and longest duration also appeared to be largely reversible. The treatment duration and dosing recommended for human use under the conditions proposed in this BLA are significantly less than those that produced the toxicities in animals. Furthermore, the toxicities observed in the animal studies can be monitored for in humans to avoid unreasonable risk

Unresolved toxicology issues (if any):
Palifermin may have a tumor promoting effect on non-hemotological malignancies. Further non-clinical studies to assess this risk are recommended and have been addressed in a post-marketing commitment.

Suggested labeling:

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY

Carcinogenicity: The carcinogenic potential of palifermin has not been evaluated in long-term animal studies.

Mutagenicity: No clastogenic or mutagenic effects of palifermin were observed in the Ames or human chromosomal aberration assays; however, such studies are generally not informative for biological products.

Impairment of Fertility: When palifermin was administered intravenously daily to male and female rats prior to and during mating, reproductive performance, fertility, and sperm assessment parameters were not affected at doses up to 100 mcg/kg/day. Systemic toxicity (clinical signs of toxicity and/or body weight effects), decreased epididymal sperm counts, and increased post-implantation loss were observed at doses ≥ 300 mcg/kg/day (5-fold higher than the recommended human dose). Increased pre-implantation loss and a decreased fertility index were observed at a palifermin dose of 1,000 mcg/kg/day.

PREGNANCY CATEGORY C

[TRADE NAME™] has been shown to be embryotoxic in rabbits and rats when given in doses that are 2.5 and 8 times the human dose, respectively.

Increased post-implantation loss and decreased fetal body weights were observed when palifermin was administered to pregnant rabbits from days 6 to 18 of gestation at IV doses ≥ 150 mcg/kg/day (2.5-fold higher than the recommended human dose). However, treatment with these doses was also associated with maternal toxicity (clinical signs and reductions in body weight gain/food consumption). No evidence of developmental toxicity was observed in rabbits at doses up to 60 mcg/kg/day.

Increased post-implantation loss, decreased fetal body weight, and/or increased skeletal variations were observed when palifermin was administered to pregnant rats from days 6 to 17 or 19 of gestation at IV doses ≥ 500 mcg/kg/day (> 8-fold higher than the recommended human dose). Treatment with these doses was also frequently associated with maternal toxicity (clinical signs and body weight effects). No evidence of developmental toxicity was observed in rats at doses up to 300 mcg/kg/day.

There are no adequate and well-controlled studies in pregnant women. [TRADE NAME™] should be used during pregnancy only if the potential benefit to the mother justifies the potential risk to the fetus.

Signatures (optional):

Reviewer Signature Barbara J. Wilcox

Supervisor Signature Martin D. Green Concurrence Yes ✓ No ___

11-1-04

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:	125103
SERIAL NUMBER:	0
DATE RECEIVED BY CENTER:	5/14/04
PRODUCT:	Palifermin
INTENDED CLINICAL POPULATION:	Mucositis/Hematologic Malignancies
SPONSOR:	Amgen
DOCUMENTS REVIEWED:	Vol. (N/A-electronic submission)
REVIEW DIVISION:	Division of Therapeutic Biological Internal Medicine Products (HFD-108)
PHARM/TOX REVIEWER:	Anita M. O'Connor, Ph.D.
PHARM/TOX SUPERVISOR:	Dave Green, Ph.D.
DIVISION DIRECTOR:	Pat Keegan, M.D.
PROJECT MANAGER:	Susan Giuliani

Date of review submission to Division File System (DFS):

TABLE OF CONTENTS

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability
Palifermin is recommended for approval.

B. Recommendation for nonclinical studies
No additional nonclinical studies are needed at this time.

C. Recommendations on labeling
No recommendations at this time.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Pharmacodynamic studies were conducted in mice and rats. These studies demonstrated that palifermin promoted epithelial cell regeneration in the salivary gland, tongue, esophagus and gastrointestinal tract when given before and/or after cytotoxic therapy. The enhancement of epithelial cell proliferation was shown by increased BrdU incorporation into cells, Ki67 immunostaining and increased target tissue(s) thickness.

The pharmacokinetics of palifermin in mice, rats, wethers and monkeys were linear and dose dependent. Terminal half-life in all these species was 1-3 hours. There was no accumulation of product (i.e., no increase in systemic exposure) with multiple dosing up to 7 days. The serum profile of palifermin in the monkey was different from the rodent; there was a plateau or "hump" effect 1-4 hours after intravenously (IV) dosing in the monkey. This effect was attributed to redistribution subsequent to rapid intravascular absorption. (The plateau effect is also seen in humans.) When given IV, volume of distribution was 50-100 ml/kg in rats, 1000-5000 ml/kg in monkeys and 2300-4000 ml/kg in wethers suggesting extensive extravascular exposure in larger species. Clearance averaged 50-165 ml/hr/kg, 450-1770 ml/hr/kg, and 800-1000 ml/hr/kg in the rat, monkey, and wether, respectively, with IV administration.

Absorption, distribution, metabolism and excretion studies demonstrated that palifermin was primarily metabolized and excreted by the kidney. A rat model showed minimal first-pass extraction of the product by the liver. In wethers, 45% of palifermin was absorbed by the lymphatic system after subcutaneously (SC) administration. Twenty-four hours after a radioactive IV injection of palifermin there was no significant accumulation in rodent tissues. Tissues with highest uptake of radioactive product over 24 hours were adrenal glands, intestine, kidneys, liver, ovaries, spleen, stomach, and trachea.

B. Pharmacologic activity

The product, palifermin, stimulates the growth of epithelial cells by binding to the keratinocyte growth factor (KGF) receptor. This receptor is found in a wide variety of tissues. KGF is a member of the fibroblast growth factor (FGF) family (specifically, FGF-7). Endogenous KGF is synthesized and released by fibroblasts and other mesenchymal cells. Endogenous KGF promotes epithelial cell healing subsequent to injury.

C. Nonclinical safety issues relevant to clinical use

It is not known if the product will enhance the growth of existing tumors, promote the growth of new tumors or protect tumors against cytotoxic therapies.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 125103/0
Sequence number/date/type of submission: STN BLA #125103/0, May 14, 2004
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: Amgen, Inc.
Manufacturer for drug substance: Amgen, Inc.
Reviewer name: Anita M. O'Connor, Ph.D.
Division name: Division of Therapeutic Biological Internal Medicine Products
HFD #: 108
Review completion date: November 1, 2004

Drug:

Trade name: Kepivance®
Generic name: palifermin
Code name: rHuKGF
Chemical name: keratinocyte growth factor
CAS registry number: 162394-19-6
Molecular formula/molecular weight: 16277.8 daltons (theoretical based on the 140 amino acid composition); structure is similar to other fibroblast growth factors (FGF) [ ] identical to endogenous keratinocyte growth factor (KGF) except for the first 23 N-terminal amino acids that are deleted in rHuKGF
Structure: (as supplied by sponsor)

Figure 1. Amino Acid Sequence of Palifermin

Relevant INDs/NDAs/DMFs: IND

Drug class: Growth Factor

Intended clinical population: Mucositis in hematologic oncology patients

Clinical formulation: The product is a sterile lyophilized — containing 6.25 mg of palifermin per 5 mL single use vial. Other ingredients are mannitol (50 mg/vial), sucrose (25 mg/vial), L-histidine (1.94 mg/vial), polysorbate 20 (.13 mg/vial)

Route of administration: Intravenous: 60 µg/kg administered as an intravenous bolus injection for 3 consecutive days prior to the initiation of high dose cytotoxic conditioning therapy, and 3 consecutive days immediately following peripheral blood stem cell (PBSC) infusion for a total of 6 doses.

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

Data reliance : Except as specifically identified below, all data and information discussed below and necessary for approval of BLA 125103 are owned by Amgen or are data for which Amgen has obtained a written right of reference. Any information or data necessary for approval of BLA 125103 that Amgen does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that Amgen does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of BLA 125103.

Studies reviewed within this submission:
Pharmacodynamics, pharmacology, pharmacokinetics, drug interactions

Studies not reviewed within this submission:
All studies in the toxicology section of the BLA

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Pharmacodynamic studies were conducted mainly in mice and rats. These studies demonstrated that palifermin promoted epithelial cell regeneration in the salivary gland, tongue, esophagus and gastrointestinal tract when given before and/or after cytotoxic therapy. The enhancement of epithelial cell proliferation was shown by increased BrdU incorporation into cells, Ki67 immunostaining and increased target tissue(s) thickness.

2.6.2.2 Primary pharmacodynamics

Mechanism of action:
Keratinocyte growth factor is a member of the FGF family. Its receptor is expressed ubiquitously. Endogenous KGF is believed to promote epithelial cell proliferation.

Drug activity related to proposed indication:

The proposed indication is .

[ ]

PRIMARY PHARMACOLOGY STUDIES

Study No.	Study Title	Route & Dose	Model	Results
R2003131	Effect of 3 Days of SC rHuKGF Administration on Salivary Gland Weight and BrdU Incorporation in Normal Male Rats	SC/0, 0.1, 0.3, 1.0 or 3.0 mg/kg of rHuKGF for 3 consecutive days	Rat	Linear dose-related treatment effect on salivary gland weight and proliferation of acinar cells of the salivary gland
R2003146	Effect of the SC Administration of rHuKGF in Protecting the Rat Parotid Salivary Gland From the Acute Effects of Radiation Induced Injury	SC/3.0 mg/kg rHuKGF 1) Control (no radiation, no rHuKGF); 2) 25 GY X-ray no rHuKGF; 3) 25 GY X-ray rHuKGF days –3 to –1; 4) 25 GY X-ray rHuKGF days 1 to 2; 5) 25 GY with rHuKGF days –3 to –1 and days 1 to 2	Rat	Treatment with 3.0 mg/kg completely reversed the radiation injury to the salivary gland; giving the drug post radiation or pre and post radiation exposure (groups 4, 5) increased salivary gland restoration of function to a greater extent than when the drug was given before radiation
R2003159	The effect of the SC administration of 3 mg/kg of rHuKGF in protecting the rat parotid and submandibular salivary glands from the chronic effects of radiation induced injury	SC/3.0 mg/kg rHuKGF 1) Normal rat without rHuKGF administration; 2) 15 GY X-ray no rHuKGF; 3) 15 GY X-ray rHuKGF days –3 to –1; 4) 15 GY X-ray rHuKGF days 1 and 2; 5) 15 GY with rHuKGF 26 weeks post irradiation 6) 15 GY x-ray, pre and post rHuKGF treatment on days –3 to –1 and days 1 and 2; 7) Sentinel group, without rHuKGF, animals were harvested at 1, 2, 3, 4, 5, and 6 months	Rat	Groups 3 and 6 were similar to controls for the following variables: salivary gland flow rate, salivary amylase, and salivary protein. It is not clear why group 4 did not respond to treatment. Giving the drug 26 weeks post radiation had no beneficial effect (group 5)
R2003308	Effects of rHuKGF on the Epithelium of the Gastrointestinal Tract of Mice Treated With Low Dose BEAM Combination Chemotherapy in Mice	Route not clear from report/5.0 mg/kg of rHuKGF Mice received BEAM therapy (BCNU, etoposide, cytosine arabinoside and melphalan) for 6 days and pretreated with vehicle or 5.0 mg/kg of rHuKGF for 3 days	Mouse	KGF pretreated mice had significantly greater epidermal thickness of the tongue and jejunum mucosal thickness than vehicle treated mice; weight loss was less in KGF treated mice
R2003309	An Exploratory Study of the Effects of rHuKGF on the BEAM	Route not clear from report/5.0 mg/kg of rHuKGF Mice given a combination	Mouse	The medium and high dose BEAM treated mice did not survive past day 7. Treating

PRIMARY PHARMACOLOGY STUDIES				
Study No.	**Study Title**	**Route & Dose**	**Model**	**Results**
	Combination of Chemotherapy in Mice	BCNU, etoposide, cytosine arabinoside and melphalan (BEAM) at 3 different levels (high, medium, low) for 6 days and 5.0 mg/kg rHuKGF either pre treatment (days –2, -1, 0), on day 7, 8 and 9 or days –2, -1, 0, 7, 8 and 9		mice pre BEAM and pre & post BEAM increased body weight significantly compared to controls. Mortality was 66% in the low dose BEAM group and 20% or less in all of the rHuKGF treated groups (P<.01)
R2003233	The Effect of rHuKGF Dosed at 5 mg/kg, 1 mg/kg, 0.5 mg/kg, or 0.1 mg/kg on Epithelium in the Oral Mucosa in BDF1 Mice Given a Single Dose of 12 GY Radiation	SC/5.0, 1, 0.5, or 0.1 mg/kg rHuKGF given after a single dose of 12 GY, 2 hours after irradiation and continued daily for 3 days	Mouse	Treatment with 5.0 mg/kg but not any of the lower doses significantly increased the epithelial thickness of the tongue, buccal mucosa, upper palate, and esophageal tissues.
R2003234	The Effect of 5 mg/kg rHuKGF for up to 4 Days Treatment in the Epithelium of the Oral Mucosa in BDF1 Mice Given a Single Dose of 12 GY Total Body Irradiation	SC/5.0 mg/kg rHuKGF or saline for 2, 3, 4, or 5 daily doses before 12 GY of total body irradiation	Mouse	Greatest increase in epithelial thickness was seen in the tongue and upper palate after 3 days of KGF treatment. For epithelial thickness of the buccal cavity the most beneficial treatment was 1 day of KGF treatment (i.e., data were not in agreement). For esophageal tissue, epithelial thickness was significantly increased at all dosing time points
R2003235	Effect of rHuKGF Mucosa in Mice Given 4 GY Radiation Over 4 Days	SC/5.0 mg/kg rHuKGF Mice were divided into 12 treatment groups of 5 mice per group and given 4 days of 4 GY radiation followed by either 2, 3, 4, or 5 doses of rHuKGF at 5.0 mg/kg. Two hours prior to harvest, mice were given their last dose of either rHuKGF or saline control. Mice were harvested 5 hours post last dose of KGF.	Mouse	Tongue and esophageal epithelial thickness increased with rHuKGF treatment in a time related trend over the four days of treatment (p<.0001); buccal mucosa epithelial thickness increased compared to controls by day 2 (p<.0001) and reached a plateau at day 2; upper palate epithelial thickness increased compared to controls in a time related trend through day 3 at which point it reached a plateau (p<.0001). Cellular proliferation also increased with treatment through day 2 and then decreased to control levels on day 3 and 4 (p<.0001)
R2003285	Effect of rHuKGF Pre and Post 5 FU Chemotherapy Treatment in a Mouse Model of Intestinal	SC/5.0 mg/kg rHuKGF on days -2, -1, 0, or days 5, 6, 7. On days 0, 1, 2, 3 the animals were injected IP with 50 mg/kg 5-FU. The control	Mouse	Only dosing with rHuKGF prior to chemotherapy enabled mice to minimize weight loss after 5-FU administration. Water and food consumption

PRIMARY PHARMACOLOGY STUDIES*				
Study No.	**Study Title**	**Route & Dose**	**Model**	**Results**
	Mucositis	group received 5-FU only plus SC saline injections on days -2, -1, 0.		were higher in the pre dosed group also (p<.001). Survival was significantly higher in this group as opposed to the group dosed with rHuKGF after chemotherapy that survived only marginally better than controls. When crypt and villus measures of duodenum tissue were evaluated after H&E staining, both crypt and villus measures were significantly increased by rHuKGF treatment after 2-3 days of treatment (p<.001).
R2003289	Early Scheduling of rHuKGF Pretreatment in a Mouse 5 FU Mucositis Model	SC/5 mg/kg rHuKGF Mice were injected with rHuKGF 5 mg/kg days for three consecutive days starting at either day -6, -5, -4 or -2. Additional groups were given 15 or 30 mg/kg rHuKGF, 30 mg/kg pegylated rHuKGF or 30 mg/kg pegylated BDNF IV as a negative pegylated protein control on day -2. A control group was given saline SC days -2, -1, 0. The animals were injected IP with 60 mg/kg 5-FU on days 1, 2, 3, 4.	Mouse	Body weights were significantly increased compared with controls with all rHuKGF treatments; more improvement was seen with the multiple SC dosing schedule compared to a single equivalent IV dose. Also, the schedule of SC dosing with multiple doses of rHuKGF prior to 5-FU therapy significantly improved survival compared to controls and a single equivalent IV dose of rHuKGF.
R2003238	Effect of rHuKGF, Cisplatin, and 5 FU on Epithelium of the Oral Mucosa in BDF1 Mice Given 5 GY Radiation	SC/5.0 mg/kg rHuKGF BDF1 mice were pre-dosed with rHuKGF on days -2 to 1 for three days prior to chemotherapy and radiation. On day 1, the mice were given Cisplatin (10 mg/kg IP) followed by administration of 5-FU (40 mg/kg IP) one hour later. Two hours after Cisplatin injection, mice were exposed to 5 GY gamma irradiation to the head and neck. On days 2 through 4, mice were given 5-FU (40 mg/kg) and irradiated with 5 GY one hour later. On day 5, mice were exposed to 5 GY gamma irradiation and treated with rHuKGF 5 hours	Mouse	RHuKGF significantly increased epithelial thickness of the tongue compared to controls (p<.0001). Cellular proliferation as measured by Ki67 immunostaining was increased on day 1 (p<.0001) and on day 6 as measured by BrdU incorporation (p<.0001). Esophagus thickness was increased on days 1 and 6 (p<.0001); cellular proliferation as measured by Ki67 and BrdU was correspondingly increased on days 1 and 6 compared to controls (p<.0001). There was an increase in the weights of liver and intestine in rHuKGF treated mice (p<.05). Glucose, cholesterol, triglycerides, blood urea nitrogen, alkaline

PRIMARY PHARMACOLOGY STUDIES				
Study No.	**Study Title**	**Route & Dose**	**Model**	**Results**
		later.		phosphatase, and amylase were measured in mice sacrificed on day 1 (prior to chemotherapy and radiation), day 6 and 8. Amylase and triglycerides were significantly increased in rHuKGF treated mice on day 1 compared to controls. Alkaline phosphatase was significantly decreased on day 1 compared to controls ($p<.03$)
R2003239	Effect of rHuKGF, Cisplatin, and 5 FU on Epithelium of the Oral Mucosa in BDF1 Mice Given 5 GY Radiation	SC/5.0 mg/kg rHuKGF Female BDF1 mice were divided into six groups. Groups were pre-dosed with rHuKGF or saline for three days prior to chemotherapy and radiation on days -2 to 0 or days -4 to -2. On day 1, the mice were given Cisplatin (10 mg/kg IP) followed by administration of 5-FU (40 mg/kg) one hour later. Two hours after Cisplatin injection, mice were exposed to 5 GY locoregional gamma irradiation to the head and neck. On days 1 through 4, mice were given 5-FU (40 mg/kg IP) and irradiated with 5 GY one hour later. On day 5, mice in groups 3 and 6 were similarly exposed to 5 GY gamma irradiation and treated with rHuKGF 5 hours later. Mice in groups 1 through 3 were evaluated for survival. Mice in groups 4 through 6 were sacrificed on day 8.	Mouse	RHuKGF increased epithelial thickness ($p<.0001$) in both ventral and dorsal tongue with both treatment schedules. Cellular proliferation was increased with both treatment schedules as measured by the Ki67 immunostaining technique. Liver and salivary gland weight were significantly increased with the –2 to 0 day treatment group relative to controls and the –4 to –2 treatment group ($p<.0001$). All rHuKGF treated groups had a greater rate of body weight recovery after chemotherapy compared to controls. Overall survival was improved with both treated groups compared to saline controls, however, the –2 to 0 day group had ~20-30% greater survival compared to the –4 to –2 day treated group.
R2003236	Effect of rHuKGF in Normal and Irradiated BDF1 Mice	SC/5.0 mg/kg rHuKGF BDF1 mice were divided into 7 dose groups of 5 mice per group. Group 1 received 5 mg/kg rHuKGF on day -2 to 4 and no radiation. Group 2 received no rHuKGF treatment or radiation. Group 3 received 12 GY with 5 mg/kg of rHuKGF given as a pre and post treatment on days -2 to 4. Group 4 received 12 GY treated with	Mouse	Epithelial thickness of the tongue was increased over controls ($p<.05$) with 2-4 days of product administration (groups 3, 5, 7). Epithelial thickness of the palate and esophagus was increased in groups 3 and 7 ($p<.05$, $p<.0001$, respectively). Buccal mucosa was not affected by treatment with palifermin. Epithelial thickness of the upper aerodigestive tract

PRIMARY PHARMACOLOGY STUDIES				
Study No.	**Study Title**	**Route & Dose**	**Model**	**Results**
		0.9% NaCl on day -2 to 4. Group 5 received 12 GY with 5 mg/kg rHuKGF given on pre treatment days -2 to 0. Group 6 received 12 GY with 5 mg/kg rHuKGF given on day -1 only. Group 7 was given 12 GY and given 5 mg/kg of rHuKGF post treatment on days 0 to 4. X-ray treatment was given on day 0.		(tongue, buccal cavity, palate, esophagus) were increased by (pooled) KGF treatment ($p<.0001$). The product caused BrdU labeled cells to increase in group 7 ($p<.0001$). KGF improved small intestinal crypt survival in mice given 12 GY in all treated groups compared to controls ($p<.0001$)
R2003273	Effect of rHuKGF on the Oral Cavity and Small Intestine in Normal and Irradiated BDF1 Mice	SC/5.0 mg/kg rHuKGF BDF1 mice were divided into 7 dose groups of 5 mice per group. Group 1 received no rHuKGF treatment or radiation. Group 2 received irradiation only. Group 3 received irradiation and rHuKGF given as a pre and post treatment on days -2 to 4. Group 4 received irradiation and rHuKGF on day -1. Group 5 received irradiation and rHuKGF given on days -2 to 0. Group 6 received irradiation and rHuKGF given on day 0. Group 7 was given irradiation and rHuKGF post treatment on days 0 to 4. Total body irradiation (12 GY X-ray treatment) was administered on day 0. On day 4, two hours prior to harvest, mice were given their last injection of either rHuKGF or 0.9% NaCl.	Mouse	Epithelial thickness of the tongue and oral cavity increased with rHuKGF treatment ($p<.0001$) compared to controls. Only two days of treatment increased epithelial thickness of the tongue only ($p<.0001$). Esophageal thickness was increased with 1-4 days of treatment ($p<.0001$). BrdU labeled epithelial cells of the esophagus were increased with 4 days of treatment ($p<.0001$). Small intestinal crypt survival was improved in all treated groups ($p<.0001$) compared to controls.
R2003274	Effect of rHuKGF on Epithelium of the Upper Aerodigestive Tract and Small Intestine in BDF1 Mice	SC/5.0 mg/kg rHuKGF mice were divided into 10 treatment groups of 5 mice per group and treated with 9% NaCl SC for 3, 5 or 6 days or rHuKGF 5 mg/kg from 1 to 6 days. A group of normal mice were also included. Tongue, esophagus, and small intestine were collected and examined for epithelial thickness, Ki67	Mouse	The dorsal thickness of the tongue tripled in a time-dependent trend with 6 days of product treatment ($p<.0001$). The thickness of the ventral tongue surface doubled and reached a plateau at 3 days ($p<.0001$). Proliferation of the tongue doubled and plateaued within 3 days of treatment ($p<.05$). The thickness of the esophagus increased 30%

PRIMARY PHARMACOLOGY STUDIES				
Study No.	**Study Title**	**Route & Dose**	**Model**	**Results**
		immunostaining area, and BrdU labeling.		within 3 days and reached a plateau (p<.0001). Total intestinal weight increased 30% within 3 days, mainly due to the increase in duodenum and jejunum weight (to a lesser extent), (p<.0001)
R2003303	Effect of rHuKGF on Mouse Tongue Ulceration Following Chemotherapy and Radiation	SC/5.0 mg/kg rHuKGF Chemotherapy was comprised of daily injections of cDDP (1 mg/kg per injection) and 5-FU (30 mg/kg/ per injection) on days 0-3 or days 7-10. The drugs were administered 30 minutes prior to irradiation. The mice were given fractionated irradiation locally to the snout as 5 daily fractions of 3 Gy on days 0-4 and 7-11. Recombinant HuKGF was given as 5 mg/kg SC daily doses before, during, or after radiotherapy. Graded test doses of radiation were given to an area of the ventral tongue on day 14. The graded test doses were used to calculate the ED50 which is the dose of radiation required to induce a mucosal ulceration in 50% of the mice. The ED50 was used as the endpoint representing radiation tolerance in mucosal tissue.	Mouse	A statistically significant (p<.003) reduction in the ulceration of mouse tongue exposed to radiation and chemotherapy occurred when the product was given: 1) days –2, +4, +11 2) days +4, +11 3) days +4, +11, +18 4) days –2, +4, +11, +18 There was no statistically significant (p<.17) effect of the product on tongue ulceration when it was given only on days –2, +4.

2.6.2.3 Secondary pharmacodynamics

SECONDARY PHARMACOLOGY STUDIES				
Study No.	**Title**	**Route & Dose**	**Model**	**Results**
R2003171	Effects of Pre-Treatment With a Single High IV Dose of rHuKGF on CPT-11 Induced	IV/30 mg/kg rHuKGF Two separate experiments were performed. The first had 13 rats per arm while the second had 15 rats per arm.	Rat	The product had no effect on survival or body weight but it improved mean diarrhea score and incidence in rats that did survive the chemotherapy (p<.06, p<.0001, respectively).

SECONDARY PHARMACOLOGY STUDIES				
Study No.	Title	Route & Dose	Model	Results
	Diarrhea in Rat	One group was given a 30.0 mg/kg dose of rHuKGF IV 3 days prior to chemotherapy with 300 mg/kg CPT-11. The second group was given CPT-11 alone. Rats were followed for 15 days post-chemotherapy.		
R2003227	Effects of Pretreatment With a Subcutaneous Dose of rHuKGF From CPT-11 Induced Weight Loss and Mortality in Mice	SC/5.0 mg/kg rHuKGF Mice received CPT-11 at either 200 mg/kg or 250 mg/kg IP for 4 days starting on day 0. Recombinant HuKGF was given as a SC pre-treatment on days -2, -1 and day 0 or as an early pre-treatment on days -4, -3, -2 prior to chemotherapy.	Mouse	rHuKGF improved body weight loss recovery after chemotherapy; there was no consistent effect on improvement of survival rate in the rHuKGF treated animals
R2003228	Effects of Pretreatment With a Single High Subcutaneous Dose of rHuKGF on CPT-11 Induced Diarrhea in Rat	SC/30.0 mg/kg rHuKGF Rats were divided into three groups. Group 1 was given saline IP on day 0. Group 2 received 325 mg/kg CPT-11 IP on day 0. The third group received 30 mg/kg rHuKGF SC on day -1 prior to CPT-11 IP on day 0.	Rat	Survival rate was improved ~40% in rats given rHuKGF prior to chemotherapy ($p<.01$); body weight loss and diarrhea were also improved in rHuKGF treated rats; peak time of diarrhea effects were postponed and scores were lower due to test article
R2003231	Effect of Pretreatment of KGF on Life Expectancy of Mice Irradiated With 12 Gy Irradiation	SC/5.0 mg/kg rHuKGF Female BDF1 mice were divided into four groups. Groups were pre-dosed with rHuKGF or saline for three days (-3 to -1) prior to radiation. On day 1, mice were exposed to 12 Gy total body irradiation and two groups subsequently post treated with rHuKGF and rMuGCSF on days 2 through 6.	Mouse	No protective effects of pre treatment with or without rMuGCSF on radiation injury.
R2003232	Effect of Crypt Survival in Mice Irradiated With 12 Gy and Given Autologous BMT	SC/5.0 mg/kg rHuKGF Female BDF1 mice were divided into two groups. Groups were pre-dosed with rHuKGF or saline for 3 days prior to total body radiation. On day 1, the mice	Mouse	Mice treated with rHuKGF had a significant increase in the number of BrdU labeled crypts ($p<.0001$) in the duodenum, jejunum, and ileum. Total small intestine wet weight was also increased in the rHuKGF group compared to controls ($p<.0003$)

SECONDARY PHARMACOLOGY STUDIES				
Study No.	Title	Route & Dose	Model	Results
		were given an autologous BMT. Mice were sacrificed on day 4. The effects of rHuKGF on crypt survival, as measured by counts of BrdU-labeled crypts, and intestinal weight were examined.		
R2003237	Effects of KGF on Survival Using 3 Doses of Irradiation	SC/5.0 mg/kg rHuKGF Female BDF1 mice were divided into six groups. Groups were pre-dosed with rHuKGF or saline for three days (-2 to 0) prior to radiation. On day 1, mice were exposed with 11 GY, 12 GY, or 13 GY radiation. On day 2, mice were given a BMT. Mice were evaluated for changes in body weight and survival.	Mouse	Mice treated with rHuKGF had less weight loss than control groups. The only difference in the survival curves for the 3 treated groups was for the group that received 13 GY, in this group survival increased relative to controls by 14.7 vs. 10.6 days ($p<.0125$).
R2003305	Effect of rHuKGF on Weight and Survival in Mice Treated With a Combination of Methotrexate and Radiation	SC/5.0 g/kg/day rHuKGF Animals were retreated with rHuKGF on day -3, -2, -1. On day 0, mice were given a single IV dose of 150 or 300 mg/kg methotrexate. One hour later mice were irradiated with 6 Gy total body irradiation. Mice were weighed daily for 12 days.	Mouse	Pre treatment with rHuKGF improved survival 60% in both methotrexate/radiation groups; weight loss was also improved in both groups given rHuKGF
R2003306	Effect of rHuKGF on Weight in Mice Following Cyclophosphamide Treatment Combined With Cisplatin or Irradiation	SC/5.0 mg/kg/day rHuKGF Animals were pretreated with rHuKGF on day -3, -2, -1. On day 0, mice were irradiated with a single dose of 5 Gy and injected with a single dose of 200 or 400 mg/kg cyclophosphamide (CP). CP was given immediately after irradiation. Mice were weighed daily until day 10 and then again at day 13.	Mouse	Treatment with rHuKGF reduced the severity of weight loss due to chemotherapy
R2003148	Effects of rHuKGF Treatment on the Survival of Mice Irradiated With 12	SC/5.0 mg/kg rHuKGF Animals were randomized into groups of 10 or 15 mice/group. The rHuKGF	Mouse	To summarize all 8 studies in this report, rHuKGF improved survival in 4/8 studies; there was no clear beneficial effect of rHuGCSF or rHuKGF.

SECONDARY PHARMACOLOGY STUDIES				
Study No.	Title	Route & Dose	Model	Results
	GY Followed by Transplantation With Bone Marrow or Peripheral Blood Progenitor Cells	(5.0 mg/kg/day) or rHuGCSF (100 µg/kg/day) were administered once daily SC. The mice were irradiated with 12 GY. Mice receiving injections of rHuKGF as a pretreatment received them on day -2, -1 and 0. Irradiation of the mice occurred on day 1, and the transplants were performed on day 2. Post treatments of rHuKGF were administered on day 3, 4 and 5 and rHuGCSF was administered as a single daily injection for 7 days beginning on day 3. In each experiment there was a control group that received only saline as a pre and/or post treatment. BMT Procedure: Female BDF1 mice were euthanized by cervical dislocation, the femurs taken and flushed with RPMI 1640 plus 1% fetal bovine serum. The suspension was centrifuged at 1100 rpm for seven minutes, the supernatant aspirated and the cells washed twice with PBS and fetal bovine serum. The cells were counted with a hemacytometer, diluted in PBS with 1% mouse serum and injected IV into irradiated mice at a dose of 2x106 cells/mouse. PBC Procedure: Male BDF1 mice were implanted with subcutaneous osmotic pumps that delivered rHuGCSF at a dose of 200 µg/kg/day. Five to 7 days after pump implantation, blood was collected into EDTA coated tubes by cardiac puncture. Blood from all mice was combined and layered onto an — gradient and spun at 2500 rpm for 45		Appears This Way On Original

SECONDARY PHARMACOLOGY STUDIES				
Study No.	Title	Route & Dose	Species/ Model	Results
		minutes. The cells were collected, washed twice in PBS, counted and diluted in PBS and injected IV into female irradiated BDF1 mice at a dose of $10x10^6$ cells/mouse.		
R2003194	The Effects of the Administration of rHuKGF in the DSS Mouse Model of Inflammatory Bowel Disease	SC/1.0 and 3.0 mg/kg rHuKGF Female SJL/L mice aged 8-10 weeks were used for this study. For disease induction, mice were given 4% DSS ad libitum as drinking water. rHuKGF was administered by SC injection at doses of 1.0 or 3.0 mg/kg in both preventative and therapeutic regimens over the course of the study.	Mouse	Survival benefit, body weight loss, and diarrhea were improved with both rHuKGF treatments; the large intestines were more histologically normal in the treated mice. In general, more improvement was seen with the 3.0 versus the 1.0 mg/kg group for all variables evaluated
R2003147	The Effects of rHuKGF in the CD45RB Immunologic Transfer Model of Inflammatory Bowel Disease	IP/3.0 mg/kg rHuKGF 3 times a week for 7 weeks Colitis like disease was induced in 18 C.B-17 SCID mice by injection of semi-syngeneic D4+CD45RB^{high} T cells isolated from donor CB6F1 mice (a cross between C57BL/6 x BALB/c). During the course of the trial, the animals were weighed twice weekly and were observed for clinical signs of illness. The animals were not treated until they had lost 10% of their original starting weight. At this point, they were regularly treated with rHuKGF or vehicle control.	Mouse	The intestines of rHuKGF treated mice were less inflamed and histologically had a more normal appearance than control animals; smaller small intestines were also evident in treated mice. There was more proliferation of the squamous epithelium of the esophagus and the livers were larger in treated mice.

2.6.2.4 Safety pharmacology

The sponsor conducted a general safety study in mice, rats and rhesus monkeys (i.e., study SNBL 39-32/Amgen Study Number 960100, " A GENERAL PHARMACOLOGY STUDY OF RECOMBINANT-HUMAN KERATINOCYTE GROWTH FACTOR (rHuKGFd23")

GENERAL PHARMACOLOGY STUDIES				
Study No.	**Title**	**Route & Dose**	**Model**	**Results**
960100	A General Pharmacology Study of Recombinant-Human Keratinocyte Growth Factor	IV/0.5, 5.0, 50.0 mg/kg rHuKGF	mice rats rhesus monkeys	No adverse effects observed for any of the species tested.

General effects: No effects

Neurological effects: No effects

Cardiovascular effects: No effects

Pulmonary effects: No effects

Renal effects: Not evaluated

Gastrointestinal effects: No effects

Abuse liability:

Other:

2.6.2.5 Pharmacodynamic drug interactions

2.6.3 PHARMACOLOGY TABULATED SUMMARY

PHARMACODYNAMIC DRUG INTERACTIONS				
Study No.	**Title**	**Route & Dose**	**Model**	**Results**

PHARMACODYNAMIC DRUG INTERACTIONS				
Study No.	Title	Route & Dose	Model	Results
IH-95-KGF-002	Exploratory Drug Interaction Study of Recombinant Human Keratinocyte Growth Factor (rHuKGF) given prophylactically in a 5-Fluorouracil /Murine Granulocyte Colony Stimulating Factor (Mu-GCSF) Murine Model of Chemotherapy	SC/5 mg/kg/day rHuKGF and .10 mg/kg Mu-GCSF All treatment groups received daily SC injections of 50 mg/kg of 5-FU with either rHuKGF alone-(pretreatment only), rMuG-CSF alone-(post treatment only) or both cytokines (pre and post 5-FU).	Mouse	Mu-GCSF caused white blood cells to increase 2-3 times the mean value of the untreated mice after chemotherapy and increase mean absolute spleen weight; body weights were improved in cytokine treated mice
R2003172	Pathology Report: Exploratory Drug Interaction Study of rHuKGF Given Prophylactically in a 5-fluorouracil/ rMuGCSF Murine Model of Chemotherapy (Toxicology Study No. IH-95-KGF-002)	SC/5 mg/kg/day rHuKGF 80 male mice were divided into 5 groups of 20. Group 1 received 5-FU and rMuGCSF. Group 2 was pretreated with rHuKGF followed by 5-FU chemotherapy with rMuGCSF treatment. Group 3 received only 5-FU chemotherapy. Group 4 received rHuKGF pretreatment followed by 5-FU chemotherapy. An additional 5 mice (group 5) served as untreated controls.	Mouse	In this study the expected gastrointestinal and/or hepatic lesions in a majority of mice did not occur (due to chemotherapy). Weight loss also did not occur as a result of chemotherapy. Survival was also high. Thus, the pathologic findings are not interpretable.
R2003173	Effects of rHuKGF Pre-and Post Treatment With and Without rHuGCSF Post Treatment in a Rhesus Macaque Model of Lethal Irradiation and Bone Marrow	IV/100, 300 µg/kg/day rHuKGF Eighteen young adult male rhesus macaques were divided into 5 groups: (1) control, (2) 100 µg/kg/day rHuKGF pre	Rhesus Monkey	There were no statistically significant differences in stool scores, oral gastric interventions, liquid intake, body weight changes and clinical chemistries in any treated group compared to controls. RHuGCSF improved neutrophil counts in group 5 compared to group 4 starting

PHARMACODYNAMIC DRUG INTERACTIONS:				
Study No.	Title	Regimen & Dose	Model	Results
	Transplant	radiation (days -3,-2,-1), (3) 300 µg/kg/day rHuKGF pre radiation , (4) 300 µg/kg/day rHuKGF pre and post radiation (3,4,5), and (5) 300 µg/kg/day rHuKGF pre and post radiation and 10 µg/kg/day rHuGCSF starting on day 1 after radiation and continuing until the absolute neutrophil counts were over 3000 µL.		around day 5 post radiation treatment. Histopathology changes were due to radiation damage. There was a suggestion of reduced diarrhea in the rHuKGF treated groups. No beneficial effects on the epithelial tissues were found upon necropsy.

Appears This Way On Original

2.6.4 PHARMACOLOGY TABULATED SUMMARY (AS SUPPLIED BY SPONSOR)

Table 2.6.3.2. Safety Pharmacology

Organ Systems Evaluated	Species/Strain	Method of Administration	Doses[a] (µg/kg or as shown)	Gender and No. Per Group	Noteworthy Findings	GLP Compliance	Study Number
Central nervous system	[redacted]CD-1(ICR) mice Crj:Wistar rats	Intravenous	500, 5000, 50000	6M	rHuKGFd23 had no effect on general behavior in mice. rHuKGFd23 had no effect on the locomotor activity or thiopental-induced sleep, nor any analgesic, anticonvulsive or proconvulsive activity in mice. rHuKGFd23 had no effect on body temperature in rats.	Yes	960100 [redacted]. 39-32)
Autonomic nervous system and smooth muscle	Guinea pigs	In vitro	5×10^{-7}, 5×10^{-6}, 5×10^{-5} g/mL	16M (total)	rHuKGFd23 had no effect on the spontaneous movements of the isolated guinea pig ileum, and there were no effects on acetylcholine-, histamine-, or barium chloride-induced contractions at any concentration.		
Respiratory and cardiovascular	Rhesus monkeys	Intravenous	500, 5000, 50000	3M (total)	rHuKGFd23 had no effect on the respiratory or cardiovascular systems, or on the electrocardiograms in monkeys.		

(Continued)

[a] Single dose unless specified otherwise

Table 2.6.3.2. Safety Pharmacology (Continued)

Organ Systems Evaluated	Species/Strain	Method of Administration	Doses[a] (µg/kg)	Gender and No. Per Group	Noteworthy Findings	GLP Compliance	Study Number
Gastrointestinal	[redacted]:CD-1(ICR) mice	Intravenous	500, 5000, 50000	6M	rHuKGFd23 had no effect on intestinal transport of charcoal in mice.	Yes	960100 ([redacted] 39-32)
Renal - Water and metabolic electrolytes	[redacted]Wistar rats	Intravenous	500, 5000, 50000	6M	5000: statistically significant decreases in concentration of Na^+ and Cl^- and total excretion of Na^+ 50000: statistically significant decreases in urinary concentrations of electrolytes. These changes were thought to be related to a slight increase in urinary volume.		

[a] Single dose unless specified otherwise

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

The pharmacokinetics of palifermin in mice, rats, wethers and monkeys were linear and dose dependent. Terminal half-life in all these species was 1-3 hours. There was no accumulation of product (i.e., no increase in systemic exposure) with multiple dosing up to 7 days. The serum profile of palifermin in the monkey was different from the rodent; there was a plateau or "hump" effect 1-4 hours after IV dosing in the monkey. This effect was attributed to redistribution subsequent to rapid intravascular absorption. (The plateau effect is also seen in humans.) When given IV, volume of distribution was 50-100 ml/kg in rats, 1000-5000 ml/kg in monkeys and 2300-4000 ml/kg in wethers suggesting extensive extravascular exposure in larger species. Clearance averaged 50-165 ml/hr/kg, 450-1770 ml/hr/kg, and 800-1000 ml/hr/kg in the rat, monkey, and wether, respectively, with IV administration.

Absorption, distribution, metabolism and excretion studies demonstrated that palifermin was primarily metabolized and excreted by the kidney. A rat model showed minimal first-pass extraction of the product by the liver. In wethers, 45% of palifermin was absorbed by the lymphatic system after subcutaneously (SC) administration. Twenty-four hours after a radioactive IV injection of palifermin there was no significant accumulation in rodent tissues. Tissues with highest uptake of radioactive product over 24 hours were adrenal glands, intestine, kidneys, liver, ovaries, spleen, stomach, and trachea.

2.6.4.2 Methods of Analysis

2.6.4.3 Absorption

Study title: A Dose-Range Pharmacokinetic Study of Recombinant Human Keratinocyte Growth Factor (rHuKGF) Following Intravenous and Subcutaneous Administration in Sprague-Dawley Rats

Study no.: KGF. 114
Facility: Amgen, Inc.
Date of study: December 29, 1995
GLP: (No)
Dose & Formulation: 3000, 1000, 300, 100, 30, and 10 *u*g/kg via intravenous and subcutaneous routes
Animals: Male Sprague-Dawley rats
Protocol: Rats were administered rHuKGF on two days of dosing, separated by 48 hours between the two doses. On the first day, rHuKGF was administered at dose levels of 3000, 1000, 300, 100, 30, and 10 ug/kg via intravenous and subcutaneous routes. Two days later, the animals were administered the same dose, but by the alternate route. Serial blood samples were collected up to 24 hours postdose on both days and an additional blood sample was taken at 48 hours for hematocrit determination.

RESULTS: There were no sequence effects, therefore the data were pooled. Pharmacokinetic parameters, as supplied by the sponsor, are:

Table 2.

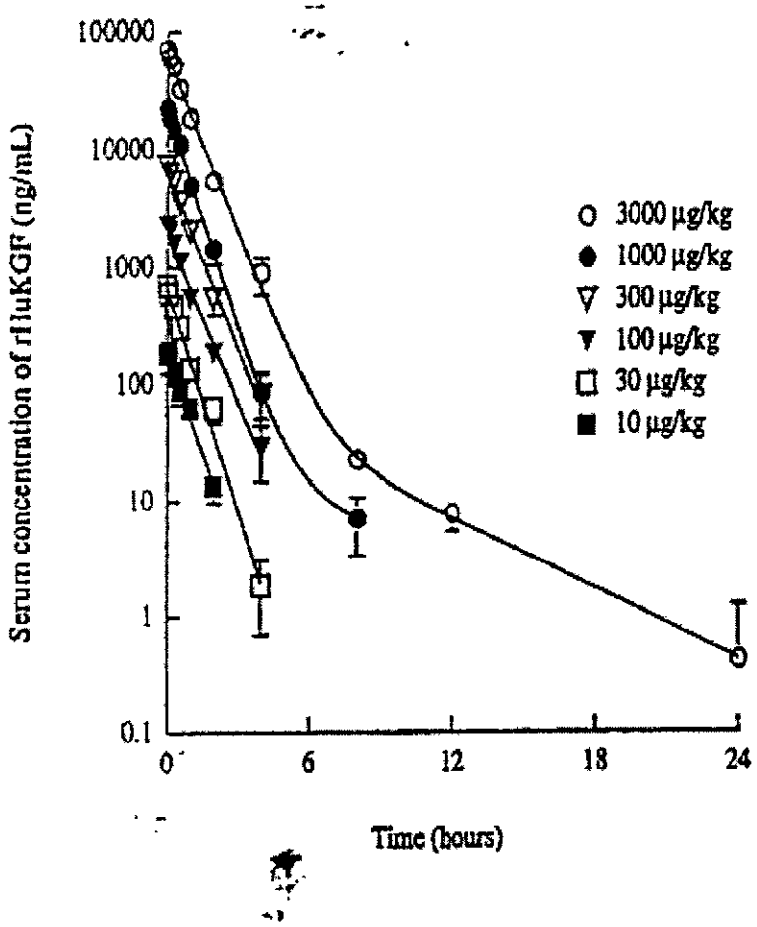
Pharmacokinetic Parameters of rHuKGF Following Intravenous and Subcutaneous Administration in the Rat (Mean ± SD, n = 4)

Parameters	Dose Levels (µg/kg)					
	3000	1000	300	100	30	10
IV dosing						
AUC (ng·h/mL)	60300 ± 7830	16600 ± 1970	6040 ± 732	1860 ± 183	519 ± 35.1	158 ± 30.1
CL (mL/h/kg)	50.4 ± 6.15	61.0 ± 6.67	50.2 ± 6.14	54.2 ± 5.10	58.0 ± 4.11	64.7 ± 10.6
V_C (mL/kg)	43.0 ± 4.57	45.1 ± 9.02	41.8 ± 4.06	44.5 ± 8.15	43.5 ± 4.09	59.5 ± 18.9
AUMC (ng·h^2/mL)	51400 ± 5880	12300 ± 2250	5090 ± 1200	1640 ± 200	406 ± 70.3	113 ± 23.4
MRT (h)	0.86 ± 0.06	0.74 ± 0.07	0.83 ± 0.10	0.88 ± 0.02	0.78 ± 0.09	0.71 ± 0.03
V_{ss} (mL/kg)	43.2 ± 6.63	45.0 ± 4.35	41.5 ± 2.09	47.6 ± 3.48	44.9 ± 3.26	46.0 ± 7.26
$t_{1/2,\alpha}$ [a] (h)	0.602	0.486	0.602	0.630	0.476	0.529
$t_{1/2,\beta}$ (h)	2.98	2.91	ND[b]	ND	ND	ND
SC dosing						
t_{max} (h)	1.13 ± 0.63	1.00 ± 0.00	1.00 ± 0.00	1.33 ± 0.58	1.00 ± 0.00	1.00 ± 0.00
C_{max} (ng/mL)	3420 ± 901	1030 ± 239	192 ± 77.1	40.1 ± 6.26	6.01 ± 3.43	0.95 ± 0.75
AUC (ng·h/mL)	11400 ± 3530	3370 ± 1360	592 ± 358	123 ± 37.7	12.0 ± 7.29	1.21 ± 1.23
CL/F (mL/h/kg)	282 ± 83.7	328 ± 106	692 ± 415	868 ± 258	4090 ± 3890	9894 ± 8540
F (% dose)	19.5 ± 5.4	20.6 ± 8.7	10.4 ± 7.3	6.7 ± 2.4	2.4 ± 1.6	0.7 ± 0.8
$t_{1/2,\alpha}$ (h)	0.485	0.543	0.121	0.274	ND	ND
$t_{1/2,\beta}$ (h)	0.672	0.775	1.76	1.71	ND	ND
$t_{1/2,\gamma}$ (h)	3.00	3.21	ND	ND	ND	ND

[a] Half-life estimates determined by curve-fitting of the average concentration data (n = 4)

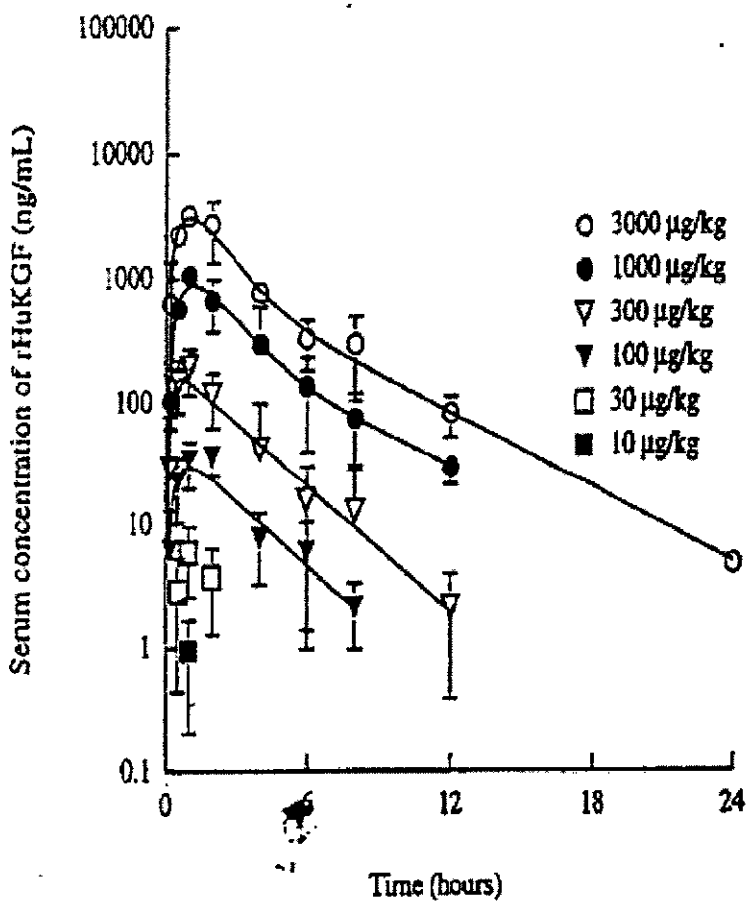
[b] ND: Not determined

FIGURE 2



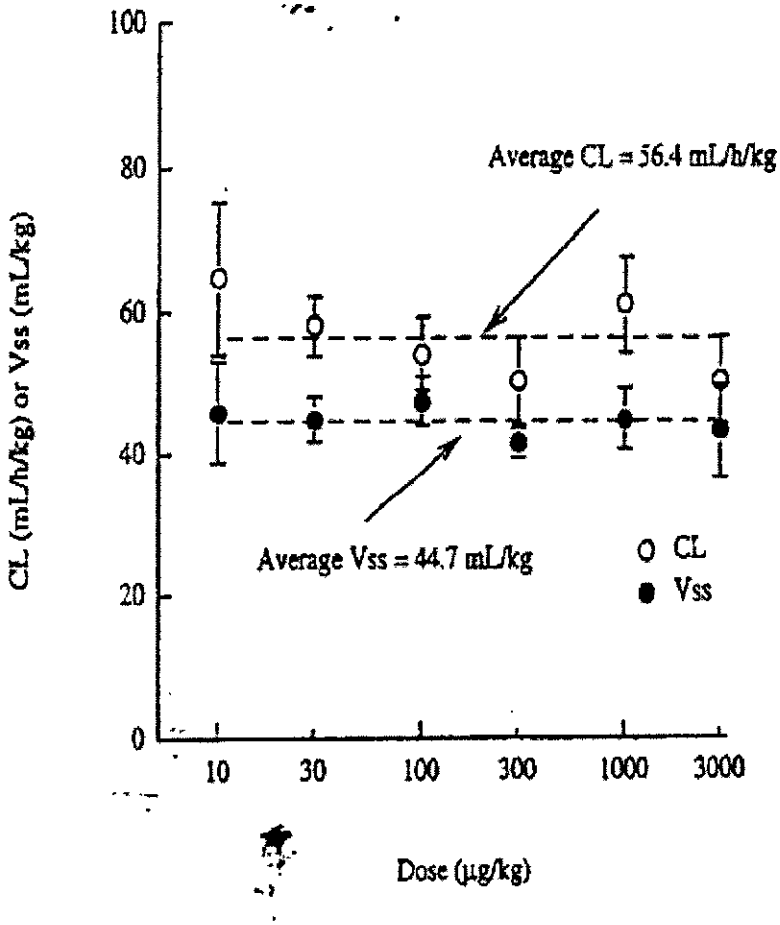
Serum concentration (mean+/-SD, n=4) of rHuKGF following intravenous doses at 10, 30, 100, 300, 1000, and 3000 µg/kg in rats. Solid line represents curve-fit of the data.

FIGURE 4



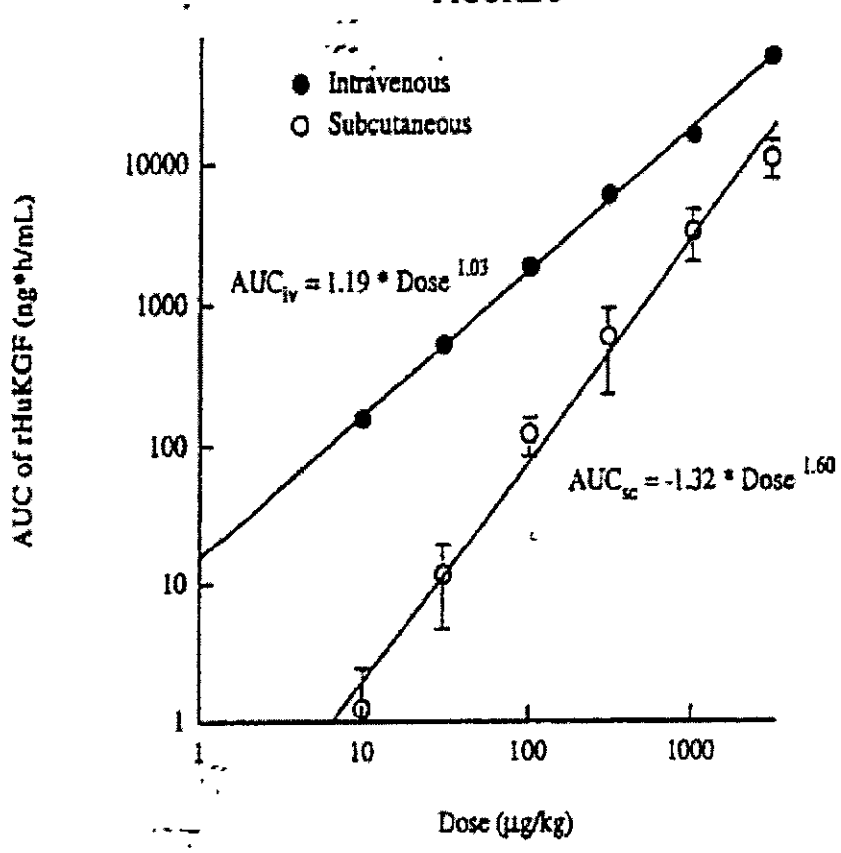
Serum concentration (mean+/-SD, n=4) of rHuKGF following subcutaneous doses at 10, 30, 100, 300, 1000, and 3000 µg/kg in rats. Solid line represents curve-fit of the data. Curve-fit was not performed for the 10 and 30 µg/kg due to insufficient data above the quantification limit.

FIGURE 3



Values of CL and Vss, as a function of the dose level, following intravenous administration of rHuKGF in the rat (mean–SD, n=4) Dotted lines represent average values over the range of doses.

FIGURE 5



Regression analysis of AUC vs. dose level of rHuKGF, following subcutaneous administration in the rat (mean+/-SD, n=4)

Absorption was dose dependent. Terminal half-lives were both approximately 3 hours with both the IV and SC administration. The volume of distribution in the rat is smaller than the blood volume of a rat (40-50 ml/kg versus 65 ml/kg, respectively). Clearance averaged 56 ml/h/kg across all IV doses. The characteristic "hump" seen in serum concentration profiles in humans and monkeys given the product intravenously is not evident in Figure 2 (above, supplied by sponsor).

CONCLUSIONS: The pharmacokinetics of the product in the rat show substantially less extracellular distribution into the blood and tissues compared to monkeys and humans. Terminal half-life was similar to that of the monkey. Dose dependent absorption (dose linear kinetics) was similar to the monkey with the exception of lack of a plateau effect 1-4 hours after rapid absorption seen in the monkey model.

Study title: Pharmacokinetic Evaluation of Five Forms of rHuKGF Following a Single Bolus Intravenous Injection in Male Sprague-Dawley Rats

Study no.: KGF.251/PK97023
Facility: Amgen, Inc.
Date of study: April 15, 1997
GLP: (No)
Dose & Formulation: Five different forms of rHuKGF; some forms were pegylated; doses were either 1.0 mg/kg or 0.1 mg/kg as a single IV bolus injection
Animals: Male Sprague-Dawley rats
Protocol: Parallel design, single IV injection of test article with pharmacokinetics followed for 72 hours

RESULTS: Data were highly variable and difficult to interpret.
CONCLUSIONS: No differences in most pharmacokinetic parameters of the five different product forms because of variability between mean estimates of pK parameters except for (decreased) clearance and (increased) volume of distribution caused by pegylation of 3/5 of the products tested.

Study title: Comparison of the Pharmacokinetics of rHuKGF in Two Different Formulations (0.0055% Tween, pH 7.0 versus 0.01% Tween, pH 6.5) in Male Sprague-Dawley Rats

Study no.: 100378
Facility: Amgen, Inc.
Date of study: February 8, 1999
GLP: (No)
Dose & Formulation: 0.3 mg/kg of 2 formulations by IV
Animals: Male Sprague-Dawley Rats
Protocol: Rats were divided into 2 cohorts (n=6/cohort) and each rat received one formulation of rHuKGF (0.3 mg/kg) on Day 1 and the alternate formulation, via the same route and same dose, on Day 3. The formulations were investigated in a two-period crossover design with a 48 hour washout period. Blood samples were collected pre-dose and at frequent time intervals post-dose from all animals. Following IV bolus dosing, the individual profiles from all rats were generally quantifiable to 4 hours post-dose.

RESULTS: The pharmacokinetics of both formulations, as supplied by the sponsor is:

Table 2: Mean (SD) Non-Comparmental Parameters[a] for Original (0.0055% Tween, pH 7.0) and New (0.01% Tween, pH 6.5) Formulations for rHuKGF Following IV Administration 0.3 mg/kg to Male Rats

Parameter	Units	Original Formulation		New Formulation	
		Mean	SD	Mean	SD
C_o	ng/mL	3200	485	3660	589
V_c	mL/kg	95.4	14.2	83.6	12.7
$AUC_{(0-t)}$	ng hr/mL	2200	337	2260	205
$AUC_{(0-\infty)}$	ng hr/mL	2220	344	2280	210
V_{ss}	mL/kg	108	10.3	102	6.21
$t_{1/2,z}$	hr	0.573	0.0501	0.591	0.0562
CL	mL/hr/kg	138	19.6	133	12.7

[a]Numbers rounded to three significant figures

Volume of distribution was approximately 100 ml/kg (i.e., higher than the blood volume in the rat of 65 ml/kg). This suggests 100% distribution into the extracellular volume and some extravascular distribution. Half-life was roughly .6 hours and clearance was approximately 135 ml/kg/hour.

CONCLUSIONS: No pharmacokinetic differences were evident between the two formulations. The data do not agree the half-life data in other studies that showed a half-life of ~ 3 hours. This could be an error in the sponsor's report.

Study title: A Crossover Bioavailability and Multiple-Dose Pharmacokinetic Study with Recombinant Human Keratinocyte Growth Factor (rHuKGF) in Rhesus Monkeys

Study no.: — 950345AM
Facility: [redacted]
Date of study: June 5, 1995
GLP : (No)
Dose & Formulation: 30 and 300 *u*g/kg, intravenous and subcutaneous
Animals: Twelve rhesus monkeys
Protocol: The protocol is a combination of a single and repeat dose pharmacokinetic study. On day 1 of the study 12 monkeys received a single dose of product at 30 or 300 *u*g/kg by either SC or IV administration. Following a 3 day washout period the animals were dosed daily from day 4 to day 10 with the same dose but the alternate route of administration. Serum blood samples were collected on days 1, 4 and 10. Trough samples were collected daily from day 5 to day 10.

RESULTS: Absorption was dose dependent. Using the IV route clearance was roughly 400 ml/h/kg and volume of distribution was 900-1200 ml/kg. Serum concentrations of rHuKGF initially declined and then plateaued at 1-2 hours after administration. This effect was more pronounced using the IV route as compared to the SC route, and at higher doses. The terminal half-life was roughly 3 hours. No accumulation of product occurred. No antibodies to the product were detected.

Table 2.

Pharmacokinetic Parameters of rHuKGF in Rhesus Monkeys, Following Intravenous and Subcutaneous Administrations at 30 µg/kg and 300 µg/kg[a]

Parameters	Intravenous					
	30 µg/kg			300 µg/kg		
AUC (ng•h/mL)	71.3	±	8.98	762	±	209
CL (mL/h/kg)	426	±	55.6	414	±	94.0
V_{ss} (mL/kg)	873	±	388	1220	±	336
MRT (h)	2.00	±	0.66	3.01	±	0.81
V_c (mL/kg)	118	±	65.8	105	±	40.9
Parameters	Subcutaneous					
	30 µg/kg			300 µg/kg		
t_{max} (h)	0.17	±	0.00	0.17	±	0.00
C_{max} (ng/mL)	0.69	±	0.58	35.0	±	22.8
AUC (ng•h/mL)	NC[b]	±	NC	134	±	53.2
F	NC	±	NC	0.18	±	0.07

[a] Mean ± SD, n=6
[b] NC: Not calculated. Serum concentrations were below the quantification limit.

Appears This Way
On Original

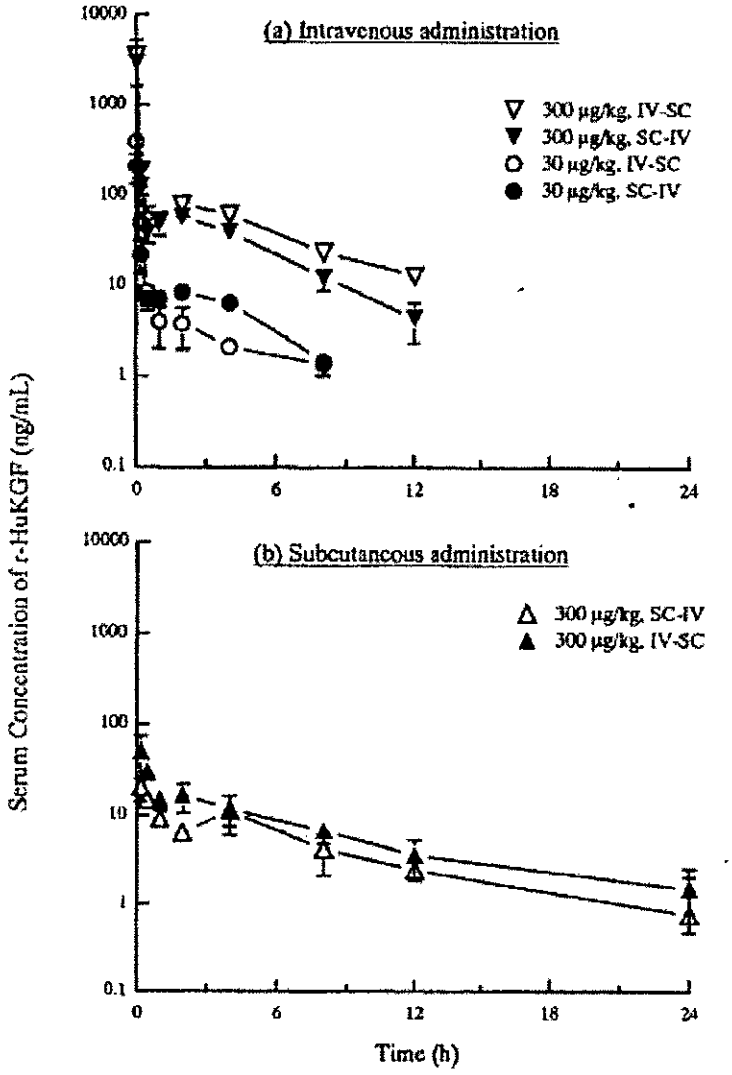


Figure 1

The effects of sequence of intravenous and subcutaneous administration on serum concentrations of rHuKGF. Rhesus monkeys were administered either the intravenous dose (IV-SC) or the subcutaneous dose (SC-IV) first (mean ± SD, n = 3).

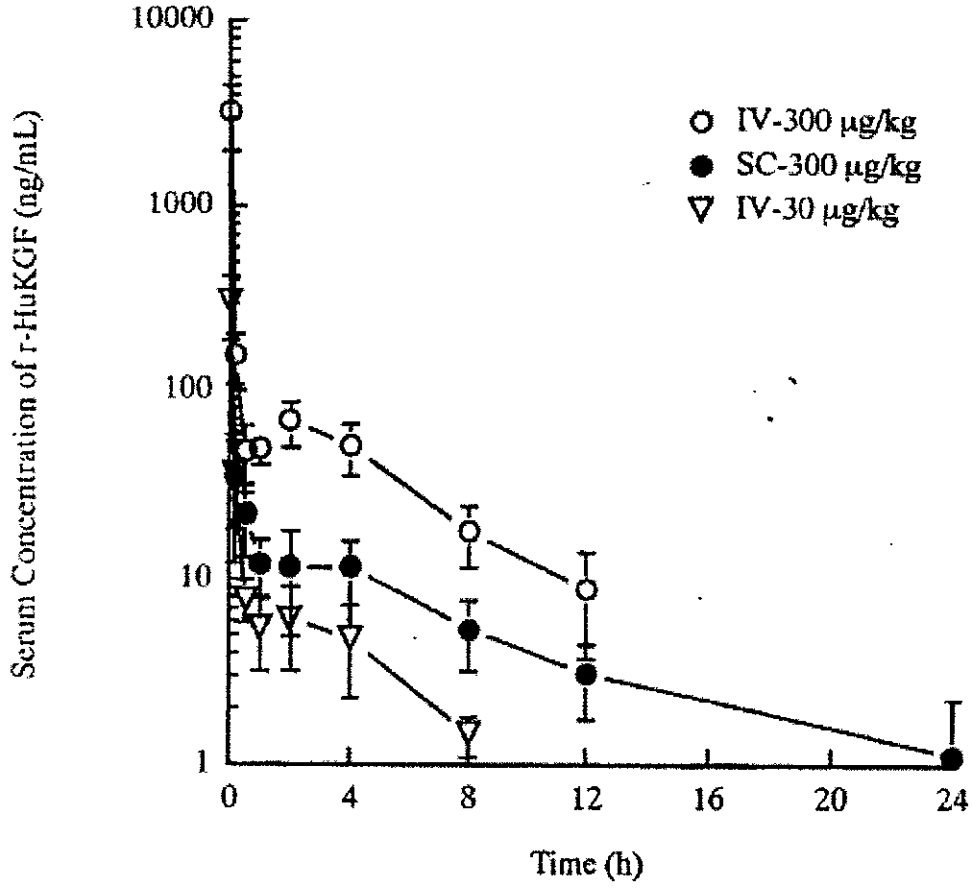


Table 3. Comparison of AUC for Male and Female Rhesus Monkeys Receiving Intravenous or Subcutaneous Administration of rHuKGF

Dose and Route	AUC (ng•h/mL)		ANOVA
	Female	Male	
30 µg/kg, iv[a]	64.3 ± 9.07	74.8 ± 7.59	NS[c]
300 µg/kg, iv[a]	678 ± 108	932 ± 313	NS
300 µg/kg, sc[b]	125 ± 67.5	166 ± 21.8	NS

[a] n = 4 for males, n = 2 for females

[b] n = 2 for males, n = 4 for females

[c] NS: Not statistically significant at $p < 0.05$

CONCLUSIONS: The pharmacokinetics of the product in monkeys has a higher volume of distribution and the serum concentration profile is markedly different than rats. The "hump" or plateau effect is not seen in the serum concentration profile of the rat. Distribution is markedly higher in the monkey compared to the rat. There was no accumulation of product.

Study title: Pharmacokinetic Study of rHuKGF Following Intravenous Administration to Female Nu/Nu Mice

Study no.: 101827
Facility: Amgen, Inc.
Date of study: January 22, 2002
GLP: (No)
Dose & Formulation: .5, 4, 15, 25 mg/kg as a single or multiple dose intravenously
Animals: Two hundred forty female Nu/Nu (athymic) mice
Protocol: Exploratory pharmacokinetic study; 6 treatment groups; animals in groups 1 to 4 received a single bolus IV administration of .5, 4, 15, or 25 mg/kg rHuKGF (n = 42/group). Animals in groups 5 and 6 received daily bolus IV administrations of .5 and 4 mg/kg rHuKGF, respectively, for 3 consecutive days (n = 36/group).

Table 9-1. PK Parameters in Nu/Nu (athymic nude) Mice After Single and Multiple IV Administrations of rHuKGF

Dose Group (mg/kg)	Observed C_{max} (ng/mL)	$AUC_{(0-\infty)}$ (ng·h/mL)	$AUC_{(0-24)}$ (ng·h/mL)	$AUC_{(0-8)}$ (ng·h/mL)	CL (mL/h/kg)	V_{ss} (mL/kg)	MRT (h)	$t_{1/2}$ (h)	AR (%)
Single Administration (Day 1)									
0.5									NA
4									NA
15									NA
25									NA
Mean	NA	NA	NA	NA	2450	5360	2.17	16.6	NA
SD	NA	NA	NA	NA	381	1860	0.626	13.1	NA
Multiple Administration (Day 3)									
0.5									
4									
Mean	NA	NA	NA	NA	2540	5640	2.23	2.60	NA
SD	NA	NA	NA	NA	191	279	0.278	0.366	NA

C_{max} = Maximum Concentration
$AUC_{(0-\infty)}$ = Area under serum concentration-time curve from time 0 to infinity
$AUC_{(0-24)}$ = Area under serum concentration-time curve from time 0 to 24 hours postdose
$AUC_{(0-8)}$ = Area under serum concentration-time curve from time 0 to 8 hours postdose
CL = Serum clearance after IV administration
MRT = Mean residence time
V_{ss} = Volume of distribution at steady state
$t_{½}$ = Terminal half-life
AR = Accumulation ratio = $AUC_{(0-24)\,day\,3}$/ $AUC_{(0-24)\,day\,1}$
NA = Not applicable

Note: Numbers are reported to 3 significant figures. The $AUC_{(0-24)}$ being slightly higher than $AUC_{(0-\infty)}$ for the 0.5-mg/kg dose group is an artifact of setting the non-quantifiable levels at the end of the sampling period to zero in the WinNonlin analysis. Back-extrapolation to time zero was not possible, thus the observed C_{max} rather than C_0 was reported.

The percent of $AUC_{(0-\infty)}$ that was attributed to $AUC_{(0-8)}$ was 99.4%, 95.5%, 95.6%, and 96.2% for the 0.5-, 4-, 15-, and 25-mg/kg dose groups, respectively.

RESULTS: For the single IV route of administration absorption was dose dependent. Clearance was 2000-3000 ml/kg/hour. Half-life was especially variable and ranged from 1 to 33 hours. For the multiple dose route of administration, clearance was in the same range as the single dose route (~2400-2700 ml/hr/kg) and absorption was dose dependent. Volumes of distribution were in the same range for the IV and SC administrations (~3700-5800 ml/kg). Half-life averaged 2.23 hours for the multiple dose group.

CONCLUSIONS: The pharmacokinetics of single versus multiple IV dosing in the mouse was similar.

Study title: A Pilot Pharmacokinetic Study of Iodine-125-labeled Recombinant Human Keratinocyte Growth Factor (rHuKGF) Following Intravenous and Subcutaneous Administration in Sprague-Dawley Rats

Study no.: KGF. 165

Facility: Amgen, Inc.
Date of study: December 29, 1995
GLP: (No)
Dose & Formulation: rHuKGF at 300 pg/kg, containing ^{125}I -rHuKGF
Animals: Male Sprague-Dawley rats
Protocol: Pilot study; male Sprague-Dawley rats were administered a single intravenous or subcutaneous dose of rHuKGF at 300 pg/kg, containing ^{125}I -rHuKGF. The serum concentrations of total radioactivity, acid-precipitable radioactivity, and rHuKGF were determined at selected time-points within 24 hours postdose.

RESULTS: Between .5 and 2 hours after IV infusion rHuKGF is rapidly degraded and at 8 hours it is not detectable. The subcutaneous data suggest that only 15% of the product is intact 8 hours after administration.

Table 1.

Serum Concentrations of Total Radioactivity, Acid-precipitable Radioactivity, and rHuKGF in the Rat Following Intravenous or Subcutaneous Administration at 300 µg/kg

Time (hours)	(A) Total radioactivity (ngEq/mL)	(B) Acid-precipitable radioactivity (ngEq/mL)	(C) Serum rHuKGF concentrations (ng/mL)	% acid-precipitable (B)/(A) x 100%	% intact protein (C)/(B) x 100%
Intravenous administration					
0.0167	6160±24.7	6270±147	6206±834	102±2.17	98.9±11.0
0.5	2780±208	2710±234	2980±639	97.3±1.39	112±31.0
2	737±63.6	486±63.1	383±132	65.8±3.37	77.2±18.3
8	228±12.8	43.0±0.52	BQL	18.9±1.08	NC
24	53.2±13.5	3.89±1.30	BQL	7.22±0.94	NC
Subcutaneous administration					
0.167	63.7±37.3	27.6±24.5	27.2±7.15*	34.1±24.5	67.5±4.21*
0.5	130±48.8	90.1±36.3	77.9±1.53*	68.7±2.93	70.2±0.11*
2	197±22.4	98.1±9.70	66.0±17.4	50.53±9.57	66.6±12.2
8	182±15.7	40.5±8.69	5.77±0.97	22.2±3.99	14.5±3.24
24	83.9±7.92	13.6±7.83	BQL	15.8±8.60	NC

BQL: Below the quantification limit — ng/mL)
NC: Not calculated
* n=2; sample for animal 170 was not included in the calculation due to non-determinable rHuKGF concentrations

CONCLUSIONS: Palifermin is rapidly degraded after both IV and SC infusion in the rat.

2.6.4.4 Distribution

Study title: Excretion, Tissue Distribution and Mass Balance of a Radiolabeled Test Article in Rats after Intravenous Administration

Study no.: 529-028/96060
Facility: [redacted]
Date of study: August 1, 1996
GLP: (Yes)

Dose & Formulation: 26.5 *u*Ci/mL of [^{125}I]-KGF in a volume of 0.792 mL/kg intravenously; final dose was 300 *u*g/kg and ~ 21 *u*Ci/kg
Animals: 12 male and 12 female Sprague-Dawley rats
Protocol: The dose solution was administered once by intravenous injection into a lateral tail vein at a body weight adjusted dosage. The rats were observed twice daily for mortality, morbidity and signs of toxicity following administration and samples were collected for up to 24 hours after dose administration. Urine and feces were collected from separate groups of 3 rats per sex at 30 minutes, 2 hours, 8 hours, and 24 hours post dose. After the final excreta collection, each rat was anesthetized by carbon dioxide inhalation, and 1 to 2 ml of blood was collected via cardiac puncture and separated to obtain whole blood, serum, and plasma. Two samples of serum were collected per animal. Rats were euthanized and protocol-specified organs were weighed, homogenized in isotonic saline, and reweighed.

RESULTS: Acid precipitable radioactivity was highest in the blood at 2 hours. Major early tissue sites of acid precipitable radioactivity were the thyroid, adrenal glands, spleen, liver, kidneys and ovaries (.5 hour). By 8 hours the product is cleared by the kidneys, and high amounts of radioactivity were found in the trachea and thyroid (only). Radioactivity was negligible in all organs at 24 hours except for the thyroid. Only the thyroid gland appeared to accumulate the radioactivity that was attributed to free radiolabelled iodide.

CONCLUSIONS: No significant accumulation of KGF in the tissues. The product is eliminated mainly by the kidneys.

Study title: Excretion, Tissue Distribution and Mass Balance of a Radiolabeled Test Article in Rats after Subcutaneous Administration

Study no.: 529-025/95010
Facility: [ ]
Date of study: July 27, 1995
GLP: (not clear-there is a quality assurance statement but no GLP statement)
Dose & Formulation: Single, mid-dorsal subcutaneous injection of prepared test article containing approximately 5-10 *u*Ci of ^{125}I labeled KGF in a volume of 0.6 ml/kg
Animals: Thirteen male and twelve female Sprague-Dawley rats
Protocol: Each rat was observed once daily for mortality, morbidity and signs of toxicity. Samples of urine, feces and cage rinses were collected at protocol-specified intervals. Blood was collected via cardiac puncture prior to euthanasia after the last sample collection interval. Blood samples were processed to obtain whole blood, serum and plasma. All rats then were euthanized by carbon dioxide asphyxiation. Protocol-designated tissues were collected at necropsy. Each organ was weighed and homogenized in isotonic saline on ice. The final weight of the homogenate then was recorded. Duplicate aliquots of homogenized tissue were analyzed for total radioactivity, and where possible, additional aliquots were taken

and analyzed for acid-precipitable radioactivity. The concentration of radioactivity (total and acid-precipitable) in whole blood, serum, plasma, urine, feces, the residual carcass and rinses also was determined.

RESULTS: Acid precipitable radioactivity was highest in the blood at 8 hours. Radioactivity concentrations in the tissues mimicked those in the previous study in terms of target organs. Maximum tissue concentrations were observed at 8 hours post dosing and highest percent radioactivity was seen in trachea, stomach, thyroid, small intestines, liver and kidneys. After 24 hours 70% and 4% of the dose were excreted by the kidneys and feces, respectively.

CONCLUSIONS: No accumulation of radioactivity in any organ besides the thyroid. The kidney is the major pathway of excretion.

2.6.4.5 Metabolism

Study title: Evaluation of the First-Pass Hepatic Elimination in Rats of N823 Recombinant Human Keratinocyte Growth Factor (N823 r-HuKGF) by Using the Accelerated Infusion Technique

Study no.: Amgen Pharmacokinetics Study: KGF.265; Amgen Pharmacokinetics Report: PK97140
Facility: Amgen, Inc.
Date of study: October 31, 1997
GLP: (No)
Dose & Formulation: The initial infusion rate, 0.14 ml/hour increased over the 3 hour infusion period to a final value of 0.76 ml/hour. Each animal received a total of 1.40 mL of the 0.04 mg/mL infusate.
Animals: Eight rats
Protocol: Group 1 (n=4) and group 2 (n=4) were infused via the femoral and hepatic portal vein, respectively.

RESULTS: This was a preliminary study. First pass extraction by the liver was estimated to be 20-30% based on C_{max} and AUC (see table below supplied by sponsor). However, this result was not exactly repeatable when a second experiment conducted (see study 100478).

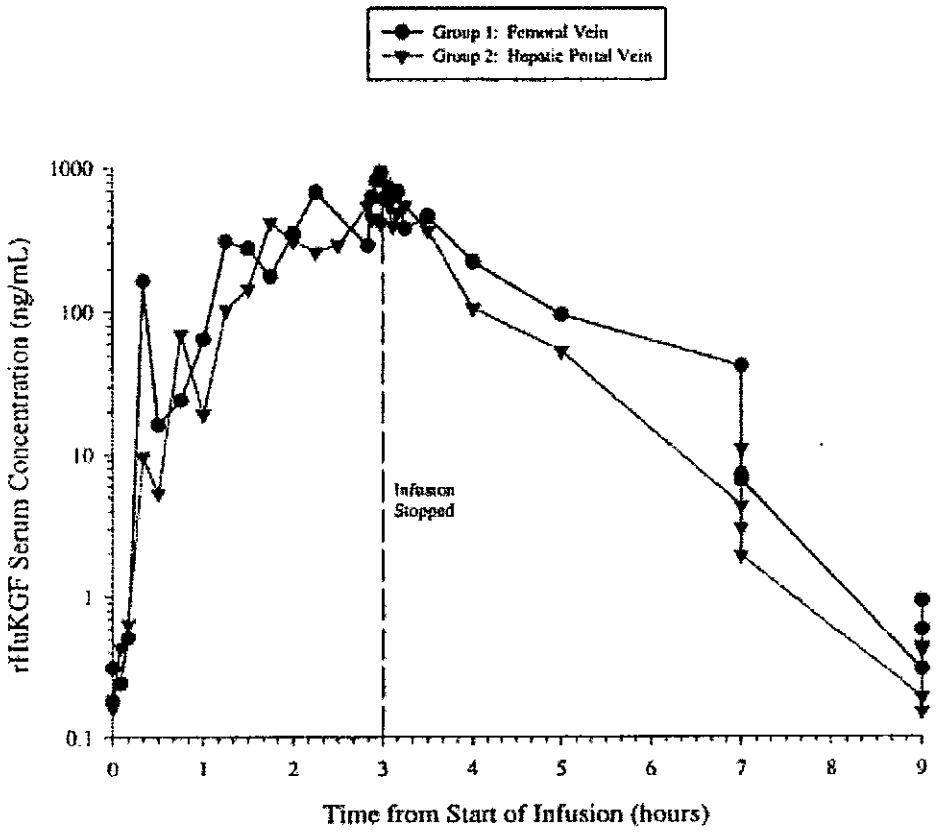


Figure 2. Naïve-Pooled Serum Concentration-Time Profiles Following Accelerated Infusion of Nδ23rHuKGF for 3 hours to Male Rats (logarithmic y-axis)

CONCLUSIONS: There was a suggestion of slight first pass extraction by the liver (20-30% based on C_{max} and AUC); the liver did not appear to be the major organ of elimination for rHuKGF.

Study title: Evaluation of the First-Pass Hepatic Elimination in Rats of Recombinant Human Keratinocyte Growth Factor (rHuKGF) by Using the Accelerated Infusion Technique

Study no.: 100478
Facility: Amgen, Inc.
Date of study: Final report is dated June 10, 1999

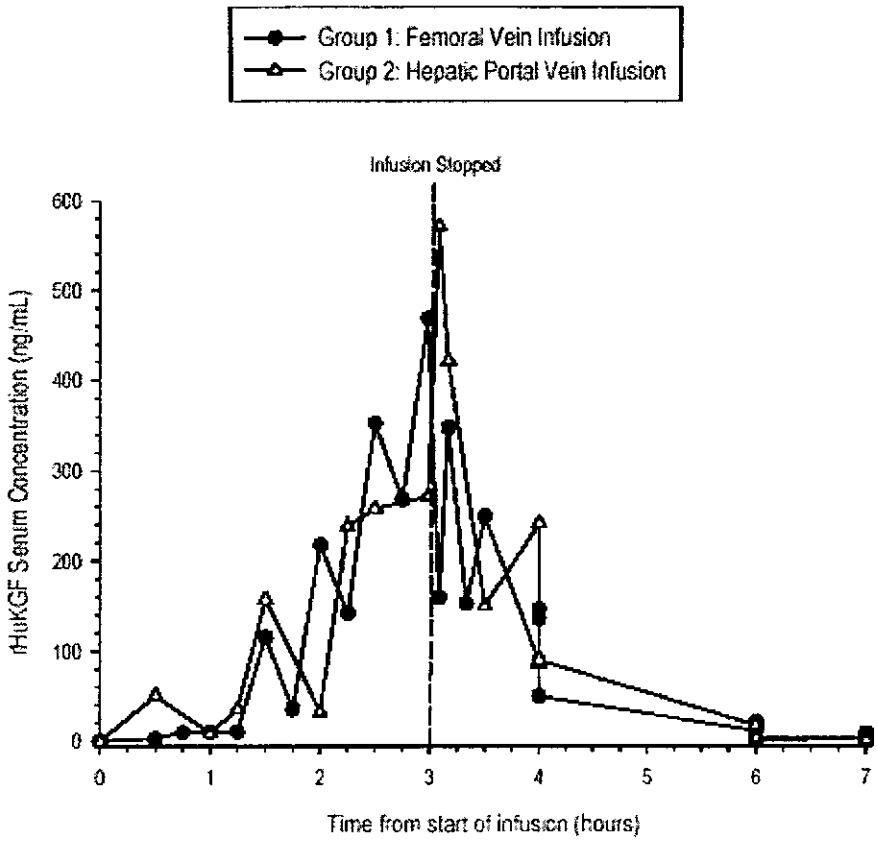
GLP : (No)
Dose & Formulation: All rats were infused for 3 hours at a rate that increased linearly from an initial value of 0.14 ml/hour to 0.76 ml/hour; total volume was 1.40 ml with a concentration of 0.04 mg/ml infusate.
Animals: Four Sprague-Dawley rats per group
Protocol: This study repeats study PK97140. The objective is the same as the previous study: to evaluate the contribution of the liver to overall clearance of the product. The pharmacokinetics of rHuKGF was studied in rats by infusing the protein via the femoral vein (group 1) and hepatic portal vein (group 2). All rats were infused for 3 hours at a rate that increased linearly from an initial value of 0.14 ml/hour to 0.76 ml/hour; total volume infused was 1.40 ml. Serum samples were collected in a sparse sampling design during the infusion period and up to 4 hours after stopping the infusion (total of 7 hours).

RESULTS: The concentration of endogenous KGF in the serum was below the limit of detection so serum samples were not corrected for baseline values. The pharmacokinetic mean group values are shown in Table 3 below, as supplied by the sponsor. For all variables, except C_{last} the ratio of the portal:femoral mean pharmacokinetic estimate was approximately 1.

Table 3: Non-Compartmental Parameters Estimated Following Accelerated Infusion of rHuKGF to Rats in Groups 1 (via femoral vein) and Group 2 (via hepatic portal vein)

Parameter	Units	Group (Route)		Ratio[a]
		Group 1: (Femoral)	Group 2: (Hepatic Portal)	
T_{max}	hr	2.98	3.08	1.03
C_{max}	ng/mL	468	571	1.22
T_{last}	hr	7	7	1.00
C_{last}	ng/mL	4.00	2.16	0 541
$AUC_{(0-7)}$	ng hr/mL	638	665	1.04
$AUC_{(0-\infty)}$	ng hr/mL	641	667	1.04
$MRT_{(0-7)}$	hr	1.55	1.45	0.934
λ_z	hr^{-1}	1 16	1.28	1.10
$t_{1/2,z}$	hr	0.598	0.543	0.907

[a] Ratio estimated as Portal:Femoral
[b] Numbers rounded to 3 significant figures



CONCLUSIONS: The liver is not a major organ of elimination for rHuKGF upon first pass extraction in rats.

2.6.4.6 Excretion

2.6.4.7 Pharmacokinetic drug interactions

2.6.4.8 Other Pharmacokinetic Studies

Study title: Determination of Pharmacokinetics of rHuKGF in Male BDF-1 Mice Following Single and Multiple Doses

Study no.: 100123
Facility: Amgen, Inc.
Date of study: April 29, 1999
GLP: (No)
Dose & Formulation: 1 or 3 daily single IV doses of 300, 1500, 5000 or 15000 *u*g/kg
Animals: BDF-1 Mice
Protocol: Male mice (n=179) were randomly assigned to 9 groups, with 3 mice in Group 1 (control group) and 22 mice in all other groups. Animals received rHuKGF at doses of 300, 1500, 5000 or 15000 *u*g/kg as either a single IV dose on

Day 1 (Groups 2, 4, 6, and 8, respectively), or as 3 once daily IV doses (Groups 3, 5, 7 and 9, respectively). Serum samples for pharmacokinetic analysis were collected by terminal sacrifice on Day 1 or Day 3 from 2 mice per group up to 24 hours post-dose. All pharmacokinetic analyses were conducted with n=2 per timepoint.

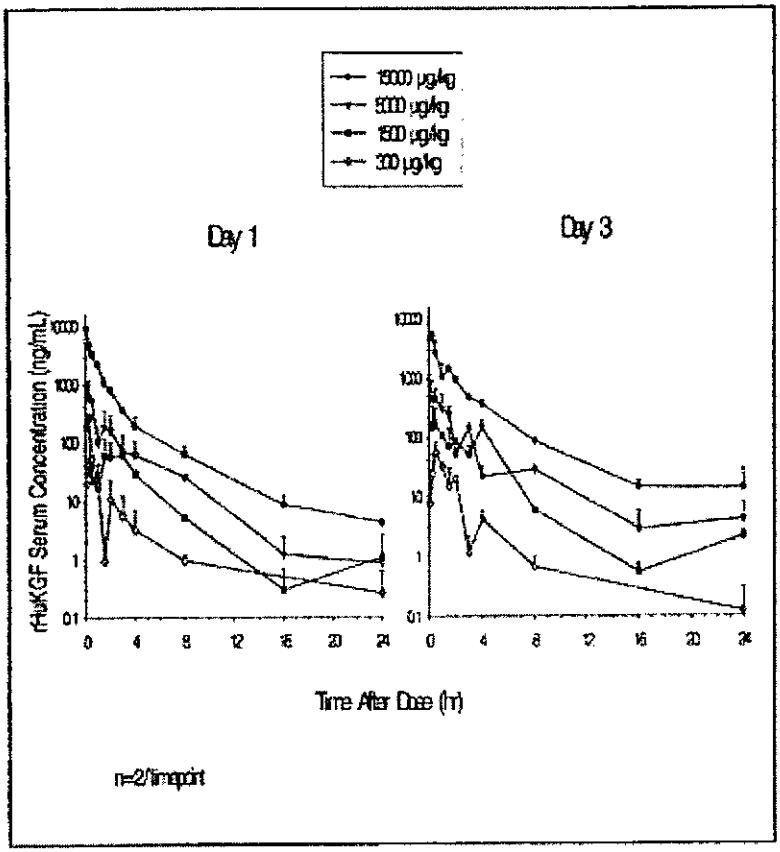
RESULTS: There were no changes in body weights due to treatment. The variability was too great to make meaningful comparisons between the pharmacokinetics of a single dose versus 3 daily doses less than 15000 *u*g/kg. Pharmacokinetic parameters for groups 8 and 9 are shown in the table below, as supplied by the sponsor:

Non-Compartmental Parameters Estimated on Days 1 and 3 Following IV Administration of rHuKGF to Groups 8 and 9 (15000 *u*g/kg)

Dose: 15000 µg/kg				
Parameter*	**Units**	**Day 1 (Group 8)**	**Day 3 (Group 9)**	**Ratio of Day 3: Day1**
C_0	ng/mL	12100	10800	**0.891**
$AUC_{(0-\infty)}$	ng hr/mL	6930	7320	1.06
V_{ss}	mL/kg	3660	4920	**1.35**
$t_{1/2,z}$	hr	4.10	2.64	**0.645**
CL	mL/hr/kg	2160	2050	**0.947**

* estimated using naïve-averaged data with n=2 per timepoint

Appears This Way
On Original



The pharmacokinetics of a single dose versus 3 daily doses appeared similar (see preceding figure and table supplied by sponsor).

CONCLUSIONS: The pharmacokinetics of rHuKGF was similar at the highest dose (15000 *u*g/kg) at both single and triple (daily) IV doses. There was no accumulation. Variability between animals was high at all doses.

Appears This Way
On Original

Study title: A Pharmacokinetic Study of Recombinant Human Keratinocyte Growth Factor (rHuKGF) in Bilaterally-Nephrectomized and Sham-Operated Sprague-Dawley Rats

Study no.: KGF.104
Facility: Amgen, Inc.
Date of study: December 29, 1995
GLP: (No)
Dose & Formulation: 300 *u*g/kg by IV
Animals: Male Sprague-Dawley rats
Protocol: Male Sprague-Dawley rats were administered intravenous doses of 300 *u*g/kg on days 1 and 3 of the study. Day 1 doses served as the normal controls for both groups of animals. On day 3, a subgroup of the animals were bilaterally-nephrectomized and the remaining animals were sham-operated. Both the day 1 single dose animals and the sham-operated animals served as controls for the nephrectomized animals. Serial blood samples were obtained during a 48-hour period following both day 1 and day 3 dosing.

Group	Number of animals	Dose (µg/kg)	rHuKGF Conc. in solution (µg/mL)	Dose Volume (mL/kg)	Day 1	Day 3
1	5	IV-300	500	0.6	Control	Nephrectomized
2	5	IV-300	500	0.6	Control	Sham

RESULTS: The pharmacokinetic data from this study are presented in the following table, as supplied by the sponsor:

Table 2.

Pharmacokinetic Parameters of rHuKGF in Nephrectomized and Sham-operated Rats, Following Intravenous Administration at 300 µg/kg

Group 1	Day 1 (control)			Day 3 (nephrectomized)			ANOVA
AUC_{0-24} (ng•h/mL)	1930	±	503	4090	±	1210	$p < 0.05$
CL (mL/h/kg)	165	±	49.7	78.7	±	24.5	$p < 0.05$
$AUMC_{0-24}$ (ng•h^2/mL)	966	±	431	2800	±	1620	$p = 0.066$
V_{ss} (mL/kg)	76.4	±	8.13	49.4	±	12.1	$p = 0.061$
MRT (h)	0.48	±	0.10	0.65	±	0.18	$p < 0.05$
V_c[a] (mL/kg)	73.3			54.7			N/A[b]
$t_{1/2,\alpha}$[a] (h)	0.286			0.430			N/A
$t_{1/2,\beta}$[a] (h)	1.77			2.70			N/A
Group 2	Day 1 (control)			Day 3 (sham-operated)			
AUC_{0-24} (ng•h/mL)	2040	±	502	1940	±	224	N/A
CL (mL/h/kg)	151	±	37.2	156	±	18.0	N/A
$AUMC_{0-24}$ (ng•h^2/mL)	1190	±	511	873	±	107	N/A
V_{ss} (mL/kg)	83.9	±	4.42	70.1	±	7.59	N/A
MRT (h)	0.57	±	0.11	0.45	±	0.00	N/A
V_c[a] (mL/kg)	67.9			64.6			N/A
$t_{1/2,\alpha}$[a] (h)	0.251			0.225			N/A
$t_{1/2,\beta}$[a] (h)	1.04			1.37			N/A

[a] Calculated from the average serum concentration data; therefore, no standard deviation was calculated.
[b] N/A: No statistical comparison was performed.

CONCLUSIONS: The absorption, clearance, and distribution data in this experiment suggest that the kidney is a major site of distribution and clearance of rHuKGF in the rat. The clearance and absorption figures indicate that 50% of the product is cleared by the kidneys. The volume of distribution data suggests that in a normal rat the volume of distribution is equal to blood volume (~65 ml/kg); in the nephrectomized rat the volume of distribution is 40% lower, suggesting 40% distribution to the kidney.

Study title: A Pilot Comparative Pharmacokinetic Study of Three Forms of Human Keratinocyte Growth Factor (rHuKGF) in Rhesus Monkeys

Study no.: PK97077
Facility: [redacted]
Date of study: April 8, 1998
GLP: (No)
Dose & Formulation: 100 *ug*/kg or 300 *ug*/kg of either N823 rHuKGF (Group 1), 20K Mono-PEG N823 rHuKGF (Group 2) or 20K Mono-PEG N816 rHuKGF (Group 3) intravenously
Animals: Nine rhesus monkeys
Protocol: The study used 3 forms of rHuKGF, two forms were pegylated (group 2, 3) and one was not (group 1). Monkeys were dosed intravenously on day 1 and day 4 of the experiment. Each group was comprised of 1 male and 2 females. Serum samples for pharmacokinetic analyses were collected up to 72 hours post dosing on both dosing days. Serum samples for antibody analysis were collected pre dosing on day 1 and on day 14. The study design, as supplied by the sponsor, is in Table 1 (below).

RESULTS: See Table 5 below, as supplied by the sponsor. None of the animals had detectable endogenous serum levels of KGF so the sponsor did not correct the pharmacokinetic data for baseline values. Clearance and half-lives were similar after the day 1 and day 4 doses (1.12 and 1.77 L/hr/kg, and 3 and 2.5 hours, respectively), for the nonpegylated form of the product. (Note: The units for clearance are L/hr/kg, not ml/hr/kg as reported in the table above.) The pharmacokinetic parameters of the pegylated forms of the product used in group 2 and 3 had characteristic longer half-lives (~8-10 hours); clearance was 5-10 times slower for the pegylated forms of the product.

Table 5: Mean Non-Compartmental Parameters Estimated on Days 1 and 4 Following Single Dose IV Administration of Nδ23rHuKGF, 20K Mono-PEG Nδ23 rHuKGF and 20K Mono-PEG Nδ16 rHuKGF on Day 1 (100 μg/kg) and Day 4 (300 μg/kg) (n = 3 per group per day)[a]

Parameter	Units	Nδ23 rHuKGF	20K Mono-PEG Nδ23 rHuKGF	20K Mono-PEG Nδ16 rHuKGF[a]
Day 1				
C_0	ng/mL	1010	1780	1550
$AUC_{(0-\infty)}$	ng hr/mL	89.0	672	320
$nAUC_{(0-\infty)}$	(ng hr/mL)/(μg/kg)	0.890	6.72	3.20
$t_{1/2\lambda}$	hr	3.07	8.36	10.9
CL	mL/hr/kg	1.12	0.151	0.316
V_{ss}	mL/kg	2.68	2.06	3.63
Day 4				
C_0	ng/mL	1380	3740	2140
$AUC_{(0-\infty)}$	ng hr/mL	176	1660	866
$nAUC_{(0-\infty)}$	(ng hr/mL)/(μg/kg)	0.588	5.54	2.89
$t_{1/2\lambda}$	hr	2.52	8.13	10.4
CL	mL/hr/kg	1.77	0.184	0.349
V_{ss}	mL/kg	5.17	2.37	4.10

[a]Animal R5604F (Group 3) not analyzed on Day 4

The serum profile for groups 1, 2, 3 is shown in the following sets of figures, as supplied by the sponsor:

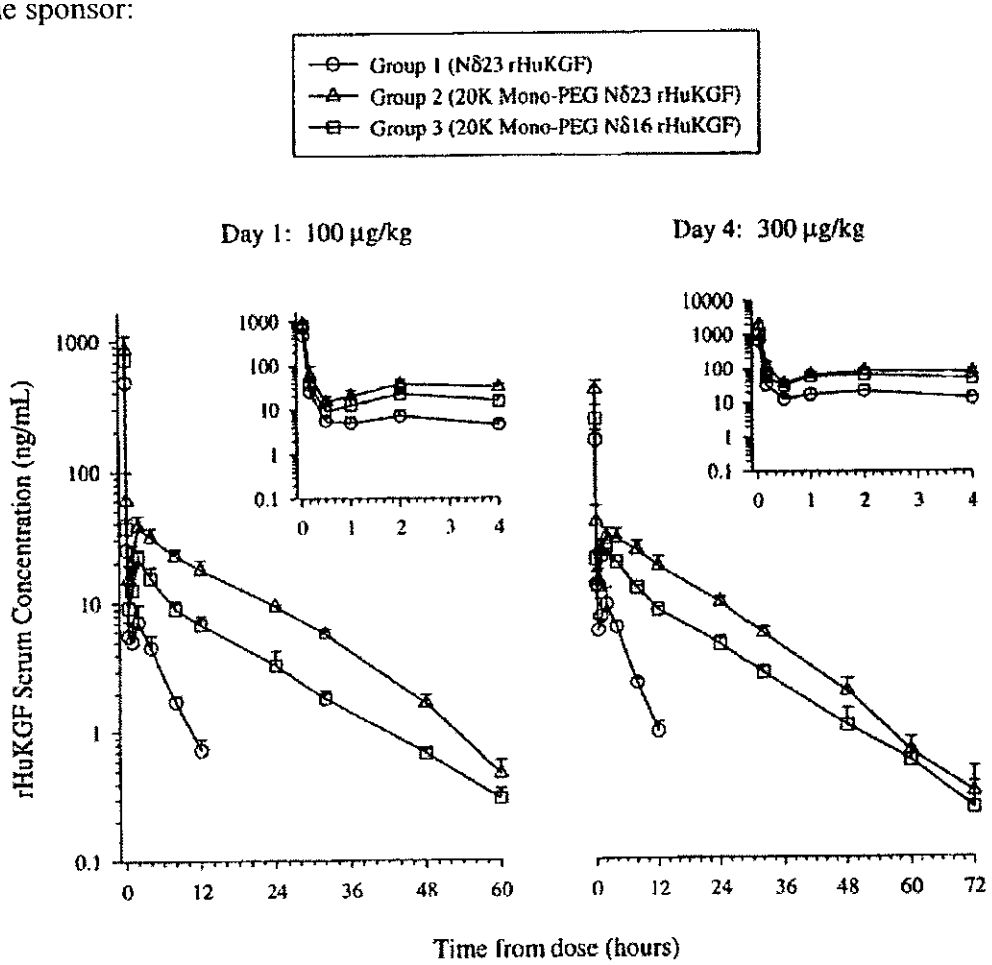


Figure 1. Mean (SD) Serum Concentration-Time Profiles on Days 1 (100 µg/kg) and 4 (300 µg/kg) Following Single Dose IV Administration of Nδ23rHuKGF, 20K Mono-PEG Nδ23 rHuKGF and 20K Mono-PEG Nδ16 rHuKGF to Rhesus Monkeys in Groups 1, 2 and 3, Respectively.

Appears This Way
On Original

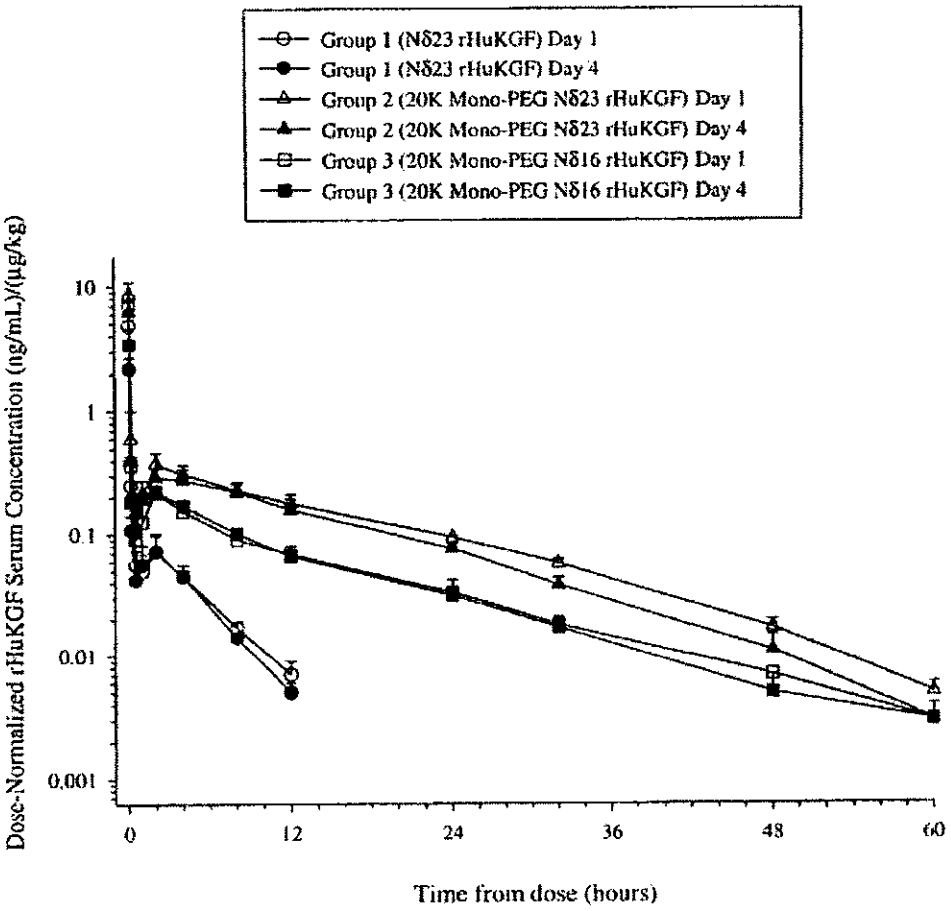


Figure 2. Mean (SD) Dose-Normalized Serum Concentration-Time Profiles on Days 1 (100 µg/kg) and 4 (300 µg/kg) Following Single Dose IV Administration of Nδ23 rHuKGF, 20K Mono-PEG Nδ23 rHuKGF and 20K Mono-PEG Nδ16 rHuKGF to Rhesus Monkeys in Groups 1, 2 and 3, Respectively.

Between 1 and 4 hours after dosing there is a plateau effect, with all forms of the product. This was also seen in another rhesus monkey study submitted (see — 950345).

CONCLUSIONS: There was no accumulation of product at the doses tested. There is a plateau effect (i.e., a redistribution phase) between 1 and 4 hours after dosing.

Study title: A Pharmacokinetic Study of Recombinant Human Keratinocyte Growth Factor (rHuKGF) in Male Sprague-Dawley Rats Following Multiple Subcutaneous Doses

Study no.: KGF.153
Facility: Amgen, Inc.
Date of study: December 29, 1995
GLP: (No)

Dose & Formulation: 30 or 300 *u*g/kg/day daily subcutaneously for 7 days
Animals: Twelve male Sprague-Dawley rats
Protocol: Rats were dosed subcutaneously with rHuKGF at either 300 or 30 *u*g/kg/day for 7 days. Serial blood samples were obtained following the day 1 and day 7 dosing. Trough blood samples were taken prior to dosing on each day. An additional blood sample was obtained at 4 hours post dose on day 3 and day 5.

RESULTS: The serum concentration profiles for animals dosed with 30 *u*g/kg/day of product were below the quantification limit. The pharmacokinetic parameters for day 1 and day 7 were similar, suggesting no accumulation of product with repeated subcutaneous dosing. (See table 2 and figure 2 below, as supplied by sponsor.)

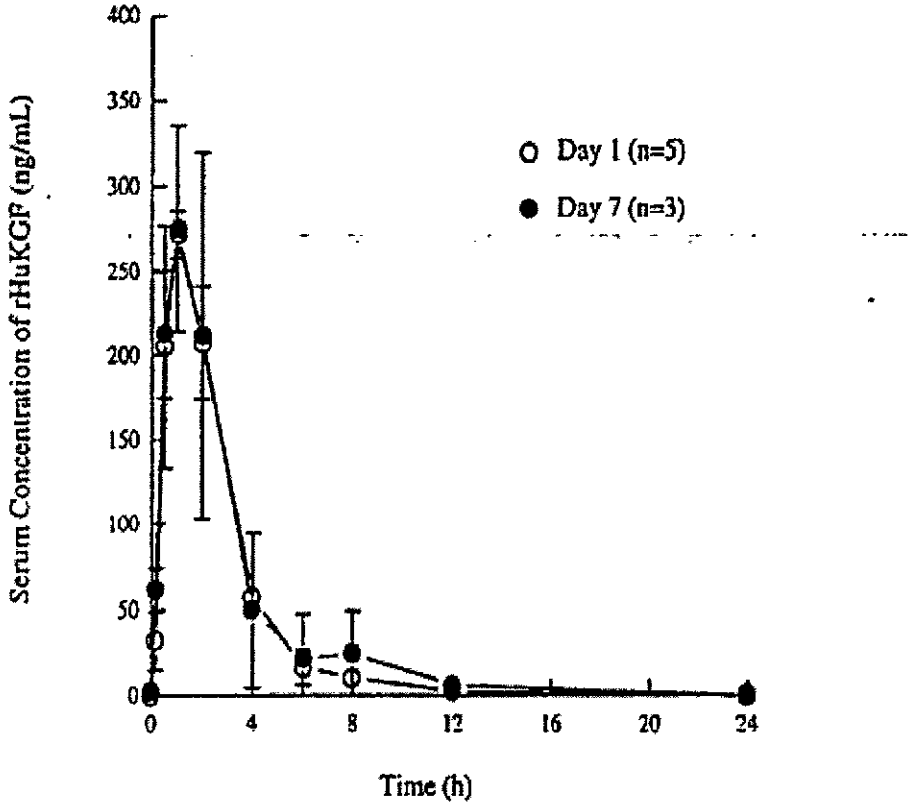
Table 2.

Comparison of Pharmacokinetic Parameters of rHuKGF on Day 1 and Day 7, Following Multiple Subcutaneous Dosing at 300 mg/kg/day

Pharmacokinetic Parameters	Day 1 (n = 5)	Day 7 (n = 3)
t_{max} (h)	1.0 ± 0.0	**0.83 ± 0.29**
C_{max} (ng/mL)	272 ± 13.8	276 ± 59
AUC (ng•h/mL)	806 ± 76.4	889 ± 457

Appears This Way
On Original

FIGURE 2



Comparison of serum concentration profiles following the first (Day 1) and the last (Day 7) dose in animals receiving daily subcutaneous doses of rHuKGF at 300 µg/kg/day (mean+/-SD).

When the data were fit to a two-compartment model with first order kinetics the 3 half-lives obtained were 0.50, 0.79, and 3.4 hours.

CONCLUSIONS: No accumulation of product with subcutaneous dosing; the terminal half-life was approximately 3.4 hours.

Study title: Pilot Single Dose Subcutaneous Pharmacokinetic Study of KGF in Male [ ] Wethers Sheep

Study no.: 101264
Facility: [ ]

Date of study: April 16, 2001
GLP: (No)
Dose & Formulation: 0.15 mg/kg as a single subcutaneous dose

Animals: 1 wether (castrated male sheep)
Protocol: Pilot study; blood samples were taken at .5 through 36 hours after dosing

RESULTS:

Table 2. Serum Concentration (pg/mL) Time Profile of KGF Following Single Dose SC Administration of 0.15 mg/kg to Male Sheep (Animal #9)

Day	Time (hr)	Concentration (pg/mL)
1	0	
1	0.5	
1	1	
1	1.5	
1	2	
1	2.5	
1	3	
1	4	
1	5	
1	6	
1	8	
1	10	
1	12	
2	24	
2	36	

QNS = Quatity not sufficient

Table 3. Non Compartmental Pharmacokinetic Parameters of KGF in Male Sheep Following Single Dose SC Administration of 0.15 mg/kg

Parameter	Units	Estimate
t_{max}	hr	2.5
C_{max}	pg/mL	3090
T_{last}	hr	10
C_{last}	pg/mL	809
λ_z	hr^{-1}	0.168
$t_{1/2,z}$	hr	4.13
$AUC_{(0-t)}$	pg*hr/mL	17000
$AUC_{(0-\infty)}$	pg*hr/mL	21500
$AUC_{(0-\infty)}/D$	pg*hr/mL/(pg/kg)	0.0001
$AUC\%_{extrap}$	% AUC	21.0
Vz/F	mL/kg	41600
Cl/F	mL/hr/kg	6980
$AUMC_{(0-\infty)}$	pg*hr*hr/mL	143000
$AUMC\%_{extrap}$	% AUMC	50.4
$MRT_{(0-\infty)}$	hr	6.66

CONCLUSIONS: None; see study 102241 for a similar study using more than 1 wether.

Study title: Bioavailability and Lymphatic Absorption of Palifermin (rHuKGF) After Subcutaneous Administration to Sheep

Study no.: 102241
Facility: [redacted]

Date of study: Final report is dated January 14, 2004, not clear when the study was actually done
GLP: (No)
Dose & Formulation: 37.5, 150 *ug*/kg
Animals: Seventeen wethers
Protocol:

Group	Route	Dose (*ug*/kg)	N	Lymph collection	Collection time points
1	Bolus SC interdigital space of hind leg)	150	5	No	0 →48 hours (blood)
2	Bolus SC (interdigital space of hind leg)	150	5	Yes (cannula in the efferent duct of the popliteal lymph node)	0→48 hours (blood) 0→24 hours (lymph)
3	IV (jugular)	37.5	5	No	0→24 hours (blood)
4	IV (jugular)	150	2	No	0→48 hours (blood)

Table 5.2. Mean (SD) Non-Compartmental Parameter Estimates[a] of palifermin in Serum of Male Sheep Following Single-Dose SC Administration

Parameter	Units	Group I		Group II		SC Summary (Groups I & II)	
		Mean	SD	Mean	SD	Mean	SD
T_{max}	hr	1.5[b, e]	(0.5 - 4)[c]	0.5[b, e]	(0.5 - 6)[c]	1[b, e]	(0.5 - 1.5)[c]
C_{max}	pg/mL	6990	5360	3450	1490	NA[d]	NA
$t_{1/2,z}$	hr	5.3[e]	0.5	4.6[e]	0.4	4.9[e]	0.5
CL/F	mL/hr/kg	3110	616	6320	1890	NA	NA
$MRT_{(0-t)}$	hr	8.4[e]	1.2	7.6[e]	0.8	8.0[e]	1.0
F	% Dose	35.7	8.1	17.9	4.1	NA	NA

[a]Rounded to three significant figures [b]Median value, [c]Range, [d]Not applicable, [e]Rounded to two significant figures

Table 8.3. Mean (SD) Subcutaneous Absorption Parameters of palifermin in Male Sheep Following Single-Dose Administration of 150 μg/kg

Parameter	Units	Group I (SC Control)		Group II (SC Peripheral)	
		Mean	SD	Mean	SD
A) Cumulative Lymph Recovery	% dose	NA[a]	NA	14.5	8.42
B) Non-Lymphatic Recovery	% dose	NA	NA	17.9	NA
C) Total SC Recovery (= A+B)	% dose	35.7[b]	NA	32.4	NA
D) % SC Dose Absorbed via Lymphatics (= A/C)	% absorbed dose	NA	NA	44.8	NA

[a]NA = Not Applicable. [b]Group I, bioavailability (F)

Appears This Way On Original

Table 8.4. Mean (SD) Non-Compartmental Parameter Estimates[a] of palifermin in Serum of Male Sheep Following Single-Dose IV Administration

Parameter	Units	Group III		Group IV		IV Summary (Groups III & IV)	
		Mean	SD	Mean	SD	Mean	SD
T_{max}	hr	0.017[b]	NA[c, d]	0.017[b]	NA[c, d]	0.017[b]	NA[c, d]
C_{max}	pg/mL	387000	145000	1040000	439000	NA	NA
$t_{1/2,z}$	hr	2.7[e]	0.4	3.3[e]	0.6	2.8[e]	0.5
CL	mL/hr/kg	789	154	1080	170	NA	NA
$MRT_{(0-\infty)}$	hr	3.0[e]	0.4	3.7[e]	0.1	3.2[e]	0.5
V_{ss}	mL/kg	2330	470	4010	540	2810	930
F	% Dose	100	NA	100	NA	100	NA

[a]Rounded to three significant figures; [b]Median value; [c]Range; [d]Not Applicable; [e]Rounded to two significant figures

RESULTS: The SC parameter estimates (groups 1 and 2) data do not agree with the exception of half-life that was approximately 4-5 hours; differences in SC pharmacokinetic parameters were probably due to the lymph collection, and animal variability. For the IV groups the pharmacokinetic data were dose linear. Absorption was dose dependent and terminal half-life was roughly 3 hours. The volume of distribution was 2810-4000 ml/kg or substantially higher than blood volume (~75 ml/kg) suggesting distribution to tissues. Clearance was 800-1000 ml/hr/kg.

CONCLUSIONS: The IV pharmacokinetic parameters are similar to those seen in the monkey. A slight "hump" or plateau was seen in the product's IV serum profile (i.e., similar to the monkey and human profile). Approximately 45% of the SC dose was absorbed by the lymphatic system.

2.6.4.9 Discussion and Conclusions

Appears This Way On Original

2.6.4.10 Tables and figures to include comparative TK summary

2.6.5 PHARMACOKINETICS TABULATED SUMMARY (AS SUPPLIED BY SPONSOR)

Table 2.6.5.3. *Pharmacokinetics: Absorption After a Single Dose*
Test Article: Palifermin

Study No	Species	Sex (N)	Formulation, Vehicle and Diluent	Sample	Dose µg/kg	Mean PK Parameters T_{max} hr	C_{max} ng/mL	$t_{1/2,z}$ hr	AUC_{0-24} ng•hr/mL	$AUC_{0-\infty}$ ng•hr/mL	CL mL/hr/kg	V_{ss} mL/kg	F %	Vol.	Page
100123[a]	Mouse	M (2/timepoint)	A, B	Serum	15000 IV	ND	12100[b]	4.10	6910	6930	2160	3660	ND		
101827[a]	Mouse[c]	F (3/timepoint)	A, C	Serum	500 IV	ND	80.8	1.08	175	173	2890	4850	ND		
	Mouse[c]	F (3/timepoint)	A, D	Serum	4000 IV	ND	1150	33.0	1510	1540	2610	8020	ND		
	Mouse[c]	F (3/timepoint)	A, D	Serum	15000 IV	ND	9890	16.6	6370	6450	2330	4890	ND		
	Mouse[c]	F (3/timepoint)	A, D	Serum	25000 IV	ND	26300	15.8	12400	12500	2000	3680	ND		
KGF 114	Rat	M (4)	A, E	Serum	10 IV	ND	ND	ND	158	ND	64.7	46.0	ND		
	Rat	M (4)	A, E	Serum	30 IV	ND	ND	ND	519	ND	58.0	44.9	ND		
	Rat	M (4)	A, E	Serum	100 IV	ND	ND	ND	1860	ND	54.2	47.6	ND		
	Rat	M (4)	A, E	Serum	300 IV	ND	ND	ND	6040	ND	50.2	41.5	ND		
	Rat	M (4)	A, E	Serum	1000 IV	ND	ND	2.91[e]	16600	ND	61.0	45.0	ND		
	Rat	M (4)	A, E	Serum	3000 IV	ND	ND	2.98[e]	60300	ND	50.4	43.2	ND		
	Rat	M (4)	A, E	Serum	10 SC	1.00	0.95	ND	1.21	ND	9890[d]	ND	0.7		
	Rat	M (4)	A, E	Serum	30 SC	1.00	6.01	ND	12.0	ND	4090[d]	ND	2.4		
	Rat	M (4)	A, E	Serum	100 SC	1.33	40.1	ND	123	ND	868[d]	ND	6.7		
	Rat	M (4)	A, E	Serum	300 SC	1.00	192	ND	592	ND	692[d]	ND	10.4		
	Rat	M (4)	A, E	Serum	1000 SC	1.00	1030	3.21[e]	3370	ND	328[d]	ND	20.6		
	Rat	M (4)	A, E	Serum	3000 SC	1.13	3420	3.00[e]	11400	ND	282[d]	ND	19.5		
KGF.153[a]	Rat	M (5)	A, E	Serum	300 SC	1.00	272	3.4	806	ND	ND	ND	ND		
100378	Rat	M (6)	G	Serum	300 IV	ND	3200[b]	0.573	2200[f]	2220	138	108	ND		
	Rat	M (6)	H	Serum	300 IV	ND	3660[b]	0.591	2260[f]	2280	133	102	ND		

See footnotes on the next page

Table 2.6.5.3. Pharmacokinetics: Absorption After a Single Dose (Continued)
Test Article: Palifermin

Study No.	Species	Sex (N)	Formulation, Vehicle and Diluent	Sample	Dose µg/kg	Mean PK Parameters T_{max} hr	C_{max} ng/mL	$t_{1/2,z}$ hr	AUC_{0-24} ng•hr/mL	$AUC_{0-\infty}$ ng•hr/mL	CL mL/hr/kg	V_{ss} mL/kg	F %	Vol.	Page
950345AM[a]	Monkey	M (4), F (2)	A, F	Serum	30 IV	0.03	304	ND	71.3	ND	425[c]	873[b]	ND		
	Monkey	M (2), F (4)	A, F	Serum	300 IV	0.03	3230	3	762	ND	414[c]	1220[b]	ND		
	Monkey	M (4) F (2)	A, F	Serum	30 SC	0.17	0.69	ND	ND	ND	ND	ND	ND		
	Monkey	M (2), F (4)	A, F	Serum	300 SC	0.17	35.0	ND	134	ND	ND	ND	18		
20010192	Human	M (13), F (3)	I	Serum	60 IV	ND	891[e]	4.50	132	134	494	2310	ND		
960189	Human	M (10), F (3)	A, D	Serum	60 IV	ND	851[e]	4.02	59.1[f]	72.3	1030	4320	ND		
20010182	Human	M (7), F (6)	I	Serum	60 IV	ND	512[e]	4.87[e]	37.8	38.2[g]	1730[e]	5320[e]	ND		

Assay: ELISA
Feeding Condition. Standard feed. Mice and rats: ad libitum access to food and water. Monkeys. food appropriate for their size and age/ ad libitum access to water
(A)Formulation. Palifermin was supplied as lyophilized powder. The powder was reconstituted with different vehicles and further diluted with different diluents. (See Table 2.6.5.17 for additional details.)
(B)Vehicle. Reconstitution solution - Diluent. Phosphate buffered saline (PBS)
(C)Vehicle. Sterile water - Diluent. Placebo
(D)Vehicle. Sterile water - Diluent. None
(E)Vehicle. Sterile water.
(F)Vehicle. Reconstitution solution - Diluent: Placebo
(G)Formulation. Lyophilized powder containing
(H)Formulation. Lyophilized powder containing
Diluent.
(I) Formulation:
Diluent. None
ND = Not Determined
[a] PK after single dose presented here. PK after repeated dose presented in the appropriate table.
[b] C_0
[c] Athymic nu/nu mouse
[d] CL/F
[e] Half-life estimates determined by curve-fitting of the average concentration data.
[f] AUC_{0-t} where t = time of last quantifiable concentration.
[g] n=9
[h] AUC_{0-t} and $AUMC_{0-t}$ were used for computation of CL and V_{ss}.

Table 2.6.5.4. Pharmacokinetics: Absorption After Repeated Doses
Test Article: Palifermin

Study No.	Species	Sex (N)	Formulation, Vehicle and Diluent	Sample	Dose µg/kg/day	Mean PK Parameters: T_{max} hr	C_{max} ng/mL	$t_{1/2}$ hr	AUC_{0-24} ng•hr/mL	AUC_{0-t} ng•hr/mL	CL mL/hr/kg	V_{ss} mL/kg	AR	Vol.	Page
100123	Mouse	M (2/time point)	A, B	Serum	15000 IV 3rd dose	ND	10800[b]	2.64	7270	7320	2050	4920	ND		
101827	Mouse[a]	F (3/time point)	A, C	Serum	500 IV 3rd dose	0.50	91.6	2.34	208	208	2400	5840	1.19		
	Mouse[a]	F (3/time point)	A, D	Serum	4000 IV 3rd dose	0.50	1020	2.86	1490	1500	2670	5440	0.990		
KGF.153	Rat	M (3)	A, E	Serum	300 SC 7th dose	0.83	276	ND	889	ND	ND	ND	1.05		

Assay: ELISA
Feeding Condition: Standard feed. Mice, rats, and sheep: ad libitum access to food and water. Monkeys: food appropriate for size and age/ad libitum access to water.
(A)Formulation: Palifermin was supplied as lyophilized powder. The powder was reconstituted with different vehicles and further diluted with different diluents. (See Table 2.6.5.17 for additional details.)
(B)Vehicle: Reconstitution solution - Diluent: Phosphate buffered saline (PBS)
(C)Vehicle: Sterile water - Diluent: Placebo
(D)Vehicle: Sterile water - Diluent: None
(E)Vehicle: Sterile water - [redacted]
(F)Vehicle: Reconstitution solution - Diluent: Placebo
(I) Formulation: [redacted] - Diluent: None
ND = Not Determined
NR = Not Reported
[a] Athymic Nu/Nu Mouse
[b] C_0
[c] Hodgkin's Disease and Non-Hodgkin's Lymphoma patients before Heme Transplantation
[d] n=12
[e] AUC_{0-t} where t = time of last quantifiable concentration

Appears This Way
On Original

Table 2.6.5.4. Pharmacokinetics: Absorption After Repeated Doses (Continued)
Test Article: Palifermin

Study No.	Species	Sex (N)	Formulation, Vehicle and Diluent	Sample	Dose µg/kg/day	Mean PK Parameters T_{max} hr	C_{max} ng/mL	$t_{1/2,z}$ hr	AUC_{0-24} ng-hr/mL	$AUC_{0-\infty}$ ng-hr/mL	CL mL/hr/kg	V_{ss} mL/kg	AR	Vol	Page
[redacted] 950345AM	Monkey	M (2), F (1)	A, F	Serum	30 IV 7th dose	ND	ND	ND	70.0	ND	ND	ND	0.99		
	Monkey	M (1), F (2)	A, F	Serum	300 IV 7th dose	ND	ND	ND	297	ND	ND	ND	0.48		
	Monkey	M (1), F (2)	A, F	Serum	300 SC 7th dose	ND	ND	ND	123	ND	ND	ND	0.80		
960189	Human[c]	M (7) F (3)	A, D	Serum	60 IV 3rd dose	ND	429[a]	3.68	58.7[c]	60.5	1220	4710	ND		
20010182	Human	M (7) F (6)	I	Serum	60 IV 3rd dose	ND	800[a]	5.71[d]	42.6	NR	NR	NR	1.06		

Assay: ELISA
Feeding Condition: Standard feed. Mice, rats, and sheep ad libitum access to food and water. Monkeys, food appropriate for size and age/ad libitum access to water.
(A)Formulation: Palifermin was supplied as lyophilized powder. The powder was reconstituted with different vehicles and further diluted with different diluents. (See Table 2.6.5.17 for additional details.)
(B)Vehicle: Reconstitution solution - Diluent: Phosphate buffered saline (PBS)
(C)Vehicle: Sterile water - Diluent: Placebo
(D)Vehicle: Sterile water - Diluent: None
(E)Vehicle: [redacted]
(F)Vehicle: Reconstitution solution - Diluent: Placebo
(I) Formulation: [redacted] - Diluent: None
ND = Not Determined
NR = Not Reported
[a]Athymic Nu/Nu Mouse
[b] C_0
[c]Hodgkin's Disease and Non-Hodgkin's Lymphoma patients before heme transplantation.
[d]n=12
[e] AUC_{0-t} where t = time of last quantifiable concentration.
See toxicokinetic results (Section 2.6.7) for additional description of multiple dose PK in the nonclinical species

Table 2.6.5.5a. Pharmacokinetics: Organ Distribution
Test Article: Palifermin; Study Number: [redacted] 529-028

Study No. [redacted] 529-028 (PK96060)

Study Design	Tissue	Mean ± SD Total Radioactivity (ng Eq/g) 0.5 hr[a]	2 hr[b]	8 hr	24 hr	Mean ± SD Acid Precipitable Radioactivity (ng Eq/g) 0.5 hr[a]	2 hr[b]	8 hr	24 hr
Rat - M(3) F(3) per time point	Blood	159 ± 18.6	242 ± 24.3	217 ± 36.8	33.5 ± 8.39	124 ± 17.4	165 ± 17.1	150 ± 34.3	24.4 ± 6.35
Radionuclide - ^{125}I	Serum	233 ± 44.9	268 ± 19.1	228 ± 40.4	40.5 ± 8.48	106 ± 21.3	57.2 ± 7.72	33.9 ± 6.04	17.2 ± 5.60
Dose (Route) - 300 µg/kg, 21 µCi/kg (IV)	Plasma	238 ± 40.7	274 ± 23.6	227 ± 38.6	41.0 ± 9.12	109 ± 16.4	63.7 ± 9.05	38.2 ± 7.06	17.8 ± 5.01
	Brain	12.4 ± 1.52	12.3 ± 1.26	8.67 ± 1.03	2.33 ± 0.516	6.45 ± 2.71	6.16 ± 0.685	3.07 ± 0.572	1.57 ± 0.754
Feeding condition - Ad libitum	Eyes	130 ± 39.6	154 ± 25.3	101 ± 29.4	14.2 ± 2.48	94.6 ± 37.8	100 ± 18.7	69.0 ± 34.1	8.27 ± 1.35
Non-radio-labeled Formulation, Lyophilized powder	Thyroid	5150 ± 651	39100 ± 21400	347000 ± 101000	306000 ± 53600	4150 ± 652	36200 ± 20200	329000 ± 95100	291000 ± 52400
	Pancreas	237 ± 35.7	283 ± 49.8	125 ± 21.6	13.5 ± 2.59	173 ± 33.6	191 ± 41.6	76.4 ± 21.9	8.57 ± 2.34
Vehicle: Reconstitution solution	Mesenteric Lymph Node	65.0 ± 17.0	118 ± 19.0	98.5 ± 20.0	14.7 ± 3.08	44.4 ± 12.1	73.9 ± 16.9	52.9 ± 14.8	8.62 ± 1.61
	Adrenal Glands	2470 ± 750	1100 ± 55.5	113 ± 17.3	12.5 ± 2.61	1810 ± 624	825 ± 69.0	63.1 ± 15.2	10.2 ± 2.51
Diluent: Placebo	Pituitary	680 ± 444	773 ± 121	129 ± 55.5	17.2 ± 14.2	657 ± 365[d]	567 ± 116	41.9 ± 17.9	9.74 ± 16.9[d]
Method Gamma counting	Thymus	57.8 ± 11.7	92.0 ± 19.2	99.2 ± 48.8	16.3 ± 10.3	35.7 ± 6.72	54.8 ± 9.84	62.8 ± 39.5	11.5 ± 8.99
	Tongue	88.8 ± 17.4	145 ± 9.95	121 ± 21.2	19.8 ± 5.49	64.0 ± 13.6	95.8 ± 8.00	83.8 ± 8.89	14.4 ± 4.38
	Trachea	288 ± 120	1660 ± 482	3820 ± 2100	654 ± 837	171 ± 82.0	1380 ± 481	3440 ± 2050	594 ± 820
	Esophagus	83.4 ± 16.7	170 ± 19.2	180 ± 45.7	33.5 ± 10.8[b]	37.7 ± 3.76	56.8 ± 2.38	64.2 ± 18.8	15.4 ± 5.49[b]
	Spleen	1230 ± 322	713 ± 67.5	126 ± 18.4	21.0 ± 3.41	1060 ± 288	595 ± 61.4	86.6 ± 11.9	15.3 ± 2.55

NA = Not available. Acid precipitation was not performed for these tissues.
[a] n=5
[b] n=4
[c] n=3
[d] n=2, SD not reported when n<3
[e] n=1; SD not reported when n<3
[f] Male only
[g] Female only

Table 2.6.5.5a. Pharmacokinetics: Organ Distribution (Continued)
Test Article: Palifermin; Study Number: [redacted] 529-028

Study No. [redacted] 529-028 (PK96060)									
		Mean ± SD Total Radioactivity (ng Eq/g)				Mean ± SD Acid Precipitable Radioactivity (ng Eq/g)			
Study Design	Tissue	0.5 hr[a]	2 hr[b]	8 hr	24 hr	0.5 hr	2 hr	8 hr	24 hr
	Heart	188 ± 55.1	211 ± 12.2	133 ± 19.7	18.3 ± 2.50	88.3 ± 28.2	85.1 ± 11.5	47.2 ± 8.16	8.71 ± 1.84
	Lungs	463 ± 76.3	310 ± 36.2	967 ± 1990	24.2 ± 7.36	226 ± 18.2	138 ± 19.8	347 ± 729	11.6 ± 3.71
	Liver	3180 ± 228	1380 ± 145	179 ± 22.9	29.8 ± 3.76	2640 ± 187	1140 ± 139	126 ± 11.8	23.6 ± 2.90
	Kidneys	3130 ± 271	2130 ± 206	327 ± 107	34.7 ± 5.72	2280 ± 302	1529 ± 163	153 ± 38.7	18.1 ± 2.52
	Bladder	121 ± 33.3	296 ± 59.0	368 ± 205	44.3 ± 27.8	57.0 ± 21.8	81.5 ± 15.8	85.5 ± 47.1	11.9 ± 5.17
	Stomach	348 ± 105	1380 ± 316	1220 ± 399	233 ± 106	176 ± 51.0	552 ± 147	312 ± 75.6	62.1 ± 23.5
	Small Intestine	246 ± 37.7	434 ± 52.8	352 ± 51.9	49.2 ± 27.9	161 ± 35.9	240 ± 17.6	147 ± 30.4	17.4 ± 7.53
	Large Intestine	134 ± 21.3	193 ± 19.8	123 ± 15.0	16.2 ± 2.04	96.6 ± 16.3	122 ± 12.9	64.1 ± 13.8	8.63 ± 0.769
	Adipose Tissue (Psoas)	37.8 ± 8.41	41.5 ± 9.47	37.0 ± 7.24	5.67 ± 1.03	26.7 ± 8.28	24.4 ± 4.86	20.8 ± 3.63	3.93 ± 0.805
	Muscle	45.6 ± 7.54	56.0 ± 3.56	53.2 ± 7.08	7.33 ± 1.86	30.2 ± 5.77	34.9 ± 3.03	25.0 ± 3.16	3.88 ± 0.946
	Bone (Femur)	131 ± 11.6	146 ± 14.5	128 ± 20.6	36.5 ± 11.0	69.5 ± 7.89	84.0 ± 8.24	57.9 ± 8.22	24.2 ± 6.79
	Carcass	76.0 ± 6.67	104 ± 6.25	84.3 ± 11.3	20.7 ± 6.12	54.9 ± 9.05	61.7 ± 2.41	41.3 ± 3.75	14.0 ± 4.51
	Prostate[f]	68.0[d]	149[e]	126 ± 14.0	20.7 ± 1.16	31.6[d]	53.2[e]	30.2 ± 3.62	5.84 ± 0.727
	Epididymus[f]	67.0[d]	115[e]	89.0 ± 7.00	18.3 ± 2.08	44.0[d]	63.6[e]	47.2 ± 6.05	10.1 ± 1.89
	Testes[f]	45.5[d]	89.0[e]	92.7 ± 13.7	18.3 ± 1.16	25.4[d]	34.0[e]	23.0 ± 3.62	6.47 ± 0.963

NA = Not available. Acid precipitation was not performed for these tissues.
[a] n=5
[b] n=4
[c] n=3
[d] n=2; SD not reported when n<3
[e] n=1; SD not reported when n<3
[f] Male only
[g] Female only

Table 2.6.5.5a. Pharmacokinetics: Organ Distribution (Continued)
Test Article: Palifermin; Study Number: [redacted] 529-028

Study No. [redacted] 529-028 (PK96060)									
		Mean ± SD Total Radioactivity (ng Eq/g)				Mean ± SD Acid Precipitable Radioactivity (ng Eq/g)			
Study Design	Tissue	0.5 hr[a]	2 hr[b]	8 hr	24 hr	0.5 hr	2 hr	8 hr	24 hr
	Injection Site	276 ± 104	527 ± 219	1080 ± 1270	187 ± 69.1	NA	NA	NA	NA
	Skin	112 ± 11.5	254 ± 13.5	300 ± 53.3	94.8 ± 56.9	NA	NA	NA	NA
	Ovaries[g]	1230 ± 601	878 ± 135	263 ± 49.8	20.7 ± 1.53	919 ± 453	647 ± 122	153 ± 17.7	12.6 ± 0.428
	Uterus[g]	152 ± 7.64	226 ± 3.51	200 ± 51.0	17.7 ± 1.53	101 ± 6.47	154 ± 8.84	130 ± 33.5	13.0 ± 0.719
	Mammary Glands[g]	104 ± 8.72	313 ± 58.9	206 ± 40.3	36.7 ± 6.66	NA	NA	NA	NA

NA = Not available. Acid precipitation was not performed for these tissues.
[a] n=5
[b] n=4
[c] n=3
[d] n=2; SD not reported when n<3
[e] n=1; SD not reported when n<3.
[f] Male only
[g] Female only

Appears This Way
On Original

Table 2.6.5.5b. Pharmacokinetics: Organ Distribution
Test Article: Palifermin; Study Number: [redacted]529-025

Study No. [redacted]529-025 (PK95010)									
Study Design	Tissue	Mean ± SD Total Radioactivity (ng Eq/g)				Mean ± SD Acid Precipitable Radioactivity (ng Eq/g)			
		0.5 hr	2 hr	8 hr	24 hr	0.5 hr	2 hr	8 hr	24 hr
Rat - M (3) F (3) per time point Radionuclide - ^{125}I Dose (Route)- 300 µg/kg; 5-10 uCi/kg (SC) Feeding condition - Ad libitum Non-radio-labeled Formulation. Lyophilized powder Vehicle: Reconstitution solution Diluent: Placebo Method: Liquid scintillation counting	Blood	131 ± 26.0	297 ± 150	433 ± 224	89.3 ± 6.31	94.6 ± 18.9	215 ± 146	338 ± 237	65.0 ± 4.95
	Serum	85.7 ± 23.9	181 ± 33.8	221 ± 17.7	62.7 ± 7.89	26.8 ± 8.05	42.9 ± 11.7	50.0 ± 13.9	22.9 ± 3.05
	Plasma	78.0 ± 23.9	148 ± 80.0	216 ± 10.8	62.5 ± 9.42	19.0 ± 8.58	46.8 ± 19.4[a]	42.6 ± 21.6	15.2 ± 3.03[a]
	Brain	2.17 ± 0.753	3.50 ± 0.548	3.17 ± 0.408	1.00 ± 0.00	0.316 ± 0.181	0.508 ± 0.144	0.391 ± 0.159[b]	0.386 ± 0.169
	Eyes	8.00 ± 2.90	21.0 ± 4.94	27.8 ± 4.83	7.17 ± 1.17	0.951 ± 0.372	2.97 ± 0.637	3.18 ± 0.571	1.48 ± 0.349
	Thyroid	242 ± 92.4	1510 ± 1070	16400 ± 1640	25900 ± 10700	NS	111[c]	NS	31400[d]
	Pancreas	15.7 ± 7.87	28.0 ± 6.16	29.0 ± 3.80	6.67 ± 1.37	2.63 ± 1.64	5.84 ± 1.45	5.20 ± 1.66	1.38 ± 0.541
	Lymph Nodes	7.50 ± 3.51	17.7 ± 2.66	29.0 ± 3.58	6.67 ± 1.37	NC	1.57 ± 1.00	4.47 ± 1.00	1.06 ± 0.388
	Adrenal Glands	10.5 ± 3.94	33.7 ± 8.24	12.8 ± 4.45	2.33 ± 0.516	6.79 ± 3.37	25.5 ± 7.62[e]	6.05 ± 2.95	0.680 ± 0.169[a]
	Heart	13.3 ± 4.13	26.2 ± 4.54	29.3 ± 3.50	8.67 ± 1.21	3.60 ± 1.13	6.21 ± 0.840	5.96 ± 1.11	2.79 ± 0.810
	Lungs	15.0 ± 5.55	32.3 ± 5.35	39.8 ± 1.47	10.8 ± 1.47	2.09 ± 1.70[a]	4.39 ± 3.73	6.24 ± 9.90	2.44 ± 0.646
	Trachea	24.3 ± 8.24	18.0 ± 5.90	218 ± 300	633 ± 682	16.5 ± 5.42	9.74 ± 4.61	194 ± 285	612 ± 665

NA = Not available. Acid precipitation was not performed for these tissues.
NC = Not calculated due to negative value for acid precipitable total sample DPM.
NS = Insufficient sample to aliquot.
[a] n=5
[b] n=4
[c] n=3
[d] n=2; SD not reported when n<3.
[e] n=1; SD not reported when n<3
[f] Male only
[g] Female only
Note: One animal had an unscheduled necropsy at [redacted] hours postdose. Data not shown.

Table 2.6.5.5b. Pharmacokinetics: Organ Distribution (Continued)
Test Article: Palifermin; Study Number: [redacted]529-025

Study No. [redacted]529-025 (PK95010)									
Study Design	Tissue	Mean ± SD Total Radioactivity (ng Eq/g)				Mean ± SD Acid Precipitable Radioactivity (ng Eq/g)			
		0.5 hr	2 hr	8 hr	24 hr	0.5 hr	2 hr	8 hr	24 hr
	Liver	42.0 ± 15.2	102 ± 17.1	53.5 ± 12.4	11.7 ± 1.51	26.4 ± 10.2	65.6 ± 13.8	21.2 ± 7.09	4.29 ± 0.747
	Kidneys	55.3 ± 19.8	150 ± 20.2	99.5 ± 11.0	21.0 ± 2.78	31.2 ± 13.0	93.1 ± 19.7	37.5 ± 10.0	5.23 ± 1.03
	Spleen	32.8 ± 9.41	63.5 ± 8.62	42.7 ± 5.09	10.0 ± 3.03	21.6 ± 6.34	38.8 ± 7.91	15.0 ± 3.80	4.02 ± 0.415[a]
	Tongue	11.0 ± 3.90	23.8 ± 6.01	35.0 ± 5.29	9.17 ± 2.71	1.79 ± 0.988	3.02 ± 1.29	2.91 ± 1.06	2.53 ± 1.80[b]
	Esophagus	4.33 ± 1.86	11.8 ± 4.22	16.7 ± 3.01	7.50 ± 3.27	0.813 ± 0.600	2.22 ± 1.26[d]	3.89 ± 2.79[a]	4.48 ± 2.79[a]
	Stomach	72.2 ± 20.2	244 ± 82.4	529 ± 117	106 ± 22.5	9.31 ± 3.27	25.1 ± 9.81	61.5 ± 13.1	15.3 ± 5.63
	Small Intestine	22.8 ± 6.71	65.0 ± 17.0	110 ± 14.4	23.3 ± 4.59	4.40 ± 1.84	13.9 ± 3.00	17.5 ± 3.09	3.93 ± 0.763
	Large Intestine	9.67 ± 3.01	24.8 ± 4.58	33.5 ± 6.29	9.50 ± 1.38	2.06 ± 1.22	6.95 ± 0.752	7.98 ± 1.22[a]	3.04 ± 0.517
	Urinary Bladder	6.67 ± 3.27	20.5 ± 7.23	26.8 ± 6.46	9.17 ± 6.74	0.627[d]	0.959 ± 1.00[b]	1.75 ± 0.973	0.806 ± 0.481[b]
	Adipose Tissue	6.33 ± 2.88	9.67 ± 1.97	11.2 ± 1.94	4.50 ± 1.76	0.870[e]	NC	0.707 ± 0.697	0.387 ± 0.229[d]
	Muscle	7.33 ± 2.88	12.5 ± 2.26	13.7 ± 1.75	3.67 ± 1.03	1.34 ± 1.17	1.43 ± 0.704[a]	1.60 ± 0.679	0.800 ± 0.130[c]
	Bone (Femur)	8.17 ± 2.86	20.3 ± 3.39	25.2 ± 2.23	6.67 ± 1.37	1.92 ± 0.536	5.03 ± 0.808	5.80 ± 1.01	2.23 ± 0.524

NA = Not available. Acid precipitation was not performed for these tissues.
NC = Not calculated due to negative value for acid precipitable total sample DPM
NS = Insufficient sample to aliquot.
[a] n=5
[b] n=4
[c] n=3
[d] n=2; SD not reported when n<3.
[e] n=1; SD not reported when n<3.
[f] Male only
[g] Female only
Note: One animal had an unscheduled necropsy at [redacted] hours postdose. Data not shown

Table 2.6.5.5b. Pharmacokinetics: Organ Distribution (Continued)
Test Article: Palifermin; Study Number: [redacted]529-025

Study No. [redacted] 529-025 (PK95010)									
Study Design	Tissue	Mean ± SD Total Radioactivity (ng Eq/g) 0.5 hr	2 hr	8 hr	24 hr	Mean ± SD Acid Precipitable Radioactivity (ng Eq/g) 0.5 hr	2 hr	8 hr	24 hr
	Remaining Carcass	20.7 ± 9.85	38.3 ± 16.4	32.3 ± 5.82	5.17 ± 0.98	7.91 ± 6.22	15.4 ± 7.96[a]	10.5 ± 4.33	1.38 ± 0.550
	Prostate[f]	4.00 ± 0.00	8.67 ± 1.53	17.7 ± 1.53	4.67 ± 0.577	0.342 ± 0.259	0.664 ± 0.195	1.99 ± 0.213	1.01[d]
	Testes[f]	6.67 ± 1.53	16.0 ± 1.73	33.3 ± 1.53	10.7 ± 0.577	NC	0.218[e]	1.49 ± 0.569	0.368 ± 0.310
	Uterus[g]	20.7 ± 8.96	28.3 ± 6.43	41.3 ± 7.57	11.7 ± 4.73	4.22 ± 1.32	3.57 ± 1.67	5.80 ± 1.84	1.38 ± 1.25
	Ovaries[g]	7.00 ± 2.65	21.7 ± 9.24	23.7 ± 3.22	5.33 ± 3.22	2.34[d]	9.17 ± 3.41	5.69 ± 0.966	0.608[d]
	Mammary Glands[g]	7.00 ± 2.65	24.0[d]	36.3 ± 4.51	8.50[d]	NA	NA	NA	NA
	Injection Site	2900 ± 529	2140 ± 770	795 ± 229	124 ± 13.7	NA	NA	NA	NA
	Skin	11.0 ± 4.10	42.8 ± 27.1	48.8 ± 11.1	16.8 ± 6.49	NA	NA	NA	NA

NA = Not available. Acid precipitation was not performed for these tissues.
NC = Not calculated due to negative value for acid precipitable total sample DPM.
NS = Insufficient sample to aliquot.
[a] n=5
[b] n=4
[c] n=3
[d] n=2; SD not reported when n<3.
[e] n=1; SD not reported when n<3.
[f] Male only
[g] Female only
Note: One animal had an unscheduled necropsy at [redacted] hours postdose. Data not shown.

Table 2.6.5.8. Pharmacokinetics: Other Distribution
Test Article: Palifermin

Study No.:	KGF 165									
Type of Study:	Pilot PK study of ^{125}I-labeled rHuKGF following IV and SC administration in Sprague-Dawley rats									
Formulation/Vehicle/Diluent:	Non-radio-labeled - Lyophilized powder/Reconstitution solution (sterile water and 0.004% Tween®20)/Placebo									
Sex (N):	M (3)					M (3)				
Dose (Route):	300 µg/kg, 2.3 x 10^7 cpm/kg (IV)					300 µg/kg, 2.3 x 10^7 cpm/kg (SC)				
Radionuclide:	^{125}I					^{125}I				
Sample:	Serum					Serum				
Method:	ELISA, Gamma counting					ELISA, Gamma counting				
	Mean Serum Concentrations									
	IV					SC				
Time (hours):	0.0167	0.5	2	8	24	0.167	0.5	2	8	24
Total Radioactivity (ng Eq/mL) = (A)	6160	2780	737	228	53.2	63.7	130	197	182	83.9
Acid-precipitable Radioactivity (ng Eq/mL) = (B)	6270	2710	486	43.0	3.89	27.6	90.1	98.1	40.5	13.6
Serum Palifermin Concentrations (ng/mL) = (C)	6206	2980	383	BQL	BQL	27.2[a]	77.9[a]	66.0	5.77	BQL
% Acid-Precipitable = (B)/(A)x100%	102	97.3	65.8	18.9	7.22	34.1	68.7	50.5	22.2	15.8
% Intact Protein = (C)/(B)x100%	98.9	112	77.2	NC	NC	67.5[a]	70.2[a]	66.6	14.5	NC

BQL = Below the quantification limit [redacted] ng/mL)
NC = Not calculated
[a] n = 2

Table 2.6.5.9. Pharmacokinetics: Metabolism In Vivo
Test Article: Palifermin

Study No.	Species	Sex (N)	Formulation, Vehicle and Diluent	Sample	Dose µg	Mean PK Parameters: T_{max} hr	C_{max} ng/mL	$t_{1/2,\lambda z}$ hr	AUC_{0-24} ng-hr/mL	$AUC_{0-\infty}$ ng-hr/mL	CL mL/hr/kg	V_{ss} mL/kg	F %	Vol.	Page
100478	Rat	M (1-4/ timepoint)	A, C	Serum	56 IV(F)[a]	2.98	468	0.598	638[c]	641	ND	ND	ND		
	Rat	M (1-3/ timepoint)	A, C	Serum	56 IV(HP)[b]	3.08	571	0.543	865[c]	667	ND	ND	ND		
KGF 265 (PK97140)	Rat	M (1-4/ timepoint)	A, B	Serum	56 IV(F)[a]	2.98	924	0.547	1550[d]	ND	ND	ND	ND		
	Rat	M (1-4/ timepoint)	A, B	Serum	56 IV(HP)[b]	3.02	778	0.549	1090[d]	ND	ND	ND	ND		

Assay: ELISA
Feeding Condition: Standard feed. Rats: ad libitum access to food and water.
(A)Formulation: Palifermin was supplied as lyophilized powder. The powder was reconstituted with different vehicles and further diluted with different diluents. (See Table 2.6.5.17 for additional details.)
(B)Vehicle: Reconstitution solution - Diluent: Phosphate buffered saline (PBS)
(C)Vehicle: Sterile water - Diluent: Placebo
ND = Not Determined
[a]Intravenous via Femoral Vein: Infusion (increasing from 0.14 mL/ hr to 0.76 mL/hr) given over 3 hr
[b]Intravenous via Hepatic Portal Vein: Infusion (increasing from 0.14 mL/ hr to 0.76 mL/hr) given over 3 hr
[c] AUC_{0-t}
[d] $AUC_{0-\infty}$

Table 2.6.5.13. Pharmacokinetics: Excretion
Test Article: Palifermin

	Study No. 529-028 (PK96060)				Study No. 529-025 (PK95010)			
Species:	Rat				Rat			
Sex (N):	M(3) F(3) per time point				M(3) F(3) per time point			
Feeding Condition:	Ad libitum				Ad libitum			
Formulation/ Vehicle/Diluent:	Non-radiolabeled - Lyophilized powder/ Reconstitution solution/Placebo				Non-radiolabeled - Lyophilized powder/ Reconstitution solution/Placebo			
Method of Administration:	IV				SC			
Dose:	300 µg/kg; 21 µCi/kg				300 µg/kg; 5-10 µCi/kg			
Assay (Analyte):	Gamma counting (^{125}I)				Liquid scintillation counting (^{125}I)			
	% Dose[a]				% Dose[a]			
Time:	0.5 hr	2 hr	8 hr	24 hr	0.5 hr	2 hr	8 hr	24 hr
Excretion Route								
Urine + Bladder Contents[b]	0.03	0.43	9.71	31.02[c]	0.25	1.92	12.35	54.62
Feces + Intestinal Contents[b]	0.66	2.47	4.46	2.19	0.78	1.98	5.04	3.92
Cage Rinse[b]	0.09	0.34	6.57	14.48	0.09	0.41	2.63	14.08
Tissue								
Blood	3.40	4.70	4.36	0.67	2.88	6.03	10.24	1.99
Tissue	72.6	63.39	49.11	19.17	9.43	28.19	42.96	19.26
Carcass	9.96	12.89	10.67	2.77	10.09	16.37	15.72	2.94
Injection Site	ND	ND	ND	ND	101.15	62.09	28.25	4.66
Total	86.74	84.22	84.87	70.29	124.67	116.98	117.18	101.47

ND = Not determined
[a] % Dose based on total radioactivity
[b] Collection from time zero to time listed.
[c] In Study 529-028, % of radioactive dose recovered in the urine only was 31.02% at 24 hours after dosing. The acid precipitable radioactivity recovered in the urine was 35.41% of total radioactivity found in the urine. The resulting administered radioactivity recovered in urine as acid-precipitable radioactivity over a 24-hour period was 11% (0.3102 * 0.3541).

Appears This Way
On Original

Table 2.6.5.16a. Pharmacokinetics: Other
Test Article: Palifermin; Study No.: KGF.104

Study No :	KGF.104
Type of Study.	A pharmacokinetic study of rHuKGF (palifermin) in bilaterally-nephrectomized and sham-operated Sprague-Dawley rats
Dose (Route):	300 µg/kg (IV) on day 1 and day 3
Species:	Sprague-Dawley Rats
Method.	Day 1 doses of palifermin served as the normal controls for both groups of animals. On day 3, rats were either nephrectomized or sham-operated, then dosed with palifermin.

			Formulation.			Mean PK Parameters							
Group	Procedure	Sex (N)	Vehicle and Diluent	Sample	Dose µg/kg	T_{max} hr	C_{max} ng/mL	$t_{1/2,z}$ hr	AUC_{0-24} ng-hr/mL	$AUC_{0-\infty}$ ng-hr/mL	CL mL/hr/kg	V_{ss} mL/kg	F %
1	Control	M (4)	A, E	Serum	300 IV	ND	ND	1.77*	1930	ND	165	76.4	ND
1	Nephrectomized	M (4)	A, E	Serum	300 IV	ND	ND	2.70*	4090	ND	78.7	49.4	ND
2	Control	M (2)	A, E	Serum	300 IV	ND	ND	1.04*	2040	ND	151	83.9	ND
2	Sham-Operated	M (2)	A, E	Serum	300 IV	ND	ND	1.37*	1940	ND	156	70.1	ND

Assay: ELISA
Feeding Condition: Standard feed. Ad libitum access to food and water.
(A)Formulation: Palifermin was supplied as lyophilized powder. The powder was reconstituted with different vehicles and further diluted with different diluents. (See Table 2.6.5.17 for additional details.)
(E)Vehicle: Sterile water and 0.004%Tween®20 solution - Diluent: Dulbecco's PBS without calcium chloride and magnesium chloride
ND = Not Determined
* Half-life estimates determined by curve-fitting of the average concentration data.

Table 2.6.5.16b. Pharmacokinetics: Other
Test Article: Palifermin; Study No.: KGF.251

Study No.:	KGF.251 (PK97023)
Type of Study:	Pharmacokinetic evaluation of four modified forms of rHuKGF and unmodified rHuKGF (Ñ23 rHuKGF = palifermin) following a single bolus intravenous injection in male Sprague-Dawley rats.
Dose (Route).	Group 1 1000 µg/kg (IV) unmodified rHuKGF (Ñ23 rHuKGF = palifermin) (Only Group 1 received palifermin. Only palifermin data are presented here.)
Species:	Sprague-Dawley Rats
Method:	Each of the five forms of rHuKGF (1 form rHuKGF/group) was administered to Sprague-Dawley rats; 2 rats per group.

		Formulation.			Mean PK Parameters							
Group	Sex (N)	Vehicle and Diluent	Sample	Dose µg/kg	T_{max} hr	C_0 ng/mL	$t_{1/2,z}$ hr	AUC_{0-72} ng-hr/mL	$AUC_{0-\infty}$ ng-hr/mL	CL mL/hr/kg	V_{ss} mL/kg	F %
1	M (2)	A, F	Serum	1000 IV	ND	10600	1 40	7430	7440	137	132	ND

Assay: ELISA
Feeding Condition: Standard feed. Food appropriate for size and age/ ad libitum acces to water.
(A)Formulation: Palifermin was supplied as lyophilized powder. The powder was reconstituted with different vehicles and further diluted with different diluents. (See Table 2.6.5.17 for additional details.)
ND = Not Determined

Appears This Way
On Original

Table 2.6.5.16c. Pharmacokinetics: Other
Test Article: Palifermin; Study No.: PK97077

Study No.:	PK97077
Type of Study:	A pilot comparative PK study of two modified forms of rHuKGF and unmodified rHuKGF (N823 rHuKGF = palifermin) in Rhesus monkeys
Dose (Route):	100 µg/kg (IV)-day 1 and 300 µg/kg (IV)-day 4 for each of two modified forms of rHuKGF and unmodified rHuKGF (N823 rHuKGF = palifermin) (Only Group 1 received palifermin. Only palifermin data are presented here.)
Species	Rhesus monkeys
Method:	Each of the three forms of rHuKGF was administered to Rhesus monkeys at dose levels of 100 µg/kg on day 1 and 300 µg/kg on day 4; each group was comprised of 1 male and 2 females.

						Mean PK Parameters							
Group	Study Day	Sex (N)	Formulation, Vehicle and Diluent	Sample	Dose µg/kg	T_{max} hr	C_0 ng/mL	$t_{1/2,z}$ hr	AUC_{0-t} ng-hr/mL	$AUC_{0-\infty}$ ng-hr/mL	CL mL/hr/kg	V_{ss} mL/kg	F %
1	1	M (1), F (2)	A, F	Serum	100 IV	ND	1010	3.07	85.8	89.0	1120	2.68	ND
1	4	M (1), F (2)	A, F	Serum	300 IV	ND	1380	2.52	171	178	1770	5.17	ND

Assay: ELISA
Feeding Condition: Standard feed. Food appropriate for their size and age/ ad libitum acces to water.
(A)Formulation: Palifermin was supplied as lyophilized powder. The powder was reconstituted with different vehicles and further diluted with different diluents. (See Table 2.6.5.17 for additional details.)
(F)Vehicle: Reconstitution solution - Diluent: Placebo
ND = Not Determined

Table 2.6.5.16d. Pharmacokinetics: Other
Test Article: Palifermin; Study No.: 101264

Study No.:	101264
Type of Study	A pilot single dose subcutaneous pharmacokinetic study of KGF (palifermin) in male merino wethers sheep
Dose (Route):	150 µg/kg (SC)
Species:	wethers sheep
Method:	Pharmacokinetic serum samples were collected following dose administration.

				Mean PK Parameters							
Sex (N)	Formulation, Vehicle and Diluent	Sample	Dose µg/kg	T_{max} hr	C_{max} ng/mL	$t_{1/2,z}$ hr	AUC_{0-t} ng-hr/mL	$AUC_{0-\infty}$ ng-hr/mL	CL/F mL/hr/kg	V_{ss} mL/kg	F %
M (1)	A, J	Serum	150 SC	2.5	3.09	4.13	17.0	21.5	6980	ND	ND

Assay: ELISA
Feeding Condition: Standard feed. Ad libitum access to food and water.
(A)Formulation: Palifermin was supplied as lyophilized powder. The powder was reconstituted with different vehicles and further diluted with different diluents. (See Table 2.6.5.17 for additional details.)
(J)Vehicle: Reconstitution solution - Diluent: None
ND = Not Determined

Appears This Way
On Original

Table 2.6.5.16e. Pharmacokinetics: Other
Test Article: Palifermin; Study No.: 102241

Study No.:	102241
Type of Study:	Bioavailability and lymphatic absorption of rHuKGF (palifermin) after subcutaneous administration to sheep.
Dose (Route):	Groups 1 and 2: 150 µg/kg (SC); Group 3: 37.5 µg/kg (IV); Group 4: 150 µg/kg (IV)
Species:	wethers sheep
Method.	Pharmacokinetic serum samples were collected following dose administration. Additionally, peripheral lymph was collected continuously from sheep in Group 2.

		Formulation Vehicle and		Dose	Mean PK Parameters							
Group	Sex (N)	Diluent	Sample	µg/kg	T_{max} hr	C_{max} ng/mL	$t_{1/2,\lambda}$ hr	AUC_{0-t} ng·hr/mL	$AUC_{0-\infty}$ ng·hr/mL	CL mL/hr/kg	V_{ss} mL/kg	F %
1	M (5)	A, J	Serum	150 SC	1.5[a]	6.99	5.3	48.9	50.0	3110[b]	ND	36
2	M (5)	A, J	Serum	150 SC	0.5[a]	34.5	4.6	24.5	25.1	6320[b]	ND	18
3	M (5)	A, F	Serum	37.5 IV	0.017[a]	387[c]	2.7	47.4	49.2	789	2330	ND
4	M (2)	A, J	Serum	150 IV	0.017[a]	1040[c]	3.3	140	140	1080	4010	ND

Assay: ELISA
Feeding Condition: Standard feed. Ad libitum access to food and water.
(A)Formulation: Palifermin was supplied as lyophilized powder. The powder was reconstituted with different vehicles and further diluted with different diluents. (See Table 2.6.5.17 for additional details.)
(F)Vehicle: Reconstitution solution - Diluent: Placebo
(J)Vehicle: Reconstitution solution - Diluent: None
ND = Not Determined
[a] Median values reported.
[b] CL/F
[c] C_0

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

Appears This Way On Original

General toxicology:

Genetic toxicology:

Carcinogenicity:

Reproductive toxicology:

Special toxicology:

2.6.6.2 Single-dose toxicity

2.6.6.3 Repeat-dose toxicity

Study title:

Key study findings:

Study no.:
Volume #, and page #:
Conducting laboratory and location:
Date of study initiation:

GLP compliance:
QA report: yes ( ) no ( )
Drug, lot #, and % purity:

Methods

Doses:
Species/strain:
Number/sex/group or time point (main study):
Route, formulation, volume, and infusion rate:
Satellite groups used for toxicokinetics or recovery:
Age:
Weight:
Sampling times:
Unique study design or methodology (if any):

Observations and times: (these parameters can be captured separately here or described in connection with each endpoint under the results section.
Mortality:
Clinical signs:
Body weights:
Food consumption:
Ophthalmoscopy:
EKG:
Hematology:
Clinical chemistry:
Urinalysis:
Gross pathology:
Organ weights (specify organs weighed if not in histopath table):
Histopathology: Adequate Battery: yes ( ), no ( )—explain
Peer review: yes ( ), no ( )

Results

Mortality:

Clinical signs:

Body weights:

Food consumption:

Ophthalmoscopy:

EKG:

Hematology:

Clinical chemistry:

Urinalysis:

Gross pathology:

Organ weights (specify organs weighed if not in histopath table):

Histopathology: Adequate Battery: yes ( ), no ( )—explain
Peer review: yes ( ), no ( )

Toxicokinetics:

Other:

Histopathology inventory (optional)

Study				
Species				
Adrenals				
Aorta				
Bone Marrow smear				
Bone (femur)				
Brain				
Cecum				
Cervix				
Colon				
Duodenum				
Epididymis				
Esophagus				
Eye				
Fallopian tube				
Gall bladder				
Gross lesions				
Harderian gland				
Heart				
Ileum				
Injection site				
Jejunum				
Kidneys				
Lachrymal gland				
Larynx				
Liver				
Lungs				

Lymph nodes, cervical				
Lymph nodes mandibular				
Lymph nodes, mesenteric				
Mammary Gland				
Nasal cavity				
Optic nerves				
Ovaries				
Pancreas				
Parathyroid				
Peripheral nerve				
Pharynx				
Pituitary				
Prostate				
Rectum				
Salivary gland				
Sciatic nerve				
Seminal vesicles				
Skeletal muscle				
Skin				
Spinal cord				
Spleen				
Sternum				
Stomach				
Testes				
Thymus				
Thyroid				
Tongue				
Trachea				
Urinary bladder				
Uterus				
Vagina				
Zymbal gland				

X, histopathology performed
*, organ weight obtained

2.6.6.4 Genetic toxicology

Study title:

Key findings:

Study no.:

Volume #, and page #:
Conducting laboratory and location:
Date of study initiation:
GLP compliance:

QA reports: yes ( ) no ( )
Drug, lot #, and % purity:

Methods

Strains/species/cell line:

Doses used in definitive study:

Basis of dose selection:

Negative controls:

Positive controls:

Incubation and sampling times:

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.):

Study outcome:

2.6.6.5 Carcinogenicity

Study title:

Key study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model:
Evaluation of tumor findings:

Study no.:
Volume #, and page #:
Conducting laboratory and location:
Date of study initiation:
GLP compliance:
QA report: yes ( ) no ( )
Drug, lot #, and % purity:
CAC concurrence:

Methods

Doses:
Basis of dose selection (MTD, MFD, AUC etc.): Species/strain:

Number/sex/group (main study):
Route, formulation, volume:
Frequency of dosing:
Satellite groups used for toxicokinetics or special groups:
Age:
Animal housing:
Restriction paradigm for dietary restriction studies:
Drug stability/homogeneity:
Dual controls employed:
Interim sacrifices:
Deviations from original study protocol:

Observation times
Mortality:
Clinical signs:
Body weights:
Food consumption:
Histopathology: Peer review: yes ( ), no ( )
Toxicokinetics:

Results
Mortality:

Clinical signs:

Body weights:

Food consumption:

Gross pathology:

Histopathology:

Non-neoplastic:

Neoplastic:

Toxicokinetics:

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title:

Key study findings:

Study no.:
Volume #, and page #:
Conducting laboratory and location:
Date of study initiation:
GLP compliance:
QA reports: yes ( ) no ( )
Drug, lot #, and % purity:

Methods

Doses:
Species/strain:
Number/sex/group:
Route, formulation, volume, and infusion rate:
Satellite groups used for toxicokinetics:
Study design:
Parameters and endpoints evaluated:

Results

Mortality:

Clinical signs:

Body weight:

Food consumption:

Toxicokinetics:

Necropsy:

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

Embryofetal development

Study title:

Key study findings:

Study no.:
Volume #, and page #:
Conducting laboratory and location:
Date of study initiation:

GLP compliance:
QA reports: yes ( ) no ( )
Drug, lot #, and % purity:

Methods

Doses:
Species/strain:
Number/sex/group:
Route, formulation, volume, and infusion rate:
Satellite groups used for toxicokinetics:
Study design:
Parameters and endpoints evaluated:

Results

Mortality (dams):

Clinical signs (dams):

Body weight (dams):

Food consumption (dams):

Toxicokinetics:

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.):

Offspring (malformations, variations, etc.):

Prenatal and postnatal development

Study title:

Key study findings:

Study no.:
Volume #, and page #:
Conducting laboratory and location:
Date of study initiation:
GLP compliance:
QA reports: yes ( ) no ( )
Drug, lot #, and % purity:

Methods

Doses:
Species/strain:
Number/sex/group:
Route, formulation, volume, and infusion rate:
Satellite groups used for toxicokinetics:
Study design:
Parameters and endpoints evaluated:

Results

F_0 in-life:

F_0 necropsy:

F_1 physical development:

F_1 behavioral evaluation:

F_1 reproduction:

F_2 findings:

2.6.6.7 Local tolerance

2.6.6.8 Special toxicology studies

Study title:

Key study findings:

Study no.:
Volume #, and page #:
Conducting laboratory and location:
Date of study initiation:
GLP compliance:
QA reports: yes ( ) no ( )
Drug, lot #, and % purity:
Formulation/vehicle:

Methods

Doses:

Study design:

Results:

2.6.6.9 Discussion and Conclusions

2.6.6.10 Tables and Figures

2.6.7 TOXICOLOGY TABULATED SUMMARY

[pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the sponsor]

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

Unresolved toxicology issues (if any):

Recommendations:

Suggested labeling:

Signatures (optional):

Reviewer Signature Anita O'Connor

Supervisor Signature Martin D. Green Concurrence Yes ✓ No ___

APPENDIX/ATTACHMENTS