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APPLICATION NUMBER

BLA 125103/0

Clinical Pharmacology and Biopharmaceutics Review

11-23-04

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW

BLA: 125103	Submission Date(s): 6/15/04, 6/22/04, 7/19/04
Brand Name	[redacted]
Generic Name	Palifermin, Recombinant Human Keratinocyte Growth Factor (KGF), rHuKGF
Reviewer	Hong Zhao
Supervisor	Martin David Green
Branch	Clinical Pharmacology / Pharmacology-Toxicology
ODE VI Division	Oncology
Sponsor	Amgen Inc.
Relevant IND(s)	BB-IND 6370
Submission Type; Code	Priority
Formulation; Strength(s)	6.25 mg preservative-free, lyophilized powder per vial to be reconstituted with 1.2 mL of sterile water for IV bolus injection (5mg/mL)
Indication	[redacted]

1 Executive Summary

1.1 Recommendation

The results of clinical pharmacology and pharmacokinetic studies support the approval of the clinical trial drug product – Palifermin.

The sponsor should evaluate the potential of palifermin binding to low molecular weight heparin since it is used in the indicated clinical sitting.

1.2 Phase IV Commitments

The following post-market commitments are made:

(1) To complete a safety pharmacology/carcinogenicity study (Study 103599) in the Tg.rasH2 mouse model to evaluate the potential for palifermin to interact with tumor growth.

(2) To complete safety, pharmacokinetics and efficacy evaluation of palifermin in pediatric patients (Study 20010133) entitled "*A Phase 1/2 Dose-escalation Study to Evaluate the Safety, Pharmacokinetics (PK), and Efficacy of Palifermin in*

Children and Adolescents with Stage 1 (Unresected) and Stage 2 B-cell Non-Hodgkin's Lymphoma (B-NHL) Undergoing Multi-agent Chemotherapy".

(3) To submit the final clinical study report for study 20030142, a phase 1 study to evaluate the pharmacokinetics of palifermin in patients with renal insufficiency.

(4) To complete and submit data from study protocol 960226, a long-term observational follow-up study of subjects previously enrolled in any palifermin study conducted in the myelotoxic therapy setting. Subjects in this study continue to be followed until death or loss to follow-up.

(5) To complete and submit data from study protocol 990123, a long-term observational follow-up study of subjects with head and neck cancer previously enrolled in palifermin studies in the fractionated chemoradiotherapy setting. Subjects in this study continue to be followed until death or loss to follow-up.

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3 Summary of Clinical Pharmacology and Biopharmaceutics Findings

In this license application, 8 clinical studies provide information about the pharmacokinetic properties of palifermin; 6 of which were conducted in healthy volunteers, and 2 studies were conducted in subjects with hematologic malignancies receiving high-dose cytotoxic therapy followed by PBPCT.

Table 1. Palifermin Studies that Provided Pharmacokinetic Information

Study #	# Subjects Received Placebo	# Subjects Received Palifermin	Range of dose Route of Administration	# Dose/subject
		Healthy Volunteers		
Study 950170[a]	3	9	1.0, 10, or 30 μg/kg, SC	1
	4	12	1.0 or 10 μg/kg, SC	3
Study 960136[a]	5	16	0.2, 1, 5, 10 or 20 μg/kg, IV	1
	10	30	0.2, 1, 5, 10 or 20 μg/kg/day, IV	3
Study 970136 (Japan)	6	18	5.0, 10, 20 μg/kg, IV	1
Study 970276[a]	6	12	20 or 40 μg/kg/day, IV	3
Study 970290 (Japan)	0	4	10 μg/kg, IV	1
Study 20010192[a]	16	63	60, 90, 120, 160, 210 or 250 μg/kg, IV	1
Study 20030142[b]	renal impairment, ongoing			
		Subjects with Hematologic Malignancies		
Study 960189[c]	4	13	60 μg/kg/day, IV	6
Study 20010182	0	13	60 μg/kg/day, IV	6

[a] These studies provided information on the pharmacodynamic properties of palifermin, buccal biopsies at baseline and at a single time point (either 48 hrs or 72 hrs) post-dose.

[b] A study in volunteers with renal impairment is ongoing and is not included in the BLA

[c] A phase 1/2 dose-escalation study in subjects with Hodgkin's disease and non-Hodgkin's lymphoma.

Mechanism of Action: Palifermin (recombinant human KGF, rHuKGF) stimulates the growth of epithelial cells from a wide variety of tissues but, due to the restricted expression of the keratinocyte growth factor (KGF) receptor, has no known direct effect on other cell types. Palifermin has been shown to substantially reduce injury to the oral and gastrointestinal (GI) tract mucosa and salivary glands in numerous models of radiation-induced and chemotherapy-induced GI injury. The protective activity of palifermin has been attributed not only to its mitogenic effect on mucosal epithelium, which can result in both increased epithelial thickness (when dosed prior to cytotoxic insult) and improved recovery (when dosed shortly after myelotoxic therapy, before overt ulceration occurred), but also to its impact on intercellular junctions (which may increase the physical integrity of the tissue) and on various cytoprotective mechanisms. Because of these properties, palifermin has the potential to prevent epithelial injury caused by myelotoxic chemotherapy or radiation, and accelerates the healing process after the cytotoxic insult has occurred.

Single-Dose Pharmacokinetics: After single IV doses of 20 to 250 μg/kg (healthy subjects) and 60 μg/kg (cancer patients) of palifermin, serum drug concentrations declined rapidly (over 95% decrease) in the first 30 minutes postdose and a slight increase or plateau in concentration occurred at approximately 1- to 4-hours, followed by a terminal decline phase. Palifermin exhibited linear pharmacokinetics with extravascular distribution and an average terminal half-life ($t_{1/2}$) of 4.5 hours (3.3-5.7 hours). On average, total body clearance (CL) appeared to be 2- to 4-fold higher, and volume of

distribution at steady state (Vss) to be 2-fold higher in cancer patients compared with healthy subjects after a 60 μg/kg single dose of palifermin.

Multiple-Dose Pharmacokinetics: Not surprisingly given the short elimination half-life, no accumulation of palifermin, as measured by area under the concentration-time curve (AUC), occurred after 3 consecutive daily doses to healthy subjects (20 and 40 μg/kg/day) or to patients with hematologic malignancies (60 μg/kg/day).

Binding Proteins: It is known that endogenous KGF is a heparin binding protein. Palifermin is a recombinant human KGF and it has been showed that palifermin binds to heparin *in vitro*. Consequently, for the labeled indication, a statement has been included in the physician information to ensure that IV lines utilizing heparin are rinsed with saline prior to administration of palifermin.

Distribution, Excretion and Elimination: Volume of distribution at steady state (Vss) for palifermin was greater than total body water volume, indicating extracellular distribution of palifermin after IV administration. This result is consistent with the KGF receptor's prevalence on all epithelial cells, and the binding of palifermin to this receptor.

Renal elimination plays a role in the clearance of radiolabeled palifermin in rats. Approximately 11% of a radioactive dose administered to rats was recovered in the urine as trichloro-acetic acid perceptible radioactivity over 24 hours, suggesting that some intact palifermin and/or smaller fragments may be excreted in the urine. Exposure to palifermin, as measured by AUC, increased by approximately 2-fold in bilaterally-nephrectomized rats compared to that observed in sham-operated rats, also suggesting that the kidneys play a role in the elimination of palifermin. It is possible that other organs may also participate in the elimination of palifermin through its binding to the KGF receptor and internalization/breakdown within epithelial cells.

Metabolism: The expected consequence of metabolism of biotechnology-derived pharmaceuticals is the degradation to small peptides and individual amino acids, and the metabolic pathways are generally understood (ICH S6). As such, classical biotransformation studies as performed for pharmaceuticals are not conducted for biologic products. Because palifermin is a biologic molecule, no *in vitro* permeability, *in vitro* metabolism, or metabolic drug-drug interaction studies were performed for palifermin. However, palifermin has been shown to bind to heparin *in vitro* since KGF is a heparin-binding member of the fibroblast growth factor (FGF) family.

Drug-Drug Interactions: There were no formal *in vivo* nonclinical and clinical pharmacokinetic drug interaction studies conducted with palifermin but possibility was addressed as part of animal model evaluations. When granulocyte colony-stimulating factor (G-CSF), which is likely to be used in the indicated patient population, was used in combination with palifermin in murine and nonhuman primate chemotherapy/ radiotherapy models, there was no evidence of a drug interaction.

Pharmacokinetics in Special Populations: No formal studies were conducted to evaluate the effect of intrinsic or extrinsic factors on pharmacokinetics (PK) of palifermin except a study in subjects with renal impairment is ongoing, based on the observation of approximately 2-fold higher exposure in bilaterally-nephrectomized rats compared to sham-operated rats. The renal impairment study is not included in the BLA. The effects of age, weight, sex, and race/ethnicity on the PK of palifermin were explored using a combined dataset from studies in healthy subjects and another combined dataset from subjects with hematologic malignancies who participated in the two clinical studies. Neither weight nor sex appeared to have a notable effect on the PK of palifermin. Age did not appear to significantly alter the CL of palifermin, although the limited numbers of elderly patients preclude definitive conclusions. The effect of race/ethnicity on PK of palifermin was inconclusive because most of the subjects who provided the PK dataset were white; however, there is little rationale for race to influence the PK of palifermin.

Inter-Individual Variability in PK Data: Intersubject variability of approximately 40% has been observed for palifermin CL and up to 70% for Vss in the studies conducted in healthy subjects. A higher level of intersubject variability in palifermin CL and Vss was observed in patients with hematologic malignancies as evidenced by the wider range of parameter values. This higher variability could be related to underlying disease/treatment.

Rationale for Dose Selection: Early preclinical and clinical data in healthy subjects suggested that at the doses tested, 3 consecutive days of palifermin administration and 40 μg/kg/day were required to produce consistent biological activity on epithelial cells. In the phase 1/2 study, there were excess dose-limiting toxicities when 80 μg/kg/day was administered both before and after cytotoxic therapy. Based on these results, the dose of palifermin (60 μg/kg/day) was selected to use in the phase 2 and the phase 3 trials. Although a more recent pharmacology study in healthy subjects suggest that palifermin may be biologically active with higher single doses, the safety and efficacy of such a dosing strategy has not been investigated for the proposed indication.

Pharmacodynamic Findings: In buccal biopsies collected from healthy volunteers, a significant increase in Ki67 staining (a >200% increase in Ki67-stained area relative to baseline, a surrogate measure of epithelial cell proliferation) was observed up to 72 hours after intravenous administration of 3 consecutive daily doses of palifermin as low as 40 μg/kg/day or at single doses of 160 to 250 μg/kg. Of note, at 48 hours after dosing, the measured proliferation was highest but most of the quantifiable palifermin concentration values were less than twice the lower limit of quantification of the assay, indicating that the pharmacologic effect persists after active drug levels have dissipated.

Exposure-Response: The measurement of epithelial cell proliferation (as assessed by Ki67 staining) in the buccal mucosa before and after palifermin administration is a useful marker for palifermin's biologic activity. In a single dose study, analysis of epithelial cell proliferation demonstrated increased epithelial response with increased palifermin exposure at doses ranging from 60 to 250 μg/kg, with a plateau above 160 μg/kg. However, a clear correlation between exposure and efficacy/safety was not apparent.

Immunogenicity: As with all therapeutic proteins, there is a potential for immunogenicity. The clinical significance of antibodies to palifermin is unknown, but could include increased or decreased palifermin clearance, lessened its activity, and/or cross reactivity with FGF family members. A sensitive electrochemiluminescence-based binding assay was performed on post-treatment sera from 645 subjects treated with palifermin in clinical studies. Of these 12 of 645 patients developed reactive antibodies to palifermin. Positive samples were further tested in a cell-based neutralization assay, and none of the samples had evidence of neutralizing activity.

Safety Profiles: In all studies, palifermin was generally well tolerated. With the exception of asymptomatic increases in lipase and/or amylase concentrations, no notable potential safety issues were identified related to laboratory evaluations or vital signs. Most of adverse events (AEs) were consistent with the pharmacologic action of palifermin on skin and oral epithelium. These events were primarily mild to moderate in severity and were reversible. Serious AEs occurred at a similar rate in patients who received palifermin (20%) or placebo (21%). The most frequently reported serious AEs in both groups were fever, GI, and respiratory related.

Comparability among Product Lots: A series of analytical tests were performed to compare a lot manufactured at commercial scale (Lot) to a clinical lot manufactured using the second-generation process (Lot). The — lots are comparable by all the analytical measures, which confirmed expected primary and higher-order structures, variant profiles and potency. No PK study was required to evaluate the comparability between these two lots.

Conclusions: Palifermin exhibits linear pharmacokinetics in the dose range of 10 to 250 μg/kg after single-dose administration to healthy volunteers. Palifermin exhibits extravascular distribution with an average terminal half-life of 4.9 hours in healthy subjects and 4.4 hours in subjects with hematologic malignancies. After a single IV dose, CL and Vss were higher in subjects with hematologic malignancies than in healthy subjects. No accumulation of palifermin occurred after multiple dosing to healthy subjects (3 consecutive daily doses of 10, 20, and 40 μg/kg) or to subjects with hematologic malignancies (3 consecutive daily doses of 60 μg/kg). No apparent differences were observed in the PK of palifermin between men and women (M/F ratio was 29/15 in healthy subjects and 13/9 in patients). In buccal biopsies taken from healthy subjects, increases in epithelial cell proliferation (a >200% increase in Ki67-stained area relative to baseline) were observed in at least 50% of subjects at doses of 40 μg/kg/day administered for 3 consecutive days and at single doses of 120 to 250 μg/kg. The proposed clinical dose (60 μg/kg/day for 3 consecutive days before and after high-dose chemotherapy with PBSCT) for patients with hematologic malignancies is within the range of doses that have shown biological activity.

4 Question-Based Review (QBR)

4.1 General Attributes

1. ***What are the highlights of the chemistry and physical-chemical properties of the drug substance, and the formulation of the drug product? What is the proposed mechanism of drug action and therapeutic indication? What is the proposed dosage and route of administration?***

Chemistry and Physical-Chemical Properties: Palifermin is a recombinant form of human keratinocyte growth factor (KGF) produced in *Escherichia coli* (*E. coli*) by recombinant DNA technology. Purified palifermin, a non-glycosylated monomeric heparin-binding protein with a molecular weight of approximately 16.3 kilodaltons, contains a sequence of 140 amino acids that is identical to a portion of endogenous KGF. The main structural difference between palifermin and the native human KGF is that the first 23 N-terminal amino acids, [ ] have been deleted (designated "delta N23"), which gives the molecule greater thermal stability than the native form, but with similar biological activity.

Formulation: Palifermin is presented as a sterile, white, preservative-free, lyophilized powder with 6.25 mg of palifermin per vial to be reconstituted with 1.2 mL of Sterile Water for Injection to yield a 5 mg/mL solution for IV bolus injection.

Table 1. Palifermin Formulation

Composition	Function	Grade	Amount per vial (mg)
Palifermin	Active ingredient	in-house	6.25
Mannitol		USP/PhEur/JP	50
Sucrose		NF/PhEur	25
L-histidine		USP/PhEur	1.94
Polysorbate 20		NF	0.13 (0.01% w/v)
[			]

Mechanism of Action: The pharmacological activity of palifermin was demonstrated in a variety of species including the mouse, rat, and monkey. Results from these studies have shown that palifermin ameliorates the effects of injury to the mucosal lining of the oral cavity and aerodigestive tract induced by radiation and/or chemotherapeutic agents. The mechanism of action appears to be bimodal: a growth-enhancing differentiation and a cytoprotective effect throughout the gastrointestinal (GI) tract, both of which result in a thickening of the epithelial tissues (sequamous epithelium of the oral cavity and glandular tissue of the intestinal column). This pharmacological response has been shown to translate clinically into protection of the barrier and absorptive functions of the oral mucosa and gut in subjects exposed to cytotoxic (radiation and/or chemotherapy) therapies.

Indication: The sponsor proposed indication for palifermin is [

] in patients with hematologic malignancies receiving myelotoxic therapy requiring hematopoietic stem cell support. The FDA suggested indication for palifermin is to decrease the incidence and duration of severe oral mucositis in patients with hematologic malignancies receiving myelotoxic therapy requiring hematopoietic stem cell support. Safety and efficacy of Palifermin have not been established in patients with non-hematologic malignancies.

Dosage and Route of Administration: Palifermin is formulated for intravenous (IV) injection, as injection-site reactions were observed in healthy subjects who received palifermin subcutaneously (SC) at single doses of 10 and 30 μg/kg and after 3 consecutive daily doses of 1.0 and 10 μg/kg. The recommended dose of palifermin is 60 μg/kg/day, administered as an IV bolus injection for 3 consecutive days before and 3 consecutive days after myelotoxic therapy for a total of 6 doses. The first 3 doses should be administered prior to myelotoxic therapy, with the third dose 24 to 48 hours before myelotoxic therapy. The last 3 doses should be administered post-myelotoxic therapy; the first of these doses should be administered after, but on the same day of hematopoietic stem cell infusion and at least 4 days after the most recent palifermin administration.

*2. **What efficacy and safety information (e.g., biomarkers, surrogate endpoints, and clinical endpoints) contribute to the assessment of clinical pharmacology and biopharmaceutics study data (e.g., if disparate efficacy measurements or adverse event reports can be attributed to intrinsic or extrinsic factors that alter drug exposure/response relationships in patients)?***

None. In buccal biopsies collected from healthy volunteers, a significant increase in Ki67 staining (a >200% increase in Ki67-stained area relative to baseline, a surrogate measure of epithelial cell proliferation) was observed up to 72 hours after administration of multiple doses of palifermin as low as 40 μg/kg/day administered for 3 consecutive days or at single doses of 160 to 250 μg/kg.

4.2 General Clinical Pharmacology

*1. **What is the basis for selecting the response endpoints, i.e., clinical or surrogate endpoints, or biomarkers (also called pharmacodynamics, PD) and how are they measured in clinical pharmacology and clinical studies?***

Oral mucositis may cause significant pain, leading to consequent requirements for opioid analgesics and parenteral nutrition and increased risk of infections, limitations on patients' ability to function, and other complications. The safety and efficacy of palifermin were established in a randomized, placebo-controlled clinical trial (n=212) and a phase 2 dose-ranging study in patients with hematologic malignancies receiving cyclophospharmide/etoposide high-dose chemotherapy/TBI (fractionated total-body irradiation) with PBPC (peripheral blood progenitor cells) support. Palifermin was administered as a daily IV injection of 60 μg/kg for 3 consecutive days prior to initiation of cytotoxic therapy and for 3 consecutive days following infusion of PBPC.

Response Endpoints: The primary endpoint of the study, the number of days during which patients experienced severe oral mucositis (WHO Grade 3/4) was significantly reduced in patients received palifermin compared to patients received placebo in the phase 3 trial (median with 25th and 75th percentile was 3 days (0, 6) vs. 9 days (6, 13)). Improvements were also observed in other endpoints including the incidence of WHO Grade 4 oral mucositis (20% vs. 62%), days of WHO Grade 2/3/4 oral mucositis (8 days (4, 12) vs. 14 days (11, 19)), and severity of oral mucositis as well as clinical sequelae, such as mouth and throat soreness and the requirement for opioid analgesia.

Pharmacodynamic Marker: In buccal biopsies collected from healthy volunteers, a significant increase in Ki67 staining (a >200% increase in Ki67-stained area relative to baseline, a surrogate measure of epithelial cell proliferation) was observed up to 72 hours after IV administration of 3 consecutive daily doses of palifermin as low as 40 μg/kg/day (Study 970276) or at single doses of 160 to 250 μg/kg (Study 20010192).

Safety Profile: In all studies, palifermin was generally well tolerated. With the exception of asymptomatic increases in lipase and/or amylase concentrations, no notable potential safety issues were identified related to laboratory evaluations or vital signs. Most of adverse events (AEs) were consistent with the pharmacologic action of palifermin on skin and oral epithelium. These events were primarily mild to moderate in severity and were eversible. Serious AEs occurred at a similar rate in patients who received palifermin (20%) or placebo (21%). The most frequently reported serious AEs in both groups were fever, GI, and respiratory related. Prophylactic administration of glucocorticoids was not required by the protocol, despite the possibility that palifermin's known effects on skin and oral mucosa (eg, erythema, tongue edema) could be mistaken for allergic or hypersensitivity reactions. No adverse events occurred within a timeframe suggestive of immunologically-mediated allergic or hypersensitivity reactions.

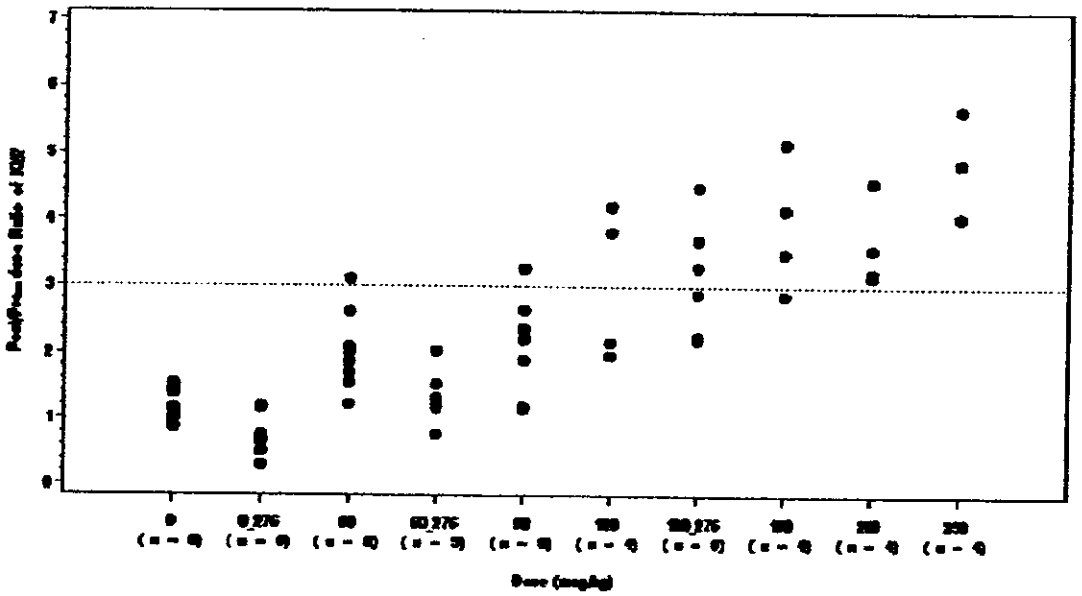
2. ***Are the active moieties in the plasma (or other biological fluid) appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationship? (if yes, refer to IV, F, Analytical Section; if no, describe the reasons)***

Serum palifermin concentrations were measured using a [ ] enzyme-linked immunosorbent assay (ELISA). Over the course of the palifermin clinical development program immunogenicity of palifermin was measured using 3 different immunoassays to test for anti-palifermin antibodies in clinical serum samples, and included a radioimmunoassay (RIA), an ELISA, and an electrochemiluminescence (ECL) assay. Each assay represents an improvement from its precursor. Samples determined to be reactive in any of these immunoassays were further tested for neutralizing activity in a cell-based assay (bioassay).

3. ***What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for efficacy and safety?***

Exposure-Response: The measurement of epithelial cell proliferation (as assessed by Ki67 staining) in the buccal mucosa before and after palifermin administration is a useful marker for palifermin's biologic activity. In Study 20010192, analysis of epithelial cell proliferation demonstrated increased epithelial response with increased palifermin exposure at single doses ranging from 60 to 250 µg/kg, with a plateau above 160 µg/kg when measured at 48 and 72 hours postdosing. At total doses of 120 µg/kg and higher, at least 50% of the subjects met the criterion for biologic activity (a >200% increase in Ki67-stained area relative to baseline, a surrogate measure of epithelial cell proliferation) when measured 24 hours after the third dose (Study 970276). The results are shown in Figure 1.

Figure 1. Ki67 Postdose to Predose Ratios after Single and Multiple Doses of Palifermin Administered to healthy Volunteers (Studies 970276 and 20010192) (from sponsor's submission)



Note: data includes Studies 970276 (at 18 to 24 hours after the last dose) and 20010192 (at 48 hours after the single dose)

Although palifermin has an average half-life of 4.9 hours in healthy subjects, significant pharmacologic effect (Ki67 staining) was observed at 48-hour postdose, with a smaller effect observed 72-hours postdose. At 48-hour postdose, 83% of the quantifiable concentration values (33 of 40) were low (below the assay detection limit of [redacted] ng/ml), suggesting that the effect of palifermin on epithelial tissue persists after active drug levels have dissipated. For biologics, persistence of effect (or a delayed effect) relative to drug concentrations is not unexpected.

A complete profiling of the concentration-response relationship of palifermin and PK/PD modeling was not feasible since a maximum of only 2 biopsies (predose and postdose) was permitted for each subject. However, the relationship between PD response (post-to-predose ratio for Ki67 staining) and exposure to palifermin, as measured by $AUC_{0\text{-inf}}$, was examined graphically using data obtained in Study 20010192 (see Figure 2). In general, increased response was observed with increased exposure.

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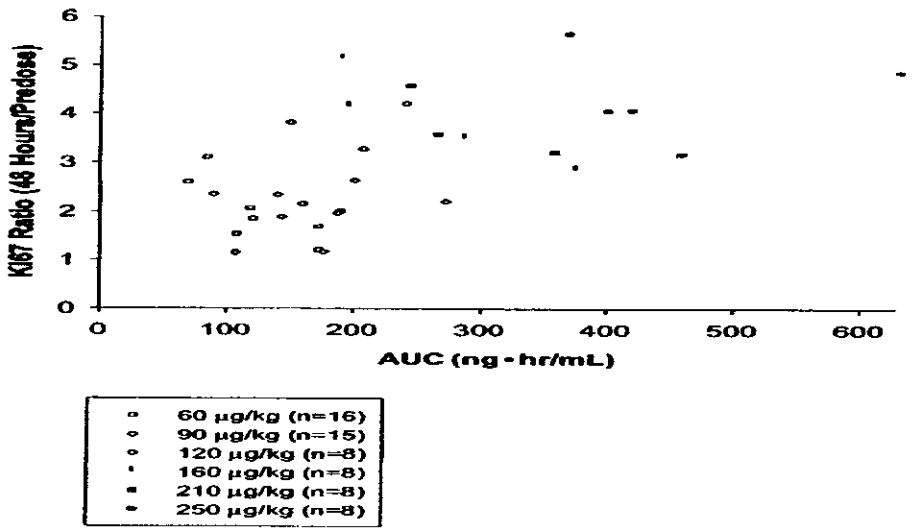
Figure 2. Pharmacodynamic Response (Ki67 Ratio) versus Exposure to Palifermin ($AUC_{0\text{-}inf}$) after Single-Dose Administration of Palifermin to Healthy Volunteers (Study 20010192) (from sponsor's submission)

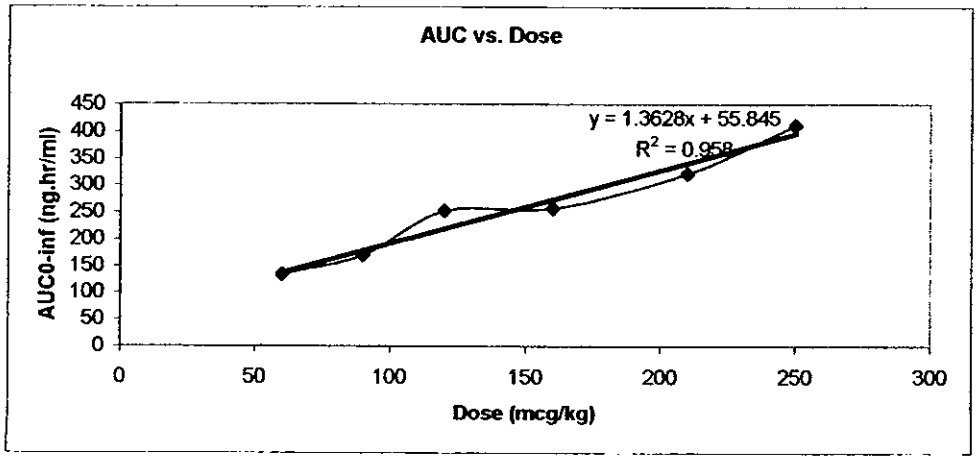


Based on the longer pharmacodynamic (PD) effect of palifermin compared with the PK, the PD endpoints appear to be more relevant to consider for dosing frequency than the PK endpoints.

a) Based on PK parameters, what is the degree of linearity or nonlinearity in the dose-concentration relationship?

Serum concentrations of palifermin increased with increased dose. AUC increased with doses in an approximately linear manner (see Figure 3). On average, exposure to palifermin, as measured by $AUC_{0\text{-}inf}$, increased approximately 3-fold for the 4-fold increase in dose administered (60 to 250 µg/kg). On average, most (>95%) of the AUC occurred in the first 24 hours postdose.

Figure 3. Plot of AUC vs. Palifermin Dose (generated by the reviewer)



b) How long is the time to the onset and offset of the pharmacological response or clinical endpoint?

The pharmacodynamic (PD) endpoints included assessments of buccal mucosal epithelial proliferation. A maximum of only 2 biopsies (predose and postdose) was permitted for each subject. Although palifermin has an average half-life of 4.9 hours in healthy subjects, pharmacologic effect (increase in Ki67-stained area relative to baseline, a surrogate measure of epithelial cell proliferation) was observed at 18 to 24 hours after the last dose in the 3 consecutive doses trial (Study 970276), and significant PD response (a >200% increase in Ki67-stained area relative to baseline) was observed at 48-hour postdose, with a smaller effect observed at 72-hours postdose in the single dose trial (Study 20010192). At 48-hour postdose, 83% of the quantifiable concentration values (33 of 40) were below the assay detection limit of [ ] ng/ml, suggesting that the effect of palifermin on epithelial tissue persists after active drug levels have dissipated.

c) Are the dose and dosing regimen consistent with the known relationship between dose-concentration-response, and are there any unresolved dosing or administration issues?

Dose Selection: Selection of the dose of palifermin (60 μg/kg/day) used in the phase 2 and the phase 3 trials was based on results from the phase 1/2 dose-escalation study (960189), and on knowledge from pharmacology in healthy volunteers. In the phase 1/2 study, although 80 μg/kg/day was well tolerated when administered only before cytotoxic therapy, there were excess dose-limiting toxicities when this dose was administered both before and after cytotoxic therapy. It was also known from pharmacology studies that a palifermin dose of 40 μg/kg/day was required to observe biological activity. Therefore, to maximize efficacy in this setting, a dose of 60 μg/kg/day was chosen to take forward in the subsequent phase 2 study (980231).

Dose Regimen: Early preclinical and clinical data in healthy volunteers suggested that at the doses tested, 3 consecutive days of palifermin administration were required to produce consistent biological activity on epithelial cells. Therefore, all early clinical studies incorporated groups of 3 consecutive daily doses and this paradigm was used in all studies in patients with hematologic malignancies receiving high-dose myelotoxic therapy. Although a more recent pharmacology study in healthy volunteers (20010192) suggests that palifermin may be biologically active with higher single doses, the safety and efficacy of such a dosing strategy has not yet been investigated for the proposed indication, and it is therefore not recommended at this time.

Dose Schedule: Preclinical and clinical data indicate that the dose schedule of palifermin administration in relation to the cytotoxic insult is important. In particular, preclinical data have shown that administration of palifermin on the same day as the cytotoxic insult resulted in a loss of the protective activity of palifermin. In clinical trials, administration of palifermin within 16 hours prior to high-dose etoposide resulted in an increased severity and duration of oral mucositis. Because of the potential sensitivity of rapidly

dividing epithelial cells to cytotoxic therapy, palifermin should be administered 24 hours before and approximately 24 hours after the administration of cytotoxic chemotherapy.

The safety and efficacy of palifermin used concurrently with chemotherapy or concurrently with radiation therapy has not been established, nor has the safety and efficacy of palifermin used in combination with radiation and chemotherapy been established when given immediately after radiotherapy, and before high dose chemotherapy starting less than 24 hours after the last fraction of radiation. To address this issue, the appropriate precautionary language has been incorporated into the proposed physician information.

4. How does the PK of the drug and its major active metabolites in healthy volunteers compare to that in patients?

On average, palifermin clearance (CL) and volume of distribution at steady state (Vss) appeared to be approximately 2- to 4-fold and 2-fold higher, respectively, in patients with hematologic malignancies, relative to healthy subjects, at the 60-μg/kg/day dose level. The cause of this difference is unclear, and may have partially resulted from intersubject and interoccasion variability in PK observed in the relatively small number of subjects who were studied, and may also be related to other variables such as age (mean age of 26 yrs for healthy subjects and 53 yrs for patients) and/or underlying disease/treatment. The elimination half-life was similar between healthy subjects and patients (4-6 hours).

a) What are the basic PK parameters?

Single Dose: The PK parameters generated from healthy subjects after IV single doses of palifermin administration are summarized in Table 2.

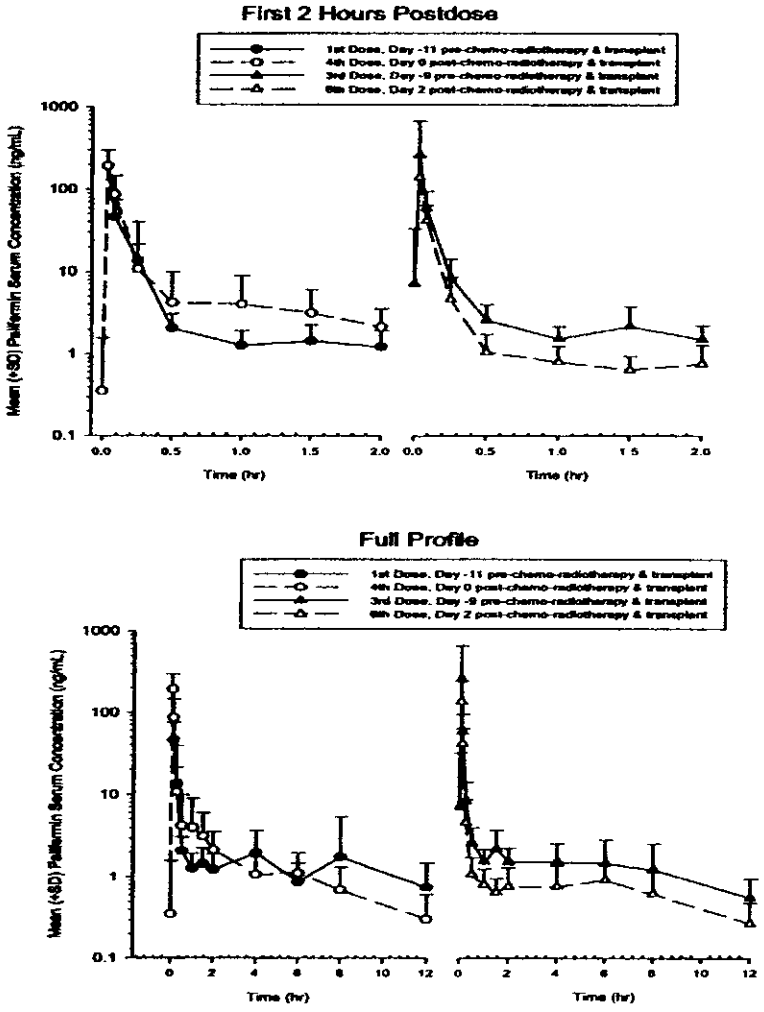
Table 2. Summary of Palifermin PK Parameters for Healthy Subjects in Study 20010192 after a Single IV Dose of Palifermin (Mean±SD)

Parameter Dose (μg/kg)	C_0 (ng/ml)	$T_{1/2}$ ((hr)	$AUC_{0\text{-}inf}$ (ng.hr/ml)	CL (ml/hr/kg)	V_{ss} (ml/kg)
60 (n=16)	891±660	4.50±0.58	134±40	494±171	2310±1600
90 (n=15)	1020±823	4.92±0.46	170±55	590±212	2900±1900
120 (n=8)	1530±1190	5.22±0.41	253±86	528±185	2790±1770
160 (n=8)	1590±1100	4.89±0.27	257±69	661±162	3130±1430
210 (n=8)	1700±1560	5.89±0.98	322±96	714±245	4630±1790
250 (n=8)	2720±2150	6.09±1.20	412±110	645±172	3570±2020

These data show that in healthy subjects serum concentrations of palifermin increased with increased dose. On average, exposure to palifermin, as measured by AUC from zero to infinity ($AUC_{0\text{-}inf}$), increased approximately 3-fold for the 4-fold increase in dose administered (60 to 250 μg/kg). On average, most (>95%) of the AUC occurred in the first 24 hours postdose. Mean $t_{1/2}$ values ranged from 4 to 6 hours across the dose levels evaluated. Mean Vss values were greater than body water, indicating extravascular distribution of palifermin. The CV% ranged from 25% to 36% for CL and from 46% to 69% for Vss.

***Three Consecutive Doses*:** Figure 4 shows that palifermin concentrations in patients received 60 μg/kg/day for 3 consecutive days before TBI and high-dose conditioning therapy and after PBSCT (6 doses total) declined rapidly (a 98% decrease on average) in the first 30-minutes postdose on all assessment days. After this rapid decline, a slight increase or plateau in concentration occurred at approximately 1- to 4-hours postdose, followed by a terminal decline phase.

Figure 4. Palifermin Concentration-time Profiles after the First and Third IV Doses (60 μg/kg/day) before Chemo- and Radiotherapy and after the Fourth and Sixth IV Doses (60 μg/kg/day) Post chemo- and Radiotherapy (Study 20010182 Part A) (from sponsor's submission)



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The concentration-time profile generated PK parameter values are summarized in Table 3. No accumulation of palifermin, as measured by AUC $_{0\text{-}24h}$ was observed after 3 consecutive daily doses. Mean $AUC_{0\text{-}t}$ values were comparable between doses 1 and 3 (last dose before chemo- and radiotherapy) and between doses 1 and 4 (first dose after chemo- and radiotherapy). The mean $AUC_{0\text{-}t}$ value after dose 6 was approximately 40% to 46% lower, on average, than that after dose 3 and dose 4, respectively. Although the reason for the lower exposure to palifermin after the sixth dose than after the third dose is not known, no antibodies to palifermin were observed in this study.

Table 3. Summary of Palifermin PK Parameters for Patients in Study 20010182 after 60-μ g/kg/day of IV Palifermin Administration (Mean±SD)

Parameter	$T_{1/2}$ (hr)	AUC_{0-t^*} (ng.hr/ml)	AUC_{0-inf} (ng.hr/ml)
First Dose (day -11, n=13)	4.87±2.02	34.3±15.9	37.8±17.9
Third Dose (day –9, n=13)	5.71±3.60	39.8±36.4	42.6±37.1
Fourth Dose (day 0, n=13)	3.27±1.27	34.8±22.5	35.8±22.4
Sixth Dose (day 2, n=13)	3.74±1.73	21.2±15.1	23.6±15.2

*The range of values for time was 8 to 24 hours for all profiles.

b) Is this a high extraction ratio or a low extraction ratio drug?

Exposure was not substantially different after administration into the hepatic portal vein versus femoral vein, indicating low hepatic extraction of palifermin in rats. No specific *in vitro* metabolism studies have been conducted with palifermin.

c) Does mass balance study suggest renal or hepatic the major route of elimination?

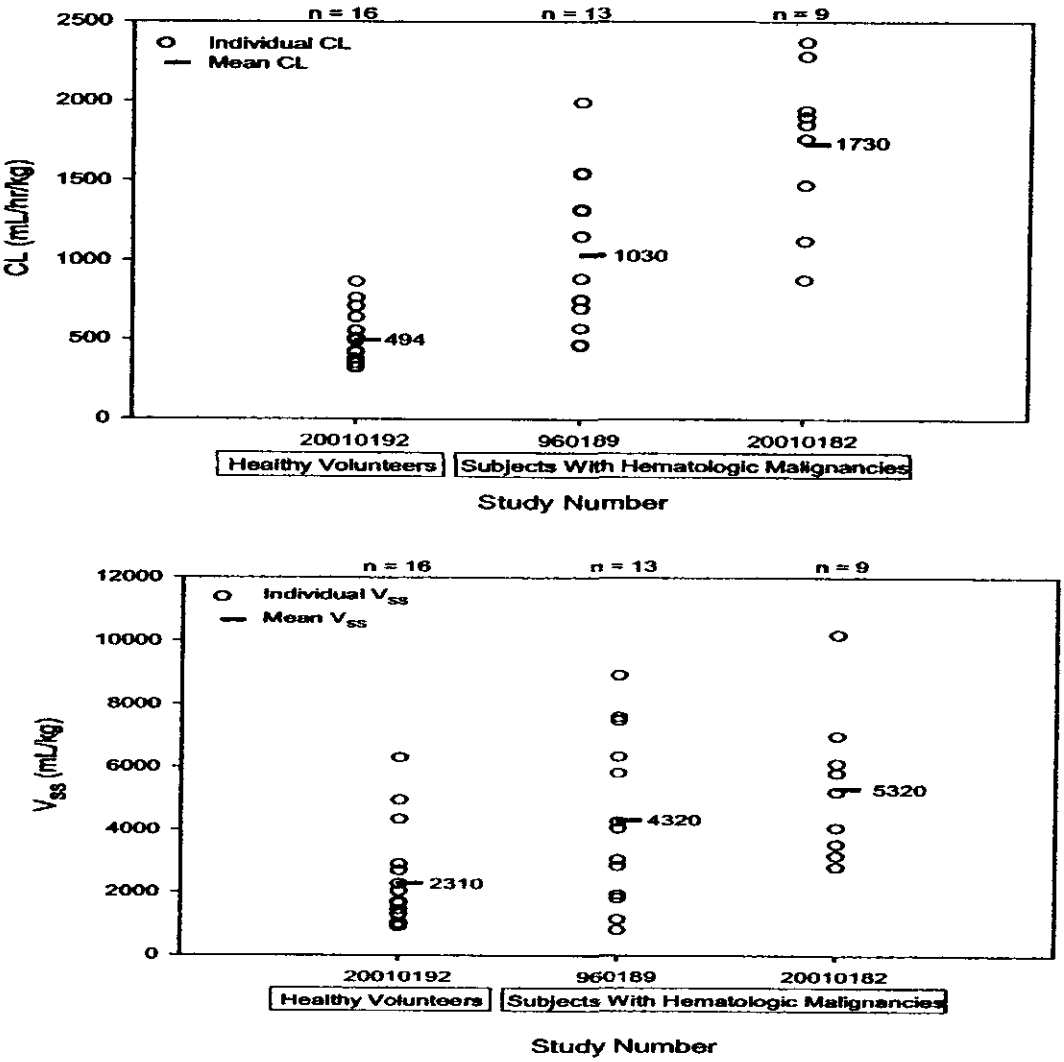
No mass balance study has been conducted for palifermin. Palifermin is a protein drug and mass balance studies are not generally performed for protein drugs because they are degraded into amino acids that then recycled into other proteins. The liver is not expected to play a major role in the metabolism of palifermin.

Renal elimination plays a role in the clearance of radiolabeled palifermin in rats. Approximately 11% of a radioactive dose administered to rats was recovered in the urine as trichloro-acetic acid perceptible radioactivity over 24 hours, suggesting that some intact palifermin and/or smaller fragments may be excreted in the urine. Exposure to palifermin, as measured by AUC, increased by approximately 2-fold in bilaterally-nephrectomized rats compared to that observed in sham-operated rats, also suggesting that the kidneys play a role in the elimination of palifermin. It is possible that other organs may also participate in the elimination of palifermin through its binding to the KGF receptor and internalization/breakdown within epithelial cells.

5. What is the inter- and intra-subject variability of PK parameters in volunteers and patients, and what are the major causes of variability?

Intersubject variability of approximately 40% has been observed in palifermin PK parameter of CL and up to 70% for Vss in the studies conducted in healthy subjects. A higher level of intersubject variability in CL and Vss was observed in patients with hematologic malignancies as evidenced by the wider range of parameter values (See Figure 5). The cause of this difference is unclear, and may have partially resulted from intersubject and interoccasion variability in PK observed in the relatively small number of subjects who were studied, and may also be related to other variables such as age (mean age of 26 yrs for healthy subjects and 53 yrs for patients) and/or underlying disease/treatment.

Figure 5. Clearance (CL, top graph) and Volume of Distribution at Steady State (Vss, bottom graph) of Palifermin after a Single IV Dose of 60 μg/kg to Healthy Volunteers (Study 20010192) and Subjects with Hematologic malignancies (Studies 960189 and 20010182) (from sponsor's submission)



4.3 Intrinsic Factors

1. ***What intrinsic factors (age, gender, race, weight, height, disease, genetic polymorphism, pregnancy, and organ dysfunction) influence exposure and/or response and what is the impact of any differences in exposure on the pharmacodynamics?***

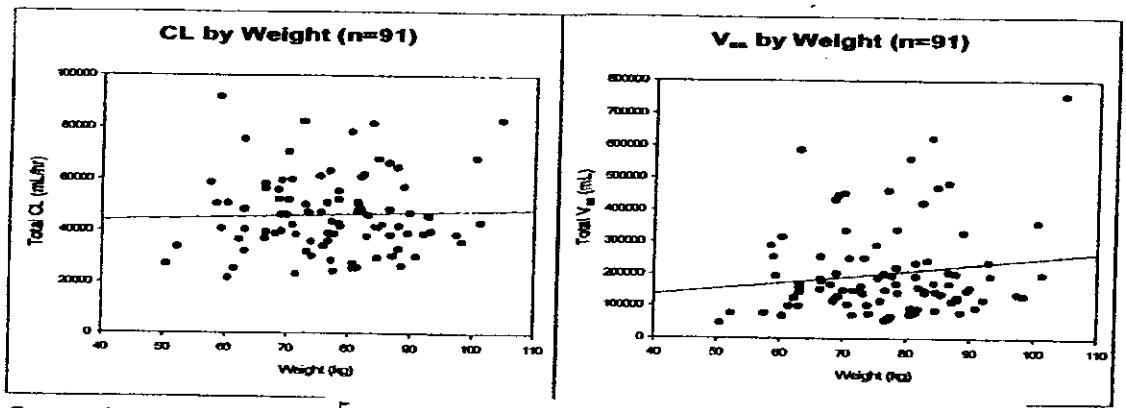
Pharmacokinetics in Special Populations: No formal studies were conducted to evaluate the effect of intrinsic or extrinsic factors on PK of palifermin except a study in subjects with renal impairment is ongoing, based on the observation of approximately 2-fold higher exposure in bilaterally-nephrectomized rats compared to sham-operated rats. The renal impairment study is not included in the BLA.

The effects of age, weight, sex, and race/ethnicity on the PK of palifermin were explored using a combined dataset from studies in healthy subjects and another combined dataset from subjects with hematologic malignancies who participated in the two clinical studies. Neither weight nor sex appeared to have a notable effect on the PK of palifermin. Age did not appear to significantly alter the CL of palifermin, although the limited numbers of elderly patients preclude definitive conclusions. The effect of race/ethnicity on PK palifermin was inconclusive because most of the subjects who provided the PK dataset were white; however, there is little rationale for race to influence palifermin PK.

Weight Effect on Palifermin PK In Healthy Subjects

Weight	Correlation
CL (n=91)	$R^2 = 0.002$, p=0.682
Vss (n=91)	$R^2 = 0.022$, p=0.157

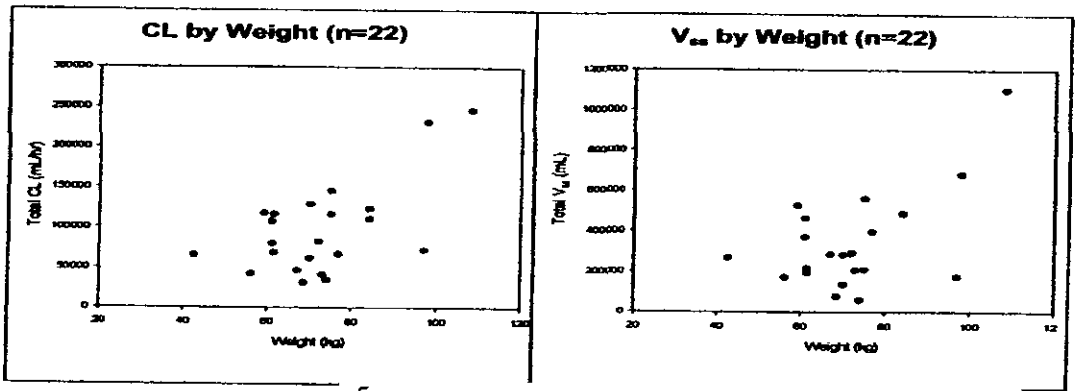
Figure 6. Clearance and Volume of Distribution at Steady State of Palifermin by Weight for Healthy Volunteers (Studies 960136, 970276, and 20010192) (Taken from the submission)



Source: documentum\Docbases\ PKDM\Clinical\FDA Submissions\BLA-NDA\Figures\ DN-AUC vs demog_HV_2-12-04.JNB\"Total CL vs Weight_current" and "Total Vss vs Weight_current" (created on 4/8/2004 4:05:27 PM)

Weight Effect on Palifermin PK in Patients

Figure 7. Clearance and Volume of Distribution at Steady State of Palifermin by Weight for Subjects with Hematologic Malignancies after 60 µg/kg Dose (Studies 960189 and 20010182) (Sponsor's plot)



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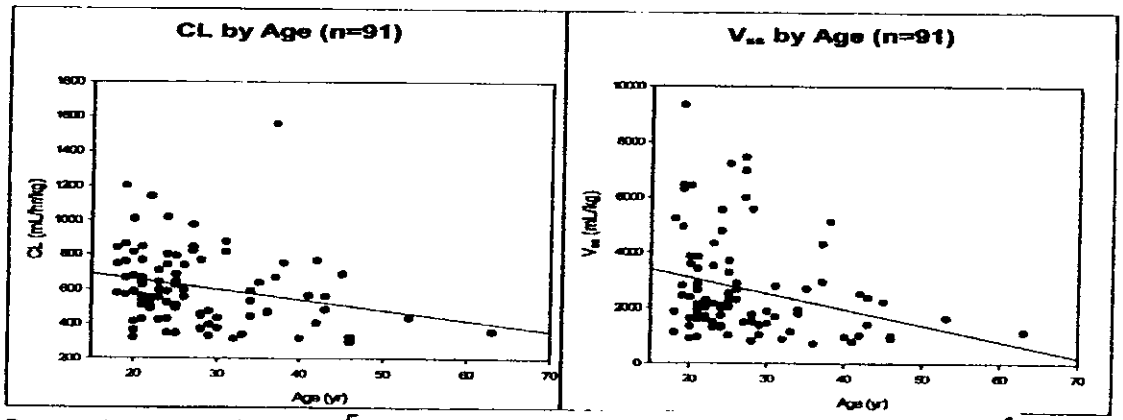
Age Effect on Palifermin PK in Healthy Subjects

Age	Correlation	Age attributed to variability
CL (n=91)	$R^2 = 0.055$, p=0.025	6%
Vss (n=91)	$R^2 = 0.077$, p=0.008	8%

Figure 8. Clearance and Volume of Distribution at Steady State of Palifermin by Age for Healthy Volunteers (Studies 960136, 970276, and 20010192) (Sponsor's plot)



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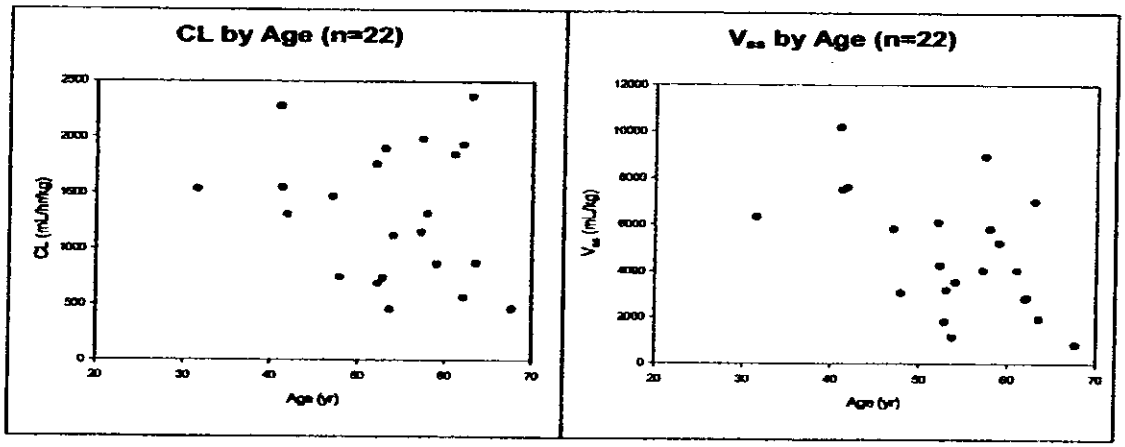
Age Effect on Palifermin PK in Patients

Figure 9. Clearance and Volume of Distribution at Steady State of Palifermin by Age for Subjects with Hematologic Malignancies after 60 μg/kg Dose (Studies 960189 and 20010182) (Sponsor's plot)



Source: documentum\Docbases\ PKDM\Clinical\FDA Submissions\BLA-NDA\Figures\ Patient PK&demog_2-12-04.JNB\"CL vs Age" and "Vss vs Age" (created on 4/8/2004 4:05:36 PM)

2. ***Based upon what is known about exposure-response relationships and their variability, and the groups studied (volunteers vs. patients); what dosage regimen adjustments, if any, are recommended for each of these subgroups (examples shown below)? If dosage regimen adjustments are not based upon exposure-response relationships, describe the alternative basis for the recommendation.***

a) Elderly

No formal PK study of palifermin was conducted in elderly patients (≥65 years). Clinical studies of palifermin did not include sufficient number of this patient population (9/409, 2%). Reported clinical experience has not identified differences in responses between the elderly and younger populations. In general, dose selection for an elderly patient should be cautious, usually starting at the low end of the dosing range, reflecting the greater frequency of decreased hepatic, renal, or cardiac function, and of concomitant disease or other drug therapy.

b) Pediatric Patients

The safety and effectiveness of palifermin in pediatric patients has not been established. As one of the post marketing commitments, the sponsor will conduct a multicenter, dose escalation study (Protocol 20010133) to evaluate the safety, pharmacokinetics and efficacy of palifermin in children and adolescents (N= 174) with Stage 1 (unresected) and Stage 2 B-cell Non Hodgkin's Lymphoma (B-NHL) undergoing multi-agent chemotherapy. The final study protocol will be submitted February 2005 and the study will be initiated by May 2005. Patient accrual will be completed by September 2007 and the study will be competed by November 2007. The final study report with revised labeling if applicable, will be submitted by February 2008.

c) Gender

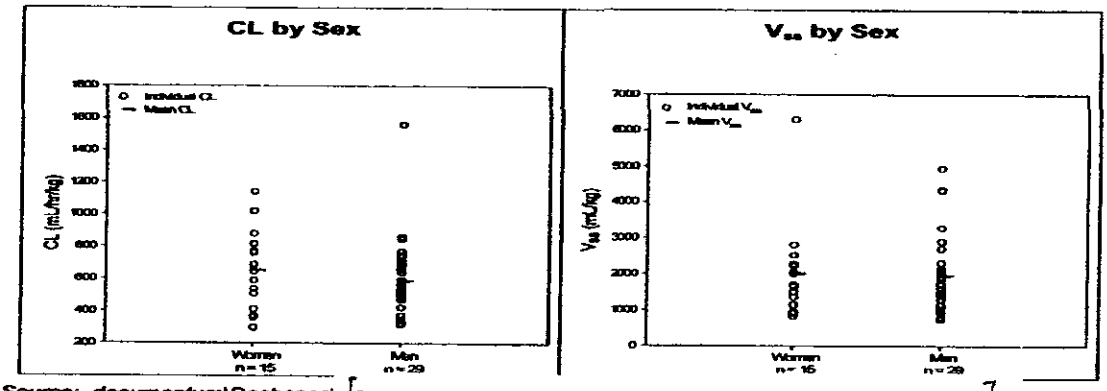
The data in the following table and the plot (taken from the submission) show that no gender-related differences were observed in the PK of palifermin at doses below 60 μg/kg. Data for doses higher than 60 μg/kg were not included in the gender analysis because majority subjects were men.

Gender Effect on Palifermin PK in Healthy Subjects

Sex	Male (n=29)	Female (=15)
CL (ml/hr/kg)	588±244	653±248
Vss (ml/kg)	1959±1086	2016±1332

*Data for doses higher than 60 μg/kg were not included because majority subjects were men.

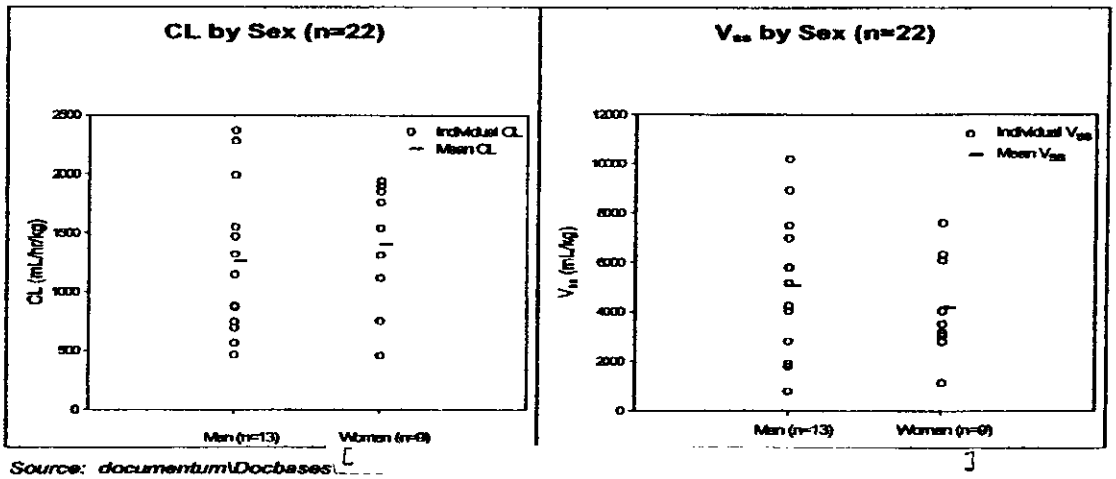
Figure 10. Individual Clearance and Volume of Distribution at Steady State of Palifermin by Sex for Healthy Volunteers (Dose Range of 10 to 60 μg/kg) (Studies 960136, 970276, and 20010192) (sponsor's plot)



Source: documentum\Docbases\ PKDMClinical\FDA Submissions\BLA-NDA\Figures\ DN-AUC vs demog_HV_2-12-04.JNB\"CL (mL/hr/kg) vs Gender" and "Vss (mL/hr/kg) vs Gender" (created on 4/8/2004 4:05:27 PM)

Gender Effect on Palifermin PK in Patients

Figure 11. Clearance and Volume of Distribution at Steady State of Palifermin by Sex for Subjects with Hematologic Malignancies after 60 μg/kg Dose (Studies 960189 and 20010182) (Sponsor's plot)



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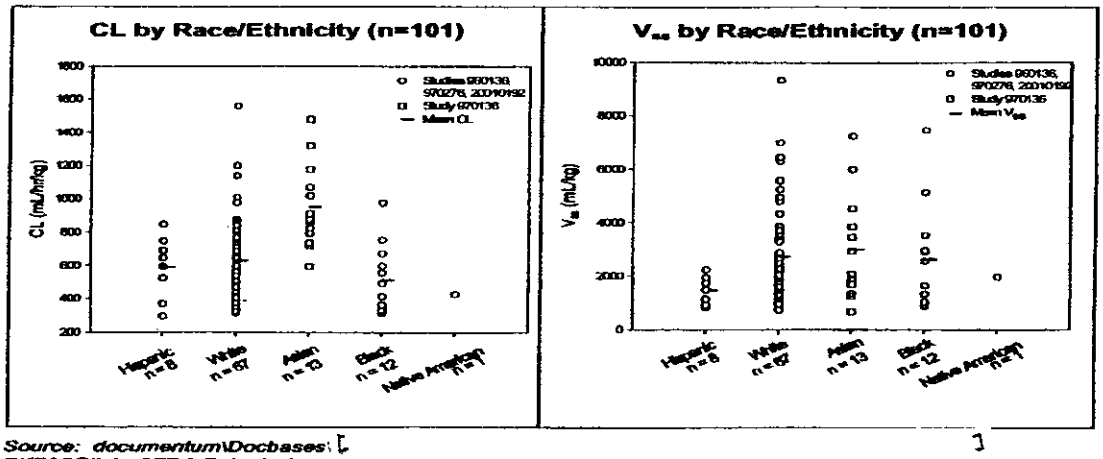
d) Race

In the population PK database from healthy subjects, most subjects were white. It was observed that Asians had 50% higher palifermin clearance than white and Hispanic had lowest Vss among the population studied. The effect of race/ethnicity on palifermin PK is inconclusive because limited number of non-white was enrolled in the PK studies.

Race/Ethnicity	White	Black	Hispanic	Asian (Japanese)
N	67	12	8	13
CL (ml/hr/kg)	629			953 (50% higher than in White)
Vss			Lowest	

Figure 12. Clearance and Volume of Distribution at Steady State of Palifermin by Race/Ethnicity for Healthy Volunteers (Studies 960136, 970276, and 20010192) (Sponsor's plot)



Source: documentum\Docbases\ PKDM\Clinical\FDA Submissions\BLA-NDA\Figures\ DN-AUC vs demog_HV_2-12-04.JNB\"CL vs Race_w/japanese" and "Vss vs Race_w/japanese" (created on 4/8/2004 4:05:27 PM)

e) Renal Impairment

In the clinical trials, subjects with hematologic malignancies were required to have normal renal and hepatic function before they could receive high-dose myelotoxic therapy requiring HSC support, consistent with standard-care practices. Based on the observation of approximately 2-fold higher exposure in bilaterally-nephrectomized rats compared to sham-operated rats, the role of renal clearance in the elimination of palifermin is being evaluated in subjects with varying degrees of renal impairment. This single dose study (20030142) is ongoing and is not included in this license application.

f) Hepatic Impairment

The liver is not expected to play a major role in the metabolism of palifermin. Studies in subjects with hepatic impairment were not performed.

g) What pregnancy and lactation use information is there in the application?

Pregnancy Category C: Palifermin has been shown to be embryotoxic in rabbits and rats when given in doses 2.5 and 8 times the human dose, respectively.

In rabbit - 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 μg/kg/day (2.5-fold higher than the recommended human dose). No evidence of developmental toxicity was observed in rabbits at doses up to 60 μg/kg/day.

In rats - 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 μg/kg/day (> 8-fold higher than the recommended human dose). No evidence of developmental toxicity was observed in rats at doses up to 300 μg/kg/day.

When palifermin was administered at IV doses up to 1,000 μg/kg in pregnant rats and up to 500 μg/kg in pregnant rabbits during gestation, palifermin levels in fetal serum and amniotic fluid were at or below the assay limit of quantitation (— ng/ml), suggesting negligible transplacental transfer. There are no adequate and well-controlled studies in pregnant women. Palifermin should be used during pregnancy only if the potential benefit to the mother justifies the potential risk to the fetus.

Lactating Women: Toxicokinetic/pharmacokinetic assessments have demonstrated negligible placental transfer of palifermin following a single high dose of 1,000 μg/kg in pregnant rats. It is not known whether palifermin is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when palifermin is administered to a nursing woman.

h) Other factors that are important to understanding the drug's efficacy and safety

Immunogenicity: As with all therapeutic proteins, there is a potential for immunogenicity. The presence of anti-drug antibodies may alter the PK of a biologic by

potentially enhancing or decreasing the clearance of the biologic. The clinical significance of antibodies to palifermin is unknown, but could include lessened activity, and/or cross reactivity with FGF family members.

Over the course of the palifermin clinical development program immunogenicity of palifermin was measured using 3 different immunoassays, each representing technical improvements over the previous one. The current assay is a sensitive electrochemiluminescence-based binding assay with a newly validated 21 CFR Part 11-compliant reader [ ] The assay was performed on post-treatment sera from 645 subjects treated with palifermin in clinical studies. Of these 12 of 645 patients developed reactive antibodies to palifermin. Positive samples were further tested in a cell-based neutralization assay; and none of the samples had evidence of neutralizing activity.

Table 4. Summary of Immunogenicity Testing Results (All subjects who received placebo or palifermin)

Immunoassay	Tested for Antibodies n (%)	Reactive[a] n (%)	Final Positive n	Bioassay Positive n
RIA	546 (51)	17 (3)	0	0
ELISA	258 (24)	110 (43)	0	0
ECL-based [ ]	317 (29)	2 (<1)	0	0
ECL-based [ ]	645	12 (2%)	2	0

[a] These were false reactive results since the samples were confirmed to be negative by subsequent assays.
All results refer to postdose samples.

The incidence of antibody positivity is highly dependent on the specific assay and its sensitivity. Additionally, the observed incidence of antibody positivity in an assay may be influenced by several factors including sample handling, timing of sample collection, concomitant medications and underlying disease. For these reasons, comparison of the incidence of antibodies to palifermin with the incidence of antibodies to other products may be misleading.

4.4 Extrinsic Factors

1. ***What extrinsic factors (drugs, herbal products, diet, smoking, and alcohol use) influence exposure and/or response and what is the impact of any differences in exposure on pharmacodynamics?***

None of the extrinsic factors including drugs, herbal products, diet, smoking, and alcohol use were studied for their influence on palifermin exposure and/or response.

2. ***Based upon what is known about exposure-response relationships and their variability, what dosage regimen adjustments, if any, do you recommend for each of these factors? If dosage regimen adjustments across factors are not based on the exposure-response relationships, describe the basis for the recommendation.***

None.

3. Drug-Drug interactions

No studies on the metabolism of palifermin have been performed in humans or in animals. Metabolism studies are not generally performed for protein drugs because they are degraded into amino acids that then recycled into other proteins. This fact has been recognized in ICH Topic S6 (Note for Guidance on Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals, dated July 16, 1997), where it is stated, "the expected consequence of metabolism of biotechnology-derived pharmaceuticals is the degradation to small peptides and individual amino acids" and that therefore classical biotransformation studies as performed for pharmaceuticals are not needed" No *in vitro* drug-drug interaction studies have been performed since P_{450} enzyme system is not expected to play any role in palifermin biotransformation. Specific drug interaction studies between palifermin and other drugs have not been performed.

a) Is there an in vitro basis to suspect in vivo drug-drug interaction?
Palifermin is a recombinant human KGF, and KGF is a heparin-binding member of the fibroblast growth factor (FGF) family. Palifermin has been shown to bind to heparin *in vitro.* In the DOSAGE AND ADMINISTRATION section of labeling, there is a statement of using saline instead of heparin to rinse the IV line prior to and after palifermin administration. The potential of palifermin binding to low molecular weight heparin should be evaluated since it is used in the indicated clinical setting.

b) Is the drug a substrate of CYP enzymes?
Not likely.

c) Is the drug an inhibitor and/or an inducer of CYP enzymes?
Not likely.

d) Is the drug a substrate and/or an inhibitor of P-glycoprotein transport processes?
Not likely.

e) Are there other metabolic/transporter pathways that may be important?
Unknown.

f) Does the label specify co-administration of another drug (e.g., combination therapy in oncology) and if so, has the interaction potential between these drugs been evaluated?
Subjects who were undergoing myeloablative therapy routinely received granulocyte colony-stimulating factor (G-CSF) after PBSCT. Possibility of the interaction between G-CSF and palifermin was addressed as part of animal model evaluations. When G-CSF was used in combination with palifermin in murine and nonhuman primate chemotherapy/radiotherapy models, there was no evidence of a drug interaction.

In clinical trials, administration of palifermin within 14 hours prior to high-dose etoposide was not efficacious and resulted in an increased severity and duration of oral mucositis. Because of the potential increased sensitivity of rapidly dividing epithelial

cells, palifermin should not be administered within 24 hours before, during infusion of, or within 24 hours after administration of myelotoxic chemotherapy.

The safety and efficacy of palifermin used concurrently with chemotherapy or concurrently with radiation therapy has not been established, nor has the safety and efficacy of palifermin used in combination with radiation and chemotherapy been established when given immediately after radiotherapy, and before high dose chemotherapy starting less than 24 hours after the last fraction of radiation. To address this issue, the appropriate precautionary language has been incorporated into the proposed physician information.

g) Are there any in vivo drug-drug interaction studies that indicate the exposure alone and/or exposure-response relationships are different when drugs are co-administered?

None.

h) Is there a known mechanistic basis for pharmacodynamic drug-drug interactions, if any?

None.

i) Are there any unresolved questions related to metabolism, active metabolites, metabolic drug interactions or protein binding?

None.

4.5 General Biopharmaceutics

1. What is the in vivo relationship of the proposed to-be-marketed formulation to the pivotal clinical trial formulation in terms of comparative exposure?

Palifermin lots used in clinical studies are listed in Table 5.

Table 5. Palifermin Lots Used in the Clinical Studies

Study #	Palifermin Lot#
950170 (SC) 960136 (IV)	2400F5
970136, 970276, 970290	24004H6
20010192	24A009082
960189	24010K8, 2400F5, 24004H6, 24006M7, 24007D8
20010182	24010J0
980231 (Phase 2)	24010C9, 24010K8
20000162 (Phase 3)	24010J0, 24A009082

Lot manufactured at commercial scale (Lot [ ]

The site and scale of manufacture for the drug substance and drug product has been changed from that used for production of material for clinical studies at Amgen Inc, Thousand Oaks, California. Drug substance at the increased scale will be manufactured at Amgen's commercial site in Boulder, Colorado, and drug product will be manufactured at [ ] The increase in scale and the

change in sites were implemented after the completion of the preclinical and clinical study program; consequently, no preclinical or clinical experience with palifermin manufactured at the new scale and sites has been accrued. The change to the manufacturing procedures required to accommodate the changes in scale and sites are considered to have minimum potential to alter the product quality.

To address comparability, the sponsor conducted a comprehensive, prospective designed analytical assessment comparing the drug substance and drug product manufactured at the commercial sites (Lot [ ] with that manufactured at the clinical site (Lot 24010). The 2 lots are comparable by all the analytical measures, which confirmed expected primary and higher-order structures, variant profiles and potency. The relative potencies determined separately using the cell-based bioassay are [ ] for the two lots, respectively.

On the basis of this assessment and in accordance with regulatory guidance on the topic of assessing the comparability of biotechnology products (ICH Q5E) additional nonclinical or clinical studies are not considered to be necessary, as changes to the quality and consequent safety or efficacy of palifermin are not expected.

a) What are the safety or efficacy issues, if any, for BE studies that fail to meet the 90% CI using equivalence limits of 80-125%?

Not applicable.

b) If the formulations are not BE, what dosing recommendations should be made that would allow approval of the to-be-marketed formulation? (e.g., dosage adjustments may be made for injectables)

Not applicable.

2. ***What is the effect of food on the bioavailability (BA) of the drug from the dosage form? What dosing recommendation should be made, if any, regarding administration of the product in relation to meals or meal types?***

Not applicable because palifermin is given via intravenous injection.

3. When would a fed BE study be appropriate and was one conducted?

Not applicable.

4. How do the dissolution conditions and specifications assure in vivo performance and quality of the product?

Not applicable.

4.6 Analytical

1. How are the active moieties identified and measured in the plasma in the clinical pharmacology and biopharmaceutics studies?

A [ ] enzyme-linked immunosorbent assay (ELISA) was utilized to quantify palifermin (rHuKGF) in human serum. For determination of anti-palifermin antibodies, 3 different immunoassays including radioimmunoassay (RIA), ELISA, and an electrochemiluminescence (ECL)-based assay were employed over the course of the palifermin clinical development program. Each assay represents an improvement from its precursor.

2. Which metabolites have been selected for analysis and why?

None.

3. For all moieties measured, is free, bound or total measured? What is the basis for that decision, if any, and is it appropriate?

Not applicable because palifermin is a protein.

4. What bioanalytical methods are used to assess concentrations?

Assay for Palifermin Concentration Determination: Serum palifermin concentrations were determined by an ELISA method. The following validation characteristics were assessed during method validation for all matrices except rat: accuracy and precision, assay range, dilutional linearity, specificity, and stability.

Table 6. Palifermin Analytical Methods and Validation Results (ELISA)

Study No. 970136, 970276, 960136, 950170, 970290		960189	20010182, 20010192
Method validation No.	[ ]		101872/102155/103674
Primary Ab	Mouse anti-KGF 1G4		
Secondary Ab	Mouse anti-KGF A1		
Calibration curve	log-log regression or 4-parameter regression depending on the specific assays		
Standard curve range (ng/ml)	[ ]		
LLOQ (ng/ml)			[ ]
Accuracy	— %		
Precision	— %		
Stability	stable for — freeze-thaw cycles		
Long-term Stability	stable for — days when stored at – —		

Stated concentrations are in 50% human serum matrix except for Studies 20010182 and 20010192.

Assays for Immunogenicity Testing: The anti-palifermin antibody immunoassay is an ELC technique that utilizes the bivalent characteristics of antibody molecule. In this assay pooled human serum (PHS) is used as a negative control, and an affinity-purified rabbit polyclonal anti-human palifermin antibody serves as the positive control. The sensitivity of this assay is validated to be — ng/ml. The mean ECL values obtained with the sample (n=2 replicates) are divided by the mean ECL values obtained with PHS (n=8 replicates) to obtain a S/N ratio.

Table 7: Criteria for Anti-Palifermin Antibody Reactivity by ECL Assay

S/N ratio	[		]
	Negative	positive below quantitation limit	reanalyzed[1]
If confirmed positive by reanalyzing and — (Post-dose/Pre-dose Ratio)			
	Positive		

[1]Samples with S/N ratios equal to or greater than — are re-analyzed in the ECL-based — assay following an [ ] by addition of 800 ng/ml unlabeled palifermin; they are confirmed positive if they show at least 50% reduction in the signal.

Cell-based Anti-Palifermin Neutralizing Antibody Bioassay: Samples that yield S/N ratio equal to or greater than 1.21 in the ECL-based immunoassay are tested in this bioassay at a 1:100 dilution for the presence of neutralizing antibodies to palifermin. The bioassay uses the murine cell line, 32D, which is transfected with a gene encoding the human KGF receptor/erythropoietin receptor chimera (KGFR/EPOR). The transfected 32D (KECA) cells respond to palifermin (pg/ml) with proliferation and are dependent upon recombinant murine interleukin-3 (IL-3) for routine culturing and growth. The palifermin- or IL-3-induced proliferation is determined by measuring [ ] into the cellular DNA. Palifermin-induced proliferation can be inhibited by pretreatment of palifermin with an anti-palifermin polyclonal neutralizing antibody.

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

_____ § 552(b)(5) Deliberative Process

✓ § 552(b)(5) Draft Labeling

Individual Study Reviews

Clinical Pharmacology and Pharmacokinetics Program

Overall Assessment of Clinical Pharmacology: In this license application, 8 clinical studies provide information about the pharmacokinetic properties of palifermin. Six (6) of 8 studies were conducted in healthy volunteers (dose range: 0.2 to 250 μg/kg, 164 subjects total) after single and multiple injections. The other 2 studies were conducted in subjects with hematologic malignancies receiving high-dose cytotoxic therapy followed by PBPCT (dose: 60 μg/kg/day, 3 consecutive daily IV administrations before high-dose myelotoxic conditioning treatment (26 patients total) and 3 consecutive daily IV administrations after PBPC (13 patients).

Table 1. Palifermin Studies that Provided Pharmacokinetic Information

Study #	# Subjects received Placebo	# Subjects received Palifermin	Range of dose	# Dose/subject
		Healthy Volunteers		
Study 950170[a]	3	9	1.0, 10, or 30 μg/kg, SC	1
	4	12	1.0 or 10 μg/kg, SC	3
Study 960136[a]	5	16	0.2, 1, 5, 10 or 20 20 μg/kg, IV	1
	10	30	0.2, 1, 5, 10 or 20 μg/kg/day, IV	3
Study 970136 (Japan)	6	18	5.0, 10, 20 μg/kg, IV	1
Study 970276[a]	6	12	20 or 40 μg/kg/day, IV	3
Study 970290 (Japan)	0	4	10 μg/kg, IV	1
Study 20010192[a]	16	63	60, 90, 120, 160, 210 or 250 μg/kg, IV	1
Study 20030142[b]		renal impairment, ongoing		
		Subjects with Hematologic Malignancies		
Study 960189[c]	4	13	60 μg/kg/day, IV	6
Study 20010182	0	13	60 μg/kg/day, IV	6

[a] These studies also obtained buccal biopsies at baseline and at a single time point (either 48 hrs or 72 hrs) post-dose.
[b] A study in volunteers with renal impairment is ongoing and is not included in the BLA
[c] A phase 1/2 dose-escalation study in subjects with Hodgkin's disease and non-Hodgkin's lymphoma.

In addition to PK information, 4 of these studies (Studies 950170, 960136, 970276, and 20010192) provided information on the PD properties of palifermin (buccal mucosal epithelial proliferation, Ki67 staining). The effect of demographic variables (age, sex, weight, and race/ethnicity) on the PK of palifermin was examined using a combined dataset from IV studies carried out in healthy volunteers in the U.S., including Studies 960136, 970276, and 20010192, and a second combined dataset from subjects with hematologic malignancies who participated in Studies 960189 and 20010182.

Overview of Human Biomaterial Studies: Because palifermin is a biologic molecule, no *in vitro* permeability, *in vitro* metabolism, or metabolic drug-drug interaction studies were performed for this program that used human biomaterials.

Overview of Studies Characterizing PK and PD of Palifermin: Palifermin was administered to healthy volunteers in 6 studies (see Table 1). Study 950170 was

conducted using the SC route of administration at 3 single-dose levels (1.0, 10, or 30 μg/kg) and at 2 multiple-dose levels (1.0 or 10 μg/kg/day) for 3 consecutive days). As a result of the number of subjects who reported injection-site reactions in this study, the SC route of administration was abandoned, and all subsequent clinical studies were conducted using the IV route of administration.

Three (3) IV studies evaluated both PK and PD of palifermin in healthy volunteers (Studies 960136, 970276, and 20010192). In all 3 studies, PD data were collected to measure epithelial cell proliferation in buccal mucosal biopsies, which were taken at baseline and at a single time point (18, 24, 48 or 72 hours) after placebo or palifermin administration.

In all of the studies mentioned above, PK assessments were conducted using frequent serial sampling. A sensitive, specific, accurate, and reproducible enzyme-linked immunosorbent assay (ELISA) was used to quantify palifermin concentrations in human serum. Subjects were monitored throughout each study for the development of anti-palifermin antibodies and to characterize any positive results.

In selective studies, the PD endpoints included measures of cellular proliferation that included assessments of buccal mucosal proliferation, as measured by Ki67 staining. Additionally, forearm epithelial proliferation and thickness was evaluated in Study 970276. No evidence of forearm epithelial proliferation was observed as a result of palifermin treatment, and all discussion of Ki67 staining therefore refers to buccal mucosal epithelial biopsies.

The studies in healthy volunteers enrolled a population of mostly young, white men, and thus is not fully representative of the patient population (in particular in terms of age).

Palifermin is formulated for intravenous (IV) injection, as injection-site reactions were observed in healthy volunteers who received palifermin subcutaneously (SC) at single doses of 10 and 30 μg/kg and after 3 consecutive daily doses of 1.0 and 10 μg/kg (Study 950170). The proposed dosing regimen for the treatment of oral mucositis associated with hematologic malignancies is 60 μg/kg/day for 3 consecutive days administered before chemo- and radiotherapy and 3 consecutive days after chemo- and radiotherapy and PBSCT. Single doses up to 250 μg/kg have been safely administered to healthy volunteers.

Distribution: In healthy volunteers and in patients with hematologic malignancies, the PK profile was similar, with a rapid decline of palifermin concentrations in the first 30 minutes post dose. After this rapid decline, a slight increase or plateau in concentrations occurred at approximately 1- to 4-hours post dose, followed by a terminal decline phase. Volume of distribution at steady state (Vss) was greater than total body water volume, indicating extracellular distribution of palifermin after IV administration. This result is consistent with the KGF receptor's prevalence on all epithelial cells, and the binding of palifermin to this receptor.

Elimination: An average terminal half-life ($t_{1/2,z}$) of approximately 4.5 hours was observed in both healthy volunteers and in patients with hematologic malignancies. On average, clearance (CL) and Vss appeared to be approximately 2- to 4-fold and 2-fold higher, respectively, in patients with hematologic malignancies, relative to healthy volunteers, at the 60-μg/kg/day dose level. The cause of this difference is unclear, and may have partially resulted from intersubject and interoccasion variability in PK observed in the relatively small number of subjects who were studied. It may also related to underlying disease/treatment.

Linearity and Accumulation: Palifermin exhibited linear PK in the dose range of 10 to 250 μg/kg in the studies conducted in healthy volunteers. Not surprisingly given the short $t_{1/2z}$, no accumulation of palifermin, as measured by area under the concentration-time curve (AUC), occurred after 3 consecutive daily doses to healthy volunteers (20 and 40 μg/kg/day) or to patients with hematologic malignancies (60 μg/kg/day).

Drug Interactions: Drug metabolism and specific drug interaction studies between palifermin and other drugs have not been performed. Subjects who were undergoing myeloablative therapy routinely received granulocyte colony-stimulating factor (G-CSF) after PBSCT; in controlled studies, no apparent difference was observed in neutrophil recovery between placebo and palifermin subjects.

Assays: A [ ] enzyme-linked immunosorbent assay (ELISA) was utilized to quantify palifermin (rHuKGF) in human serum. For determination of anti-palifermin antibodies, 3 different immunoassays including radioimmunoassay (RIA), ELISA, and an electrochemiluminescence (ECL)-based assay were employed over the course of the palifermin clinical development program. Each assay represents an improvement from its precursor.

Safety profiles: In all studies, palifermin was generally well tolerated. Most adverse events were mild to moderate in severity, and no deaths occurred. With the exception of asymptomatic increases in lipase and/or amylase concentrations, no notable potential safety issues were identified related to laboratory evaluations. Likewise, no notable safety issues were identified related to vital signs and no evidence of antibody formation was observed.

Pharmacokinetics

In Healthy Volunteers

Study 950170: This was the first-in-human palifermin study, and was the only study in which the SC route administration was evaluated. Because of the number of subjects who reported injection-site reactions, the study was closed early after the completion of 3 of the 7 planned single-dose cohorts (1.0, 10, and 30 μg/kg) and 2 of the 7 planned multiple-dose cohorts (1.0 and 10 μg/kg/day).

Subject Demographics: N=28 (7 placebo, 21 palifermin), 71% White, 57% females, ages between 19 to 42 years. No subject was withdrawn due to AEs.

PK Results:

Table 1. Summary of Palifermin PK Parameters for Healthy Volunteers in Study 950170 (Mean±SD)

Parameter		1 μg/kg	10 μg/kg	30 μg/kg
C_{max} (ng/ml)	single-dose (n=3)	ND	0.494±0.225	1.69±0.37
	Multi-dose Day 1 (n=6)	ND	0.391±0.086	
	Day 3 (n=6)	ND	0.640±0.259	
AUC_{0-t} (ng.hr/ml)	single-dose	ND	2.56±0.48	10.7±4.8
	Multi-dose Day 1	ND	1.32±0.53	
	Day 3	ND	2.60±1.06	

Safety Results: Though mild in severity and not requiring treatment, all (100%) of the subjects in the 30 μg/kg single-dose cohort and the 10-ug/kg multiple-dose cohort had injection-site reactions after SC administration of palifermin and the incidence of these events increased with increasing dose. An external pathologist examined the biopsy tissues from 3 of the subjects who experienced injection-site reactions and determined that the observed effects were the consequence of the biologic activity of palifermin on epithelial tissue at the site of injection, and were not the result of an allergic reaction.

Study 960136: This was a phase 1, dose-escalation, single-center study to determine the safety, tolerability, pharmacokinetic profile, and biologic activity (pharmacodynamics) of IV palifermin in healthy volunteers after a single dose and after 3 consecutive daily doses (0.2, 1.0, 5.0, 10, 20 μg/kg).

Subject Demographics: 61 subjects (15 placebo, 46 palifermin), 70% white, 57% males, 18 to 45 years of age, two withdraws, one in 0.2 μg/kg single dose group due to poor vein status, the other in 5 μg/kg/day group due to development of mild periorbital and conjunctival petechiae after receiving 2 of 3 doses.

PK Results:

Table 2. Summary of Palifermin PK Parameters for Healthy Volunteers in Study 960136 (Mean±SD)

Parameter	C_{max} (ng/ml)	T_{max} ((hr)	AUC_{0-t} (ng.hr/ml)	CL (ml/hr/kg)	V_{ss} (ml/kg)
Single dose (μg/kg)					
10 (n=2)	68.0	0.03	12.2	855	1800
20 (n=3)	168±49	0.03	34.0±4.3	595±81	1980±589
Multi-dose (μg/kg/day)	Day 1				
10 (n=5)	74.9±11.2	0.03	15.2±3.0	679±126	1530±589
20 (n=6)	143±63	0.03	25.6±10.5	905±391	1950±1240
	Day 3			AUC_{0-t} ratio (Day 3/Day 1)	
10 (n=5)	68.8±17.1	0.03	11.2±1.7	0.75±0.08	
20 (n=6)	102±60	0.03	17.8±6.0	0.73±0.17	

Three palifermin-treated subjects (21104, 0.2 μg/kg, 13002, 5 μg/kg; and 24006, 10 μg/kg) had baseline serum [palifermin concentrations of 0.774, 0.444 and 2.329 ng/ml,

respectively, which were above the detection limit [1 ng/ml). At 24 hours postdose, the concentrations were similar to the predose value in these 3 subjects, whereas other subjects had generally non-quantifiable concentrations at 24 hours post dose. These three subjects were not included in the PK data analyses. The full PK profiles could not be characterized for doses of 0.2, 1.0 and 5.0 mg/kg due to concentration values below the detection limit.

PD Results: Notable epithelial proliferative activity was not observed after palifermin single dose administrations. One subject met the criterion for epithelial proliferation (a ≥200% [3-fold] increase from baseline in Ki67-stained area) at multiple doses of 20 μg/kg/day for 3 consecutive days.

Safety Results: Overall, the incidence of adverse events did not appear to increase with increased dose in the single-dose or multi-dose cohorts and was generally similar between placebo and palifermin subjects.

Table 3. Reported at Least 1 Adverse Event

Treatment	Single-dose cohorts	Syncope	Multi-dose cohorts	Headache
Placebo	0/5	0	4/10 (40%)	2/10 (20%)
Palifermin	4/16 (25%)	2	15/30 (50%)	3/30 (10%)

In both syncope cases, reported as a vasovagal reaction (associated with venipuncture), was mild in severity and was not considered by the investigator to be related to treatment. The events lasted no longer than 5 minutes and occurred on the day of dosing.

Study 970136: This was a phase 1, randomized, double-blind, placebo-controlled, dose-escalation, single-center study to evaluate the safety, tolerability, and pharmacokinetics of single IV doses of 5-, 10-, or 20-μg/kg palifermin or matching placebo administered to healthy *Japanese men*.

Subject Demographics: Twenty-four (24) Japanese men (6 on placebo, 18 on palifermin) of 20 to 35 years of age completed the study.

PK Results:

Table 4. Summary of Palifermin PK Parameters for Healthy Volunteers in Study 970136 (Mean±SD)

Parameter Dose (μg/kg)	C_0 (ng/ml)	$T_{1/2}$ ((hr)	AUC_{0-t} (ng.hr/ml)	CL (ml/hr/kg)	V_{ss} (ml/kg)
5 (n=6)	64.2±27.2	2.40±0.90	5.06±1.28	1030±211	1720±278
10 (n=4)*	130±57	2.47±0.49	13.2±2.7	779±145	1470±641
20 (n=6)	212±81	4.67±0.75	19.2±5.4	1110±284	3080±1090

*The 2 subjects who were suspected of having been misdosed and having approximately 80% to 100% lower concentration values than the mean values were excluded from the mean calculation. These two subjects were redosed in Study 970290.

Summary: After single IV administration of palifermin, systemic exposure increased in an approximately dose-proportional manner between the 5- and 20-μg/kg dose cohorts. As dose increased by 4-fold (from 5 to 20 μg/kg), initial concentration (C_0) increased by 3.3-fold and AUC increased by 3.8-fold. The $t_{1/2}$ values ranged from 2.40 to 4.67 hours. Mean

Vss values were greater than total body water, indicating extravascular distribution of palifermin. The CV% ranged from 19% to 26% for CL and from 35% to 44% for Vss after a single administration of 10 and 20 μg/kg doses.

Safety Results: One laboratory abnormality was reported. A subject in the 20-μg/kg palifermin cohort had a mild, asymptomatic elevation in amylase concentration 48 hours after palifermin administration with the maximum elevation on Day 3 (<2 times the upper limit of normal).

Study 970290: This was a phase 1, open-label, single-center study to evaluate the pharmacokinetics of palifermin after IV administration of a single 10-μg/kg dose to 2 healthy Japanese men who were suspected to having been misdosed in Study 970136. Two subjects from the same dosing cohort in Study 970136 who had typical serum palifermin concentrations were included in this study as controls.

Subject Demographics: Four (4) Japanese men of 21 to 26 years of age completed the study.

PK Results:

Table 5. Summary of Palifermin PK Parameters for Healthy Volunteers in Study 970290 and Compared with Those in Study 970136 after 10 μg/kg Single IV Dose of Palifermin (Mean±SD)

Parameter	C_0 (ng/ml)	$T_{1/2}$ ((hr)	AUC_{0-t} (ng.hr/ml)	CL (ml/hr/kg)	V_{ss} (ml/kg)
Study 970290 (n=4)	116±73	4.55±2.37	10.8±4.1	1030±365	2790±1520
Study 970136 (n=4)	130±57	2.47±0.49	13.2±2.7	779±145	1470±641
Subject 141 (125)	223	3.95	16.0	624	1490
Subject 142 (122)	103	3.63	12.2	822	2150
Subject 143 (124)	60.8	8.00	8.04	1240	4980
Subject 144 (128)	76.8	2.63	7.08	1410	2540

*The 2 subjects who were suspected of having misdosed in Study 970136 (Subjects 122 and 125) were excluded from the mean calculations.

Summary: The 2 subjects who were suspected of having been misdosed in Study 970136, had concentration-time profiles in this study that were not markedly different from those of the 2 control subjects in this study. These results support the hypothesis that the unexpected observations in Study 970136 were likely caused by dosing error rather than by extremely high intersubject variability.

Safety Results: No adverse events were reported.

Study 970276: This was a phase 1, dose-escalation, single-center study to determine the safety, tolerability, pharmacokinetic profile, and pharmacodynamics (biologic activity) of palifermin versus placebo in healthy volunteers after a once-daily IV injection of placebo or palifermin for 3 consecutive days. Subjects received palifermin (20 or 40 μg/kg/day) or matching placebo.

Subject Demographics: Eighteen (18) subjects (6 on placebo, 12 on palifermin) were enrolled in this study, 78% white, 50% men, 18 to 63 years of age. One subject in 20-µg/kg/day cohort was withdrawn due to hematuria observed in a predose sample.

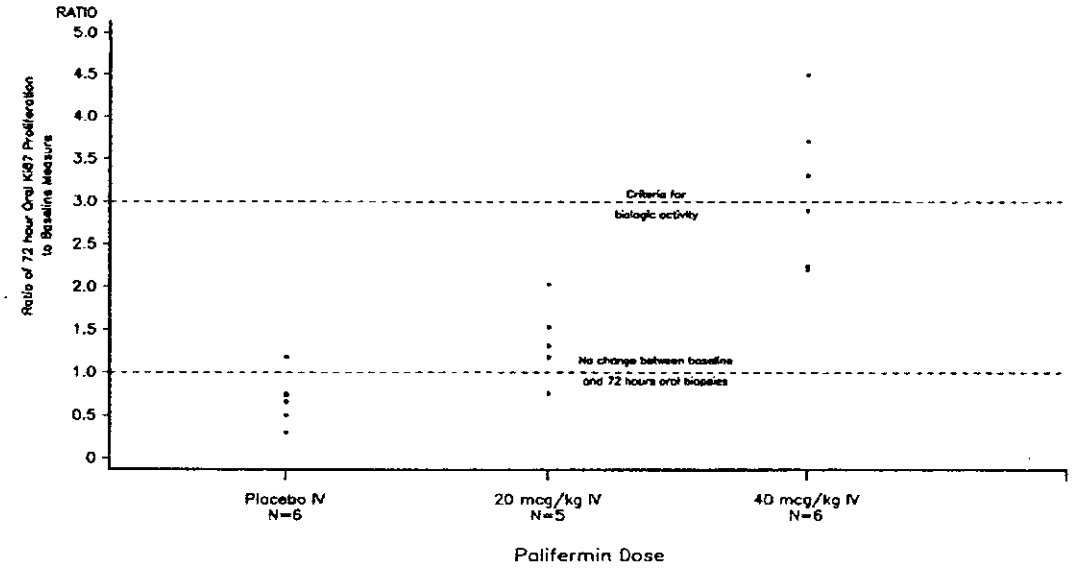
PK Results:

Table 6. Summary of Palifermin PK Parameters for Healthy Volunteers in Study 970276 (Mean±SD)

Parameter Dose	C_0 (ng/ml)	$T_{1/2}$ (hr)	$AUC_{0\text{-}24h}$ (ng.hr/ml)	$AUC_{0\text{-}inf}$ (ng.hr/ml)	CL (ml/hr/kg)	V_{ss} (ml/kg)
20 µg/kg/day						
Day 1(n=6)	275±132	3.31±0.59	36.5±11.7	36.7±11.9	596±194	1890±651
Day 3 (n=5)	601±878	6.21±2.69	41.4±20.6	41.9±20.3	558±222	1620±1110
40 µg/kg/day						
Day 1 (n=6)	861±531	3.78±0.26	84.6±29.1	85.3±29.2	509±142	1650±607
Day 3 (n=6)	797±457	21.4±16.0	73.7±23.9	76.1±23.1	563±148	2800±1660

PD Results: In the buccal mucosa, increased response with increased dose was observed in the Ki67-staining analysis. At 72-hours after the first dose (24-hours after the final dose), 3 of 6 subjects who received 40 µg/kg/day palifermin for 3 consecutive days met the predefined criterion for epithelial proliferation. (a ≥ 200% [3-fold] increase from baseline in Ki67-stained area).

Figure 1. Ratio of Palifermin Ki67 Oral Activity at 72 Hours Compared with Baseline (Study 970276)



Safety Results:

Table 7. Reported Adverse Events in Study 970276

AE	Placebo	Palifermin 20 µg/kg /day	40 µg/kg/day
At least 1 AE	3/6 (50%)	8/12 (67%)	83%
Most frequent AEs			
Back pain	0%	50%	
Erythema	0%	33%	
Elevated amylase and/or lipase			3 women

Table 7. (cont.) Elevation of Serum Amylase and /or Lipase in Study 970276

Serum Amylase Level (U/L) (Serum Lipase Levels U/L)
Normal range: 16 to 108 U/L, 7 to 60 U/L

	Baseline Day 3	Day 4	Day 5	Day 7
Subject 2001	28	66	79	63
Subject 2004	45 (18)	60 (30)	138 (131)	(112)44
Subject 2005	49 (16)	109 (66)	130 (91)	(80)39

The pharmacology of palifermin (epithelial cell proliferation, including pancreatic and salivary epithelial cells) would have predicted elevated amylase and lipase concentrations. Because of the elevated amylase and lipase levels, in conjunction with mild to moderate back pain, a decision was made not to continue dose escalation (up to 80 μg/kg/day).

Study 20010192: This was a phase 1, randomized, double-blind, placebo-controlled, dose-escalation, single-center study to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of palifermin versus placebo in healthy volunteers. Subjects received single IV doses of palifermin ranging from 60 to 250 μg/kg, or matching placebo.

Subject Demographics: A total of 84 subjects of 18 to 53 years of age were enrolled and of which 79 received placebo or palifermin (16 on placebo, 63 on palifermin). 76% white, 96% men.

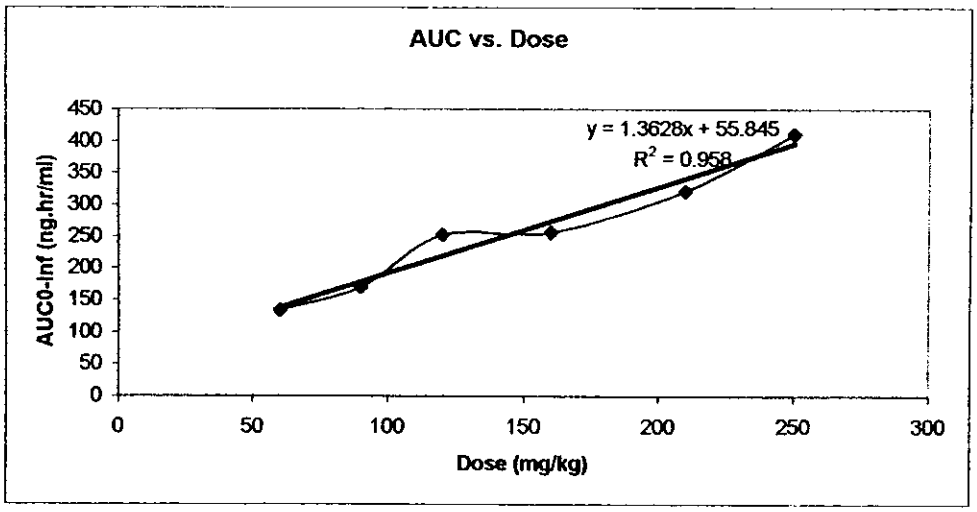
PK Results: Serum palifermin concentrations declined rapidly within the first 30 minutes after IV administration of palifermin at all dose levels. This decline was followed by an increase or plateau in concentration between 1.5 and 4 hours postdose. After 6 hours postdose, a consistent decline in concentration was observed over the remainder of the sampling time (up to 24 hours).

Table 8. Summary of Palifermin PK Parameters for Healthy Volunteers in Study 20010192 after a Single IV Dose of Palifermin (Mean±SD)

Parameter Dose (μg/kg)	C_0 (ng/ml)	$T_{1/2}$ ((hr)	$AUC_{0\text{-}inf}$ (ng.hr/ml)	CL (ml/hr/kg)	V_{ss} (ml/kg)
60 (n=16)	891±660	4.50±0.58	134±40	494±171	2310±1600
90 (n=15)	1020±823	4.92±0.46	170±55	590±212	2900±1900
120 (n=8)	1530±1190	5.22±0.41	253±86	528±185	2790±1770
160 (n=8)	1590±1100	4.89±0.27	257±69	661±162	3130±1430
210 (n=8)	1700±1560	5.89±0.98	322±96	714±245	4630±1790
250 (n=8)	2720±2150	6.09±1.20	412±110	645±172	3570±2020

Serum concentrations of palifermin increased with increased dose. On average, exposure to palifermin, as measured by AUC from zero to infinity ($AUC_{0\text{-}inf}$), increased approximately 3-fold for the 4-fold increase in dose administered (60 to 250 μg/kg). On average, most (>95%) of the AUC occurred in the first 24 hours postdose. Mean $t_{1/2}$ values ranged from 4 to 6 hours across the dose levels evaluated. Mean Vss values were greater than body water, indicating extravascular distribution of palifermin. The CV% ranged from 25% to 36% for CL and from 46% to 69% for Vss.

Figure 2. Plot of AUC vs. Dose



PD Results:

Figure 3. Ratio of Palifermin Ki67 Activity at 48 Hours Compared with Baseline (Study 20010192)

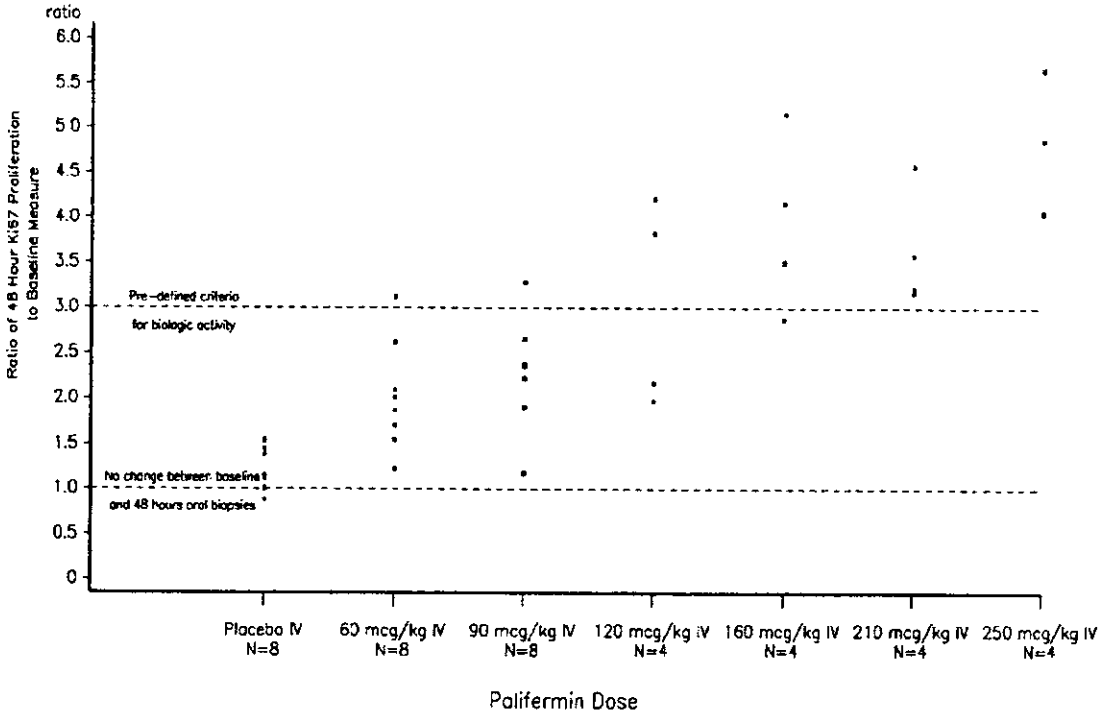
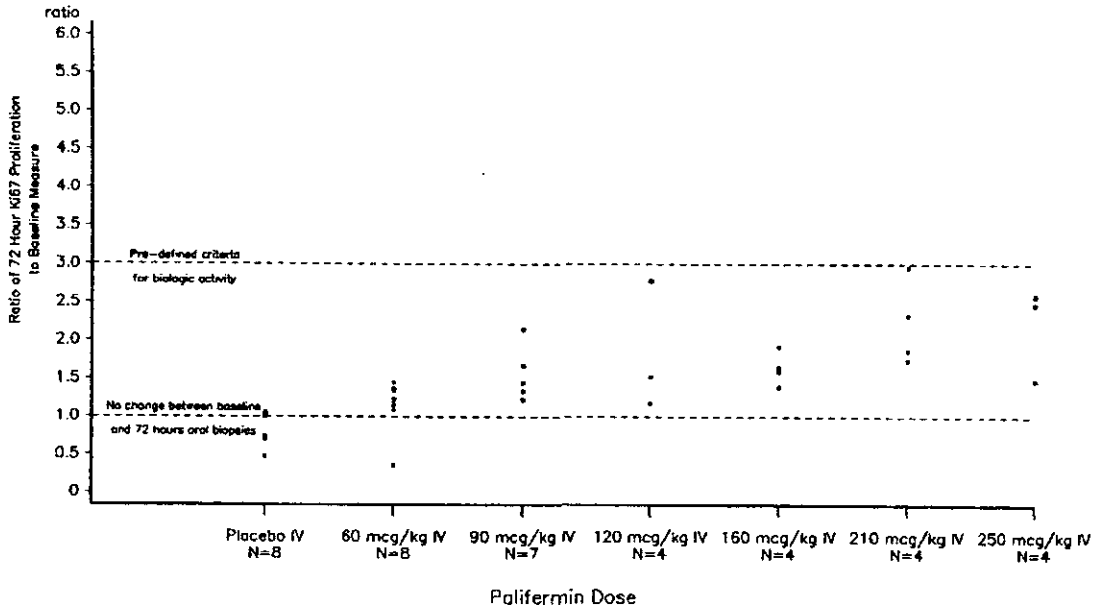


Figure 4. Ratio of Palifermin Ki67 Activity at 72 Hours Compared with Baseline (Study 20010192)



In the buccal mucosa, increased response with increased dose was observed in the Ki67-staining analyses. Figure 2, taken from the submission) shows that at 48 hours after palifermin administration, 11 of 12 subjects who received 160-, 210- or 250-μg/kg palifermin met the predefined criterion for epithelial proliferation (a ≥ 200% [3-fold] increase from baseline in Ki67-stained area). Figure 3, taken from the submission shows that at 72 hours after palifermin dose, a lesser dose-response was observed. In the 210- to 250-μg/kg cohorts, 5 of 8 subjects had a 100% to 199% increase in Ki67 staining at 72 hours postdose.

Safety Results: Ninety-four (94%) of subjects (15 of 16) who received placebo and 97% of subjects (61 of 63) who received palifermin reported at least 1 AE. The most frequent reported AEs (placebo, palifermin) were injection-site (access) pain (31%, 33%), headache (25%, 32%), increased amylase (6%, 27%), erythema (25%, 25%), and injection-site erythema (0%, 21%).

In Patients

Studies 960189 and 20010182 evaluated the tolerability of palifermin in subjects with Hodgkin's disease and non-Hodgkin's lymphoma undergoing high-dose chemotherapy, and in subjects with hematologic malignancies undergoing TBI and high-dose chemotherapy followed by PBSCT, respectively. Both of these studies incorporated the same dose and schedule as the phase 2 (Study 980231) and the pivotal phase 3 (Study 20000162) studies (60 μg/kg/day IV palifermin once daily for 3 consecutive days before high-dose conditioning chemotherapy and 60 μg/kg/day IV palifermin once daily for 3 consecutive days after PBSCT.

Study 960189: This was a phase 1/2 dose-escalation study to evaluate the safety and tolerability of palifermin compared with placebo in subjects with Hodgkin's disease and non-Hodgkin's lymphoma undergoing high-dose chemotherapy with Autologous PBPC transplantation (PBSCT). Subjects received from 5- to 80-μg/kg/day palifermin or placebo for 3 days before high-dose chemotherapy or for 3 days before and 3 days after high-dose chemotherapy as a conditioning regimen for PBSCT.

Subject Demographics: Nineteen subjects of 22 to 67 years of age were enrolled into the 60-μg/kg/day cohort (4 on placebo, 15 on palifermin), of which 13 subjects participated in PK assessments. Most subjects were white (95%), and most were men (73%).

PK Results: The pharmacokinetic properties of palifermin were characterized after the first (day -11) and third (day –9) doses for the cohort who received 60-μg/kg/day for 3 days before and for 3 days after high-dose chemotherapy.

Table 9. Summary of Palifermin PK Parameters for Patients in Study 960189 after 60-μ g/kg/day of IV Palifermin Administration (Mean±SD)

Parameter	C_0 (ng/ml)	$T_{1/2}$ (hr)	AUC_{0-t}* (ng.hr/ml)	AUC_{0-inf} (ng.hr/ml)	CL (ml/hr/kg)	V_{ss} (ml/kg)
Dose 1(n=13)	851±914	4.02±0.62	69.1±34.5	72.3±34.2	1030±482	4320±2690
Dose 3 (n=10)	429±272	3.68±0.75	58.7±28.0	60.5±27.6	1220±566	4710±2870

* The value of t (time of last quantifiable concentration) ranged from 12 to 24 hours for dose 1, and 12 to 36 hours for dose 3.

After IV administration, serum palifermin concentrations declined rapidly within 30-minutes postdose, increased or plateaued between 1- and 6-hours postdose, and then gradually decreased over the remainder of the sampling time.

The similarity between mean AUC_{0-t} and AUC_{0-inf} indicated that most of the exposure to palifermin occurred within the first 12- to 24- hours postdose. No accumulation of palifermin was observed during the multiple-dosing regimen. Mean $t_{1/2}$ was approximately 4 hours. Mean V_{ss} values were greater than total body water, indicating extravascular distribution of palifermin. The CV% was 47% for CL, 62% for V_{ss}, and 50% for AUC_{0-t} after the first dose.

Study 20010182: This was a phase 1, open-label, single-center study to evaluate the pharmacokinetics, safety, tolerability, and efficacy of palifermin after subjects with hematologic malignancies received 60 μg/kg/day IV palifermin once daily for 3 consecutive days before TBI and high-dose conditioning therapy and 60 μg/kg/day IV palifermin once daily for 3 consecutive days after PBSCT (6 doses total).

Subject Demographics: Thirteen (13) subjects of 18 to 63 years of age received palifermin. Most subjects were white (85%), and men (54%).

PK Results:

Figure 5. Palifermin Concentration-time Profiles after the First and Third IV Doses (60 μg/kg/day) before Chemo- and Radiotherapy and after the Fourth and Sixth IV Doses (60 μg/kg/day) Post chemo- and Radiotherapy (Study 20010182 Part A)

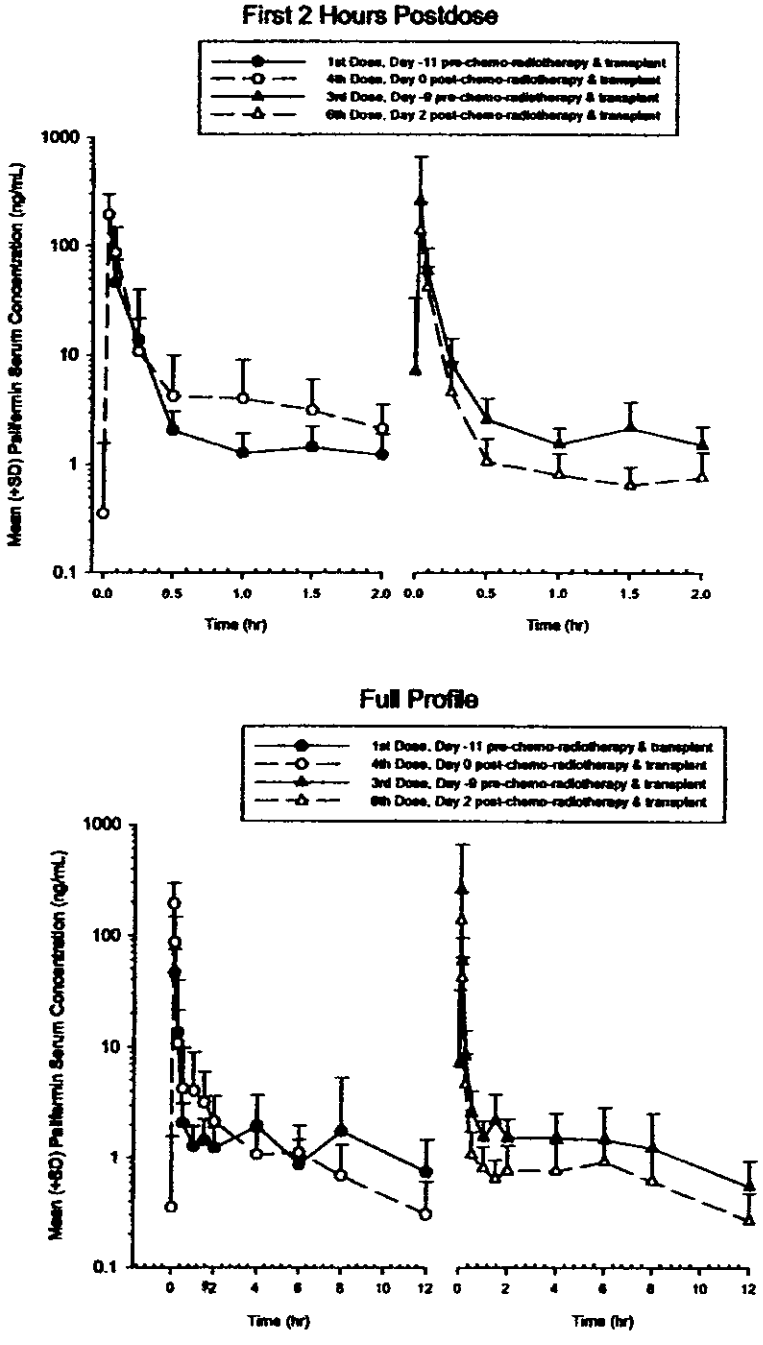


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Table 10. Summary of Palifermin PK Parameters for Patients in Study 20010182 after 60-μ g/kg/day of IV Palifermin Administration (Mean±SD)

Parameter	$T_{1/2}$ (hr)	AUC_{0-t} (ng.hr/ml)	AUC_{0-inf} (ng.hr/ml)
First Dose (day -11, n=13)	4.87±2.02	34.3±15.9	37.8±17.9
Third Dose (day –9, n=13)	5.71±3.60	39.8±36.4	42.6±37.1
Fourth Dose (day 0, n=13)	3.27±1.27	34.8±22.5	35.8±22.4
Sixth Dose (day 2, n=13)	3.74±1.73	21.2±15.1	23.6±15.2

Summary: Palifermin concentrations declined rapidly (a 98% decrease on average) in the first 30-minutes postdose on all assessment days. After this rapid decline, a slight increase or plateau in concentration occurred at approximately 1- to 4-hours postdose, followed by a terminal decline phase.

No accumulation of palifermin, as measured by AUC $_{0\text{-}24h}$. Mean AUC_{0-t} values were comparable between doses 1 and 3 (last dose before chemo- and radiotherapy) and between doses 1 and 4 (first dose after chemo- and radiotherapy). The mean AUC_{0-t} value after dose 6 was approximately 40% to 46% lower, on average, than that after dose 3 and dose 4, respectively. Although the reason for the lower exposure to palifermin after the sixth dose than after the third dose is not known, no antibodies to palifermin were observed in this study.

Safety Results: In general, the nature, incidence, and severity of adverse events (AEs) were consistent with expectations for a patient population with hematologic malignancy undergoing high-dose chemotherapy and TBI followed by PBSCT. Most AEs were those associated with myeloablative chemo- and radiotherapy or hematologic malignant disease. All subjects reported at least 1 AE. Diarrhea was reported in all patients and nausea in 92% of patients. One subject (8%) experienced a serious AE (myocardial infarction), which was considered to be related to palifermin. Transit, asymptomatic elevations in serum amylase and lipase were common, with 11 subjects (84%) having elevations in amylase or lipase or both. No subject was withdrawn from the study due to AEs, and no deaths occurred during the study.

Adverse Events Reported in Clinical Trials

Table 11. AEs Reported in Clinical Trials

AE	Palifermin	Placebo	AE	Palifermin	Placebo
Skin rash	40%	40%	Increase in serum lipase	10%	6%
Pruritis	36%	24%	Increasing in serum amylase	62%	54%
And/or erythema	32%	21%	(both reversible, mild elevation)		
Whole body	28%	21%	Grade 3 or 4 increasing lipase	10%	6%
And/or facial edema	12%	7%	Grade 3 or 4 increasing amylase	38%	31%
Abdominal pain	39%	38%	Abnormal vision	5%	4%

Comparison of PK Properties of Palifermin in Healthy Volunteers and in Subjects with Hematologic Malignancies

On average, CL and Vss appeared to be higher (approximately 2- to 4-fold and 2-fold, respectively) in subjects with hematologic malignancies compared with healthy volunteers at the 60-µg/kg/day dose level (see Figures 6 below). Additionally, a higher level of intersubject variability in CL, and Vss was observed patients than in healthy volunteers.

Figure 6. Clearance (CL, top graph) and Volume of Distribution at Steady State (Vss, bottom graph) of Palifermin after a Single IV Dose of 60 µg/kg to Healthy Volunteers (Study 20010192) and Subjects with Hematologic malignancies (Studies 960189 and 20010182)

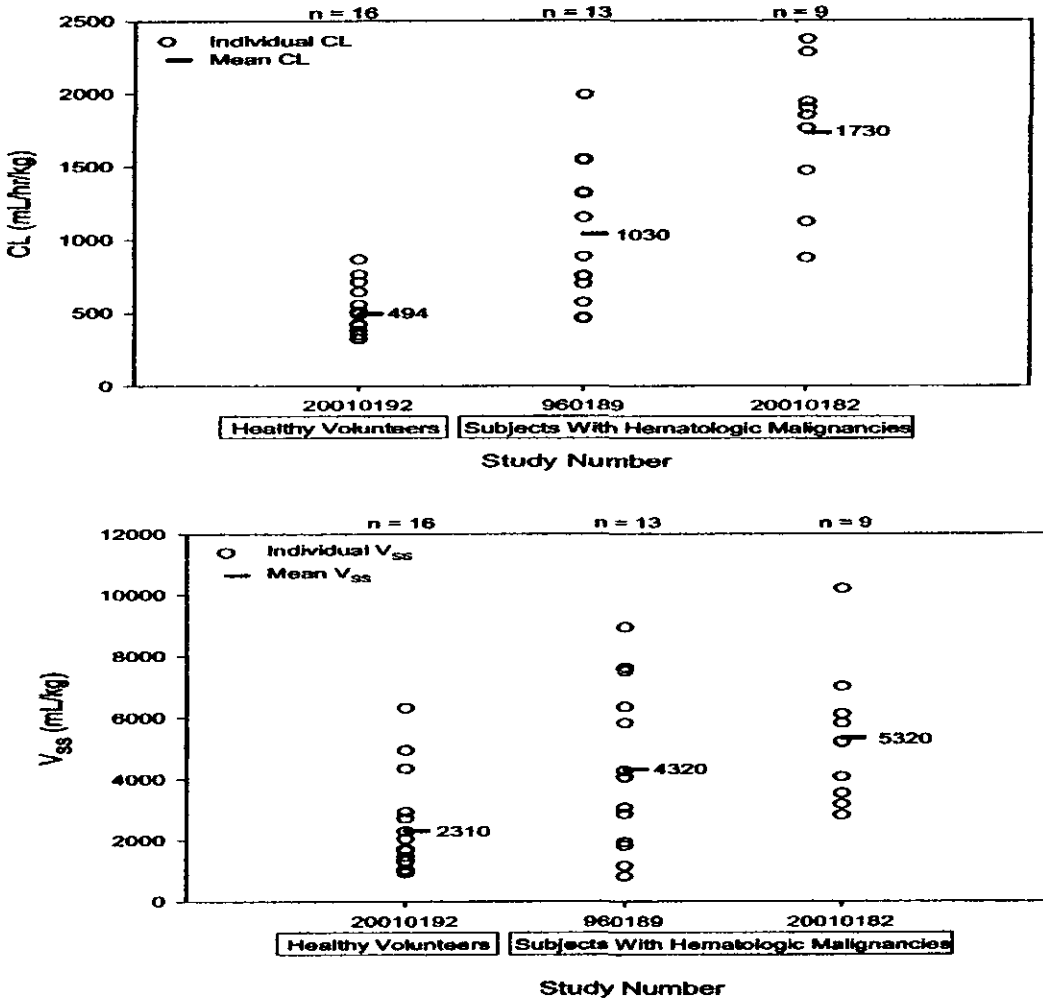


Table 12. Demographic Characteristics for Subjects Received a Single IV Dose of 60 µg/kg Palifermin

Study	Subject type	n (M/F)	Age (yrs)	Weight (kg)	Race
20010192	Healthy	16 (13/3)	26 (19-46)	77 (58-92)	12White/4Black
960189	Patients	13 (9/4)	53 (32-68)	69 (43-97)	12W/1Asian
20010182	Patients	13 (7/6)	52 (18-63)	78 (48-108)	11W/2B

Table 13. Disease and Previous Therapy Characteristics of Palifermin-treated Subjects Included in PK Assessments (Studies 960189 and 20010182)

Study	Non-HDL	Hodgkin's Disease	Chemotherapy	Radiotherapy
960189 (n=13)	13 (100%)	0 (0%)	13 (100%)	13 (100%)
20010182 (n=13)	11 (85%)	2 (15%)	13 (100%)	3 (23%)

Effect of Demographic Variables on the PK of Palifermin

Overview: Neither sex, age nor weight had a notable effect on the pharmacokinetics of palifermin. The effect of race/ethnicity on palifermin PK is inconclusive because limited number of non-white subjects was enrolled in the PK studies.

Healthy Volunteers

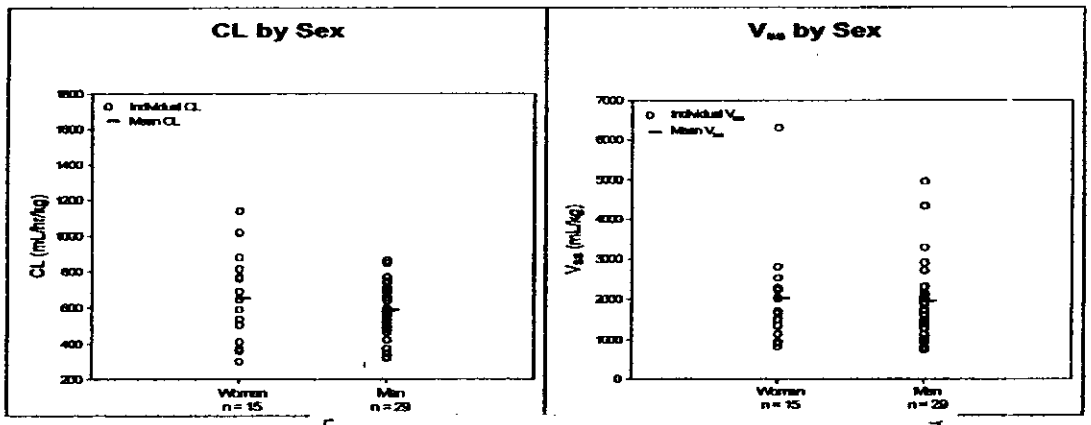
Sex: In the analysis of CL and Vss against dose and sex, only doses from 10 to 60 μg/kg are included, as all the subjects in the higher dose groups were men.

In Healthy Subjects

Sex	Male (n=29)	Female (=15)
CL (ml/hr/kg)	588±244	653±248
Vss (ml/kg)	1959±1086	2016±1332

*Data for doses higher than 60 μg/kg were not included because majority subjects were men.

Figure 7. Individual Clearance and Volume of Distribution at Steady State of Palifermin by Sex for Healthy Volunteers (Dose Range of 10 to 60 μg/kg) (Studies 960136, 970276, and 20010192)



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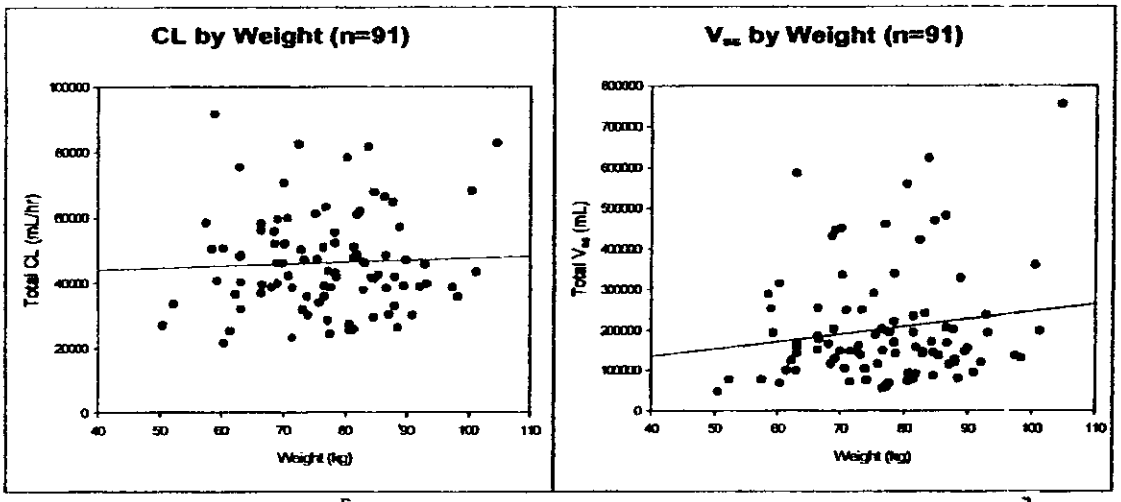
Weight	Correlation
CL (n=91)	$R^2 = 0.002$, p=0.682
Vss (n=91)	$R^2 = 0.022$, p=0.157

Figure 8. Clearance and Volume of Distribution at Steady State of Palifermin by Weight for Healthy Volunteers (Studies 960136, 970276, and 20010192)



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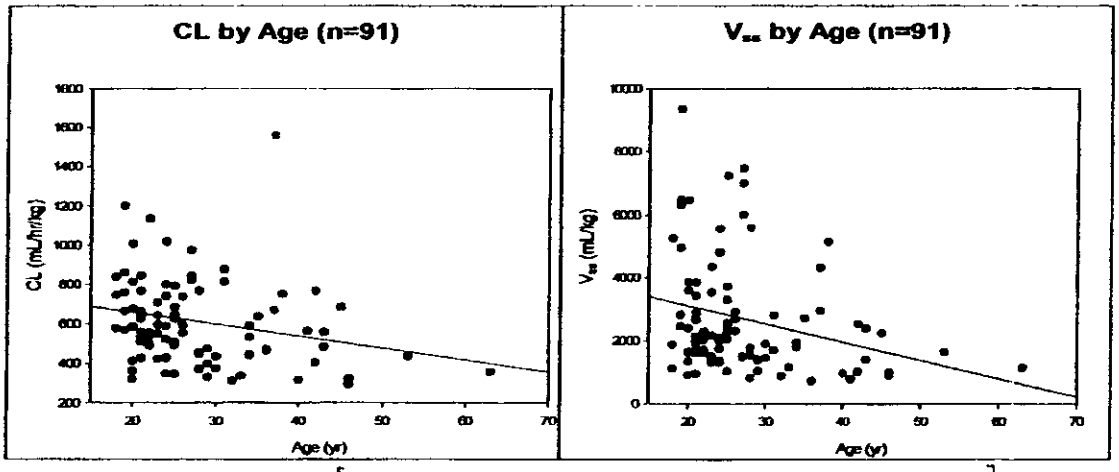
Age	Correlation	Age attributed to variability
CL (n=91)	$R^2 = 0.055$, p=0.025	6%
Vss (n=91)	$R^2 = 0.077$, p=0.008	8%

Figure 9. Clearance and Volume of Distribution at Steady State of Palifermin by Age for Healthy Volunteers (Studies 960136, 970276, and 20010192)



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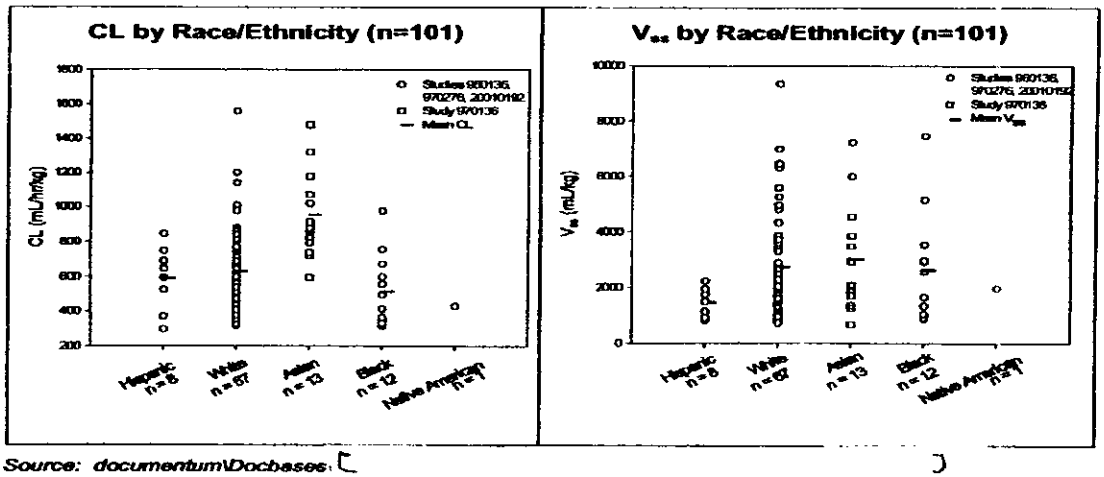
Race/Ethnicity	White	Black	Hispanic	Asian (Japanese)	
N		67	12	8	13	
CL (ml/hr/kg)		629			953	50% higher in Asian than in White
Vss		Similar				

Figure 10. Clearance and Volume of Distribution at Steady State of Palifermin by Race/Ethnicity for Healthy Volunteers (Studies 960136, 970276, and 20010192)



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Patients with Hematologic Malignancies

Pharmacokinetic parameters were available from 22 patients with hematologic malignancies who received 60 µg/kg/day of palifermin. Because of the limited number of data points, the effects of sex, age, and weight on the PK in the patient population were examined graphically by plotting the CL and Vss of palifermin versus these demographic variables. Since 91% of patients studied were white, race/ethnicity effect could not be evaluated. Similar to observations seen in healthy subjects, a high degree of overlap in CL and Vss was observed between men and women.

Figure 11. Clearance and Volume of Distribution at Steady State of Palifermin by Sex for Subjects with Hematologic Malignancies after 60 µg/kg Dose (Studies 960189 and 20010182)

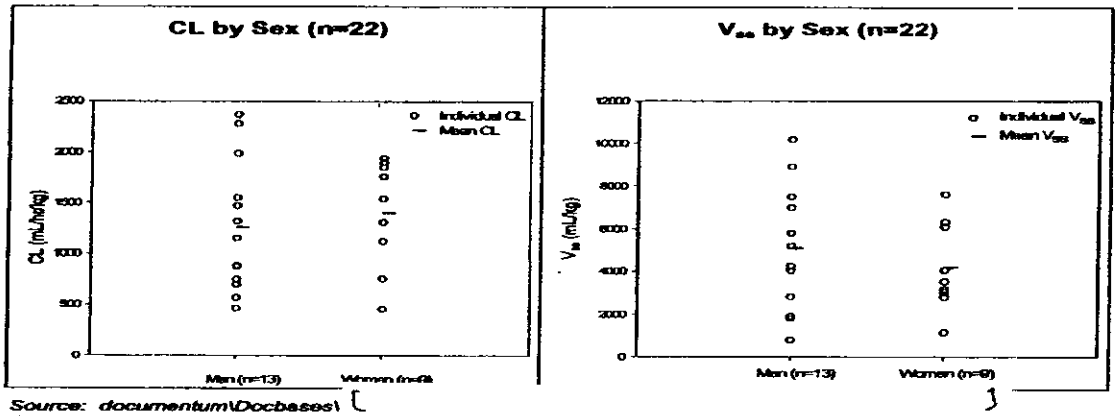

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Figure 12. Clearance and Volume of Distribution at Steady State of Palifermin by Age for Subjects with Hematologic Malignancies after 60 μg/kg Dose (Studies 960189 and 20010182)

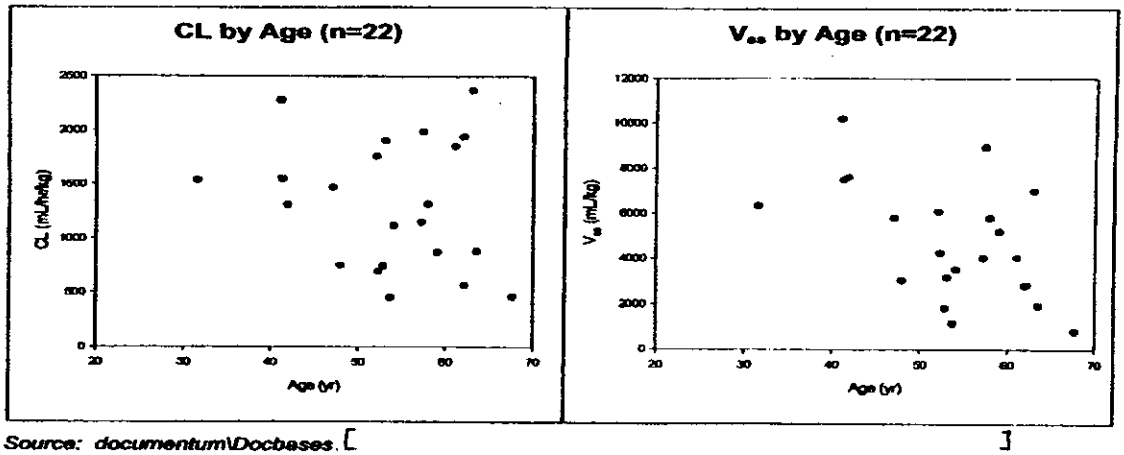

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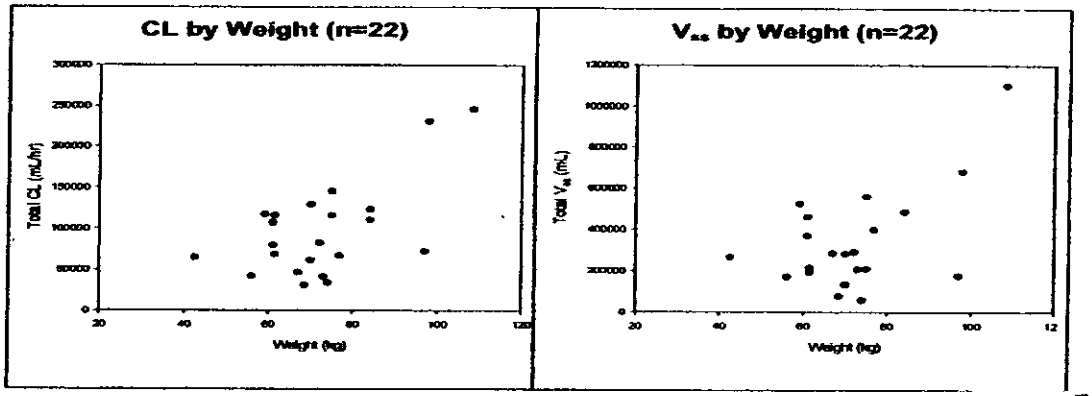
Figure 13. Clearance and Volume of Distribution at Steady State of Palifermin by Weight for Subjects with Hematologic Malignancies after 60 ug/kg Dose (Studies 960189 and 20010182)



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Pharmacokinetics in Special Populations

No formal clinical studies in patients with hepatic impairment, renal impairment or in pediatric populations were conducted.

Pediatric Patients: The safety and effectiveness of palifermin in pediatric patients has not been established. As one of the post marketing commitments, the sponsor will conduct study protocol 20010133, a 174 pediatric patient, multicenter, dose escalation study to evaluate the safety, pharmacokinetics and efficacy of palifermin in children and adolescents with stage 1 (unresected) and stage 2 B-cell Non Hodgkin's Lymphoma (B-NHL) undergoing multi-agent chemotherapy. The final study protocol will be submitted February 2005 and the study will be initiated by May 2005. Patient accrual will be completed by September 2007 and the study will be competed by November 2007. The final study report with revised labeling if applicable, will be submitted by February 2008.

Elderly Population: Insufficient numbers of patients have been studied; among 409 patients with hematologic malignancies who received palifermin in clinical studies, 9 (2%) were ≥ age 65.

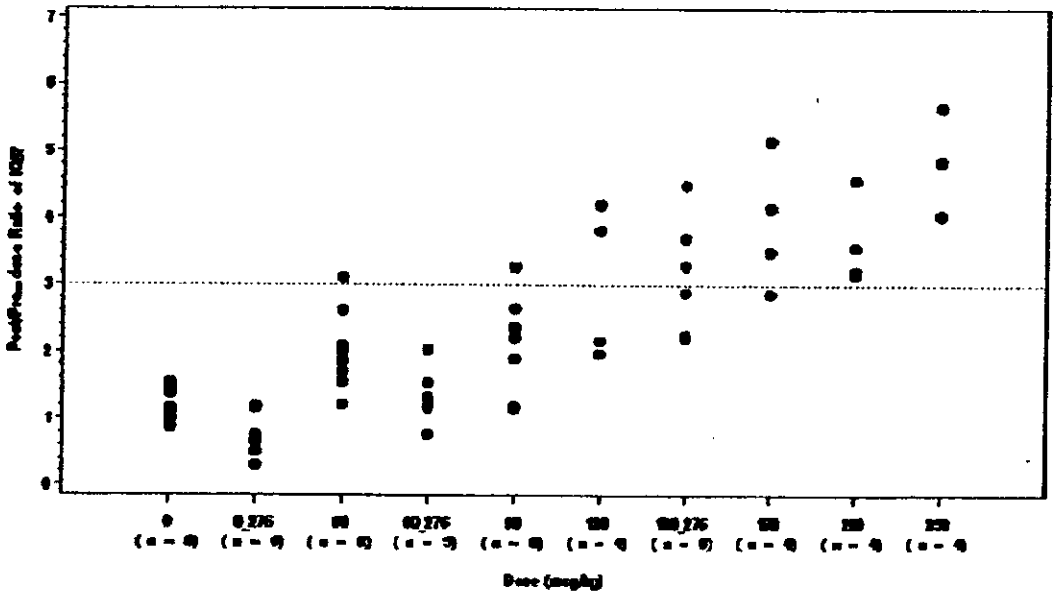
Hepatic Impairment: No specific *in vitro* metabolism studies have been conducted with palifermin. Hepatic impairment studies have not been conducted for the palifermin clinical program.

Renal Impairment: A pharmacokinetic study in bilaterally-nephrectomized rats showed an approximately 2-fold increase in AUC relative to sham-operated rats, suggesting a potential for alteration in pharmacokinetics and tolerability in renally impaired humans. As a result, although subjects with hematologic malignancies (the population for which palifermin is indicated) are required to have normal renal function before they can receive myeloablative therapy, the role of renal clearance in the elimination of palifermin is being evaluated in subjects with varying degrees of renal impairment. This single dose study is ongoing and is not included in this license application.

Exposure-Response

The pharmacodynamic endpoints included measures of cellular proliferation that included assessments of buccal mucosal epithelial proliferation (Studies 950170, 960136, 970276, 20010192). Because Study 950170 was the only SC study in the palifermin clinical development program, and because the only dose at which biologic activity was observed (20 μg/kg/day) in Study 960136 was lower than the proposed clinical dose, these 2 studies are not included in this analysis.

Figure 14. Ki67 Postdose to Predose Ratios after Single and Multiple Doses of Palifermin Administered to healthy Volunteers (Studies 970276 and 20010192)



Note: data includes Studies 970276 (at 18 to 24 hours after the last dose) and 20010192 (at 48 hours after the single dose)

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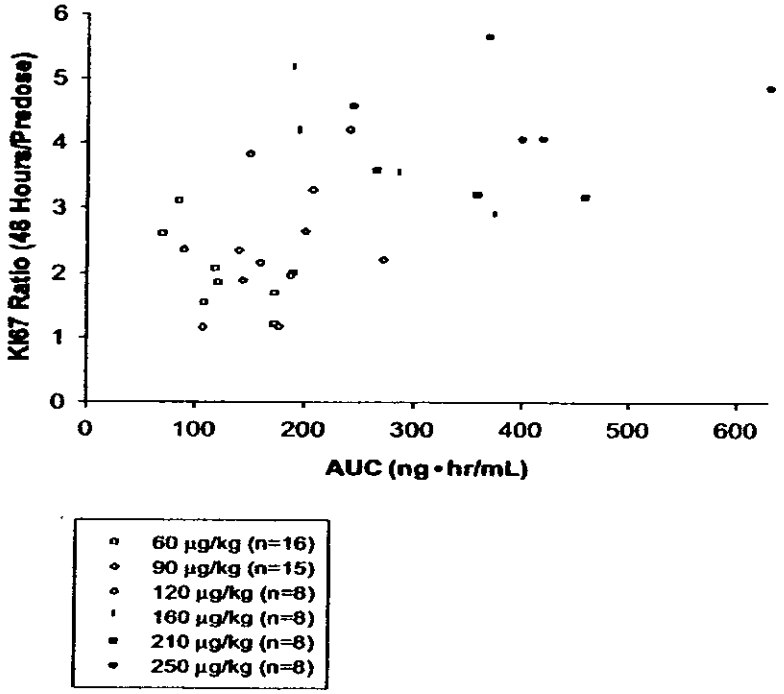
Figure 14 shows that the pharmacodynamic response increased with dose. At total doses of 120 µg/kg and higher, at least 50% of the subjects met the criterion for biologic activity. A complete profiling of the concentration-effect relationship of palifermin and PK/PD modeling was not feasible since a maximum of only 2 biopsies (predose and postdose) was permitted for each subject. However, the relationship between PD response (post-to-predose ratio for Ki67 staining) and exposure to palifermin, as measured by AUC0-inf, was examined graphically using data obtained in Study 20010192 (Fig 15). In general, increased response was observed with increased exposure.

Figure 15. Pharmacodynamic Response (Ki67 Ratio) versus Exposure to Palifermin (AUC0-inf) after Single-Dose Administration of Palifermin to healthy Volunteers (Study 20010192)



Although palifermin has an average half-life of 4.9 hours in healthy subjects, significant pharmacologic effect (Ki67 staining) was observed at 48-hour postdose, with a smaller effect observed 72-hours postdose. At 48-hour postdose, 83% of the quantifiable concentration values (33 of 40) were low (below [ ] ng/ml), suggesting that the effect of palifermin on epithelial tissue persist after active drug levels have dissipated.

Immunogenicity

Assays Used for determining Anti-Palifermin Antibodies

See clinical overview P47. Over the course of the palifermin clinical development program immunogenicity of the molecule was measured using 3 different immunoassays to test for anti-palifermin antibodies in clinical serum samples, and included a radioimmunoassay (RIA), an enzyme-linked immunosorbent assay (ELISA), and an electrochemiluminescence (ECL)-based assay. Each assay represents an improvement

from its precursor. Samples determined to be reactive in any of these immunoassays were further tested for neutralizing activity in a cell-based assay (bioassay).

RIA: The RIA was used for detection of anti-palifermin antibodies in clinical serum samples. Despite the fact that this platform has the ability to detect low amount of immunoglobulin (Ig)G, it is unable to detect other types of Igs, and low-affinity antibodies may be lost during the required washing procedures. In addition, because of the use of iodinated Protein A, nonspecific binding to the experimental wells potentially reduced the specificity of the assay, thereby enhancing the possibility of false-positive results.

ELISA: As a result of the RIA limitations, an ELISA was developed and was validated. Although the ELISA was an improvement over the RIA since it detects all Igs, similar to the RIA, certain low-affinity antibodies could have been lost during the necessary washing procedures.

ECL-based Assay: The current immunoassay used for the palifermin clinical program is an ECL-based assay. This assay is capable of detecting all Igs without necessitating vigorous wash steps that could cause the loss of low-affinity antibodies.

Bioassay: After implementation of the bioassay for characterizing samples that were considered reactive by immunoassay, an attempt was made to test all earlier reactive samples for neutralizing activity. None of the samples that were tested yielded positive results for anti-palifermin antibodies.

Immunogenicity Results

Table 14. Types of Immunoassays Used to Analyze Samples from Palifermin Clinical Studies

Immunoassay	RIA only	RIA and ELISA	ELISA only	ECL-based Assay
Study #	950170	950225	990119	20000162
	960136	950275	--	20010182
	970136	960189	--	20010192
	970276	970149	--	--
	970290	980231	--	--

Table 15. Immunogenicity Testing Results (All subjects who received placebo or palifermin)

Immunoassay	Tested for Antibodies n (%)	Reactive[a] n (%)	Final Positive n	Bioassay Positive n
RIA	546 (51)	17 (3)	0	0
ELISA	258 (24)	110 (43)	0	0
ECL-based	317 (29)	2 (<1)	0	0
ECL-based	645	12	2	0

[a] These were false reactive results since the samples were confirmed to be negative by subsequent assays.
All results refer to postdose samples.

According to the sponsor, currently, all available samples from completed studies are being retested using the ECL-based assay with a newly validated 21 CFR Part 11-compliant reader, which is considered to be the state-of-art method for the detection of anti-palifermin antibodies. Samples from studies 950170, 960136, 970276, and 970290

had been disposed as per Amgen SOPs and were not available for re-analysis by the ECL-based assay.

Table 16. Results of ECL-Based Assay and Bioassay

	ECL-based	Bioassay
Study 950225/950275/950226 (n=141)	n=2[1]	Negative
Study 960189 (n=244)	All negative	NT
Study 970149 (n=43)	All negative	NT
Study 980231 (n=135)	n=1[2]	Negative
Study 990119 (n=76)	All negative	NT
Study 20000162 (n=210)	n=9[3] ( ), n=1 ( )	All negative
Study 20010182 (n=13)	n=2 ( )	All negative
Study 20010192 (n=78)	n=4 [4]	All negative

[1] One subject was on placebo. [2]Failed immunodepletion to confirm positive. [3]Three were on placebo and 6 palifermin treated. The one with S/N ratio >1.59 failed immunodepletion to confirm positive. [4]One was on placebo.

Conclusion

Of the 964 subjects tested by the ECL-based assay, only 12 (1%) palifermin-treated patients tested positive. Of these 12 subjects, ten were below the quantitation limit for anti-palifermin antibodies and 2 were with S/N ratio greater than but upon immunodepletion failed to confirm positive. None of these subjects showed any neutralizing activity in the bioassay. Development of anti-palifermin antibodies was not observed in any of the studies reported in this BLA submission.

It should be noted that in the high-dose myelotoxic therapy setting, patients are inherently immunosuppressed. As such, development of antibodies is less likely than in healthy volunteers or in patients receiving less immunosuppressive regimens. Anti-palifermin antibody testing was also performed for patients enrolled in the solid tumor studies and in studies of healthy volunteers. No binding or neutralizing activity was observed in these populations.

Prophylactic administration of glucocorticoids was not required by the protocol, despite the possibility that palifermin's known effects on skin and oral mucosa (eg, erythema, tongue edema) could be mistaken for allergic or hypersensitivity reactions. No adverse events occurred within a timeframe suggestive of immunologically-mediated allergic or hypersensitivity reactions.

Overall Conclusions

Based on the results of clinical PK studies, the following conclusions are made:

- Palifermin exhibits approximately linear PK in the dose range of 10 to 250 μg/kg after IV single-dose to healthy volunteers.

- Palifermin exhibits extravascular distribution. Compared with healthy subjects, subjects with hematologic malignancies had larger average volume of distribution (Vss, 4320 to 5230 vs. 2310 ml/kg), higher average clearance (CL, 1030 to 1730

vs. 494 ml/hr/kg), and shorter average elimination half-life ($t_{1/2z}$, 4.4 vs. 4.9 hours).

- No accumulation of palifermin was observed after multiple dosing (3 consecutive daily doses of 10, 20, 40 or 60 μg/kg/day).

- Sex, age or weight does not have a notable effect on the pharmacokinetics of palifermin. The effect of race/ethnicity on palifermin PK is inconclusive because limited number of non-white subjects was enrolled in the PK studies.

- In buccal biopsies taken from healthy subjects at 72 hours or 48 hours postdose, increases in epithelial cell proliferation (a ≥ 200% increase in Ki67-stained area relative to baseline) were observed in at least 50% of subjects at doses of 40 μg/kg/day administered for 3 consecutive days and at single doses of 120 to 250 μg/kg. In general, increased response was observed with increased dose (exposure).

- The proposed clinical dose (60 μg/kg/day for 3 consecutive days before and after high-dose chemotherapy with PBSCT) for patients with hematologic malignancies is within the range of doses that have shown biological activity.

- Anti-palifermin antibodies were measured using 3 different immunoassays, each representing technical improvements over the previous one. A bioassay was used to test samples that were considered reactive by immunoassays. Neither binding nor neutralizing activity against palifermin has been detected to date for any samples in any of the study populations. No adverse events occurred within a timeframe suggestive of immunologically-mediated allergic or hypersensitivity reactions.

General Biopharmaceutics

The site and scale of manufacture for the drug substance and drug product has been changed from that used for production of material for clinical studies at Amgen Inc, Thousand Oaks, California. Drug substance at the increased scale will be manufactured at Amgen's commercial site in Boulder, Colorado, and drug product will be manufactured at [ ] The increase in scale and the change in sites were implemented after the completion of the preclinical and clinical study program; consequently, no preclinical or clinical experience with palifermin manufactured at the new scale and sites has been accrued. The change to the manufacturing procedures required to accommodate the changes in scale and sites are considered to have minimum potential to alter the product quality.

To address comparability, Amgen conducted a comprehensive, prospective designed analytical assessment comparing the drug substance and drug product manufactured at the commercial sites in Boulder, Colorado and [ ] with the drug substance and drug product manufactured at the clinical site in Thousand Oaks,

California. The assessments have demonstrated that the proposed commercial material is comparable to the clinical material.

On the basis of this assessment and in accordance with regulatory guidance on the topic of assessing the comparability of biotechnology products (ICH Q5E) additional nonclinical or clinical studies are not considered to be necessary, as changes to the quality and consequent safety or efficacy of palifermin are not expected.

Analytical

Palifermin Concentration Assay

The method used for all matrices was a [ ] enzyme-linked immunosorbent assay (ELISA). A mouse anti-human KGF monoclonal antibody (1G4) was [ ] Recombinant HuKGF was used to prepare standards (STDs) and quality controls (QCs). Samples were added to the plate and the immobilized antibody bound any rHuKGF present. After [ ], a [ ] mouse anti-human KGF monoclonal antibody (A1) was added to the wells. Following [ ], a mixture of [ ] was added to the wells. After the final mixture of substrate solution [ ] was added to the wells. The [ ] reaction, measured as the optical density, developed in proportion to the amount of rHuKGF bound to the antibody — on the plate. The color development was stopped with [ ] and the intensity of the color was measured at — nm with reference at — nm wavelength.

Calibration curve: The calibration curve was obtained by plotting the optical density versus the concentration of palifermin standards using a log-log regression model or a 4-parameter regression model depending on the specific assays.

The following validation characteristics were assessed during method validation for all matrices except rat: accuracy and precision, assay range, dilutional linearity, specificity, and stability.

Accuracy and precision: Inter-assay accuracy was assessed by determining the percent analytical recovery (%AR) observed in the analyses of serum quality control samples (QCs). Inter-assay precision was assessed by determining the variability (%CV) observed in the serum QCs. For all validated analytical methods, the accuracy ranged from [ ] and the inter-assay precision ranged from [ ]

Stability: The stability studies demonstrated that palifermin was stable for — freeze-thaw cycles in human serum, and for — freeze-thaw cycles in rabbit and rhesus monkey serum. The long-term stability studies demonstrated that palifermin was stable in rhesus monkey serum for — days and was stable in human serum for — days when stored at a nominal temperature of -— °C.

Sensitive, specific, accurate and reproducible analytical methods were developed to quantify rHuKGF in mouse, rat, rabbit, monkey, and human serum. Over the course of the various studies, the analytical methods were refined to improve assay performance and throughput. The sponsor claims that these improvements have minimal impact on the interpretation of results across studies since the monoclonal antibody pairs were constant, thereby providing consistent specificity.

Table 17. Palifermin Analytical Methods and Validation Reports (ELISA)

Study No. 970136, 970276, 960136, 950170, 970290		960189	20010182, 20010192
Method validation No.			101872/102155/103674
Standard curve range (ng/ml)			
LLOQ (ng/ml)			
Primary Ab	Mouse anti-KGF 1G4		
Secondary Ab	Mouse anti-KGF A1		

Stated concentrations are in 50% human serum matrix except for Studies 20010182 and 20010192.

Immunogenicity Assays

The immunoassays that have been used for the detection of anti-palifermin antibodies include radioimmunoassay (RIA), ELISA, and ECL-based . Since the reader used for the ECL-based assay was not 21 CFR Part 11 compliant, a new anti-palifermin antibody assay was developed and validated when the became available (March 2004). The ECL-based immunoassay technology offers improved sensitivity, specificity and throughput relative to other immunoassay platforms. Most importantly, the ECL platform facilitates the detection of low-affinity antibodies through minimal washing steps.

ECL-Based Immunoassay: The anti-palifermin antibody immunoassay is an ELC technique that utilizes the bivalent characteristics of antibody molecule. In this assay pooled human serum (PHS) is used as a negative control, and an affinity-purified rabbit polyclonal anti-human palifermin antibody serves as the positive control. The sensitivity of this assay is validated to be — ıg/ml. The mean ECL values obtained with the sample (n=2 replicates) are divided by the mean ECL values obtained with PHS (n=8 replicates) to obtain a S/N ratio.

Table 18. Criteria for Evaluation of Anti-Palifermin Antibody Reactivity Using ECL- Assay

S/N ratio			
	Negative	positive below quantitation limit	reanalyzed[1]
	If confirmed positive by reanalyzing and — (Post-dose/Pre-dose Ratio)		
	Positive		

[1]Samples with S/N ratios equal to or greater than — are re-analyzed in the ECL-based — assay following an by addition of 800 ng/ml unlabeled palifermin; they are confirmed positive if they show at least 50% reduction in the signal.

Cell-based Anti-Palifermin Neutralizing Antibody Bioassay: Samples that yield S/N ratio equal to or greater than — in the ECL-based immunoassay are tested in this bioassay at a 1:100 dilution for the presence of neutralizing antibodies to palifermin. The bioassay uses the murine cell line, 32D, which is transfected with a gene encoding the human KGF receptor/erythropoietin receptor chimera (KGFR/EPOR). The transfected 32D (KECA) cells respond to palifermin (100 pg/ml) with proliferation and are

dependent upon recombinant murine interleukin-3 (IL-3) for routine culturing and growth. The palifermin- or IL-3-induced proliferation is determined by [ ] into the cellular DNA. Palifermin-induced proliferation can be inhibited by pretreatment of palifermin with an anti-palifermin polyclonal neutralizing antibody.

Samples are preincubated with 100 pg/ml palifermin. Proliferation of the KECA cells is decreased in proportion to the quantity of neutralizing antibodies present in the sample. Samples with neutralizing activity towards palifermin-induced proliferation are then tested for their ability to inhibit KECA cell proliferation induced by — pg/ml of IL-3. Only samples that do not inhibit IL-3-induced proliferation of the KECA cells can be considered as having specific anti-palifermin activity. The sensitivity of this bioassay is validated to detect neutralizing activity associated with — ng/ml of the anti-palifermin polyclonal antibody in undiluted human serum.

A subject is positive for development of anti-palifermin neutralizing antibodies if the following criteria are met for a post dose sample: (1) the sample has a confirmed positive result in the immunoassay; (2) the samples inhibits the palifermin-induced proliferation of the KECA cells to below a pre-established assay threshold derived during assay validation; (3) The sample shows a 1.30-fold greater inhibition of palifermin-induced proliferation than that observed with the baseline sample from the same subject; and (4) the sample does not inhibit the IL-3-induced proliferation of the KECA cells below an assay threshold derived during assay validation.

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