SCIENTIFIC DISCUSSION

1.   Introduction

Oral mucositis

Oral mucositis is a significant problem in patients receiving chemotherapy or radiotherapy. Estimates of oral mucositis incidence among cancer therapy patients range from 40% of those receiving standard chemotherapy to 76% of bone marrow transplant patients [1]. Virtually, all patients who receive radiotherapy to the head and neck area develop oral complications. Cancer therapy-induced mucositis occurs when radiation or chemotherapy destroys rapidly proliferating cells such as those lining the mouth, throat, and gastrointestinal tract. In the setting of high-dose myelotoxic therapy, mucositis is a frequent, extremely painful and debilitating complication [2]. Mucositis can arise along the entire gastrointestinal tract and can be associated with significant morbidity, including: pain, specifically in the oral cavity, pharynx, and esophagus (esophagitis), requiring opioid analgesia; difficulty or inability to swallow, because of ulcerations in the oral cavity, pharynx, and esophagus, leading to a compromised nutritional status necessitating parenteral feeding and hydration; infection due to the breakdown of the epithelial barrier and exacerbated by accompanying neutropenia (when present); difficulty or inability to speak; nausea, vomiting, and diarrhoea, which may require intravenous (i.v.) rehydration and may predispose the patient to severe electrolyte and acid-base disorders; gastrointestinal bleeding due to ulceration at any site of the gastrointestinal tract, but typically in the esophagus or stomach. These manifestations diminish the quality of life of patients with cancer and can interfere with management of the primary disease, e.g., requiring dose reductions or treatment delays [3]. The effect of mucositis on length of hospital stay, admissions for fluid support or treatment of infection, and alteration of optimum anticancer treatment have significant clinical and economic consequences [4].

Normally, cells of the mouth undergo rapid renewal over a 7- to 14-day cycle. Both chemotherapy and radiotherapy interfere with cellular mitosis and reduce the regenerative ability of the oral mucosa. Typical sequelae of cytotoxic agents include epithelial hyperplasia, collagen and glandular degeneration, and epithelial dysplasia [5]. Direct stomatotoxicity usually is seen 5 to 7 days after the administration of chemotherapy or radiotherapy. In non myelosuppressed patients, oral lesions heal within 2 to 3 weeks [6]. The pathophysiology of mucositis has been described as a complex biologic process that occurs in four phases: from day 0 to 5 of therapy (1) an inflammatory or vascular phase, during which cytokines are released from the epithelial tissue and submucosal vascularity is increased and (2) an epithelial phase, during which atrophy and ulceration of the epithelial occurs, then from days 6 to 12 of therapy (3) an ulcerative or bacteriologic phase, the most complex and symptomatic phase, which usually coincides with the patient's period of maximum neutropenia, and finally from day 12 to 16, (4) a healing phase, which consist of renewal in epithelial proliferation and differentiation, with normalization of the patient's peripheral white blood cell count and reestablishment of the local microbial flora [7].

The underlying pathophysiology of oral mucositis is essentially the same regardless of the type of insult that causes it [8], but the severity and duration of the mucositis is conditioned by the type of insult (e.g., alkylating agents, including busulfan, antimetabolites, mitotic inhibitor etc.), intensity (high-dose cytotoxic therapy, standard-dose chemotherapy), and duration (multi-cycle chemotherapy *versus* fractionated radiotherapy for HNC treatment). Indirect factors include myelosuppression, immunosuppression, reduced secretory IgA and infections [9]. Many different grading systems exist (such as that proposed by the National Cancer Institute [10]), allowing the assessment of mucositis severity and the construction of a treatment plan. Grading systems are mostly based on two or more clinical parameters, including erythema, pain, and problems with eating.
Erythema is normally the first manifestation of mucositis and starts about 5 days after initiation of chemotherapy and may be followed by oedema and ulceration. Severe mucositis is usually defined as grade 3 and 4 according to the WHO classification where grade 3 signifies ulcers requiring liquid diet. The effective prevention of mucositis requires a comprehensive patient examination to identify potentially complicating oral disease before the cancer therapy begins [11].

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Prevention and treatment of mucositis

Currently no approved treatments are available to prevent oral mucositis induced by chemotherapy and/or radiotherapy [12-14]. Interventions are primarily supportive and are aimed at palliating symptoms such as pain (from topical anesthetics and coating agents to opioid analgesics), addressing inability to eat and drink (parenteral feeding and hydration), reducing local trauma (dental care), and decreasing the risk of secondary infection (prophylactic antibiotics). Although benzydamine hydrochloride (a topically-applied agent with anti-inflammatory, anesthetic, and antimicrobial properties) has recently been recommended by the Multinational Association of Supportive Care in Cancer (MASCC) for the prevention of radiation-induced oral mucositis in patients with HNC receiving moderate-dose radiotherapy (< 50 Gy), benzydamine hydrochloride is not approved for the prevention of radiation-induced oral mucositis, and it has not shown efficacy in other settings. A number of other experimental approaches to preventing oral mucositis have been studied, including topical and systemic granulocyte or granulocyte-macrophage colony-stimulating factors (G-CSF or GM-CSF), laser and cryotherapies, and radioprotectants such as amifostine. To date, all of these approaches have failed to conclusively demonstrate any benefit in reducing oral mucositis.

Treatment breaks and dose reduction are often used to manage severe oral mucositis in the settings of fractionated radiation therapy (with or without concomitant chemotherapy) and multicycle chemotherapy. Although the value of maintaining dose intensity remains somewhat controversial, increased relative dose-intensity has been shown to be of benefit in specific settings (e.g., adjuvant treatment of breast cancer) and an association between maintaining standard doses and improved survival has been demonstrated for some cancers. In the setting of high-dose myelotoxic therapy, oral mucositis is unlikely to result in treatment breaks or dose reductions since the cytotoxic therapy is consolidated in a short but intense time frame and since oral mucositis generally occurs only after completion of therapy. However, although efficacy is the primary consideration in choosing a therapeutic regimen for an individual patient, toxicities (including oral mucositis) may also influence the choice of treatment regimens, which could impact on disease outcome.

About the product

Palifermin (also referred to during the development as rHuKGFΔ23, rHuKGFd23, Palifermin Amgen or Kepivance), is a purified recombinant truncated form of human keratinocyte growth factor (rHuKGF).

Keratinocyte growth factor (KGF), first described in 1989 as a growth factor that stimulates the proliferation of mouse keratinocytes, is a member of the fibroblast growth factor (FGF) family (specifically, FGF-7) [15, 16]. Endogenous KGF is synthesized and released by fibroblasts and other mesenchymal cells, and is the ligand for the KGFR. Keratinocyte growth factor exhibits strict specificity of action for epithelial cells because its receptor (KGFR) is expressed almost exclusively by these cells. Expression of KGFR has been demonstrated in a wide variety of tissues, including upper and lower gastrointestinal tract, lung, urogenital tissues, skin, mammary gland, kidney, and cornea [15-17]. Keratinocyte growth factor is widely expressed by mesenchymal cells of tissues in which KGFR is expressed by epithelial cells, consistent with a paradigm of KGF-mediated, mesenchymal-epithelial, paracrine interactions. A general role for KGF in the organism's response to tissue injury is suggested by findings of increased KGF mRNA levels within human dermal wounds and in the intestines of patients with inflammatory bowel disease [18, 19]. These observations suggest that increased KGF production is a physiological response to injury of epithelial tissue.

Palifermin is being developed to reduce the severity and duration of oral mucositis and related clinical sequelae and improve patient functioning in patients with hematologic malignancies who receive myeloablative therapy (radiotherapy and/or chemotherapy) with autologous peripheral blood stem cell transplant (PBSCT). These treatment regimens have been shown to be effective in controlling disease in patients with hematologic malignancies and therefore are frequently used in this patient population. Conditioning regimens may consist of high-dose chemotherapy alone or with total body irradiation (TBI). Depending upon the disease, various chemotherapy regimens are used, including etoposide and cyclophosphamide, BEAM (BCNU, etoposide, cytarabine, and melphalan), CBV (cyclophosphamide, carmustine, and etoposide) and ICE (ifosfamide, carboplatin, and etoposide).

2.   Part II: Chemical, pharmaceutical and biological aspects

Introduction

Composition

Kepivance is presented as a white lyophilised powder for solution for injection, in a single use type I glass vial, closed with a silicone stopper. Each vial contains 6.25 mg of palifermin. The medicinal product will be reconstituted prior to administration with 1.2 ml sterile water for injection, to yield a 5 mg/ml solution.

Table 1: Composition of the medicinal product

Component[a]	Function	Amount per Vial (mg)
Palifermin	Active Ingredient	6.25
Mannitol	Bulking Agent	50
Sucrose	Stabiliser	25
L-histidine	Buffer	1.94
Polysorbate 20	Stabiliser	0.13
Hydrochloric Acid	pH Adjustment	Titrate

Active Substance

Palifermin, which is a recombinant Human Keratinocyte Growth Factor is produced in *Escherichia coli* (*E.coli*). Palifermin is a nonglycosylated, monomeric protein of 140 amino acids. Compared to the endogenous human keratinocyte growth factor (HuKGF) of 163 amino acids, it is truncated by 23 amino acids at the N terminus. Palifermin is reported to have a comparable *in vitro* and *in vivo* activity to endogenous HuKGF.

Description of Manufacturing Process and Process Controls

Fermentation

Palifermin is produced intracellularly (i.e. not secreted) in a soluble active form. The production fermentation is terminated and then the harvesting procedure starts. The cells are concentrated and lysed. Cell debris is then removed.

The process conditions were considered defined in sufficient detail.

Purification

Several chromatography steps are employed in purification of palifermin to remove or reduce the level of host cell-derived proteins, nucleic acids and endotoxins and palifermin–related variants. After the first chromatography step, palifermin undergoes an oxidation step, for formation of one disulfide link.

Control of materials

Raw Materials and Reagents

Raw materials conform to compendial requirements apart from those for which in-house specifications are set:

Animal Derived Materials

Trypticase peptone and glycerol of animal origin were used in the preparation of the Master Cell Bank (MCB) in February 1995. These materials comply with the requirements of the TSE Note for Guidance [20] referred to in Directive 2001/83/EC [21]. When the Working Cell Bank (WCB) was created, no trypticase peptone was used, and the glycerol used was from a non-animal derived source.

Development Genetics

Palifermin is expressed by an *E. coli* host cell system carrying a plasmid vector genetically modified to direct the production of palifermin. The entire coding sequence was chemically synthesized using optimal *E. coli* codons. The production strain was cultured to establish the Master Cell Bank (MCB).

Cell Bank System and Testing

A two-tiered cell bank is used, which ensures the production capacity for the life of the product. The MCB has been laid down and a WCB has been prepared. The procedures for production of the cell banks were considered acceptably described.

Cell bank characterisation

The MCB and WCB have been characterised, according to ICH guidelines, for identity, safety and stability. The cell bank specifications and test results are presented. The characterization was acceptable and the analytical methods were satisfactorily described.

Genetic stability

Acceptable stability has been shown for the MCB and WCB respectively.

Controls of Critical Steps and Intermediates

For critical parameters, i.e. those suitable for decision to accept or reject a batch, limits have been set. The acceptance limits will be re-evaluated and potentially updated when a larger data set is available.

Process Validation and/or Evaluation

The validation studies that have been carried out were satisfactory.
Process validation was performed with four commercial scale batches, derived from corresponding fermentation batches.

Manufacturing Process development

During development of the manufacturing process, different process development stages in different manufacturing sites have been documented. The applicant has provided data to demonstrate comparability of commercial and clinical trial batches.

Characterisation

Palifermin has been characterised using physico-chemical and biological assays.

Impurities

Host cell-related impurities, such as DNA, host cell proteins and endotoxins are controlled via the purification process during manufacture.
Process-related impurities are removed during the concentration/diafiltration step.
The expression system and reagents used are not considered to raise concerns as regards adventitious agents. The bioburden control was found acceptable.

Control of Active Substance

All of the non-pharmacopeial methods used for testing of the active substance against the defined specification have been validated to evaluate their reproducibility, linearity, sensitivity, precision, accuracy, specificity, robustness and ruggedness, generally in accordance with the ICH Q2A and Q2B guidance, where ICH standard criteria were not met an alternative approach was used. Validation was made, in addition for QC analysis of active substance, by using the sample compositions relevant for the specified IPC analyses.
Batch data was reviewed for 7 clinical batches, and for 7 commercial scale batches.
Active substance specifications were established based on historical data (including clinical trial lots), assay variability, and active substance shelf-life. Justifications were presented for each test attribute.
The active substance will be stored and transported in containers conforming to Ph.Eur requirements.
Chemical resistance and extractable testing has been performed in accordance with Ph.Eur test methods to ensure that any potential leaching of components from the container closure is within

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acceptable limits as defined by relevant pharmacopoeial and/or industry standards. Integrity studies have been performed to confirm the quality of fit between the bottle and the closure.

Stability

Based on the real time stability data a shelf life for the active substance has been accepted.

Medicinal Product

Pharmaceutical Development

The manufacturing process for the final product consists of a dilution of the active substance to the desired concentration followed by sterile filtration and filling into vials. The manufacturing process for the final product has been validated and is controlled by in process controls and product release specifications. The quality of the finished product is ensured by analyses of the product by a combination of physico-chemical and biological methods.

During development of the manufacturing process, different process development stages in different manufacturing sites have been documented. The applicant has provided data to demonstrate comparability of commercial and clinical trial batches.

Process validation has been made for the commercial scale production. The validation program evaluated the following aspects of the process: Media fill, Manufacturing process, Mixing, Hold time, Fill weight, Lyophilizer cycle, Filter, Cleaning, sanitization and sterilization, Inspection, Sub-visible particle determination and Transportation.

Conventional excipients are used and material specifications are in place.

Control of Medicinal Product

For most analytical methods, reference is made to the method validation presented in the Active substance section. Remaining non-pharmacopeial methods used for testing of the medicinal product have been validated essentially in accordance with the relevant guidance provided in ICH guidance.

Batch analysis data were presented for 20 batches of medicinal product manufactured at both clinical and commercial scale, the latter including four consecutive medicinal product lots manufactured at Baxter Pharmaceutical Solutions (BPS), which were used to validate the commercial scale manufacturing process.

The glass (Type I) vial and stopper (chlorobutyl) comply with relevant Ph.Eur monographs. Studies to monitor physicochemical aspects of the elastomeric stopper, extraction characteristics of the rubber closure and functional suitability indicate that the container system has no adverse impact on medicinal product. The USP biological reactivity test for elastomeric materials was also performed successfully.

Stability of the product

The medicinal product stability program was provided.

All commercial-scale batches were stable when stored at 2- 8 °C for 15 months. Clinical trial batches were stable when stored at 2- 8 °C for 60 and 48 months, respectively.

The stability data provided support the applicant's proposed shelf life as mentioned in the SPC.

The results of studies after reconstitution confirm, as also shown in the pharmaceutical development, that the stability of the reconstituted medicinal product directly correlates with temperature. The recommended storage conditions for the reconstituted medicinal product is 2° to 8°C. The duration of storage should be kept to a minimum as the medicinal product formulation does not contain a preservative.

The results indicate that the medicinal product is photolabile both when lyophilized and when reconstituted, with significant decreases in main peak percentages when evaluated by SE-, CE-, and RP-HPLC. The lyophilized medicinal product should therefore be stored at 2° to 8°C and protected from light.

The medicinal product stability protocol for palifermin stored at 2° to 8ºC will continue for up to 48 months. In addition, the sponsor proposes to perform stability testing on a minimum of 1 commercial lot of medicinal product per year, except for the event that no product is produced during a given year

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3.   Part III: Toxico-pharmacological aspects

The main structural difference between palifermin and the endogenous protein is that the first 23 N-terminal amino acids, including two cysteine residues, have been deleted (designated "delta N23"). In the non-clinical pharmacology studies, different truncated forms of rHuKGF were used, and for these studies the term "rHuKGF" refers to any of these forms. Safety pharmacology, pharmacokinetics and toxicology studies were performed with the delta N23 form. In these studies, evaluated lots were representative of, or identical to, the clinical formulations.

Safety pharmacology studies, and with a few exceptions, toxicology studies were performed in accordance with Good Laboratory Practice (GLP) regulations. Non-GLP toxicology studies were some non-pivotal repeat-dose toxicity studies, range-finding embryo-foetal toxicity studies, and studies addressing the effect of palifermin on the growth of human tumours in athymic nude mice. Most toxicokinetics studies were stated to be GLP compliant.

Pharmacology

The non-clinical pharmacology program was designed to evaluate the use of rHuKGF in the treatment of epithelial/mucosal tissue injury induced by radio- and/or chemotherapy. Non-clinical pharmacology studies with rHuKGF were conducted in a variety of species including the mouse, rat, and monkey, with the majority being conducted in mice. Doses used in the pharmacology studies ranged from 0.1 mg/kg to 30 mg/kg. The primary route of administration was subcutaneous.

Physical chemistry

Palifermin has demonstrated mitogenic activity commensurate with native KGF [22]. The importance (related to mitogenic activity) of KGF N-terminal amino acid residues 15 to 27 has been specifically assessed [23]. The bioactivity of the KGF truncation mutants lacking amino acid residues 1-15 through 1-24 was comparable to that of full length KGF. The KGF protein structure, including glycosylation and other post-translational modifications has been described in the literature [24, 25].

- *Primary pharmacodynamics (in vitro/in vivo)*

Primary pharmacodynamics studies evaluated epithelial protection by exogenously administered rHuKGF. A total of 16 non-clinical studies of direct relevance for the proposed indication were submitted. The animal models used in these studies included rat models of acute and chronic salivary gland toxicity (xerostomia) induced by radiation, murine models of oral mucosal toxicity induced by radiation or combination chemotherapy/radiation and mouse and non human primate models of lethal irradiation and transplantation with bone marrow or peripheral blood progenitor cells (mice only).

The affinity of rHuKGF for mouse and human KGFR has been reported in the literature [26, 27]. rHuKGF showed affinity of 200 pM for human KGFR and 25 to 400 pM for the mouse (determined by Scatchard analysis).

The primary pharmacodynamics studies demonstrated that in radiation-induced xerostomia models in rats, administration of rHuKGF preserved salivary secretion. The various studies showed rHuKGF increased salivary gland weight, increased salivary protein and amylase secretion, improved lag time for salivary flow and increased salivary flow rate. The histological lesions induced by the radiation were also largely ablated by the administration of rHuKGF.

In murine mucositis models, the parameters investigated included epithelial thickness of tongue, buccal mucosa, oesophagus, duodenum, jejunum and ileum as well as crypt number and weight. In these models, administration of rHuKGF ameliorated the effects on the digestive tract induced by radiation and / or chemotherapy. In addition, BrdU-labeling data demonstrated that rHuKGF promoted the proliferation of the entire gastrointestinal epithelium, including the oral mucosa. The effects of rHuKGF on chemotherapy-induced weight loss and mortality were also investigated. In some studies, administration of rHuKGF reduced weight loss and in some cases increased survival.

In the primary pharmacodynamic studies, the subcutaneous route was generally used (as opposed to the clinical, i.v., route) and the schedules varied. However, in general, pre-treatment with rHuKGF, or pre- and post-radiation/chemotherapy treatment with rHuKGF appeared to be the more effective schedules, although the benefit of post-treatment administration was not clearly demonstrated.

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The kinetics of epithelial cell proliferation after the administration of a single s.c. dose of palifermin has been studied in mice. Administration of palifermin significantly enhanced the BrdU incorporation (a measure of S-phase) in the mucosa of the mouse tongue, esophagus and jejunum 24 hours after treatment. At 48 hours, BrdU incorporation in the mucosa of the tongue and esophagous was decreased to levels that were significantly below control and gradually recovered to control levels within 4 to 5 days. In the jejunum, BrdU incorporation to about the same levels as in control was observed at 48 hours.

In a second study, cell proliferation was followed after 3 consecutive injections of palifermin given 24 hours apart. The peak of cell proliferation after 1, 2, or 3 injections of palifermin always happened 24 hours after the first injection; 48 hours after a single injection or after the last injection of a series of 2 or 3 administrations of palifermin, there was a consistent drop in cell proliferation to a level that was significantly below the homeostatic level of cell proliferation seen in the epithelium of control mice.

- *Secondary pharmacodynamics*

A series of studies were conducted to investigate the additional effects of rHuKGF in animal models of disease and injury. In a model of chemotherapy-induced diarrhoea, rats were administered irinotecan hydrochloride (CPT-111) at a single i.p. dose of 300 mg/kg. This dose had previously been determined to induce diarrhoea in rats without significant mortality. Administration of rHuKGF (30 mg/kg i.v.) 3 days before CPT-111 decreased the severity of the CPT-111-induced diarrhoea as well as the mean daily diarrhoea incidence. There was no significant decrease in mortality or in weight gain or loss. A similar study showed that early pre-treatment (days -4, -3, -2) with rHuKGF (5 mg/kg s.c.) decreased the rate of mortality and hastened recovery of weight in mice treated with CPT-111 for 4 days (days 0-4) at 200 mg/kg i.p. Similar effects were not seen in mice dosed with CPT-111 at 250 mg/kg i.p. A single high dose of rHuKGF (30 mg/kg) given s.c. on day -1 prior to CPT-111 administration reduced rat mortality, decreased the severity of diarrhoea, and postponed the onset of diarrhoea by two days. Pre-treatment of mice for three days (-3 to -1) with 5 mg/kg rHuKGF s.c. prior to radiation (12 GY) and subsequent post-treatment with rHuKGF and recombinant Murine Granulocyte Colony Stimulating Factor (rMuGCSF) on days 2 to 6 had no effect on survival or body weight in comparison with the irradiated and saline-treated control. However in another study, pre-treatment of mice with rHuKGF (5 mg/kg s.c.) on days -3 to -1 prior to irradiation (12 GY) and autologous bone marrow transplantation (BMT) increased the number of surviving crypts in the duodenum, jejunum and ileum, and increased the weight of the duodenum and jejunum when compared with saline-treated controls.

In a similar study investigating the effects of pre-treatment with rHuKGF (5 mg/kg s.c. on days -2 to 0) on survival and body weight in mice receiving radiation (either 11, 12 or 13 GY) and BMT, rHuKGF did not prevent weight loss, and improved survival only in mice receiving 13GY.

Mice pre-treated with rHuKGF (5 mg/kg/day s.c. on days -3, -2 and -1) before methotrexate (single i.v. dose of 150 mg or 300 mg) and total body irradiation (6 GY) improved survival and weight loss nadirs compared with saline-treated controls. Mice pre-treated with rHuKGF (5 mg/kg/day s.c. on days -3, -2 and -1) before radiation (5 GY) and cyclophosphamide (200 or 400 mg/kg) administration lost less weight than animals that did not receive rHuKGF. Mice were pre-treated with rHuKGF (5 mg/kg/day s.c. on days -2, -1 and 0) before irradiation (12 GY) on day 1 and BMT on day 2. Post-treatment rHuKGF was administered on days 3, 4 and 5, and rHuGCSF (200 µg/kg/day) for 7 days beginning on day 3. Pre-treatment with rHuKGF generally improved survival compared with controls (saline-treated), in which mortality was high. In a dextran sodium sulphate (DSS) model of inflammatory bowel disease in mice, rHuKGF was administered S.C. at 1 or 3 mg/kg every second day from either day 0 or day 4. Both doses improved survival, decreased body weight loss and diarrhoea, with the higher dose appearing to improve symptoms more than the lower dose. In another model of IBD, the immunologic transfer murine model, colitis-like disease was induced in SCID mice by injection of semi-syngeneic CD4+CD45RBhigh T cells isolated from donor mice. Treatment with rHuKGF (i.p.) or saline began when they had lost 10% of their body weight. Disease progression, weight loss and mortality were similar in both groups, but differences were seen at necropsy, with large intestines appearing more normal on gross examination and less inflamed on microscopic examination in the rHuKGF-treated group. rHuKGF treated mice had larger livers and smaller small

intestines compared with control mice, and had lower blood levels of segmented neutrophils (increased segmented neutrophils, increased white blood cell count, decreased blood protein and decreased cholesterol are associated with disease progression in this model). There was also increased proliferation of the squamous epithelium of the oesophagus in the rHuKGF treated animals.

Safety pharmacology

A safety pharmacology study (960 100) was conducted to investigate the effect of palifermin on general behaviour, central nervous system (CNS), autonomic nervous system and smooth muscle, respiratory, cardiovascular, gastrointestinal and renal systems. Mice, rats and Rhesus monkeys were used, with rHuKGFd23 administered intravenously at doses of 0.5, 5.0 and 50 mg/kg. Guinea pig ileum was tested *in vitro*, at concentrations of 5×10^{-5} g/ml, 5×10^{-6} g/ml and 5×10^{-7} g/ml.

Effects on general behaviours

In a modified Irwin test in mice [28], rHuKGFd23 had no effect on general behaviour.

CNS effects

Central nervous system tests included effects on locomotor activity, thiopental-induced sleep, acetic acid-induced writhing and convulsions in mice and body temperature in rats.

In mice, rHuKGFd23 had no effect on locomotor activity, the latency or duration of thiopental-induced sleep or on acetic acid-induced writhing. Furthermore, the same doses had no effect on the incidence of tonic convulsions or mortality following a maximal electroshock, or on the time to onset of tonic convulsions, duration of the tonic convulsions, or time until death in mice treated with 150 mg/kg of pentylenetetrazol. It also had no effect on the incidence of clonic convulsions, and did not induce tonic convulsions in mice treated with a sub-convulsive dose of pentylenetetrazol (50 mg/kg).

In rats, rHuKGFd23 had no effect on body temperature.

Autonomic nervous system and smooth muscle effects

Autonomic nervous system and smooth muscle effects were investigated in isolated guinea pig ileum. Recombinant HuKGFd23 had no effect on spontaneous movement of the isolated ileum, and no effects on contractions induced by acetylcholine, barium chloride or histamine.

Respiratory and cardiovascular effects

Respiratory and cardiovascular effects were investigated in anaesthetised rhesus monkeys.

Recombinant HuKGFd23 had no effect on respiratory rate, diastolic or systolic blood pressure, heart rate, femoral artery blood flow or the elecrocardiogram.

Gastrointestinal effects

Recombinant HuKGFd23 had no effect on the transit of a charcoal meal in mice.

Renal effects

Renal effects were investigated in saline-loaded rats. At all doses, there was a statistically significant decrease in the concentration of sodium excreted. In the 5 and 50 mg/kg groups, there was also a reduction in the concentration of chloride excreted. At the high dose (50 mg/kg), the concentration of potassium excreted was also decreased.

Pharmacodynamic drug interactions

Two studies (IH-95-KGF-002 / R2003172 and R2003173) addressed the potential interaction between rHuKGF and granulocyte colony stimulating factor (G-CSF).

The first study investigated the potential interactions between rHuKGF pre-treatment (50 mg/kg s.c. on days -2, -1 and 0) with 5-FU administration and subsequent s.c. administration of recombinant murine granulocyte colony stimulating factor (rMuG-CSF) in male BDF1 mice.
The decreased body weights and reduction in mean platelet counts induced by 5-FU (whether or not it was followed by rMuG-CSF administration) were diminished by rHuKGF pre-treatment.

On histopathological examination, the mucosal caeco-colic damage induced by 5-FU was not as severe as was expected from previous studies, and appeared to be aggravated in some animals by the administration of rMuG-CSF. Pre-treatment with rHuKGF decreased the incidence and severity of this damage. Recombinant HuKGF had minimal or no effect on the bone marrow lesions induced by 5-FU. The second study investigated the effect of rHuKGF and/or rHuG-CSF on the clinical course of the response of rhesus macaques exposed to 12 GY single dose total body irradiation with and without bone marrow autografts. Monkeys received either vehicle or rHuKGF (100 or 300 μg/kg/day) on days -3, -2 and -1, or rHuKGF at 300 μg/kg/day on days -3, -2, -1, 3, 4 and 5. An additional group received pre- and post-irradiation rHuKGF and rHuG-CSF (10 μg/kg/day) starting on day 1 until neutrophil counts were above 3000/μl. In all the rHuKGF-treated groups, recovery of platelets was accelerated and serum cholesterol was significantly lower. Neutrophil recovery in the group that received rHuG-CSF was accelerated. There were no statistically significant differences in any of the other parameters assessed, including clinical observations, in life haematology and clinical chemistry, and histology. The rHuKGF-treated groups tended to have a reduced incidence of diarrhoea.

Pharmacokinetics

The pharmacokinetics of palifermin has been characterized after single-dose intravenous (i.v.) and/or subcutaneous (s.c.) administrations, and during multiple-dose administrations to rats (i.v and s.c.), monkeys (i.v. and s.c.), and mice (i.v.). The pharmacokinetic parameters of palifermin after a single intravenous dose are summarized in Table 7.

Methods of analysis

An enzyme-linked immunosorbent assay (ELISA) was developed for the measurement of rHuKGF in serum. Validation reports were provided for the assays in rhesus monkey, rabbit and human serum. Acceptable data were provided for the assays in rat serum.

Absorption-Bioavailability

Exposure to palifermin increased approximately dose-proportionally in rats (dose range of 10 to 3000 μg/kg; study KGF.114), monkeys (dose range of 30 to 300 μg/kg; study SBI 950345AM) and mice (dose range of 0.5 to 25 mg/kg; study 101827) after single i.v. administration of palifermin.

No accumulation of palifermin was observed in monkeys receiving 7 consecutive daily i.v. doses of 30 or 300 μg/kg/day or in mice after 3 consecutive daily i.v. doses of 0.5 and 4 mg/kg.

Distribution

The average volumes of distribution of palifermin in rats, monkeys and mice following a single i.v. dose were 45, 1050 and 5400 ml/kg, respectively. In mice and monkeys, distribution exceeds the plasma volume.

Tissue distribution of palifermin was examined after a single i.v. dose of 300 μg/kg ^{125}I-rHuKGF to rats (study MPI 529-028). At 0.5 and 2 hours post-dose, the highest percentages of total radioactivity were found in the liver, skin, carcass, kidney, small intestine, and blood. At 8 hours post-dose, total radioactivity was distributed mainly in skin (22 %), carcass (11%), thyroid (7%) and stomach (6%). At 24 hours post-dose, only skin (7%), thyroid (9%), and carcass (3%) had appreciable levels of radioactivity.

The i.v. administration of 300 μg/kg ^{125}I-rHuKGF to rats, in a study comparing the presence of total and acid precipitable radioactivity in serum (study KGF.165), showed that already at 8 hours post-dose, more than 80% of serum radioactivity was not acid-precipitable, representing free iodide and/or breakdown fragments of palifermin.

There was negligible placental transfer of palifermin following a single i.v. dose of 1000 μg/kg to pregnant rats, on day 19 of gestation. One fetal serum sample at 30 minutes post-dose had a concentration of 0.269 ng/ml, whilst all other fetal serum samples and all amniotic fluid samples were less than the lower LOQ of 0.250 ng/ml (study 970138). There was no evidence of placental transfer of palifermin following a single i.v. dose of 500 μg/kg to pregnant rabbits, on day 18 of gestation. All fetal serum and amniotic fluid samples were below the LOQ of 0.20 ng/ml (study 970021).

No plasma protein binding studies have been conducted.

Metabolism

Two studies (KGF.265 and 100478) were conducted in rats to evaluate the first-pass hepatic elimination of palifermin. In both studies, palifermin was administered to a group of 4 rats *via* a 3-hour infusion either into the hepatic portal vein or into the femoral vein. Blood samples were collected during the first 9 or 7 hours respectively, after the start of the infusion.

Similar values were obtained when infusion occurred *via* the femoral or hepatic portal vein for time to peak concentration (2.98 and 3.02 hours respectively) and terminal half-life (0.547 and 0.549 hours respectively). In study KGF.265, there was a 20% to 30% reduction in Cmax and AUC_{0-9} in animals infused *via* the hepatic portal vein compared with the femoral vein. However there was inter-animal variation in the femoral vein group, and the study was stopped in the hepatic portal vein group due to excessive bleeding, making difficult the interpretation of the results.

In study 100478, similar Cmax and AUC_{0-7} values were obtained for the two groups.

In vitro metabolism studies have not been conducted.

Excretion

Average clearance (CL) values were approximately 56, 420, and 2500 ml/hr/kg in rats, monkeys, and mice, respectively.

The mean terminal half-life was about 3 hours in rhesus monkeys after single and 7 consecutive daily i.v. administrations of 30 or 300 µg/kg (study SBA 950345AM).

In mice, the terminal half-life values were about 1, 33, 17, and 16 hours after the single doses of 0.5, 4, 15 and 25 mg/kg, respectively. Given that there was no accumulation on repeated dosing and that most of the exposure occurred in the first 8 hours post-dose, the effective half-life of palifermin in mice was considered to be closer to the mean residence time (MRT) of 2-3 hours (the MRT values were 1.68, 3.08, 2.10, and 1.84 hours for the 0.5, 4, 15 and 25 mg/kg dose groups, respectively).

The total radioactivity excreted over 24 hours in urine (including bladder contents) and faeces (including intestinal contents) of rats was approximately 31% and 2%, respectively, after a single i.v. dose of 300 µg/kg ^{125}I-rHuKGF (study MPI 529-028).

About 11% of the administered radioactivity was recovered in the urine as acid-precipitable radioactivity (composed of intact or smaller acid-precipitable fragments of palifermin) over 24 hours.

In bilaterally nephrectomised rats (study KGF.104), exposure to palifermin (AUC) increased about 2-fold compared with sham-operated controls.

Studies to examine the excretion of palifermin in milk have not been conducted.

Table 7. Pharmacokinetics of palifermin after a single intravenous dose

Study	Species	n	Dose (µg/kg)	AUC (ng*h/ml)	C_{max}[a] (ng/ml)	CL (ml/h/kg)	V_{ss} (ml/kg)	$t_{1/2,z}$ (h)
101827	mouse		500	175	80.8	2890	4850	1[b]
			400	1510	1150	2610	8020	33[b]
			1500	6370	8890	2330	4890	17[b]
			25000	12400	26300	2000	3680	16[b]
KGF.114	rat	4	10	158	181	64.7	46.0	ND
		4	30	519	694	58.0	44.9	ND
		3	100	1860	2296	54.2	47.6	ND
		4	300	6040	7237	50.2	41.5	ND
		4	1000	16600	22810	61.0	45.0	2.9
		4	3000	60300	70375	50.4	43.2	3
SBI 950345AM	Rhesus monkey	6	30	71.3	304	426	873	ND

Abbreviations: a: Observed at 1 minute post-injection (rat) or 2 minutes post-injection (rhesus); b: The values for mean residence time (the weighted average of the half-life values for the different phases of the concentration curve) were between 1-3 hours; AUC = area under the plasma concentration-time curve; CL = clearance; C_{max} = maximal observed plasma concentration; ND: not determined; $t_{1/2,z}$: terminal half-life; V_{ss}: Volume of distribution at steady state.

Pharmacokinetic drug interactions

Pharmacokinetic drug interactions studies have not been conducted. See discussion on non-clinical aspects, pharmacokinetics.

Toxicology

Palifermin was evaluated single-dose and repeat-dose in rats and Rhesus monkeys. In repeat-dose studies rats were treated up to 28 days, monkeys were treated up to 28 days and within two cycles of three days. Reproductive and developmental studies were conducted in rats and rabbits. Other toxicity studies included the assessment of genotoxicity, antigenicity, hemolysis and the evaluation of the potential of palifermin to enhance the growth of epithelial-derived human tumor cells expressing the KGFR in several *in vitro* and *in vivo* studies.

All toxicology studies were conducted with rHuKGFΔ23, the same molecule used in all clinical studies and intended for marketing.

Exposure multiples mentioned are based on systemic exposure observed in hematological transplant patients (n = 23; studies 960189 and 20010182). In these patients, i.v. treatment with 60 µg/kg/day for 3 consecutive days was associated with a mean maximal concentration of 638.7 ng/ml and a mean AUC of 48.03 ng.h/ml observed after the third dose of palifermin (for these patients, day 3 t ranged from 12 to 24 hours).

Single-Dose Toxicity

No mortality was observed at doses up to 30 mg/kg (i.v. or s.c.) in rats or up to 50 mg/kg i.v. in monkeys. These doses were 500- and 833-times, respectively, the clinical dose (based on mg/kg). The major finding in rats was an enlarged thymus (30 mg/kg). In monkeys, reversible facial and skin reddening were observed (≥10 mg/kg). Histological effects consistent with the pharmacological activity of palifermin, namely acanthosis of the skin and hyperplasia of ileum goblet cells (≥10

mg/kg), and hyperplasia of stomach mucous cells and intestinal goblet cells (50 mg/kg), were observed in monkeys (necropsied 14 days after treatment).

Repeat-Dose Toxicity (with toxicokinetics)

- Pivotal studies were conducted in rats and rhesus monkeys *via* the i.v. and s.c. routes. The design and the main findings of these studies (i.v. administration) are shown in the table below:

Study ID	Species/Sex/ Number/Group	Dose/Route/ Duration	NOEL/ NOAEL	Major findings
100148 (WIL-120072)	Rat/ 3M+3F/group	1 mg/kg/day i.v. and s.c. 7 days		↑ serum lipase; ↑ serum amylase; ↑ C-reactive protein; ↓ insulin (males); thickened mucosal stomach lining; enlarged and/or pale thyroid glands; ↑ liver weight; microscopic changes in liver and pancreas
T-95-KGF-002 (MO16-95)	Rat/ 15M+15F/group	0, 30, 100, 300, 1000 µg/kg/day i.v. 28 days (+ 28 day recovery)	NOAEL: 30 µg/kg/day	All doses: enlarged liver, ↑ total protein, globulin, albumin, calcium, cholesterol and triglycerides; ↑ kidney weights (F only) ≥ 100 µg/kg/day: ↑ reticulocyte and platelet counts; hyperplastic/ hypertrophic changes in GI tract; urinary bladder epithelial hyperplasia; ↓ thymus size and weight (thymic gland lymphoid depletion) ≥ 300 µg/kg/day: ↓ RBC, Hb, HCt; ↑ number and size of thyroid follicles 1000 µg/kg/day: ↑ incidence of centrilobular apoptosis
SBL 39-35	Rhesus monkey/ 3/group (1M+2F or 1F+2M)	0, 500, 1000, 2000, 4000 µg/kg/day i.v. 3 days	NOAEL: < 500 µg/kg/day	All doses: reddening of facial skin; acanthosis in nipple, injection site, upper lip, buccal mucosa, tongue, mammary gland skin and oesophagus; goblet cell hyperplasia in small and large intestines; oedema/ haemorrhage in the submucosa of the urinary bladder; haemorrhage in the mucosal epithelium of the urinary bladder
SBL 39-36	Rhesus monkey/ 3/group (1M+2F or 1F+2M)	0, 500, 1000, 2000, 4000 µg/kg/day i.v 2 cycles of 3 days with 5 non-dosing days between cycles	NOAEL: < 500 µg/kg/day	All doses: reddening of facial skin; acanthosis in nipple, injection site, upper lip, buccal mucosa, tongue, mammary gland skin, oesophagus, palm and vagina; goblet cell hyperplasia in small and large intestines; low total cholesterol; oedema/ haemorrhage in the submucosa of the urinary bladder; ↑ bladder weight ≥ 1000 µg/kg/day: ↓ food consumption; ↓ zymogen granules in pancreatic acinar cells

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| T-95-KGF-005 (SBL 39-27) | Rhesus monkey/ 5 sex/group (3/sex/group for 1 and 10 µg/kg groups) | 0, 1, 10, 30, 100, 300 µg/kg/day

i.v

28 days (+ 28 day recovery) | NOEL: 1 µg/kg/day

NOAEL: 100 µg/kg/day | ≥ 10 µg/kg/day: marked involution of the thymus; ↓ thymus weights (F)

≥ 30 µg/kg/day: thickening of buccal mucosa in oral cavity, tongue or oesophagus; acanthosis in mucosa of tongue, buccal mucosa, oesophagus, skin of scalp and injection site; hyperplasia of mucous cells in the stomach, goblet cell hyperplasia in the small and large intestine

≥ 100 µg/kg/day: ↓ RBC, Hb, HCt, ↑ α1-globulin, ↑ serum amylase; ↑ submandibular gland weight

300 µg/kg/day: swollen lips, squama of skin, pale oral mucosa; ↓ food consumption and body weight; 1M sacrificed moribund; ↓ total cholesterol, Ca²⁺, total protein and albumin; hypertrophy of acinar cells of submandibular gland |

Abbreviations: F = Female; Hb = Hemoglobin; HCt = Hematocit; i.v. = intravenous; M = Male; NOAEL: no observed adverse effect level; NOEL: no observable effect level; RBC = Red Blood Cells; s.c. = subcutaneous. Symbols: ↓: decrease; ↑: increase.

In the 7-day rat study (100148, WIL-120072), the microscopic changes in the liver and pancreas consisted of cytoplasmic basophilia of hepatocytes, hyperplasia in bile duct epithelial cells and ductal hyperplasia in the pancreas. There was no evidence of pancreatitis or pancreatic necrosis that could explain the increases in serum lipase and amylase.

In female mice, receiving i.v. rHuKGF once weekly for 6 weeks (study 101623), the Maximum Tolerated Dose (MTD) was 25 mg/kg/injection. In male and female mice, receiving i.v. rHuKGF on 3 consecutive days/week for 6 weeks (study 100929), the MTD was 5 mg/kg/injection. Increased stomach weight and/or histological findings of hyperplasia and hyperkeratosis of the stomach were observed.

The 28-day repeated dose s.c. studies were conducted in rats (T-95-KGF-001, MO15-95) and monkeys (SBL 39-26). Doses were 0, 30, 100, 300 and 1000 µg/kg/day, with a 28-day recovery period and 0, 1, 10, 30, 100 and 300 µg/kg/day, respectively. Findings were similar to those observed in the i.v. studies. In the rat study, reversible dilation of the glandular mucosal crypts was seen at 30 µg/kg/day, so a no observable effect level (NOEL) was not established. In monkeys, the NOEL was 30 µg/kg/day.

Two preliminary studies were conducted in cynomolgus monkeys using the i.v. (study SBL 39-27-10) or s.c. (study SBL 39-26-10) routes. Doses were 30, 300 and 1000 µg/kg/day. Reddening of the skin of the face and lower belly including scrotum, acanthosis/hyperkeratosis and were observed.

Toxicokinetics

An overview of toxicokinetic data from several non-clinical studies was provided. Toxicokinetics data from the pivotal 28-day i.v. repeat-dose toxicity study in rats were not provided.
No differences between sexes were observed. Increases in exposure were observed in the 28-day i.v. monkey study and in a rabbit embryo/fetal developmental study, in which the presence of anti-rHuKGF antibodies was detected. Antibodies were also formed in the 28-day rat s.c. study and in the rabbit embryo/fetal developmental study. The antibody response was not associated with any adverse events.

Genotoxicity

Although the standard battery of genotoxicity studies was not required for palifermin, since the development of palifermin was initiated before ICH S6 [29] guideline, palifermin was evaluated in the core battery of mutagenicity studies (conducted in accordance with ICH S2A [30] and S2B [31]). Palifermin was negative in *in vitro* bacterial and mammalian mutagenicity assays, negative in the *in vitro* chromosome aberration assay, and negative in the *in vivo* mouse bone marrow micronucleus assay.

Carcinogenicity

Carcinogenicity studies have not been conducted. However, a number of *in vitro* and *in vivo* assays were conducted to investigate the potential of palifermin to enhance the growth of KGFR-expressing human tumour cells (see other toxicity studies).

Reproductive and Developmental Toxicity (with toxicokinetics)

In a segment I reproductive toxicity study in rats (25/sex/group), palifermin administered i.v. at doses of 100 to 1000 µg/kg/day (study T-095-KGF-015, 1902-012), two weeks prior to mating and throughout cohabitation in male, and 1 week prior to mating and to gestational day 7 in female, resulted in adverse effects in male and/or female reproductive performance, offspring numbers and viability, for doses higher than 100 µg/kg/day. In pregnant rats (study 970138, 100101), C_{max} and AUC increased with increasing dose on gestation days 6 and 19 after daily i.v. administrations of 50, 100, 300 and 1000 µg/kg of palifermin.

In an embryo-fetal developmental toxicity study in rats (25/group), palifermin administered i.v. at doses of 100 to 1000 µg/kg/day (study 970132, WIL-120056) resulted in mammary gland hyperplasia at all doses, with decreased fetal weight and increased post-implantation loss at the high dose.

In rabbits, i.v. administration at doses of 5 to 150 µg/kg/day (study 101728, 529-048) resulted in increased post-implantation loss, reduced litter size, and reduced fetal body weights at the high dose. In both species, maternal effects were seen at doses producing embryo-fetal toxicity. Toxicokinetics were evaluable and accumulation was seen. In this study, C_0 and AUC increased with increasing dose on gestation day 6 and 18 and higher exposure was observed on day 18 compared to day 6 for the 60 and 150 µg/kg/day dose groups.

In pregnant rabbits (study 970021, 97105), antibodies were detected in all drug-treated groups (single i.v. administration at doses of 15 to 500 µg/kg/day) as early as gestation day 12, with most animals positive by gestation day 18. The incidence of antibody formation was similar across palifermin dose groups.

Studies to assess the prenatal and postnatal toxicity of palifermin have not been conducted. Studies to assess the toxicity of palifermin in offspring (juvenile animals) were not conducted either.

Local tolerance

A local tolerance study (T-95-KGF-014) was performed in rabbits following administration *via* i.v., i.m. and s.c. routes. Minimal to mild haemorrhage and cellular infiltration of mainly mononuclear cells and/or oedema were observed at 48 hours post-dose, following i.v. or i.m. injection. All signs of irritation resolved by 14 days following treatment.

Other toxicity studies

Haemolytic potential of palifermin was investigated in four *in vitro* studies using rat, rhesus monkey and/or human blood with formulated rHuKGF and rHuKGF placebo. The formulations tested were not haemolytic in human blood, although they were in rat and rhesus monkey blood.

To evaluate the potential of palifermin to enhance the growth of KGFR-expressing tumors, a series of *in vitro* and *in vivo* studies were conducted in accordance with ICH S6 [29]. *In vitro* cell proliferation studies were used to determine a number of human tumour cell lines from various tumour types that were relatively sensitive to rHuKGF-induced proliferation. These rHuKGF-sensitive cell lines were squamous cell carcinoma (FaDu and Detroit-562 [head and neck cancers]), lung (EKVX), colon (HT-29), prostate (DU 145 and 22Rv1), breast (UISO-BCA-1) and were selected for use in *in vivo* xenograft studies.

The effects of i.v. treatment with rHuKGF on 3 consecutive days, each week, for 3 to 6 weeks were evaluated in an athymic mouse tumour xenograft model. Based on the results of an i.v. dose range-finding study in nude mice, doses of 150, 500, 1500, and 4000 µg/kg/injection were selected for the xenograft studies.

Treatment with rHuKGF at doses of 1500 and 4000 µg/kg/day significantly increased the growth rate of FaDu xenografts. The NOEL dose of 500 µg/kg/day for the FaDu xenograft study was associated with an AUC_{0-24} (study 101827) that was approximately 4.3 times the clinical AUC_{0-t}.

In contrast, rHuKGF did not increase the growth rate of the other 6 tumour types selected from the *in vitro* studies (Detroit-562, EKVX, HT-29, DU 145, 22Rv1 or UISO-BCA-1 xenografts). The NOEL for these cell lines (4000 µg/kg/day) was associated with an AUC_{0-24} (study 101827) that was approximately 31 times the clinical AUC_{0-t}.

Hyperplasia/hyperkeratosis of the stomach and/or increased stomach weight provided evidence of the pharmacological activity of rHuKGF and therefore of systemic exposure to the drug in these xenograft studies.

rHuKGF's effects on clonal growth and expansion of 35 human solid tumor cell lines (including head and neck, lung, stomach, colorectal, breast, glioblastoma, neuroblastoma, germ cell, kidney, prostate, and osteosarcoma cell lines) and 22 human lymphoma or leukaemia cell lines (including non-Hodgkin's, Hodgkin's, and myeloma cell lines) has been described in the literature [32]. The growth of 30 of the solid tumours was not significantly modulated by rHuKGF at 0.1 to 100 ng/ml. Colony growth of 5 of the solid tumour cell lines (2 lung, 1 stomach, 1 colorectal, and 1 breast) was significantly stimulated by rHuKGF by up to 2-fold. Clonal growth of the 22 human tumour cell lines derived from leukaemias or lymphomas was not influenced by rHuKGF. Several non-responding solid tumour cell lines showed expression of KGFR.

In addition, the potential of palifermin to protect tumour cells against the cytotoxic effects of chemotherapy or radiation was evaluated in a xenograft model in nude mice and the results published.

To investigate the potential interaction between palifermin treatment and 5-FU chemotherapy with regard to tumour response, athymic mice were inoculated with HT-29 or FaDu (human colon or human squamous cell carcinoma, respectively, expressing the KGFR) tumour cells [33]. The ability of 5-FU to inhibit tumour growth was not affected by palifermin.

The potential effects of palifermin on the *in vivo* survival and proliferation of radiation-treated tumour cells were studied in two murine mouse models [19]. Female athymic mice were used as recipients for human squamous cell carcinoma xenografts expressing the KGFR (FaDu, Detroit 562, and A431), and female Balb/c mice were used as recipients for mouse melanoma B16 tumors that do not express the receptor for KGF. Mice were treated with a dose of palifermin (1000 µg/kg/day) that had been previously shown to protect intestinal stem cells *in vivo*. Palifermin neither stimulated the growth of, nor changed the radiation sensitivity of the KGFR-expressing human tumour xenografts or the KGFR-negative mouse melanoma grafts.

Antigenicity
Passive or active immunisation with palifermin produced an expected anaphylactic response in guinea pigs, while a weak response was observed in one rat passively sensitised with mouse sera (actively sensitised with palifermin in presence of adjuvant).

Studies to assess dependence, metabolites or impurities toxicity, as well as immunotoxicity studies have not been conducted.

Ecotoxicity/environmental risk assessment
An environmental risk assessment was provided. Patients would be expected to metabolise palifermin fully and excretion of intact, biologically active protein was considered to be negligible. Breakdown products are also not considered to remain in the environment for any length of time given their susceptibility to degradation by many environmental microflora. For these reasons, exposure to concentrations of palifermin in the environment is not expected to pose an environmental concern.

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Discussion on the non-clinical aspects

Non-clinical reports and bibliographic references, a comprehensive factual synopsis of findings and the implications for the safe use of palifermin have been provided following agreed Common Technical Document recommendations [34].

Pharmacodynamics

Palifermin is a truncated form of the endogenous human KGF, produced in *E. coli*. The truncated non-glycosylated protein was demonstrated to be comparable to the endogenous protein in terms of activity at the KGFR. Epithelial protection by exogenously administered rHuKGF has been demonstrated in appropriate animal models of gastrointestinal injury induced by radiation or chemotherapy, oral mucosal toxicity induced by radiation or chemoradiotherapy, and acute and chronic salivary gland toxicity (xerostomia) induced by radiation. However, when palifermin treatment was delayed until radiation-induced ulcers were manifest, the duration of ulceration was slightly prolonged in palifermin-treated mice compared with mice that received placebo [35]. A lack of protection was observed when palifermin was given on the same day as the cytotoxic insult. From a theoretical point of view, it is conceivable that growth factor activation of epithelial cells at a time point close to a cytotoxic insult would actually increase the number of cells being affected by cytotoxicity, thus worsening the mucositis (see clinical study 980231).

Different schedules have been investigated and generally, pre- and pre-post radiation/chemotherapy treatment with rHuKGF appeared to be more successful than post-injury administration. However, it was not clear how the schedules or doses for investigation were chosen and the route of administration was generally subcutaneous rather than the intended clinical i.v. route. Although data suggested a positive effect of palifermin treatment after irradiation, there was no non-clinical evidence for the pre-post treatment protocol being superior to the pre-only treatment protocol. The issue was discussed further, and was considered resolved from the non-clinical perspective. The non-clinical data were used as a guide for the schedule and timing of doses in the clinical studies.

In order to investigate whether the administration of palifermin prior to the administration of S-phase specific drugs, such as ara-C, might increase the toxicity of these drugs as regards KGF receptor positive normal tissues, the kinetics of epithelial cell proliferation has been studied in mice, using BrdU incorporation as a measure of S-phase. Such measurements could not be performed in humans, as the data available on the kinetics of epithelial cell proliferation in humans after palifermin administration were limited to Ki67 staining, which is not specific to S-phase. BrdU-labeling data demonstrate proliferation of the entire gastrointestinal epithelium, including the oral mucosa. The data provided in mice showed that dosing with chemotherapy agents within 24 hours after a single injection of palifermin could be deleterious because it would affect the cells in S-phase. However, dosing within 24 hours after a series of 3 injections of palifermin given over a 3-day period would not be deleterious because it occurred past the peak of S-phase cell proliferation induced by palifermin on epithelial cells. As a consequence, a warning has been included in section 4.4 of the SPC to recommend that palifermin is not administered within 24 hours before, during infusion of, or within 24 hours after administration of cytotoxic chemotherapy.

Secondary pharmacodynamics studies investigated other animal models to demonstrate that palifermin has activity in ameliorating epithelial damage in a wider range of conditions. Pharmacological effects of palifermin were evident in the gastrointestinal tract of mice and rats receiving radiation therapy and/or chemotherapeutic agents. In some studies, survival was improved, as well as the preservation of the intestinal epithelial morphology. Dextran sodium sulfate (DSS) and CD45RB models of inflammatory bowel disease demonstrated palifermin had a positive effect in ameliorating symptoms and histological lesions.

Safety pharmacology studies have investigated the effects of rHuKGF on general behaviour, the CNS, autonomic nervous system and smooth muscle, respiratory, cardiovascular, gastrointestinal and renal systems. Recombinant HuKGF administered i.v. at doses of 0.5, 5.0 and 50 mg/kg to mice, rats or Rhesus monkeys had no effect on any of these systems. Renal effects were not considered clinically relevant due to the absence of a clear dose response and the absence of significant changes in total urinary excretion of electrolytes. In vitro, concentrations of 5×10^{-5} g/ml, 5×10^{-6} g/ml and 5×10^{-7} g/ml had no effect on spontaneous or induced contractions of isolated guinea pig ileum.

In pharmacodynamic drug interaction studies, in which mice were treated with 5-FU or rhesus monkeys were irradiated and then transplanted with bone marrow, rHuKGF pre-treatment reduced the

effects of 5-FU in mice relative to controls either with or without GCSF, and in rhesus monkeys, the neutrophil recovery in groups that received GCSF was accelerated. GCSF is likely to be used in the indicated patient population. These finding showed that rHuKGF did not adversely affect haematological recovery and the absence of negative interactions between rHuKGF and rHuGCSF. No other *in vivo* drug interaction studies have been conducted.

Pharmacokinetics

The increase in exposure of palifermin was approximately dose-proportional following a single i.v. dose in mice, rats and monkeys.

Average clearance (CL) values were approximately 56, 420, and 2500 ml/hr/kg in rats, monkeys, and mice, respectively, and the effective half-life ($t_{1/2}$) ranged from approximately 1 to 4 hours in the 3 species. Large volumes of distribution were observed in mice (5400 ml/kg) and monkeys (1050 ml/kg) after i.v administration, indicating extravascular distribution of palifermin. This was also the case in humans, where the Vss was about 2000 ml/kg in healthy volunteers. A lower volume of distribution was observed in rats (45 ml/kg) compared with other toxicology species and humans. However, pharmacology and toxicology studies in rat showed evidence for a pharmacological response with a clear dose response pattern. There were no signs of toxicity events not related to the pharmacology of palifermin (see Toxicology).

Palifermin did not accumulate after 3 consecutive daily doses to mice and 7 consecutive daily doses to rhesus monkeys in pharmacokinetic studies, nor in 3-day and 11-day (intermittent) toxicology studies conducted in monkeys, nor in an embryo/fetal developmental toxicology study in rats. Accumulation is not expected as the half-life of palifermin was relatively short in the animal species (see Toxicokinetics), but increased exposure after multiple dosing was observed in some of the toxicological studies. This observation coincided with the presence of anti-palifermin antibodies.

In rats following i.v. administration of ^{125}I-rHuKGF, liver, skin, carcass, kidney, small intestines, blood, stomach, and thyroid contained the highest percentage of total radioactivity.

Placental transfer of rHuKGF in pregnant rats and rabbits was negligible, with fetal serum and amniotic fluid levels below the limit of quantification of the assays. This suggested that only negligible amounts of rHuKGF crossed the placenta. No studies have been conducted to examine the excretion of palifermin in milk. The SPC states that palifermin should not be administered to women who are breast-feeding (see SPC, section 4.6).

Plasma protein binding studies have not been conducted. As KGF is a heparin binding protein, which also interacts with heparin sulphate proteoglycans (HSPG) that are present on cell surfaces and in the extracellular matrix, rHuKGF could also interact with heparin and HSPG. However, as heparin did not interfere with the ELISA for rHuKGF in human serum, the absence of protein binding studies was considered acceptable. A statement was included in the SPC to ensure that i.v. lines that are maintained with heparin are rinsed with sodium chloride solution prior to and after palifermin administration, since palifermin has been shown to bind to heparin *in vitro* (see discussion on clinical pharmacokinetics and SPC, section 6.2: Incompatibilities).

The absence of conventional metabolism studies was considered acceptable for such compounds as they would be expected to be broken down into small peptides and amino acids.

Comparison of total radioactivity, acid-precipitable radioactivity and rHuKGF concentrations suggest that palifermin undergoes substantial metabolism, particularly after subcutaneous administration (possibly pre-systemically at the injection site). Exposure in rats was similar whether rHuKGF was administered *via* the femoral or hepatic veins so the hepatic route was not considered as a major pathway of elimination in rats.

Palifermin was predominantly eliminated via the renal route in rats, as shown in the distribution and excretion studies conducted with ^{125}I-rHuKGF. This was supported by the increased exposure to palifermin seen in bilaterally nephrectomised rats, as well as the low hepatic extraction. Other organs may participate in the elimination of palifermin through its binding to the KGF receptor and internalisation/breakdown within epithelial cells.

Pharmacokinetic drug interactions studies have not been conducted. Some of the non-clinical pharmacology studies have involved administration of chemotherapeutic agents as well as rHuKGF to

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model the clinical situation, but relevant pharmacokinetic interaction data can only be obtained clinically.

Given the documentation and justifications provided, the non-clinical pharmacokinetic requirements were considered fulfilled.

Toxicology

Toxicology studies were conducted in mice, rats, guinea pigs, rabbits, and monkeys. The two species selected for the pivotal repeat-dose toxicity studies, rats and monkeys, were deemed appropriate for investigating the non-clinical safety of palifermin as they were responsive to the biological actions of rHuKGF, i.e. widespread epithelial proliferation was seen in both species. Adequate presentation of the results and conclusions have been provided, in agreement with applicable requirements and guidelines [21, 36, 37].

Safety margins were calculated based on the highest intended clinical dose of 60 µg/kg/day administered i.v. daily for 3 consecutive days. However, in most of the toxicology studies, a NOEL could not be identified due to the pharmacological effects of palifermin being observed in all treated groups. In single-dose toxicity studies, no mortality was observed in rats at doses up to 30 000 µg/kg (i.v or s.c) or in rhesus monkeys at doses up to 50 000 µg/kg (i.v). Based on administered dose (mg/kg), these doses are 500- and 833-times the clinical doses of 60 µg/kg/day, respectively. In the 28-day i.v. monkey study, a NOEL was established (1 µg/kg/day), but systemic exposure at this dose could not be calculated as rHuKGF levels were generally lower than the limit of quantification in the samples taken. At the no observed adverse effect level (NOAEL of 100 µg/kg/day), at which there was no overt toxicity or irreversible changes in the 28-day monkey i.v. study, the systemic exposure was 8 times the clinical exposure. In the 11-day intermittent i.v. monkey study, exposure at 500 µg/kg/day (at which there was no overt toxicity) was 15 times the clinical exposure.

Generally the effects observed in the repeated-dose studies were attributable to the pharmacological activity of rHuKGF and were reversible. These effects of KGF, which include the epithelial proliferation of skin, GI tract, mammary gland ducts, bladder urothelium and pancreatic ducts and hepatocyte proliferation in the liver have also been reported in published literature. The thymic gland lymphoid depletion and/or involution seen in rats and monkeys, and increases in the number and size of thyroid particles seen in rats were discussed. The thyroid changes in rats were not reversible. Thymic involution may have been stress-related and was reversible. Reports in the literature suggest that rHuKGF preserves normal thymopoiesis and thymic microenvironments during experimental graft-versus-host disease in mice [38] and plays a role in the development and function of thymic epithelium [39]. The thymus was enlarged and increased in weight in the acute rat i.v. and s.c. studies at a rHuKGF dose of 30 000 µg/kg. KGF has been shown to contribute to the growth of artificial human thyroid tissues *in vitro* [40].

The cornea expresses the KGFR, but no ophthalmic effects were seen in the toxicology studies either on ophthalmological examination or on histopathological evaluations of the eye in rats or monkeys.

From toxicokinetic evaluations, dose-related increases in exposure have been shown in pregnant rats and rabbits. Accumulation of palifermin has been observed on gestational day 18 in the mid- and high-dose groups in rabbits. Antibodies against rHuKGF were detected in the rat (28-day s.c.), monkey (28-day i.v.) and pregnant rabbit (single i.v. administration). These observations suggest that the antibodies reduced clearance, sustaining rHuKGF concentrations in the body.

The range and type of genotoxicity studies routinely conducted for small molecules are not applicable to biotechnology-derived pharmaceuticals, and therefore, these were not deemed necessary for this assessment, in agreement with applicable guidelines [29]. However, the standard battery of genotoxicity tests [30, 31] have been conducted. As expected, rHuKGF was negative in these studies.

Given the intended patient population and the short-term clinical treatment regimen, rodent carcinogenicity studies were not deemed appropriate [29], and therefore, were not conducted with palifermin. The absence of long-term carcinogenicity studies was considered acceptable.

The potential of rHuKGF to enhance the growth of KGFR-expressing tumours was investigated In accordance with ICH S6. Solid tumours of epithelial origin may express the KGFR, therefore, administration of pharmacological doses of rHuKGF for the treatment of mucositis in patients with solid tumours could theoretically produce adverse tumour outcomes (enhanced growth and/or protection from cytotoxic therapies). Haematological malignancies, the indication for this application, do not express the KGFR and rHuKGF would not be expected to have any such effect on them.

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However, patients treated with chemotherapy and/or radiotherapy for haematological or solid malignancies have been shown to develop secondary tumours as a result of the cytotoxic therapy, which may be haematological (typically associated with chemotherapy) or solid (typically associated with radiotherapy), despite the nature of the original tumour. A series of *in vitro* and *in vivo* studies was performed to address these concerns. Recombinant HuKGF increased the proliferation of some cancer cells *in vitro* that expressed KGFR, but did not enhance proliferation of cell lines derived from haematological malignancies that do not express the KGFR. Seven human tumour cell lines that were sensitive to rHuKGF-induced proliferation (including head and neck squamous cell carcinomas, lung, colon, prostate and breast) were investigated in xenograft models in athymic nude mice. Five of these seven cell lines had been shown previously to express KGFR mRNA. It is assumed that the other two cell lines also expressed mRNA but that the analytical methods employed were not sufficiently sensitive to detect it.

Intermittent treatment (3 consecutive days/week for 4 to 6 weeks) with rHuKGF had no effect on tumour growth in six of the seven xenograft models in athymic nude mice, although it increased the growth rate of the other (FaDu, squamous cell carcinoma) tumour type. Therefore *in vitro* proliferation does not necessarily correspond with *in vivo* proliferation in this model.

There are two references in the published literature that have investigated the potential of rHuKGF to alter the sensitivity of tumour cells to chemotherapy (5-FU) or radiotherapy (fractionated radiation) [19, 33]. Under the conditions of these studies, rHuKGF neither stimulated the growth of, nor changed the sensitivity of the KGFR-expressing human tumour cells xenografts to radiation or chemotherapy.

Given the results with the FaDu xenograft, a hypothetical risk of tumour stimulation in patients with tumours expressing the KGFR cannot be ruled out. Human haematological malignancies do not express the KGFR. However as patients undergoing high dose chemotherapy/radiation therapy have an increased risk of secondary malignancies (either haematological or solid in origin), the theoretical potential of rHuKGF to enhance the growth of secondary solid tumours needs to be considered (see SPC section 5.3). The possibility of expression of KGFR on haematological tumours, or their induction by pathological situations has been discussed. Although complete reassurance is not possible, the available evidence suggests that KGFR is not present on 22 haematological tumour cell lines. Pathological situations such as inflammation would be unlikely to induce KGFR in cells that do not normally express them. This will be investigated *in vitro*, post-authorisation by incubating haematopoietic cell lines with a panel of inflammatory cytokines and analysing KGFR expression at different time points.

In a fertility/general reproductive toxicity study conducted in rats (100, 300, or 1000 µg/kg/day), mating and fertility were unaffected at 100 µg/kg/day. At higher doses ($\geq$ 300 µg/kg/day), palifermin treatment was associated with adverse effects on male and/or female reproductive performance and offspring numbers and viability. However, these effects were not selective, as clinical signs of toxicity were also observed in all groups (e.g. clinical observations and/or body weight effects). The 300 and 1000 µg/kg/day doses were associated with AUC values that were approximately 9.7 and 27 time, respectively, the clinical AUC_{0-t}. The NOAEL (100 µg/kg/day) for mating/fertility effects was associated with an AUC that was approximately 2.5 times the clinical AUC_{0-t}.

In i.v. embryo/fetal developmental toxicity studies in rats and rabbits, palifermin treatment was associated with developmental toxicity (increased post-implantation loss, reduced litter size, and/or reduced fetal weight) at doses of 1000 and 150 µg/kg/day, respectively. These doses were associated with systemic exposures (based on AUC) that were greater than or equal to 15 time (based on extrapolated AUC) and 4.2 times, respectively, the anticipated clinical exposure. Because the developmental effects in these studies were seen only concomitantly with maternal effects, rHUKGF did not appear to be a selective developmental toxicant in rats. No adverse developmental effects were observed in rats and rabbits at doses of up to 300 and 60 µg /kg/day, respectively. These NOAEL doses were associated with systemic exposures (based on AUC) up to 9.7 and 2.1 times, respectively, the anticipated clinical exposure. Peri- and postnatal development has not been studied. Adequate information and warnings are provided in the SPC (Section 4.6 and 5.3). No studies in juvenile animals have been conducted. A paediatric development program has been planned.

A local tolerance study was performed in rabbits *via* the i.v., i.m. and s.c. routes. Signs of irritation were observed at 48 hours post-dose following i.v. or i.m. injection. These signs were typical of those

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following injection of recombinant protein, and reversed 14 days after treatment. It is recommended, in section 4.2 of the SPC, that palifermin is not administered subcutaneously due to poor local tolerability.

The haemolytic potential of various formulations of rHuKGF was investigated and generally appeared non-haemolytic in human blood but haemolytic in animal blood.

Antigenicity studies are considered of little value for protein products as they are generally positive and not considered predictive of antibody formation in humans (ICH S6). However, as the development of palifermin was initiated prior to availability of the ICH S6 guideline, an antigenicity study was performed in mice, rats, and guinea pigs. The expected anaphylactic response was seen in guinea pigs, while a weak response was observed in one rat passively sensitized with mouse sera (actively sensitized with palifermin in the presence of adjuvant).

Palifermin was considered unlikely to pose a risk to the environment.

4. Part IV: Clinical aspects

Introduction

Based on the information provided, all trials were conducted according to the principles of GCP.
One pivotal phase III and one phase II randomized, multicenter, double-blind, placebo-controlled studies were performed in patients with hematologic malignancies undergoing fractionated TBI and high-dose chemotherapy with peripheral blood progenitor cell transplant (PBPC) support. A total of 375 patients, receiving 60 µg/kg of palifermin for 3 consecutive days before conditioning and 3 consecutive days after PBSCT, were enrolled (see table 8). Supportive studies included a phase I, open-label, uncontrolled, single-center, pharmacokinetic study. This was performed in 13 patients with hematologic malignancies, receiving the same myelotoxic therapy and the same dose and schedule of palifermin. Two other studies were performed in patients receiving fractionated radiotherapy with concomitant chemotherapy or cyclic chemotherapy treatment for solid tumors.

Table 8: Main studies submitted for clinical efficacy

Study	Brief Description	Regimen	Primary Objective
20000162	Phase 3, randomized, double-blind, placebo-controlled	60 µg/kg/day 3 consecutive days before TBI/CT 3 consecutive days after PBPCT	Duration (days) of severe (grade 3 or 4) oral mucositis determined using the WHO toxicity scale 'Oral Toxicity' (for the mITT population)
980231	Phase 2, randomized, double-blind, placebo-controlled	60 µg/kg/day - 3 consecutive days before (pre) TBI/CT, - or 3 consecutive days before TBI/CT and 3 consecutive days after PBPCT (pre-post)	
20010182 Part A	Phase 1 (PK), open-label, uncontrolled, single-institution	60 µg/kg/day 3 consecutive days before TBI/CT 3 consecutive days after PBPCT	Clearance and volume of distribution of palifermin at steady state, as computed using noncompartmental PK analyses.

Abbreviations: mITT = modified intent-to-treat population; PBPCT = peripheral blood progenitor cell transplant; TBI = total body irradiation; CT = chemotherapy; WHO = World Health Organization; pre-post = before TBI/CT and after PBPCT dosing schedule for palifermin.

Palifermin treatment should be supervised by a physician experienced in the use of anticancer therapies (see section 4.2 of the SPC). The recommended dose of palifermin is 60 µg/kg/day, administered as an intravenous bolus injection for three consecutive days before and three consecutive days after myelotoxic therapy for a total of six doses. The first three doses should be administered prior to myelotoxic therapy, with the third dose 24 to 48 hours before myelotoxic therapy. The last three doses should be administered post myelotoxic therapy; the first of these doses should be

administered after, but on the same day of haematopoietic stem cell infusion and at least four days after the most recent palifermin administration (see SPC section 4.2).

A paediatric development programme was at an early stage at the time of the application with a protocol being planned. No further information was included in the application.

Pharmacology

- Analytical methods

In all of the studies mentioned above, pharmacokinetic assessments were conducted using frequent serial sampling. A validated enzyme-linked immunosorbent assay (ELISA) was used to quantify palifermin concentrations in human serum.

- Pharmacokinetics

Six studies, investigating the pharmacokinetic properties of palifermin were conducted in healthy volunteers (studies 950170, 960136, 970136, 970276, 970290 and 20010192) and 2 studies were conducted in patients with hematologic malignancies receiving high-dose cytotoxic therapy followed by PBPCT (studies 20010182 and 960189).

- Absorption – Bioavailability

Palifermin is intended for intravenous administration, and absorption studies are not relevant.

The site and scale of manufacture for the active substance and medicinal product has been changed during development (see 3.2 Quality aspects). The proposed commercial material was considered comparable to the clinical material and therefore, additional non-clinical or clinical studies were not considered necessary.

- Distribution

The distribution of palifermin has been investigated after a single i.v. dose of 60 µg/kg/day to healthy volunteers (study 20010192) and to patients with hematologic malignancies (study 960189 and 20010182). Palifermin had a volume of distribution of about 2 l/kg in healthy volunteers and 4-5 l/kg in patients. After administration, plasma concentrations rapidly declined during the first 30 minutes. A slight increase or plateau in concentrations was then observed at approximately 1 to 4 hours post-dose, followed by a terminal decline phase.

The plasma concentration time profile was in several studies modelled using a three-compartment model with a lag time for the redistribution from compartment 3 to the central compartment (plasma). The redistribution lag-time was estimated between 3 and 5 hours.

Plasma protein binding of palifermin has not been studied.

- Elimination

After a single i.v. dose of 60 µg/kg to healthy volunteers (study 20010192) and patients with hematologic malignancies (studies 960189 and 20010182), palifermin clearance was about 500 ml/h/kg in healthy volunteers and 2-3-fold higher in patients. Terminal half-life was about 4 to 5 hours in both healthy volunteers and patients.

In vitro studies to assess the metabolism of palifermin have not been performed. Non-clinical studies revealed low hepatic extraction of palifermin.

- Dose proportionality and time dependency

Pharmacokinetics in healthy volunteers

A phase I, randomized, double-blind, placebo-controlled, dose-escalation, single center study (study 950170) evaluated the safety, tolerability, PK/PD of single and multiple s.c. doses of palifermin administered to healthy volunteers. 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/day) and 2 of the 7 planned multiple-dose cohorts (1.0 and 10 μg/kg/day).

A randomized, double blind, placebo-controlled study (study 960136) evaluated the safety, tolerability, PK/PD of palifermin administered i.v. to healthy volunteers. Palifermin was administered in sequentially enrolling dose cohorts of 0.2, 1.0, 5.0, 10, and 20 μg/kg/day either as a single bolus i.v. injection or as a daily bolus i.v. injection for 3 consecutive days. In the single-dose cohorts, subjects were to be randomized in a 3:1 ratio to receive palifermin or placebo on study day 1. All subjects received a buccal mucosal biopsy at baseline and at 46 to 50 hours after administration of investigational product (day 3). In the multiple-dose cohorts, subjects were to be randomized in a 6:2 ratio to receive palifermin or placebo on day 1, 2, and 3. All subjects received a buccal mucosal biopsy at baseline and at 18 to 24 hours after the last administration of investigational product (day 4).

Sixty-one subjects were enrolled (15 placebo, 46 palifermin). Most subjects were white (70%), and most were men (57%); subjects were 18 to 45 years of age.

The full pharmacokinetic profiles could not be characterised for subjects who received 0.2-, 1.0-, and 5.0-μg/kg palifermin because many of the concentration values were below the detection limit. Palifermin exhibited linear pharmacokinetics after single i.v. administration of 10 and 20 μg/kg. No accumulation of palifermin was observed after 3 consecutive daily administrations of 10 and 20 μg/kg. Half-life values of 3 to 5 hours were estimated at the 20-μg/kg dose. Mean volume of distribution at steady state (Vss) values were greater than total body water), indicating extravascular distribution of palifermin [41]. Between-subject variability (CV%) was 14% for clearance and 60% for Vss after a single i.v. administration of 20-μg/kg palifermin.

A randomized, double-blind, placebo-controlled study (AMJ-9701), in which sequentially enrolled dose cohorts of healthy volunteers received a single i.v. dose of 5, 10, or 20 μg/kg of palifermin or placebo, assessed the tolerability and PK of palifermin.

Eight subjects were to be enrolled into each of the 3 cohorts and were randomized in a 6:2 ratio to receive injection of palifermin or placebo, respectively. Japanese men were enrolled (6 placebo, 18 palifermin) and completed the study. Subjects were 20 to 35 years of age.

Serum palifermin concentrations declined rapidly within the first 30 minutes after administration of palifermin at all dose levels. This decline was followed by an increase or plateau in concentration between 1.5 to 6 hours post-dose. After 6 hours, a consistent decline in concentration was observed.

Exposure increased in an approximately dose-proportional manner between the 5- and 20-μg/kg dose cohorts. The half-life values associated with the terminal phase (t½,z) ranged from 2.40 to 4.67 hours. Mean Vss values were greater than total body water. The CV% ranged from 19% to 26% for clearance and from 35% to 44% for Vss after a single administration of 10 and 20 μg/kg doses.

A randomized, double-blind, placebo-controlled, multiple-dose, dose-escalation study (970276) evaluated the safety, tolerability, PK/PD of palifermin administered i.v. to healthy subjects. Palifermin was administered in sequentially enrolling dose cohorts of 20 and 40μg/kg, once daily for 3 consecutive days. Dose escalations were to begin no earlier than day 21 of the previous lower dose cohort. Nine subjects were planned for each cohort and were to be randomized in a 2:1 ratio to receive palifermin or placebo (6 palifermin, 3 placebo). Eighteen subjects were enrolled and 17 completed the study. Most subjects were white (78%), and 50% of subjects were men; subjects were 18 to 63 years of age.

Serum palifermin concentrations declined rapidly within the first 30 minutes after administration at both dose levels. This decline was followed by an increase or plateau in concentration between 1.5- and 4-hours postdose. A consistent decline in concentration was observed beyond 6-hours postdose. Palifermin exhibited an approximately dose-proportional increase in AUC in the dose range of 20 to 40 μg/kg/day, and did not accumulate after 3 daily doses. An average of approximately 98% of the exposure, as measured by AUC, occurred in the first 24 hours postdose. The mean $t_{½,z}$ was approximately 3.5 hours for both dose cohorts on day 1. A longer $t_{½,z}$ was observed on day 3 after injection of the 40-μg/kg dose. The average range of values for mean residence time ranged from 2.6 to 4.7 hours for both cohorts on the 2 assessment days, suggesting that the effective half-life of palifermin was in this range of values.

Similar mean CL values were observed between the dose groups. Mean Vss values were greater than total body water. The CV% ranged from 28% to 33% for CL and from 34% to 37% for Vss after the first administration of palifermin.

A randomized, double-blind, placebo-controlled study (20010192) evaluated the safety, tolerability, PK and PD of escalating single doses of palifermin administered i.v. to healthy subjects. Palifermin was administered in sequentially enrolling dose cohorts of 60, 120, 160, 210, and 250 µg/kg. Subjects were randomized in a 4:1:4:1 ratio to receive palifermin and buccal mucosal biopsy at 48 hours, palifermin and biopsy at 72 hours, placebo and biopsy at 48 hours, or placebo and biopsy at 72 hours. Seventy-nine subjects received placebo or palifermin (16 placebo, 63 palifermin). Most subjects were white (76%), and most were men (96%); subjects were 18 to 53 years of age. Serum palifermin concentrations declined rapidly within the first 30 minutes after i.v. 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.

Serum concentrations of palifermin increased with increased dose. On average, exposure to palifermin increased approximately 3-fold for the 4-fold increase in dose administered (60 µg/kg to 250 µg/kg). Mean CL and Vss values (CV% [25%-36%] and [46%-69%], respectively) did not vary substantially over the examined dose range, indicating approximately linear pharmacokinetics. On average, most (> 95%) of the $AUC0-\infty$ occurred in the first 24 hours postdose. Mean $t_{1/2,z}$ values ranged from approximately 4 to 6 hours across the dose levels evaluated. The CV% ranged from 25% to 36% for CL and from 46% to 69% for Vss.

Pharmacokinetics in patients

Part A of an open-label, single-center study (20010182) characterised the PK profile of 60 µg/kg/day palifermin administered i.v. for 3 consecutive days before TBI/high-dose chemotherapy conditioning treatment (study days -11, -10, and -9) and for 3 consecutive days after PBPC transplantation (study days 0, 1, and 2). The conditioning therapy consisted of fractionated TBI for a total dose of 12 Gy in 8 fractions over 4 days, followed by high-dose chemotherapy with etoposide and cyclophosphamide. Filgrastim (5 mg/kg/day) was administered daily from day 0 after
PBPC transplantation until absolute neutrophil count (ANC) recovery was achieved (ANC > 1.0 x 109/l for 3 consecutive days, ANC > 10.0 x 109/l for 1 day, or day 21, whichever occurred first).

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 concentrations occurred at approximately 1- to 4-hours postdose, followed by a terminal decline phase. No accumulation of palifermin, as measured by AUC from time zero to 24 hours after administration (AUC_{0-24}), was observed upon 3 consecutive daily doses of 60 µg/kg (Table 9). 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 (last dose before chemo- and radiotherapy) and dose 4 (first dose after chemo-radiotherapy), respectively.

Table 9: Arithmetic mean (SD) PK parameters by dose and study day after i.v. administration
of 60 µg/kg of palifermin to patients (Study 20010182, Part A)

Parameters [a] Dose Day	n	C_0 (ng/mL)	AUC_{0-t} [b] (hr•ng/mL)	AUC_{0-24} (hr•ng/mL)	CL (mL/hr/kg)	Vss (mL/kg)	$t_{1/2,z}$ [c] (hr)
1st Dose (Day –11)	13	512 (319)	34.3 (15.9)	37.8 (17.9)	1730 (497)	5320 (2330)	4.87 (2.02)
3rd Dose (Day –9)	13	800 (1790)	39.8 (36.4)	42.6 (37.1)	NR	NR	5.71 (3.60)
4th Dose (Day 0)	13	323 (194)	34.8 (22.5)	35.8 (22.4)	2030 (862)	3870 (2080)	3.27 (1.27)
6th Dose (Day 2)	13	372 (583)	21.2 (15.1)	23.6 (15.2)	NR	NR	3.74 (1.73)

Abbreviations: a = Predose values were excluded from PK analysis for days -9, 0, and 2 to enable estimation of C0; b = The range of values for t was 8 to 24 hours for all profiles except one, in which t was 2 hours; c = n=9-12 for $t_{1/2,z}$ values; AUC_{0-t} = area under the concentration-time curve from time 0 until the last quantifiable concentration; C_0 = estimated initial concentration; AUC_{0-24} = area under the concentration-time curve from time zero to 24 hours after administration CL = clearance; NR = Not reported; $t_{1/2,z}$ = half-life associated with the terminal phase; Vss = Volume of distribution at steady state. Notes: clearance and Vss were reported only after the first dose administration during the 3-day multiple dosing regimen.

A phase I/II randomized, double-blind, placebo-controlled, dose-escalation study (960189) was conducted to evaluate the safety and tolerability of palifermin in patients with Hodgkin's disease and non-Hodgkin's lymphoma undergoing high-dose chemotherapy with autologous PBPC transplantation (PBSCT). Patients 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.

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. Nineteen subjects were enrolled into this cohort (4 placebo, 15 palifermin). Most subjects were white (95%), and most were men (73%); subjects were 22 to 67 years of age.

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 AUC0-t values were similar (< 20% change) after the first and third dose. Mean $t_{1/2,z}$ was approximately 4 hours, and was similar to MRT. The CV% was 47% for CL, 62% for Vss, and 50% for AUC_{0-t} after the first dose.

Special populations

Impaired renal function

An open-label, single-dose study (20030142) was conducted to assess the PK of palifermin after i.v. administration in subjects with various degrees of renal function. Subjects were selected for participation based upon level of renal function. Groups were established as follows:
- Group 1 (normal renal function, creatinine clearance [CLcr] > 80 ml/min)
- Group 2 (mild chronic kidney disease, CLcr: 50 to 80 ml/min)
- Group 3 (moderate chronic kidney disease, CLcr: 30 to 49 ml/min)
- Group 4 (severe chronic kidney disease, CLcr < 30 ml/min)
- Group 5 (subjects with end-stage renal disease, receiving hemodialysis)
All subjects received a single i.v. dose of 90 µg/kg on day 1. Thirty-one subjects, from 20 to 77 years old, were enrolled and received palifermin. The majority were men (81%), white (94%).
The difference in mean clearance for groups 2, 3, 4 and 5 relative to group 1 was less than 25% (mean [SD] CL values were 495 [119], 387 [118], 318 [39], 365 [119] and 406 [98] ml/hr/kg, respectively), with one subject excluded from group 4 due to substantially different PK parameter values.

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Impaired liver function

Studies assessing the pharmacokinetic profile of palifermin in patients with hepatic impaired function have not been performed.

Effect of demographic variables

The effect of gender, race, weight and age on the pharmacokinetics of palifermin was examined graphically using a combined dataset from studies performed in healthy volunteers, and a second combined dataset from studies performed in patients with hematologic malignancies. In addition, an analysis of the effect of race on the pharmacokinetics of palifermin was conducted using a dataset from the healthy volunteer studies combined with data from study 970136 (Japan).
CL and V_{ss} were similar between male and female, or between Caucasian, Black and Hispanic populations. The overall mean (SD) CL values for men and women were 588 (244) and 653 (248) ml/hr/kg, respectively. Mean (SD) V_{ss} values for men and women were 1959 (1086) and 2016 (1332) ml/kg, respectively. The mean CL was approximately 50% higher in Asian subjects (953 ml/hr/kg) compared with White subjects (629 ml/hr/kg). Weight did not affect the pharmacokinetics of palifermin.
No PK data were available in children, nor in patients above the age of 70.

Pharmacokinetic interaction studies

Specific drug interaction studies between palifermin and other drugs have not been performed.

Discussion on pharmacokinetics

Data from five randomised, double-blind and placebo-controlled studies have been provided from healthy volunteers. However these included mostly young, white men and were thus not representative of the patient population, particularly in terms of age. The dose and schedule of palifermin evaluated in the target population were chosen for consistency with the pivotal phase III study.
Palifermin exhibited linear pharmacokinetics in the dose range of 10μg to 250μg. Palifermin has a large volume of distribution (approximately 2 l/kg in healthy volunteers and 4-5 l/Kg in patients) and the distribution was rapid. The Vss was greater than the total body water implying extravascular distribution after i.v. administration. After i.v. administration, plasma concentrations rapidly decline during the first 30 minutes. After this rapid decline, a slight increase or plateau in concentrations occurred at approximately 1-4 hours post-dose, followed by a terminal decline phase. This transient increase in concentration might have been related to redistribution of palifermin out of the tissues. The average terminal half life was similar in healthy volunteers and patients with haematalogical malignances (approximately 4.5 hours) and no accumulation of palifermin occurred after 3 consecutive doses in both groups (20 and 40 μg/kg in healthy volunteers and 60 μg/kg in patients). Nevertheless, inter-subject variability of approximately 69% was observed in CL and Vss in both healthy volunteers and patients. Palifermin clearance was about 500 ml/h/kg in healthy volunteers and 2-3-fold higher in patients (mean clearance of about 1300 ml/h/kg). Given the size of the molecule (140 amino acids, 16.3 kD), filtration in the kidney with subsequent catabolism in the renal cells may contribute to the elimination. Time dependency in the pharmacokinetics was observed with, on average, a 40% to 46% lower AUC after dose 6 than that after dose 3 (last dose before chemo- and radiotherapy) and dose 4 (first dose after chemo-radiotherapy), respectively. The higher CL and Vss observed in patients with haematological malignancies and the lower AUC of palifermin observed after repeated dosing with cytotoxic agents have been discussed. Internalization of the KGF/KGF receptor (KGFR) complex has been demonstrated *in vitro* in cell systems expressing the KGF receptor [42, 43]. Therefore, clearance of palifermin may be mediated by receptor internalization and subsequent intracellular breakdown. If patients had a higher level of KGF receptor (as occurs with injury [44]), they could exhibit higher clearance of palifermin. In particular, if exposure to TBI and chemotherapy resulted in an increased expression of the receptor, this could lead to a higher clearance and thus reduced exposure in patients as compared to healthy volunteers. This suggested that inter- and intra-patients differences in KGF receptor density might be an important factor in PK variability

of palifermin. The main pharmacokinetics properties have been described appropriately in section 5.2 of the SPC. The full results from the ongoing single dose study 20010182 Part B will be provided upon completion, post authorisation.

European PK Guidelines recommend to assess the absence of interaction between two drugs indicated to be used in association [45]. Nevertheless, *in vitro* studies to assess the metabolism of palifermin and specific drug interaction studies between palifermin and other drugs have not been performed. The applicant has justified this by stating that pharmacokinetic interactions with medicinal products (small molecules) commonly administered in the patient population are not anticipated since the metabolism and elimination mechanisms differ and are not considered to impact on each other. Moreover, in controlled studies, no apparent difference was observed in neutrophil recovery between placebo and palifermin in patients undergoing myeloablative therapy who routinely received granulocyte colony-stimulating factor (G-CSF) after PBSCT. However, keratinocyte growth factor is a heparin binding protein; it also interacts with heparin sulfate proteoglycans (HSPG), which are present on cell surfaces and in the extracellular matrix. Palifermin, being a truncated version of KGF, is likely to interact with heparin and HSPG. To date there are no *in vitro* (or *in vivo*) data on the effect of KGF binding to heparin on the biological activity of heparin itself e.g. binding to Antithrombin III. Oncology patients are at increased risk of developing thromboembolism, which involves anticoagulation treatment with unfractionated or low-molecular weight heparin and progression to oral anticoagulant therapies. In patients with hematologic malignancies undergoing high dose cytotoxic therapy followed by PBSC support, the systemic use of heparin is extremely unlikely due to the patient thrombocytopenic status. However, heparin is generally used to maintain the central line open; therefore if heparin is used to maintain an i.v. line, saline should be used to rinse the line prior to and after palifermin administration. This information is adequately addressed in section 6.2 of the SPC. Moreover, section 4.5 of the SPC includes information that *in vitro* data suggest that palifermin binds to heparin. To date, the clinical relevance is unclear (see SPC, section 4.5). *In vitro* and *in vivo* studies in healthy volunteers to evaluate the drug-drug interaction of palifermin with heparin are planned and results will be assessed post-authorisation.

Due to injection-site reactions observed in a phase I study in which palifermin was administered subcutaneously, section 4.2 of the SPC states that palifermin should not be administered subcutaneously due to poor local tolerability. The pharmacokinetic profile of palifermin has been evaluated in subjects with various degrees of renal function. Mild to moderate renal impairment (creatinine clearance 30-80 ml/min) did not influence the pharmacokinetics of palifermin. In patients with severe renal impairment (creatinine clearance <30 ml/min), clearance was decreased by 22% (n=5). In patients with end-stage renal disease (requiring dialysis) palifermin clearance was decreased by 10% (n= 6). These figures have been included in section 5.2 of the SPC. From these results, it has been considered that dose adjustment in patients with renal impairement was not necessary (see SPC, section 4.2).

The pharmacokinetic profile of palifermin has not been evaluated in patients with hepatic impairment. This information is adequately mentioned in section 4.2 and 5.2 of the SPC. Neither weight nor gender had a notable effect on the PK of palifermin. Age did not significantly alter the clearance of palifermin although the limited numbers of elderly patients preclude definitive conclusions. The effect of race could not be assessed due to a majority of White subjects included in the studies. The PK profile in paediatric population and in elderly (age above 70 years) has not been assessed. This information has been adequately included in section 4.2 and 5.2 of the SPC.

In conclusion, the pharmacokinetics of palifermin have been adequately investigated and reported, in agreement with applicable guidelines and requirements [21, 46].

Pharmacodynamics

Plasma concentration and effect relationship

Ki67 is a nuclear antigen strictly associated with cell proliferation. Because the Ki67 protein is present during all active phase of the cell cycle but absent during the resting phase, it is used as a marker of proliferation in human cells [47]. 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.

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Ki67 staining was defined as the primary measure for epithelial cell proliferation, and a 200% (3-fold) or greater increase in Ki67 staining was defined as the cut off value for biologic response. The use of this threshold for biologic response was based on non-clinical data (see non-clinical primary pharmacodynamics), which demonstrated that, at doses known to prevent oral mucositis in animal models, the increase in Ki67 staining in the buccal mucosa at 48 hours after palifermin administration was at least 3 times greater than at baseline.

Mitotic figure counting, another mean of assessing cellular proliferation, involves counting the number of mitotic cells in a tissue section to determine degree of proliferation as part of the Scarff-Bloom-Richardson classification [48]. Mitotic figure counts were used as secondary marker of biologic activity. A response of >150% was considered to be a strong indicator of epithelial proliferation.

In study 960136 (see clinical pharmacokinetics), the pharmacodynamic assessments of buccal mucosal epithelial proliferation, as measured by Ki67 staining and mitotic figure counts were based on buccal mucosal biopsies performed at baseline and at day 3 for single-dose cohorts, and baseline and day 4 for multiple-dose cohorts.
When palifermin was administered as a single injection (doses 0.2 - 20µg/kg), notable epithelial proliferative activity was not observed at the doses and time points tested. At multiple doses of 20 µg/kg/day for 3 consecutive days, 1 subject met the criterion for epithelial proliferation.

In study 970276 (see clinical pharmacokinetics), the pharmacodynamic assessments of buccal mucosal and forearm epithelial proliferation and thickness, as measured by Ki67 staining and mitotic figure counts, were based on biopsies performed pre-dose and 24 hours after the last treatment.
In the buccal mucosa, increased response with increased dose was observed in the Ki67-staining analyses. 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.

In study 20010192 (see clinical pharmacokinetics), pharmacodynamic assessments of buccal mucosal epithelial proliferation and epithelial thickness were based on buccal mucosal biopsies performed pre-dose and 48 or 72 hours post-dose.
In the buccal mucosa, increased response with increased dose was observed in the Ki67-staining analyses. At 48 hours after dose administration, 11 of 12 subjects who received 160-, 210- or 250-µg/kg palifermin met the predefined criterion for epithelial proliferation. At 72-hours after dose administration, a lesser dose-response relationship was observed. In the 210- to 250-µg/kg cohorts, 5 of 8 subjects had a 100% to 199% increase in Ki67 staining.

Immunogenicity

Subjects were monitored throughout the palifermin clinical development program for the development of anti-palifermin antibodies and to characterise any positive results.
A total of 964 patients (321 placebo; 643 palifermin) were tested for anti-palifermin antibodies using an ECL-based MSD assay. Of the 964 subjects tested by the ECL-based MSD assay, only 12 (2%) palifermin treated patients and 5 (2%) placebo treated patients tested positive and were all below the quantitation limit for anti-palifermin antibodies. None of these patients showed any neutralizing activity in the bioassay. Development of anti-palifermin antibodies was not observed.

Discussion on pharmacodynamics

Epithelial cell proliferation was assessed by Ki67 immunohistochemical staining in healthy subjects. The pharmacodynamic response increased with dose of palifermin and, at total doses of 120 µg/kg and higher, at least 50% of the subjects met the criterion for biologic activity. Although an average half-life of 4.9 hours was observed in healthy volunteers, a pharmacologic effect was observed at 48-hours post-dose, with a smaller effect observed 72-hours post-dose. At 48 hours after dosing, most of the quantifiable palifermin concentration values were less than twice the lower limit of quantification of the assay, indicating that the pharmacologic effect persisted after active drug levels have dissipated.

For biologics, persistence of effect (or a delayed effect) relative to drug concentrations is not unexpected.

Based on the longer PD effect of palifermin compared with the PK, the PD endpoints appeared more relevant to consider for dosing frequency than the PK endpoint. In the multiple dose study, 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 (see section 5.1, pharmacodynamic properties of the SPC). However, in the dose escalation study in healthy volunteers, using the dose of 60 µg/kg, PD response was observed only in 1/8 subjects compared with 2/4 who responded to a dose of 120 µg/kg and 4/4 subjects responded to doses of 210 and 250 µg/kg at 48 hours. No data with multiple doses were available for 60 µg/kg at similar time points, to critically assess PD response of the proposed dosage.

No long-term immunogenicity data were available (beyond 2-3 months) to determine the safety of the product. Immunogenicity will be addressed and data provided for assessment by the CHMP through periodic safety update reports (see list of post-authorisation follow-up measures).

For the assessment of pharmacodynamic interactions with myeloablative chemo-radiotherapy, S-phase specific cytotoxic drugs such as ara-C and other growth factors (or inhibitors), see clinical safety.

Clinical efficacy

Dose response studies

Palifermin has been used to reduce the incidence, severity, and duration of oral mucositis and relevant clinical sequelae at a dose of 60 µg/kg for 3 consecutive days before conditioning treatment and for 3 consecutive days after PBPC infusion (referred to as pre-post dosing).

These dose and regimen were initially selected based on the tolerability observed in the phase I/II, dose-escalation study 960189 in patients (see Pharmacokinetic).

The phase II study 980231(see main studies) further evaluated these dose and regimen, as well as a regimen that excluded dosing after PBPC infusion (pre dosing only). Results from this study showed that palifermin at the dose of 60 µg/kg/day was well tolerated and provided efficacy regardless of whether pre or pre-post dosing was used. However, because the outcomes for most of the efficacy endpoints were numerically better with pre-post dosing than with pre dosing, pre-post administration was chosen for use in the pivotal phase III study 20000162 (see main studies).

Main studies

Study 20000162 was a phase III, multicenter, randomized, double-blind, placebo-controlled, parallel-group trial, examining the safety and the efficacy of palifermin compared with placebo in patients with hematologic malignancies who were receiving myeloablative conditioning treatment with autologous PBPC transplantation [49].

Patients and methods

Study participants – eligibility criteria

Patients who were scheduled to undergo TBI plus high-dose chemotherapy followed by autologous PBPC transplantation for the treatment of the following hematologic malignancies: non-Hodgkin's lymphoma, Hodgkin's disease, acute myelogenous leukaemia, acute lymphoblastic leukaemia, chronic myelogenous leukaemia, chronic lymphocytic leukaemia or multiple myeloma were assessed for eligibility. Eligibility requirements included Karnofsky performance score (KPS) $\geq 70\%$, age ≥ 18 years, minimum of 1.5 x 10^6 CD34$^+$ cells/kg cryopreserved for infusion.

Treatments

Eligible patients were randomized to receive placebo or 60 µg/kg/day of palifermin for 3 consecutive days, starting 3 days before the conditioning regimen (TBI plus high-dose chemotherapy), and 3 additional daily doses post PBPC infusion on day 0 (day of PBPC infusion), day 1, and day 2 (see figure 1).

The investigational products consisted of either 6.25-mg single-dose vials of lyophilized palifermin for reconstitution with 1.2 ml water for injection using a dose of 60 µg/kg/day by i.v. bolus injection or placebo, presented in vials containing all the ingredients of lyophilized powder except for palifermin, reconstituted with 1.2 ml sterile water for injection. Dose reduction of the investigational product could be made on days 0, 1, or 2 only if, by study day 0, a patient's weight had decreased by 10% or more from baseline. No dose escalations were permitted.

The conditioning therapy consisted of TBI for a total dose of 12 Gy in 6, 8, or 10 fractions over 3 or 4 days, followed by high-dose chemotherapy (etoposide and cyclophosphamide). Etoposide was administered at the dose of 60 mg/kg as a single i.v. infusion over 4 hours on day 4. Cyclophosphamide was administered at a total dose of 100 mg/kg given in 1 dose over 1 hour on day 2. Patients were to receive hydratation at the rate of 3.0 l/m^2/day beginning 4 hours before and continuing 24 hours after cyclophosphamide. In addition, continuous bladder irrigation for 24 hours and/or treatment with mesna (2-mercaptoethane sulphonate sodium) was to be considered [50].

Patients received autologus PBPCs collected after mobilization by either cytokines or chemotherapy with or without cytokines.

Filgrastim (5 µg/kg/day) was administered from day 0 after transplant until neutrophil recovery (absolute neutrophil count [ANC] > 1.0 x 10^9/l for 3 consecutive days or > 10 x 10^9/l for 1 day, or day 21, whichever occurred first).

Figure 1 - Study schema for study 20000162
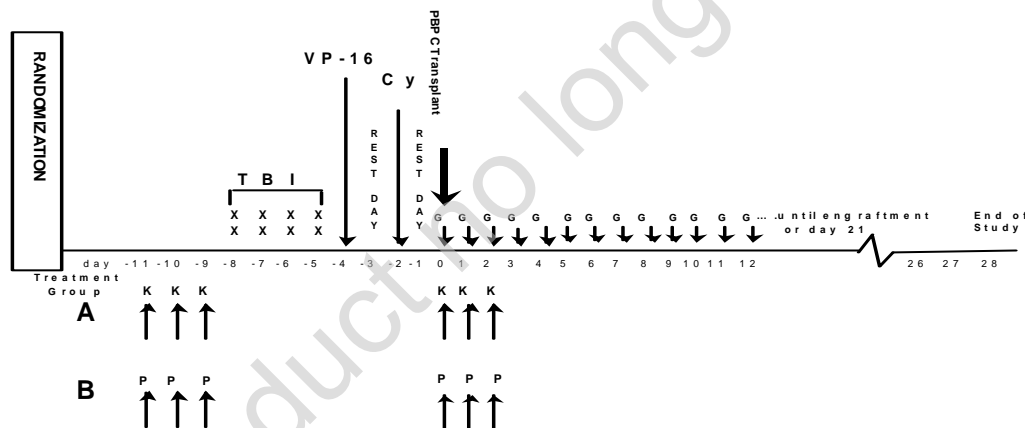
Abbreviations: Treatment group A: K = palifermin; Treatment group B: P = placebo; TBI = fractionated total body irradiation; VP-16 = etoposide; Cy = cyclophosphamide ; Conditioning Regimen: TBI / etoposide (VP-16) / cyclophosphamide (Cy); G = Filgrastim.

Objectives / Endpoints

The primary objective of this study was to evaluate the efficacy of palifermin in reducing the duration of severe oral mucositis (World Health Organisation [WHO] grade 3 or 4) in patients with haematological malignancies who were undergoing PBPC transplantation after fractionated total body irradiation (TBI) and high dose chemotherapy.

The primary endpoint was the duration of severe oral mucositis calculated as the total number of days a patient experienced grade 3 or 4 oral mucositis determined using the WHO toxicity scale "Oral Toxicity". Mean duration of severe oral mucositis for each treatment group was calculated as the average duration for all patients in that treatment group. If a patient did not experience any WHO grade 3 or 4 oral mucositis, the duration of severe oral mucositis was assigned 0 days.

The secondary objectives were to evaluate:
- patient's daily assessment of mouth and throat soreness (patient-reported outcome [PRO]),
- use of parenteral or transdermal opioid analgesics (in mg morphine equivalents),

- incidence of grade 4 oral mucositis determined using the WHO scale,
- duration (days) of grade 2, 3, and 4 oral mucositis determined using the WHO scale,
- duration of oral mucositis determined by Western Consortium of Cancer Nursing Research (WCCNR) scale, descriptor lesions grades 2 and 3, and Radiation Therapy Oncology Group (RTOG) scale grades 3 and 4,
- the safety of palifermin.
The WHO oral mucositis scale provides the following definitions: Grade 1 = soreness and erythema; Grade 2 = erythema, ulcers, patients can swallow solid diet; Grade 3 = ulcers, extensive erythema, patients cannot swallow solid diet; Grade 4 = mucositis to the extent that alimentation is not possible

Sample size

The null-hypothesis for study was that palifermin and placebo recipients will experience the same duration (in days) of severe oral mucositis. The alternative hypothesis was that the duration of severe oral mucositis in the two treatment groups would be different. The sample size was based on detecting a 3-day difference in the duration of severe oral mucositis (WHO grade 3 or 4) between the palifermin and placebo groups. The estimated common standard deviation obtained from the phase II study 980231 was 6.6 days. Based on these assumptions, the two sample independent t-test with 90% power and 5% significant level (2-sided) yields approximately 105 patients per group for a total of 210 patients. The primary analysis method, though different from the t-test approach, was not expected to have lower power since the stratified analysis was expected to be more efficient.

Randomization

Eligible patients were randomly assigned to one of two groups, palifermin or placebo (allocation ratio 1:1). Treatment assignment was blinded for the duration of the study. The type of randomization was a stratified permuted-block design randomization based on two factors: study center (approximately 15 centres in North America) and type of hematologic malignancy (NHL, Hodgkin's disease, multiple myeloma, or any acute or chronic leukaemia).

Blinding (masking)

This study was a double-blind trial. Sealed emergency unblinding envelopes were provided. A patient's treatment assignment was only to be unblinded when knowledge of the treatment was essential for the future medical management of the patient. Unblinding for any other reason was considered a protocol deviation.
Palifermin administration may cause transient elevations in serum amylase and lipase in about 1 of 5 patients. These analyses were therefore performed by a central laboratory that was to reveal results to the study centre, only upon request by the investigator, in the event of a clinical emergency, and otherwise not before study completion.
Approximately 1 of 4 patients who received palifermin experienced oral and skin-related toxicities that peaked at 36 hours after the last injection and usually resolved within 3 days from onset. These events could in theory bias the investigators. Sensitivity analyses were therefore conducted censoring patients with these events.

Statistical methods

The primary efficacy analysis set was the modified intent-to-treat (mITT) analysis set, which was defined as all randomized patients who received at least 1 dose of investigational product and who were analyzed according to their randomized treatment assignment.
The per-protocol efficacy analysis set included all patients who complied with the study protocol with respect to the entry criteria, treatment procedures, and guidelines.
The statistical analysis on the primary endpoint, duration of severe mucositis (WHO grade 3 or 4), was based on the generalised Cochran-Mantel-Haenszel (CMH) method. A 95% confidence interval on the treatment difference was also provided. The primary efficacy analysis was adjusted by study center or both study center and type of haematological malignancy. Centers with less than 6 patients were pooled into 1 center and formed a single stratum.
Resolution of severe oral mucositis was defined as the first time oral mucositis was decreased to WHO grade 2 or less after the last reading of a WHO grade 3 or 4. For patients who died or withdrew from the study, resolution of severe mucositis was defined as 2 consecutive assessments of WHO grade 2 or

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less after the last reading of a WHO grade 3 or 4; the resolution date was the first of the 2 consecutive days with WHO grade 2 or less.

Three PRO endpoints were defined. Two were from the "Oral Mucositis Daily Questionnaire" (OMDQ): 1/ mouth and throat soreness and 2/ daily activities related to mouth and throat soreness. The third endpoint was the physical well-being domain from the "Functional Assessment of Cancer Therapy" (FACT-G) quality of life questionnaire.

Results

Participant flow and numbers analysed

Study 20000162 screened 245 patients. A total of 214 patients (107 each randomized to receive placebo or palifermin) were enrolled from 13 centers (United States). Of the 214 subjects who were randomized, 1 patient randomized to receive placebo and 1 patient randomized to receive palifermin did not receive investigational product. The remaining 212 patients (106 from each treatment group) received investigational product and formed the evaluation subsets for efficacy (mITT) and safety results. The mITT population was defined as those patients who received at least one dose of investigational product. In the placebo group, 103 patients completed the study (3 patients discontinued) and 104 in the palifermin group (2 patients discontinued). The highest-enrolling center enrolled 40 patients (19%).

The percentage of patients with at least 1 important protocol deviation was 25% (26/106) in the palifermin group and 32% (34/106) in the placebo group. However, the mean number of important protocol deviations was similar for both groups.

Recruitment

The first patient was enrolled on 23 March 2001 and the last end-of-study visit was on 23 October 2002.

Conduct of study

The original protocol of this study was amended once. Amendment 1 allowed a third oral mucositis assessment tool (RTOG scale) to be implemented and the establishment of the Safety Monitoring Committee. In addition, this amendment extended the time window for screening patients from 21 to 42 days for some evaluations and added steroid rinses as a proscribed therapy.

Baseline data

Demographic and disease baseline characteristics are shown in Table 10. The proportions of men enrolled (n = 131) were higher than the proportions of women (n = 81). Most patients had been diagnosed with Non-Hodgkins Lymphoma (65% of the placebo group and 68% of the palifermin group).

Table 10. Baseline characteristics (mITT populations)

	Placebo (n=106)	Palifermin Pre-Post (60 µg/kg/day) (n=106)
Sex		
Male	72 (68%)	59 (56%)
Female	34 (32%)	47 (44%)
Race		
Caucasian	89 (84%)	78 (74%)
Black	7 (7%)	11 (10%)
Hispanic	7 (7%)	11 (10%)
Asian	1 (1%)	4 (4%)
Other	2 (2%)	2 (2%)
Age (years)		
Mean (SD)	46.4 (11.7)	46.2 (12.1)
Median	49.0	47.5
Q1,Q3	38.0, 54.0	37.0, 57.0
Min,Max	19.0, 68.0	18.0, 69.0
ECOG Performance Status		
0	86 (81%)	88 (83%)
1	20 (19%)	18 (17%)
Type of Diagnosis		
Non-Hodgkins Lymphoma	69 (65%)	72 (68%)
Hodgkins Disease	23 (22%)	21 (20%)
Multiple Myeloma	9 (8%)	11 (10%)
Leukemia	5 (5%)	2 (2%)
Patients with Prior Chemotherapy		
Yes	106 (100%)	106 (100%)
Patients with Prior Radiotherapy		
Yes	9 (8%)	13 (12%)
No	97 (92%)	93 (88%)
G-CSF		
Yes	105 (99%)	105 (99%)
Median number of days of exposure	13	12
Mobilization		
Cytokines Only	30 (28%)	26 (25%)
Chemotherapy Only	0 (0%)	1 (1%)
Cytokines and Chemotherapy	76 (72%)	79 (75%)

Efficacy results

For the mITT population, the mean (SD) duration of WHO grade 3 or 4 oral mucositis in the palifermin group was 64% lower than in the placebo group (3.7 [4.1] days *versus* 10.4 [6.2] days, respectively). The reduction was statistically significant (p<0.001) and was reproducible across study centers, underlying disease, and number of radiotherapy fractions used in the TBI conditioning regimen.

The Lachenbruch's 2-part model was performed as a sensitivity analysis [51], to consider both the incidence of WHO grade 3 or 4 oral mucositis and the duration for those patients who developed this toxicity. Palifermin reduced both the duration (44% decrease in mean duration of severe oral mucositis in the palifermin group [5.9 days] compared with the placebo group [10.6 days], p < 0.001) and the incidence of severe oral mucositis (63% patients in the palifermin group *vs*. 98% patients in the placebo group, p < 0.001).

Results of the per-protocol efficacy analysis set were similar to the results obtained for the mITT population.

The secondary assessment of mouth and throat soreness showed a lower score (indicating less soreness) in the palifermin group *versus* the placebo group, with a reduction of 38% of the mean (SD) AUC, or a reduction in mean daily AUC score of 0.49 (mean [SD] AUC was 32.6 [20.8] in the palifermin group *versus* 52.5 [22.4] in the placebo group, p < 0.001).

The median cumulative dose of opioid analgesic used for oral mucositis was 211.6 mg in the palifermin group compared with 534.9 mg in the placebo group (p < 0.001). The incidence of opioid use was 78% for the palifermin group and 97% for the placebo group (p < 0.001). The mean (SD) cumulative dose of opioid analgesics for patients with pain caused by mucositis was 893.4 (1934.7) mg in the palifermin group and 1179.9 (1715.4) mg in the placebo group.

The mean (SD) duration of WHO grade 4 oral mucositis for the mITT population was 3.9 (5.1) days for the placebo group and 0.7 (1.7) days for the palifermin group. The median (min, max) duration was 2.0 (0, 37.0) days for the placebo group and 0 (0, 9.0) days for the palifermin group.

The incidence of WHO grade 4 oral mucositis was 62% of patients in the placebo group and 20% of patients in the palifermin group (p < 0.001). The mean (SD) duration of WHO grade 4 oral mucositis for these patients was 6.2 (5.2) days for the placebo group and 3.3 (2.3) days for the palifermin group. The median (min, max) duration was 6.0 (1.0, 37.0) days for the placebo group and 2.0 (1.0, 9.0) for the palifermin group. The mean (SD) duration of WHO grade 2, 3, or 4 oral mucositis was statistically significantly shorter for patients in the palifermin group compared with the placebo group (mean (SD) was 15.7 (7.8) vs. 8.4 (5.8), p < 0.001, respectively).

Positive correlations were seen between the severe oral mucositis duration using the WHO scale and that using the RTOG scale (Spearman correlation, r = 0.72) and between the severe oral mucositis duration using the WHO scale and that using the WCCNR scale (Spearman correlation, r = 0.71). The results of both scales were consistent with the WHO scale.

A summary of efficacy results for study 20000162 is shown in table 11.

Ancillary analyses

Hematology variables were recorded between study day 5 and 21, and maximum daily temperatures were recorded between study day 8 and 21. Since all patients received myeloablative therapy, virtually all patients (104 [98%] placebo, 106 [100%] palifermin) experienced severe neutropenia, defined as ANC < 500 x 10^6/l. Febrile neutropenia, defined in the protocol as an ANC of < 500 x 10^6/l with a concurrent temperature of at least 38.5^oC, was experienced by 97 (92%) patients who received placebo and 79 (75%) patients who received palifermin (p < 0.001).

Twenty-five percent of patients in the placebo group and 15% of patients in the palifermin group reported at least 1 incidence of a bloodborne infection. For both febrile neutropenia and infections, the time to haemopoietic recovery was similar between groups.

The total number of days of parenteral feeding for the mITT population was 761 days for the placebo group and 459 days for the palifermin group. Forty-three percent of patients in the placebo group and 11% of patients in the palifermin group needed parenteral feeding as a result of the severity of their oral mucositis. The total number of days of parenteral feeding for these patients was 569 days for the placebo group and 158 days for the palifermin group.

Table 11. Efficacy results for study 20000162

	Placebo (N = 106)	Palifermin 60 µg/kg/day (N = 106)	p-value [b]	95% CI
WHO Grade 3 or 4 - Duration (days)				
mean (SD) - mITT population	10.4 (6.2)	3.7 (4.1)	< 0.001	(5.3, 8.1)
Median (25th, 75th persentile) - mITT population	9 (6, 13)	3 (0, 6)	<0.001	
WHO Grade 3 or 4 - Incidence - n (%)	104 (98)	67 (63)	< 0.001	(25%, 45%)
WHO Grade 3 and 4 - Duration (days)				
mean (SD) - affected patients	10.6 (6.1)	5.9 (3.6)	< 0.001	
median (25th, 75th persentile) - affected patients	9 (6, 13)	6 (3, 8)		
WHO Grade 4 - Incidence - n (%)	66 (62)	21 (20)	< 0.001	(30%, 54%)
WHO Grade 4 - Duration (days)				
mean (SD) - mITT population	3.9 (5.0)	0.7 (1.7)	< 0.001	
mean (SD) - affected patients	6.2 (5.2)	3.3 (2.3)		
WHO Grade 2, 3, or 4 - Incidence - n (%)	105 (99)	97 (92)	0.010	(1%, 13%)
WHO Grade 2, 3 or 4 - Duration (days)				
mean (SD) - mITT population	15.7 (7.8)	8.4 (5.8)	< 0.001	(5.4, 9.2)
median (25th, 75th persentile) - mITT population	14 (11, 19)	8 (4, 12)	<0.001	
mean (SD) - affected patients	15.8 (7.7)	9.1 (5.4)		
Patient-reported mouth and throat soreness				
VDS scale (AUC) [a]				
mean (SD)	52.5 (22.4)	32.6 (20.8)		(14.1, 25.7)
median (Min:Max)	46.8 (0:110)	29.0 (0:98)	< 0.001	
i.v. or t.d. opioid analgesic use [c]- Incidence - n (%)	103(97)	83 (78)	< 0.001	(10%, 28%)
Cumulative dose of opioid analgesics - mITT population				
mean (SD)	1146.5 (1701.5)	699.5 (1747.8)	< 0.001	(-17.6, 911.6)
median (min:max)	534.9 (0:9403)	211.6 (0:9418)		
(25th, 75th persentile)	(269, 1429)	(3, 558)		
Days of opioid analgesic use - mITT population				
mean (SD)	11.8	6.7		
median (min:max)	11 (0:32)	7 (0:28)	< 0.001	
(25th, 75th persentile)	(8, 14)	(1, 10)		
Maximum Severity (Worst WHO Grade)				
mean (SD)	3.6 (0.6)	2.7 (0.9)	<0.001	(0.7, 1.1)
median (min:max)	4.0 (0:4)	3.0 (0:4)		
Supplemental Feeding - Incidence - n (%)	58 (55)	33(31)	< 0.001	(11%, 37%)
Febrile Neutropenia - Incidence - n (%)	97 (92)	79 (75)	< 0.001	(7%, 27%)

<u>Abbreviations</u>: VDS = verbal descriptive scale; i.v. = intravenous; t.d. = transdermal; [a] Likert-type scale (0 = no soreness; 4 = extreme soreness); [b] All p-values were calculated for the comparison with placebo using a generalized CMH test based on the standardized mid-ranks (modified Ridit scores) within each stratum. All analyses of incidence used the mITT population, defined as those subjects who received at least one dose of investigational product (1% of subjects randomized did not receive investigational product); [c] morphine mg equivalent.

Study 980231 was a phase II, multicenter, randomized, double-blind, placebo-controlled, 3-arm, parallel-group trial, examining the safety and the efficacy of palifermin on the severity and duration of oral mucositis and related sequelae in patients with hematologic malignancies who were undergoing TBI and high-dose chemotherapy with autologous PBPC transplantation.

Patients and methods

Study participants – eligibility criteria

Patients who were scheduled to undergo TBI plus high-dose chemotherapy followed by autologous PBPC transplantation for the treatment of the following hematologic malignancies: non-Hodgkin's lymphoma, Hodgkin's disease, acute myelogenous leukaemia, chronic lymphocytic leukaemia or multiple myeloma were assessed for eligibility.

Eligibility requirements included Karnofsky performance score (KPS) $\geq$ 70%, age range 12 to 65 years, minimum of 1.5×10^6 CD34$^+$ cells/kg cryopreserved, ANC $> 1 \times 10^9$/l, platelet count $> 100 \times 10^9$/l (for patients scheduled to start the conditioning regimen soon after apheresis, a platelet count $< 100 \times 10^9$/l but $> 50 \times 10^9$/l was acceptable).
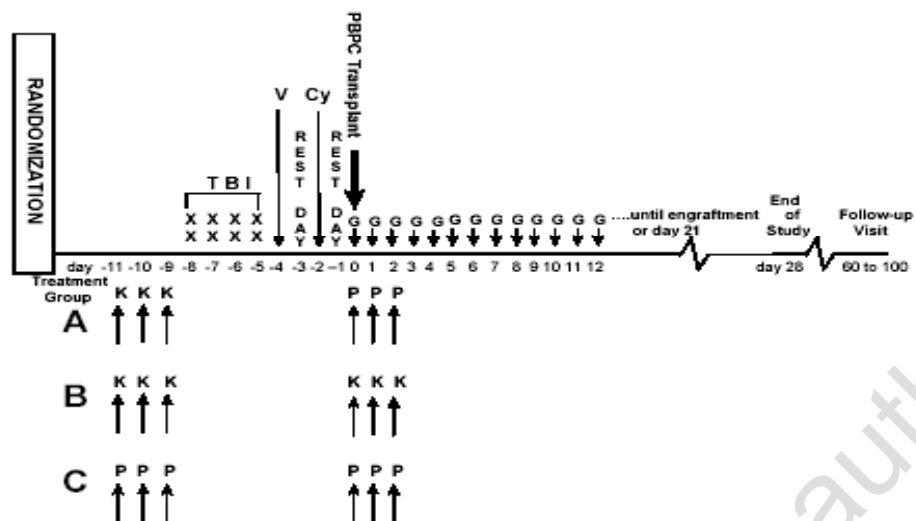
Treatments

Eligible subjects were randomised in a 1:1:1 ratio to one of the three treatment regimens in which they received 7 doses of investigational product (60 µg/kg/day of palifermin or placebo):
- "pre-post" dosing of palifermin: palifermin was administered for 3 consecutive days before the conditioning regimen began (fractionated TBI and high-dose chemotherapy), and on the day of the last TBI fraction immediately after administration of the last radiation fraction, and for 3 consecutive days beginning immediately after PBPC infusion.
- "pre" dosing of palifermin: palifermin was administered for 3 consecutive days before the conditioning regimen began, and on the day of the last TBI fraction immediately after administration of the last radiation fraction, and placebo administered for 3 consecutive days beginning immediately after PBPC infusion.
- placebo was administered for 3 consecutive days before the conditioning regimen began, and on the day of the last TBI fraction immediately after administration of the last radiation fraction, and for 3 consecutive days beginning immediately after PBPC infusion.

Further to an amendment to the protocol, the dose of investigational product administered on the day of the last TBI fraction was eliminated, which resulted in a 6-dose treatment schedule (see figure 2). This amendment was based on a review of animal data and on concerns of eliminating benefit or worsening of mucosal injury when the time period between palifermin and chemotherapy administration was shorter than 24 hours.

For administration of investigational products, conditioning therapy, autologus PBPCs and filgrastim, see study 20000162. Cyclophosphamide was administered as a single dose of 75 or 100 mg/kg infused i.v. over 1 hour on day 2. Patients received hydratation as described in study 20000162.

Figure 2 - Study schema for study 980231

Abbreviations: K = palifermin; P = placebo; Treatment group A: palifermin pre; Treatment group B: palifermin pre-post; Treatment group C: placebo; TBI = fractionated total body irradiation; VP-16 = etoposide; Cy = cyclophosphamide ; Conditioning Regimen: TBI / etoposide (VP-16) / cyclophosphamide (Cy); G = Filgrastim.

Objectives / Endpoints

The primary objective of this study was to evaluate the effects of palifermin on oral mucositis induced by myeloablative radiation (TBI) and high-dose chemotherapy in patients with hematologic malignancies. The primary endpoint was the duration (days) of grade 3 or 4 oral mucositis (severe oral mucositis) measured by the WHO toxicity scale.

The secondary objectives were to evaluate:
- the incidence of severe oral mucositis,
- the oral mucositis area under the curve (AUC) for all severity grades,
- the AUC of t.d. or i.v. opioid analgesic use (actual daily doses converted to equianalgesic units),
- the PRO assessment for palifermin, specifically the domain of mouth and throat soreness,
- the incidence and duration of grade 2, 3, or 4 diarrhea, as determined using the revised National Cancer Institute common toxicity criteria (NCI CTC) scale,
- the duration (days) of febrile neutropenia defined as ANC < 1.0 x109/L, fever 38.5°C,
- the duration (days) of treatment with i.v. antifungals or i.v. antibiotics for febrile neutropenia or documented infections (microbiologically or clinically) caused by bacteria or fungi,
- the incidence of intestinal absorption and permeability abnormalities expressed as the ratio of differential sugar probes, such as lactulose to mannitol excreted in the urine (for subjects at specific centers only),
- the safety of palifermin.

Sample size

The sample size calculation was based on a hypothesis test on the primary endpoint between a palifermin group and the placebo group. This test was expected to have a 80% power to detect a desired treatment effect with a 5% Type I error rate. Average of 10 days of WHO grade 3 or 4 oral mucositis with a standard deviation of approximately 7.5 days, were expected in this population. A 50% reduction in duration of severe oral mucositis was assumed to be clinically meaningful. Based on a 2-sample independent t-test, a sample size of 37 patients per treatment group was calculated for the study.

Randomization

The randomization was stratified by center. Centers with different conditioning regimens were considered as different centers. Patients were randomised in a 1:1:1 ratio to receive one of the three arms within each stratum. Patients randomisation information was supplied by an interactive voice response system vendor to Amgen after the database is locked.

Blinding (masking)

See study 20000162.

Statistical methods

The protocol defined a mITT population as the analysis set for the primary endpoint. This population included all patients who received at least one dose of investigational product. Evaluations were conducted using two analysis sets: patients randomised under the original protocol to the 7-dose schedule and patients randomised under the amended protocol to the 6-dose schedule. If a patient did not experience any WHO grade 3 or 4 oral mucositis, the duration of severe oral mucositis was assigned 0 days. The mean duration of severe oral mucositis was the average duration across all patients in each treatment group.

All statistical tests were 2-sided at the 0.05 significance intervals. Point estimates of interest were accompanied by 2-sided 95% confidence intervals. The primary endpoint, duration of WHO grade 3 or 4 oral mucositis was based on the generalised Cochran-Mantel-Haenszel (CMH) method stratified by study center. Centers with less than 6 patients were pooled into 1 center and formed a single stratum.

For patients who completed the study, resolution of severe oral mucositis was defined as 1 assessment of WHO grade 2 or less oral mucositis after the last assessment of a WHO grade 3 or 4. For patients who died or withdrew from the study before the end-of-study visit, oral mucositis was only to be considered resolved if the patients had 2 consecutive assessments of WHO grade 2 or less after the last assessment of a WHO grade 3 or 4. Patients without resolution of WHO grade 3 and 4 oral mucositis were given the mean duration of severe oral mucositis among patients who experienced at least the same duration of severe oral mucositis as the patients whose oral mucositis was not resolved.

Missing daily oral mucositis assessments with adjacent values were imputed with the worse of the 2 adjacent values. As a worst-case sensitivity analysis, the analysis was repeated with the missing data in the palfermin group being replaced by the worst adjacent value, and the missing data in the placebo group replaced by the best of the adjacent values.

An interim analysis was to be performed after one-half of the patients were enrolled and had completed the study. This planned interim analysis was performed for 55 patients randomized to the amended, 6-dose schedule, and was conducted at an alpha level of 0.001. Dissemination of the results of the planned interim analysis was limited to Amgen senior management (including members of the internal Safety Committee) and those members of the palifermin team not directly involved in study conduct and management. Members of the internal Safety Committee were unblinded at the interim but were blinded to new data collected after the interim analysis. The results of the planned interim analysis suggested no safety concerns with the 6-dose schedule and presented no reasons to discontinue the study.

Results

Participant flow and numbers analysed

A total of 169 patients (52 placebo, 117 palifermin) were randomized at 12 centers (North America). Six patients (1 placebo, 5 palifermin) never received investigational product. Of the 163 patients who received investigational product, 34 were randomized under the original protocol to receive 7 doses of investigational product (11 placebo, 12 palifermin pre, 11 palifermin pre-post) and 129 patients were randomized under the amended protocol to receive 6 doses of investigational product (40 placebo, 43 palifermin pre, 46 palifermin pre-post).

Patients who had enrolled shortly before the decision to change the schedule was made, but had not yet received the extra dose were treated under the 6-dose schedule. For the purpose of analysis, such patients were considered to have been randomised to the 6-dose schedule. Analyses of efficacy and safety were performed separately for the 7-dose schedule (data not shown) and the 6-dose schedule.

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In the 6-dose schedule, the total number of patients with at least one important protocol deviation was lower in the placebo (22 [55%] of 40 patients) and palifermin pre groups (25 [58%] of 43 patients) compared with the palifermin pre-post group (32 [70%] of 46 patients). However, the mean number of important deviations was similar in all 3 groups.

Recruitment

The first patient was randomized on 23 February 1999, and the last patient completed the study on 24 July 2000.

Conduct of study

There were two amendments to the original protocol of this study. Amendment 1, allowed for the inclusion of patients 12 years and older, patients with multiple myeloma, and patients with a tandem-transplant regimen. Amendment 2 adopted a 6-dose treatment schedule (see treatment).

Baseline data

Demographics and disease baseline characteristics are shown in Table 12.

Table 12. Baseline characteristics (6-dose schedule)

| | | Palifermin (60 μg/kg/day) | |
	Placebo (n=40)	Pre (n=43)	Pre-Post (n=46)
Sex			
Male	20 (50%)	30 (70%)	26 (57%)
Female	20 (50%)	13 (30%)	20 (43%)
Race			
Caucasian	30 (75%)	30 (70%)	33 (72%)
Black	5 (13%)	7 (16%)	4 (9%)
Hispanic	4 (10%)	3 (7%)	7 (15%)
Asian	1 (3%)	2 (5%)	1 (2%)
Other	0 (0%)	1 (2%)	1 (2%)
Age (years)			
Mean (SD)	42 (13)	46 (12)	44 (12)
Median	44	50	44
Q1,Q3	30, 55	34, 55	35, 55
Min,Max	18, 63	18, 65	18, 64
ECOG Performance Status			
0	28 (70%)	22 (51%)	27 (59%)
1	12 (30%)	20 (47%)	18 (39%)
2	0 (0%)	1 (2%)	0 (0%)
Unknown	0 (0%)	0 (0%)	1 (2%)
Type of Diagnosis			
Acute Lymphoblastic Leukemia	1 (3%)	0 (0%)	2 (4%)
Acute Myelogenous Leukemia	2 (5%)	0 (0%)	7 (15%)
Hodgkin's Disease	14 (35%)	5 (12%)	8 (17%)
Non-Hodgkins Lymphoma	18 (45%)	30 (70%)	28 (61%)
Multiple Myeloma	5 (13%)	8 (19%)	1 (2%)
Patients with Prior Chemotherapy			
Yes	40 (100%)	43 (100%)	46 (100%)
Patients with Prior Radiotherapy			
Yes	4 (10%)	6 (14%)	6 (13%)
No	35 (88%)	37 (86%)	40 (87%)
Unknown	1 (3%)	0 (0%)	0 (0%)
Mobilization			
Cytokines Only	2 (5%)	7 (16%)	10 (22%)
Cytokines and Chemotherapy	38 (95%)	36 (84%)	36 (78%)

Efficacy results

For the mITT population, the mean (SD) duration of WHO grade 3 or 4 oral mucositis in the palifermin pre group was decreased by 39.5% compared with the placebo group (5.2 [6.1] days *versus*

8.6 [8.2] days); the reduction was statistically significant (p = 0.003). The mean (SD) duration of WHO grade 3 or 4 oral mucositis in the palifermin pre-post group (4.7 [5.7] days) was also statistically significantly reduced (45.3%) relative to the placebo group (p = 0.004).

The Lachenbruch's 2-part model considered the incidence of WHO grade 3 or 4 oral mucositis and the duration for those patients who developed this toxicity. Statistically significant improvements for both parameters were observed using this model, for both palifermin treatment groups compared with the placebo group (p = 0.0219 palifermin pre, 0.0297 palifermin pre-post).

Results related to the 7-dose scheduled are not shown (see discussion on clinical efficacy).

The secondary assessment of severity and incidence of oral mucositis showed statistically significant difference in the percentages of patients with grade-4 mucositis (14 [33%] patients in the palifermin pre- group and 12 [26%] patients in the palifermin pre-post group, experienced grade 4 mucositis *versus* 20 [50%] patients in the placebo group, p = 0.029 and 0.025, respectively). The differences in incidence were not statistically significant for patients who developed WHO Grade 2, 3, or 4 mucositis.

The improvement in mouth and throat soreness among patients receiving palifermin translated into improvements in all related daily activities were assessed. The mean AUC limitation score for the overall study period was reduced (function was improved) in the all-palifermin group relative to the placebo group by a range of 17% (sleeping) to 27% (talking). Improvements were statistically significant for drinking (23%; p = 0.005) and eating (24%; p = 0.022).

Similar percentages of subjects in the placebo and palifermin groups experienced NCI CTC grade 3 or 4 diarrhea during the study (30% placebo, 26% palifermin pre, 24% palifermin pre-post).

Fewer patients in each of the palifermin groups (16% palifermin pre, 20% palifermin pre-post) received i.v. antifungal medications during the study than in the placebo group (30%). For patients who received i.v. antifungals, the mean (SD) duration of use was shorter in the palifermin pre-post group (5.3 [3.8] days) and longer in the palifermin pre- group (10.7 [8.2] days) than in the placebo group (6.5 [2.7] days). Approximately the same percentages of patients in placebo and palifermin groups received i.v. antibiotics during the study (93% placebo, 88% palifermin pre, 89% palifermin pre-post). The mean (SD) duration of use of i.v. antibiotics was similar for all treatment groups (11.1 [6.2] days placebo, 11.1 [6.1] palifermin pre, 11.3 [6.0] days palifermin pre-post).

A summary of efficacy results for study 980231 is shown in table 13.

Table 13. Efficacy results for study 980231

| | Placebo (N = 40) | Palifermin (60 µg/kg/day) | |
		Pre (N = 43)	Pre-post (N = 46)
WHO Grade 3 or 4 - Duration - (days), mITT population			
Mean (SD)	8.6 (8.2)	5.2 (6.1)	4.7 (5.7)
p- value [b] (95% CI)		0.003 (0.3, 6.5)	0.004 (0.9, 6.9)
WHO Grade 3 or 4 - Incidence - n (%)	32 (80)	31 (72)	31 (67)
p- value [b] (95% CI)		0.184 (-10%, 26%)	0.159 (-5%, 31%)
WHO Grade 3 or 4 - Duration - (days), affected patients			
Mean (SD)	10.8 (7.7)	7.2 (6.0)	6.9 (5.7)
WHO Grade 4 - Incidence - n (%)	20 (50)	14 (33)	12 (26)
p-Value [b] (95%)		0.029 (-4%, 38%)	0.025 (4%, 44%)
WHO Grade 4 - Duration - (days), mITT population			
Mean (SD)	2.6 (3.5)	1.4 (2.8)	1.5 (3.7)
p- value [b]		0.026	0.022
Mean (SD), affected patients	5.1 (3.4)	4.4 (3.4)	5.5 (5.7)
WHO Grade 2, 3 or 4 - Incidence - n (%)	39 (98)	41 (95)	39 (85)
p- value [b] (95% CI)		0.634 (-5%, 11%)	0.099 (2%, 24%)
WHO Grade 2/3/4 - Duration - (days), mITT population			
Mean (SD)	17.7 (11.3)	10.8 (8.4)	11.1 (9.7)
p- value [b] (95% CI)		0.002 (2.6, 11.2)	0.001 (2.1, 11.1)
Mean (SD), affected patients	18.2 (11.1)	11.4 (8.3)	12.8 (9.3)
Patient-reported mouth and throat soreness			
VDS scale (AUC) [a]			
Mean (SD)	45.0 (20.7)	36.5 (18.7)	34.9 (22.5)
p- value [b] (95% CI)		0.054 (0, 17)	0.021 (1, 19.2)
Maximum severity (worst WHO Grade)			
Mean (SD)	3.3 (0.8)	3.0 (0.9)	2.8 (1.0)
p -value [b] (95% CI)		0.013 (-0.1, 0.7)	0.013 (0.1, 0.9)
Median (Min:Max)	3.5 (1:4)	3.0 (1:4)	3.0 (1:4)
I.v. or t.d. opioid analgesics use [c] - Incidence - n (%)	38 (95)	35 (81)	37 (80)
p- value [b]		0.045	0.072
Cumulative dose of i.v. or t.d. opioid analgesics			
mITT population			
Mean (SD)	1163 (1776)	505.3 (859.1)	514.4 (922.2)
Median (Min:Max)	523.9 (0:8472)	216.7 (0:4574)	204.9 (0:5213)
p-value [b]		0.002	0.004
Supplemental Feeding - Incidence - n (%)	13 (33)	17 (40)	15 (33)
p-value [b]		0.356	0.602
Febrile Neutropenia - Incidence - n (%)	37 (93)	31 (72)	35 (76)
p-value [b]		0.007	0.439

<u>Abbreviations</u>: VDS = verbal descriptive scale; i.v. = intravenous; t.d. = transdermal; [a] Likert-type scale (0 = no soreness; 4 = extreme soreness); [b] All p-values were calculated for the comparison with placebo using a generalized CMH test based on the standardized mid-ranks (modified Ridit scores) within each stratum. All analyses of incidence used the mITT population, defined as those subjects who received at least one dose of investigational product (4% of subjects randomized did not receive investigational product); [c] morphine mg equivalent.

Ancillary analyses

Five (9%) patients in the palifermin pre- group and 2 (4%) patients in the palifermin pre-post group had a blood-borne infection *versus* 1 (2%) patient in the placebo group. Sepsis was the most common blood-borne infection (1 [2%] patient in the placebo group, 3 [5%] patient in the palifermin pre-group).

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Supportive studies

In study 20010182 (Part A), 13 patients were enrolled and received palifermin (see pharmacokinetics). All patients had WHO grade 2, 3, or 4 oral mucositis during the study. Seven (54%) patients had grade 3 or 4 oral mucositis and 3 (23%) patients had grade 4 oral mucositis. The mean (SD) duration of WHO grade 3 or 4 oral mucositis was 3.6 (4.6) days for all patients and was 6.7 (4.3) days for those who experienced it. The mean (SD) duration of WHO grade 4 oral mucositis was 0.7 (1.4) days for all subjects and was 3.0 (1.0) days for those who experienced it.

A randomized, double blind, placebo-controlled, phase I study (950225, Part B) was aimed to obtain preliminary evidence of efficacy of palifermin, in 64 patients with colorectal carcinoma treated with 5-Fluorouracil and leucovorin, on amelioration of chemotherapy-induced oral mucositis and diarrhea, when administered at a dose of 40 µg/kg i.v. for 3 consecutive days before each of the 2 cycles of 5-FU/leucovorin chemotherapy. No patients in either treatment groups had a grade 4 oral mucositis. The greatest difference between the two groups was in the proportions of patients with grade 2 or 3 oral mucositis, which was lower in patients who received palifermin than in patients who received placebo during cycle 1 (29% palifermin, 61% placebo) and cycle 2 (11% palifermin, 47% placebo) of chemotherapy.

A phase II study (990119) was performed to evaluate the effect of palifermin on the duration of grade 2 or higher of oral mucositis (phase A) in patients with locally advanced head and neck cancer receiving concomitant chemoradiotherapy with standard or hyperfractionated radiation therapy. This was a randomized study (2:1, palifermin:placebo) with stratification on tumor type and schedule of radiation.

A phase I/II study (970149) of escalating doses was aimed to determine the safety and tolerability of i.v. administration of palifermin in patients receiving concomitant chemoradiotherapy for head and neck cancer.

Discussion on clinical efficacy

The indication was supported by one pivotal phase III and one phase II study. In the phase III pivotal study, assessing a pre-post 6-dose regimen of palifermin, the statistically significant reduction seen in the mean duration of WHO grade 3 and 4 oral mucositis, the primary endpoint, was supported by statistically significant reductions in duration of oral mucositis as assessed by all other oral mucositis rating scales used. These included the Radiation Therapy Oncology Group (RTOG) acute radiation morbidity scoring criteria for mucous membranes and the Western Consortium of Cancer Nursing Research (WCCNR) oral mucositis staging system, which measures only the anatomical changes associated with oral mucositis. The use of more than one scale to measure the efficacy endpoint has shown consistency in the measurement of the primary endpoint. To assess the impact of oral mucositis on functioning as reported by the patient, a patient-reported outcomes daily questionnaire (oral mucositis daily questionnaire [OMDQ]), investigating mouth and throat soreness and its impact on daily functional activities (sleeping, swallowing, eating, drinking, and talking) was used. However, validation activities for the OMDQ used in the phase II and III studies were not totally completed prospectively and were carried out retrospectively. Nevertheless, the correlation between assessor-based measures of improvement in oral mucositis (assessments by medical professionals using the WHO scale) and subjective measures (PRO assessments by patients) was high, as demonstrated by comparing daily WHO scores and daily PRO scores across all patients.

In the phase II study, the results obtained form the 35 patients randomised to receive 7 doses of palifermin did not suggest any improved efficacy for palifermin compared to placebo, and in many analyses the trend favoured the placebo group. Further to an amendment to the protocol, the dose of investigational product administered on the day of the last TBI fraction was eliminated, which resulted in a 6-dose treatment schedule. This amendment was based on a review of animal data and on concerns of eliminating benefit or worsening of mucosal injury when the time period between palifermin and chemotherapy administration was shorter than 24 hours. An appropriate warning has been included in section 4.4 of the SPC.

The analysis of data from patients randomised to receive 6 doses of palifermin showed that the duration of WHO grade 3 or 4 oral mucositis, the primary efficacy endpoint, was numerically reduced in patients who received palifermin, either pre- or pre-post treatment, compared with patients who received placebo. This result was reproducible across study centers, underlying hematologic disease, and number of radiotherapy fractions used in the conditioning regimen. The differences seen were robust to the problems of missing efficacy observations, even using the worst-case imputation techniques which penalised the active groups compared to placebo. While the incidence of grade 3 and 4 oral mucositis was reduced in both palifermin groups compared with the placebo group, the differences were not statistically significant. Diarrhoea being commonly related to the conditioning regimen used in these studies, the incidence and duration of diarrhoea was assessed as a secondary endpoint considering the potential of palifermin to reduce chemotherapy-induced diarrhoea in mice. However no clinically or statistically significant differences were observed between the placebo and palifermin groups for these endpoints.

Although the study was not designed to statistically compare efficacy endpoints between the 2 groups receiving palifermin (*i.e.* palifermin pre- *versus* pre-post), the outcomes for most of the efficacy endpoints (including the primary endpoint) were not significantly better in the palifermin pre-post group than the palifermin pre- group. No clinical or non-clinical evidence for the pre-post protocol to be superior to the pre-only protocol has been provided. Dosing with growth factors after highly mutagenic chemo-radiotherapy may salvage otherwise dying cells with chromosomal injuries, and caution must be exercised if this is not clearly needed, especially as no long term safety data are available (see clinical safety discussion and SPC section 4.4). Therefore, the applicant has agreed to provide, post-authorisation, prospective clinical trial data to confirm the efficacy of palifermin relative to placebo when given either pre-high dose chemotherapy only *versus* pre- and post-high dose chemotherapy dosage schedules, by determining the incidence of oral mucositis (WHO grades 2, 3 and 4).

The observation that the greatest benefit is observed in patients who receive chemo-radiotherapy giving a high incidence of mucositis, as opposed to those receiving chemotherapy alone was confirmed in a small randomized, double-blind, placebo-controlled, dose-escalation study (990750) conducted in patients with hematologic malignancies undergoing high-dose chemoradiotherapy followed by allogeneic hematopoietic stem cell transplantation.

The incidence of febrile neutropenia was significantly reduced in the palifermin group compared with the placebo group in both the phase II and phase III studies, suggesting that preserving integrity of the mucosal barrier may help to reduce the incidence of systemic infections during profound myelosuppression.

Palifermin anti-mucositis activity in patients with colorectal cancer has been observed. In two clinical studies conducted in patients with locally advanced HNC receiving fractionated radiotherapy combined with chemotherapy, efficacy of palifermin has not been demonstrated.

Clinical safety

Patient exposure

The palifermin clinical program consists of 17 studies conducted between 1995 and 2003, in the US, Europe, Canada, Australia, and Japan. A total of 1168 patients were treated, i.e. received at least 1 dose of investigational product (382 with placebo and 786 with palifermin). Six pools of data have been analysed for the integrated analysis of safety (see table 14).

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Table 14. Overview of palifermin safety pools

Setting	Safety Pool	Description	n (palifermin, placebo)	Studies included
Myelotoxic therapy	A	Patients receiving recommended dose and schedule in myelotoxic therapy studies	(152, 146)	980231, 20000162
	B **Primary safety pool**	All patients from myelotoxic therapy studies	(409, 241)	960189, 980231, 20000162, 20010182 Part A
	C	Long-term data from the primary safety pool (study 960226)	(408, 242)	960189, 980231, 20000162, 20010182 Part A
Other settings	D	Patients with advanced head and neck cancer receiving fractionated chemoradiotherapy	(113, 46)	970149, 990119
	E	Long-term data from pool D (study 990123)	(113, 46)	970149, 990119
	F	Long-term data from patients with colorectal cancer receiving cyclic chemotherapy (study 950226)	(82, 63)	Combined 950225/950275, 950226

Safety results of 6 studies in healthy volunteers (n = 210) have been also analysed. No pooling of these studies for safety has been done. Adverse events (AE), deaths, serious adverse events, and other significant safety parameters are presented for the primary safety pool (pool B, which included all patients who received at least 1 dose of investigational product in myelotoxic therapy studies). The primary safety pool included patients receiving a total of 3, 6, or 7 per-protocol doses. The median number of doses in both treatment groups was 6. The median average daily dose by weight was 60 µg/kg/day in each treatment group. Patients were enrolled from 31 study centers. The 4 highest-enrolling centers enrolled 40% of the patients. Equivalent proportions of patients (3%) in both treatment groups discontinued the study prematurely. Death was the cause of early discontinuation for 3 patients in the placebo group and 1 patient in the palifermin group. Five (1%) patients in the palifermin group, *versus* none the placebo group, discontinued due to withdrawn consent or administrative/investigator decision.

Adverse events

AE are presented by the time of their first occurrence relative to the administration of chemotherapy. The pre-chemotherapy period was defined as beginning at the initiation of investigational product to the day before the initiation of chemotherapy (day - 11 to day - 4), and the post-chemotherapy period was defined as beginning on the day of the initiation of chemotherapy to the end of study (from day- 3 to day 28). AE in the primary safety pool are summarized in table 15. The incidence of AE was lower in the pre-chemotherapy period (79% placebo, 84% palifermin) than in the post-chemotherapy period (100% of patients in both groups). AE related to treatment were more frequent in the palifermin group both pre- and post-chemotherapy. Few treatment-related adverse events were serious (1% placebo, 2% palifermin). Very few patients in either group (1% placebo, 2% palifermin) discontinued investigational product due to adverse events.

Table 15. Adverse events in the primary safety pool

Preferred term	Pre-chemotherapy		Post-chemotherapy		Total	
	Placebo (n=241)	Palifermin (n=409)	Placebo (n=240)	Palifermin (n=405)	Placebo (n=241)	Palifermin (n=409)
Adverse events - n(%)	190 (79)	344 (84)	240 (100)	404 (100)	241 (100)	407 (100)
Serious adverse events	5 (2)	9 (2)	45 (19)	76 (19)	50 (21)	83 (20)
Treatment-related adverse events - n(%)	25 (10)	154 (38)	53 (22)	156 (39)	71 (29)	231 (56)
Serious adverse events	0 (0)	1 (0)	2 (1)	7 (2)	2 (1)	8 (2)
Study discontinuation due to AE - n(%)	2 (1)	2 (0)	0 (0)	2 (0)	2 (1)	4 (1)
Discontinuation of investigational product due to AE - n(%)	2 (1)	4 (1)	1 (0)	3 (1)	3 (1)	7 (2)
Deaths on study - n(%) [a]	0 (0)	0 (0)	3 (1)	2 (0)	3 (1)	2 (0)

[a] Up to 30 days after the last dose of investigational product.

Adverse events that occurred ≥5% more often in palifermin than placebo overall were rash, fever, pruritus, erythema, edema, mouth/tongue thickness or discolouration, pain, arthralgia, and granulocytopenia (table 16).

Table 16. AE occurring with ≥5% higher incidence in palifermin than placebo (primary safety pool)

Body system Preferred term	Placebo n(%) = 241	Palifermin n(%) = 409
Number of Subjects Reporting Adverse Events	214 (89)	388 (95)
Body as a whole	126 (52)	256 (63)
Fever	82 (34)	159 (39)
Oedema	50 (21)	114 (28)
Pain	27 (11)	65 (16)
Gastrointestinal	20 (8)	68 (17)
Mouth/Tongue Thickness or Discoloration[a]	20 (8)	68 (17)
Hematologic	18 (7)	52 (13)
Granulocytopenia	18 (7)	52 (13)
Musculo-skeletal	13 (5)	40 (10)
Arthralgia	13 (5)	40 (10)
Skin and appendages	156 (65)	320 (78)
Rash[b]	120 (50)	255 (62)
Pruritus	57 (24)	145 (35)
Erythema	52 (22)	131 (32)
Special senses	20 (8)	65 (16)
Taste Altered[c]	20 (8)	65 (16)

[a] Mouth/tongue thickness or discoloration included the preferred terms of lesion oral, tongue discoloration and tongue disorder. [b] Rash included the preferred terms of rash, rash erythematous, rash maculo-papular and rash purpuric. [c] Taste altered included the preferred terms of taste loss and taste perversion.

The overall incidence of severe adverse events was similar in the two treatment groups in the primary safety pool (35% placebo, 39% palifermin). The most common of these events among patients receiving palifermin were nausea (placebo 10%, palifermin 11%), anorexia (placebo 7%, palifermin 4%), and vomiting (placebo 7%, palifermin 7%).

AE considered to be related to investigational product occurred at a greater incidence in the palifermin group (56%) than the placebo group (29%). The incidence in the palifermin group was similar in the pre-chemotherapy (38%) and post-chemotherapy (39%) periods. The most common treatment-related events in the palifermin group were rash (23% *versus* 10% in the placebo group), erythema (18%

versus 4% in the placebo group) and mouth/tongue thickness or discoloration (13% *versus* 3% in the placebo group), see details in table 17.

Table 17. Adverse reactions occurring with ≥ 5% higher incidence with palifermin than placebo

System organ class	Undesirable effect
	Very common (> 1/10)
Nervous system disorders	Taste perversion
Gastrointestinal disorders	Mouth/tongue thickness or discolouration
Skin and subcutaneous tissue disorders	Rash, pruritus and erythema
Musculoskeletal and connective tissue disorders	Arthralgia
General disorders and administration site conditions	Oedema, pain and fever

The AE assessed as potentially associated with palifermin and likely related to its pharmacologic activity were oral epithelium, skin, and visual related-AE, pancreatitis and AE related to filgrastim. A higher proportion of the patients in the palifermin group had oral-related adverse events compared with patients in the placebo group during the first dosing period (14% placebo, 29% palifermin), and proportions were similar for both groups during the second dosing period (18% placebo, 19% palifermin). The median time to onset of oral events in the first treatment period was about 5 days in the palifermin group and the duration was about 5 days. Corresponding figures for the second period was 8 and 4 days, respectively. Seven patients discontinued palifermin treatment (*versus* 1 patient in the placebo group) due to skin AE (2 cases of oedema, 2 cases of erythema, 1 case of flushing, 1 case of pruritus, 1 case of rash, 2 cases of maculopapular rash). Median time to onset during the first period was 4 days in the palifermin (*versus* 7 days for placebo) with a similar median duration of 3 and 4 days in the placebo and palifermin groups, respectively. In the second dosing period, the median time to onset of skin-related adverse events in the 2 groups was similar (8 days for the placebo group and 7 days for the palifermin group), with a similar median duration of 5 and 6 days in the placebo and palifermin groups, respectively. Because of the presence of the KGFR on the eye lens [53] and the documented increased risk of cataracts associated with TBI [54, 55], two studies (20000162, 20010182) included slit-lamp eye examinations performed by ophthalmologists at baseline and during a follow-up visit between days 28 and 60, in addition to the reporting of visual-related AE from the primary safety pool. More patients in the palifermin group reported eye irritation (4 [1%] patients *versus* 1 [< 1%] patient in the placebo group). Abnormal, essentially blurred, vision was reported by 13 (3%) patients *versus* 7 (3%) in the palifermin and the placebo groups, respectively. Additional data from studies 20010182 and 20030142 did not show evidence of an increase in lens opacities in patients treated with palifermin. Clinical manifestations potentially associated with pancreatitis were reported with similar frequencies in the placebo and the palifermin groups.

Dose limiting toxicity

In study 960189 (see clinical pharmacokinetics), a total of 264 patients were enrolled and 262 patients received the investigational product (85 placebo, 177 palifermin).

Eight patients had adverse events that met the protocol-defined criteria for dose-limiting toxicities (any non-hematological adverse event of WHO [or CTC for diarrhea] grade ≥ 3 considered by the investigator to be possibly, probably, or definitely related to palifermin). One patient, each in the placebo, the 20-μg/kg/day palifermin pre-post, and the 60-μg/kg/day palifermin pre- groups, and 5(36%) patients in the 80-μg/kg/day palifermin pre-post group, met the protocol-defined stopping rule (dose escalation was to be stopped if more than 4 of 12 [33%] subjects had a dose-limiting toxicity [DLT] at the same dose and dosing schedule). The 60-μg/kg/day palifermin pre-post dose/schedule was determined to be the maximum tolerated dose (see table 18).

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Table 18- Summary of dose limiting toxicities by Cohort

Cohort	Cohort description	Palifermin		Placebo	
		Patients (n)	DLT (n)	Patients (n)	DLT (n)
1	5 µg/kg Pre	12	0	6	0
2	20 µg/kg Pre	12	0	6	0
3	40 µg/kg Pre	12	0	6	0
4	20 µg/kg Pre-Post	12	1	6	0
5	60 µg/kg Pre	12	0	6	1
6	40 µg/kg Pre-Post	12	0	6	0
7	80 µg/kg Pre	12	0	6	0
8	**80 µg/kg Pre-Post**	14	5	7	0
9	R60 µg/kg Pre*	20	1	10	0
10	R80 µg/kg Pre*	45	0	21	0
11**	**60 µg/kg Pre-Post**	15	0	4	0

*R = Repeat; ** Patients in cohorts 1 to 10 use BEAM in chemotherapy regimen, and patients in cohort 11 use BEAM or BuMel.

Serious adverse events and deaths

The incidence of deaths on study was 3 (1%) in the placebo group and 2 (0%) in the palifermin group. Reported causes of death included veno-occlusive disease (VOD), sepsis, pneumonia and respiratory insufficiency. Deaths were not considered to be related to study treatment. With respect to VOD, the KGF receptor is not known to be expressed in hepatic sinusoidal endothelia.

The overall incidence of serious adverse events was the same (20%) in both treatment groups. Three serious events occurred in more than 1% of patients receiving palifermin: fever (7 [3%] placebo, 19 [5%] palifermin); sepsis (2 [1%] placebo, 7 [2%] palifermin); and hypotension (4 [2%] placebo, 7 [2%] palifermin).

Laboratory findings

In the primary safety pool, granulocytopenia was reported as an adverse event in a higher percentage of patients who received palifermin (13%) compared with patients who received placebo (7%). However a statistically significant reduction in febrile neutropenia was observed in the pivotal trial in the palifermin treated patients compared to placebo treated patients. An increased incidence of granulocytopenia in both studies 990119 and 970149 (fractionated chemoradiotherapy setting in patients with advanced head and neck cancer) was observed among patients receiving palifermin.

However the proportion of patients who experienced clinically significant infections was the same in the placebo and palifermin treated groups (11%). In the hematologic malignancy setting, the analysis of individual patients' ANC time trajectories did not identify any differences between treatment groups, and ANC recovery curves were super-imposable. Time-trajectory plots for ANC values for patients who did and did not have granulocytopenia were similar between the two treatment groups and between the patients with or without reported granulocytopenia. In the primary safety pool, only 14 patients (5 in the placebo group, 9 in the palifermin group) were ≥ 65 years old, only one patient from the palifermin group experienced granulocytopenia. In the fractionated chemoradiotherapy studies, 2/10 (22%) patients in the placebo group and 8/10 (62%) patients in the palifermin group who were ≥ 65 years had granulocytopenia reported as an AE. In the multicycle chemotherapy setting, 9/36 (25%) patients in the placebo group and 11/39 (28%) patients in the palifermin group who were ≥ 65 years had granulocytopenia reported as an AE.

In the primary safety pool, 54% (101/186) of patients in the placebo group and 62% (184/298) of patients in the palifermin group had grade increases from study baseline in serum amylase values. More patients in the palifermin group (17%) had grade 4 increases from baseline compared with patients in the placebo group (11%). Mean (50.72 IU/l placebo, 53.12 IU/l palifermin) baseline serum amylase values were similar between treatment groups. Mean (291.77 IU/l placebo, 434.95 IU/l palifermin) peak values were higher in patients who received palifermin compared with patients who received placebo. Three patients (1 placebo and 2 palifermin) had serum amylase values greater than 4500 IU/l on study day -5. In these patients, the baseline values were within normal range and the end-of-study values were lower than baseline. Clinical sequelae, i.e. abdominal and/or back pain, potentially associated with elevated amylase levels occurred in 1 of these 3 patients. A patient who

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received 60 µg/kg palifermin pre-post had transient mild back pain. This patient had no reports of acute pancreatitis. Increased amylase levels were found to be predominantly of salivary gland origin.

In the placebo group, 23% (39/170) of patients and 28% (76/274) of patients in the palifermin group had grade increases from study baseline in serum lipase values. Mean baseline lipase values were higher in patients who received palifermin (59.87 IU/l) compared with patients who received placebo (43.08 IU/l). Overall, mean values increased, and peak values were observed in both treatment groups on the study day-6 evaluation, by the day 0 (PBSCT) evaluation, mean values decreased to below baseline values for palifermin and were similar between treatment groups. By approximately day 28 after PBSCT, in patients who received placebo, mean values increased from day 0 and were slightly increased from the baseline values. In patients who received palifermin, mean values had also increased from day 0, but were slightly decreased from the baseline values. One patient (not one of the 2 patients previously described with very high amylase levels) who received 60 µg/kg palifermin pre-post had a serum lipase values of 1980 U/l (baseline), 8350 U/l (day -4 [peak value]), 266 U/l (day 4), and 92 U/l (day 41) and experienced abdominal pain, potentially associated with elevated lipase levels. The patient had no reports of acute pancreatitis.

Study 960189 included thyroid function tests (thyroid-stimulating hormone [TSH], T3 [triiodothyroxine], and T4 [thyroxine]) at baseline and approximately 30 days and then 60 to 100 days after transplantation. A total of 59 patients in the placebo group and 138 patients in the palifermin group had pre-study and post-transplant data for TSH. More women were included in the palifermin group (42%) compared with placebo (21%). No significant differences in either baseline, post-transplant or follow-up TSH values were observed between both groups. In the pivotal study (20000162), the incidence of reported thyroid related adverse event was low and similar between the two groups. With regard to immunogenicity, see clinical pharmacodynamics.

Long term safety

Long-term safety information has been provided from study 990123 (long-term follow-up study for patients enrolled in studies conducted in the fractionated chemoradiotherapy setting, i.e. studies 970149 and 990119), study 950226 (long-term follow-up study for patients enrolled in studies conducted in the multicycle chemo setting, i.e. studies 950225 and 950275) and study 960226 (long-term follow-up study for patients enrolled in studies conducted in the hematology transplant setting, i.e. studies 960189, 980231, 20000162, and 20010182).

In the multicycle chemotherapy setting data are available from follow-up of 145 subjects who participated in the parent study (950225), 48 of whom subsequently participated in an open-label extension (Study 950275). The median duration of follow up was 13.4 months (12.6 months for the prior placebo group, 14.3 months for the prior palifermin group). Kaplan-Meier curves for overall survival, time to disease progression, and progression-free survival were superimposable between the prior placebo and prior palifermin groups.

In the fractionated chemoradiotherapy setting, the median follow-up time was 38.6 months (range 1.2 to 64.3 months) for the placebo group and 19.5 months (range 0.4 to 71.3 months) for the palifermin group. The disease progression rates were higher for the palifermin treated patients compared with placebo (27% *versus* 13%, respectively). The observed death rates were (24% placebo, 27% palifermin).

In the hematology transplant setting, follow-up data of 23.1 months (range 0.2 to 83.8 months) for the placebo group and 23.8 months (range 0.8 to 81.6 months) for the palifermin group, showed that overall survival, disease progression, progression-free survival, and the incidences of second malignancies remained similar between palifermin and placebo groups, and were in the range expected for this patient population. The overall Kaplan-Meier survival curves remained similar for both groups. A divergence on the Kaplan-Meier curves for progression-free survival, with more progressions occurring on the palifermin arm, was observed beyond the 18-month timepoint. The overall proportion of patients whose disease has progressed was 32% of patients receiving placebo and 37% of patients who received palifermin. However a non-parametric log rank analysis has been conducted on the overall survival and progression-free survival data, which did not show statistically significant differences between palifermin and placebo treated groups at this point in time during the follow up. From the currently available data, a difference in progression free survival could not be

ruled out. The overall proportion of patients with second malignancies remained the same (6%) in both treatment groups.

Safety in special populations

Within the primary safety pool, 2% of patients in each treatment group (5/241 placebo, 9/409 palifermin) were ≥65 years old. Although differences were seen in incidences of some AEs, no conclusive evidence suggested that the AE profile was different in patients ≥65 years old who received palifermin compared with patients <65 years old. A total of 86% of patients in the placebo group and 82% of patients in the palifermin group were Caucasian. Percentages of patients of other races were as follows: black (7% placebo, 8% palifermin), Hispanic (5% placebo, 7% palifermin), and other (2% placebo, 3% palifermin). A higher percentage of patients in the placebo group (68%) were men compared with patients in the palifermin group (59%). No differences in the types or incidences of AE were observed between the groups of different races, or between the placebo and palifermin groups for men or women. Safety data from the use of palifermin in paediatric patients or in pregnant women have not been provided.

Safety related to drug-drug interactions and other interactions

No safety data related to drug-drug interactions and other interactions have been reported. Since an increased risk of bacterial infections has been associated with more severe oral mucositis in several studies [56, 57], the use of myelopoietic growth factors was standardized in the palifermin program conducted in the high dose cytotoxic therapy. Filgrastim administration was mandated from day 0 onward after PBSC infusion, daily until neutrophil recovery. The median number of days of filgrastim exposure in the integrated safety set (241 placebo, 409 palifermin) was 12.0 days in the placebo group and 11.0 in the palifermin group. A potential interaction between filgrastim and palifermin has been discussed (see discussion on clinical safety).

Post marketing experience

No post marketing experience data have been available.

Risk management plan

Post marketing surveillance will be monitored through collection and analysis of spontaneous AE reports, with special attention to malignancies (relapse of pre-existing malignancies and development of new malignancies).

Moreover, 650 patients who received investigational product (including 339 patients who received palifermin) in the studies conducted in the haematological malignancies setting have been enrolled into the long term follow up study 960226 and will be followed until death or loss to follow up. Patients will be followed for survival, tumor progression and secondary malignancies. The sample size has been calculated, assuming the background rate for secondary malignancies is 2% per year [58-62], to observe approximately 23 and 39 secondary malignancies with 5-year and 10-year follow-up, respectively (the 95% CI for the annual incidence of secondary malignancies is 1.6% with 5 years and 1.2% with 10 years of follow-up). Long term safety data will be reported annually, until 2015. The available Center for International Blood and Marrow Transplant Research (CIBMTR) databases will be used to identify cases of suspected secondary malignancies and cancer relapse.

Discussion on clinical safety

Because of the distribution of KGF receptors on epithelial tissues, some adverse effects, in particular skin and oral adverse events, possibly related to palifermin's pharmacologic action on KGFR-expressing tissues, were observed. These skin (e.g., rash, pruritus, erythema, and oedema) and oral (e.g., mouth/tongue discoloration or thickness, and taste disorders) adverse events, were reversible, usually mild in severity, and infrequently led to discontinuation of dosing. The median time to onset was 6 days after the first of 3 consecutive daily doses, with a median duration of 5 days. The association of these events with palifermin treatment was more apparent before administration of

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cytotoxic therapy. However, after cytotoxic therapy, a high level of background toxicities has complicated accurate interpretation of the data.

Other than oral and skin related adverse events, the overall short term safety profile of palifermin in the myelotoxic therapy clinical trial population was similar to that of placebo. Deaths and serious adverse events occurred in a similar proportion of patients in both treatment groups and were typical of those commonly affecting this patient population.
Analysis of hematology laboratory values over time showed a similar pattern of post-transplant hematopoietic recovery, indicating that palifermin did not interfere with hematopoietic reconstitution, and the proportions of patients receiving transfusions were similar in the two treatment groups.

Increase in amylase and lipase was observed consistently across all patient groups with wide variations and high levels, although overt signs of pancreatitis were not associated consistently with these increases and no case of pancreatitis was reported (see SPC, section 4.8). Since the reasons for excess reporting of granulocytopenia in the palifermin group *versus* the placebo group, which were not supported by daily absolute neutrophil count (ANC) measurements, are unknown, together with inconsistencies in adverse events reporting procedures between different clinical settings, granulocytopenia will be kept under review post-marketing. Higher incidence of granulocytopenia was observed in patients receiving palifermin with age ≥ 65 years. However, due to the small number of patients, results for the rate of granulocytopenia in these age subsets were inconclusive.

The occurrence of visual adverse events after palifermin administration is of theoretical concern since KGF receptors have been shown to be present on the lens of the eye, i.e., the cornea and crystalline lens. Based on the available data, the risk associated with the administration of palifermin and the development of cataracts or worsening of pre-existing cataracts, was low. Visual adverse events identified through post-marketing surveillance will be included in periodic safety update reports. A warning has been included in the SPC (see section 4.4) as regard to the absence of knowledge on the long term effects on the lens of the eye.

Pre-clinical studies have indicated irreversible thyroid changes in rat (studies T95-KGF-001 and T95-KGF-002). Justification for not testing thyroid function was based on the probability that the effects seen in rat thyroid may be an indirect result of the pharmacological effects of palifermin on rat liver and the absence of similar findings in the monkey studies. In addition a Phase I clinical study did not suggest significant evidence of thyroid toxicity and the incidence of thyroid related adverse events reported form the pivotal trial was low and similar between the two treatment groups. The justification provided was considered acceptable and the CHMP concluded that palifermin did not have an effect on thyroid function in humans at the doses and schedule tested.

No safety data related to drug-drug interactions and other interactions have been reported. The potential interaction between filgrastim and palifermin has been discussed. The applicant has presented several arguments to support the absence of risk of interaction: Biologically, G-CSF and KGF bind to different receptors expressed by different cells and tissues. In addition, in *in vitro* experiments, palifermin had no effect on G-CSF induced CFUs (data not provided), and in the clinical trials conducted in the hematologic settings, the kinetics of ANC recovery were similar between treatment groups, indicating that palifermin did not affect filgrastim activity. Morover, the anti-mucositis activity of palifermin, observed in patients with metastatic colorectal cancer receiving multi-cycle chemotherapy, where filgrastim administration was low (approximately 15%), supported the lack of interaction of filgrastim with palifermin activity on oral mucosa protection from cytotoxic insults. Even if a potential interaction with filgrastim cannot be completely ruled out, the risk for palifermin to interact with other medicinal products was considered low and appropriate information has been included in section 4.5 of the SPC.

Safety and efficacy of palifermin has not been evaluated in the elderly (see section 4.2 and 5.2 of the SPC). Due to the small number of patients ≥65 years old, no conclusive evidence suggested that the AE profile was different in patients ≥65 years old who received palifermin compared with patients <65 years old. No differences in the types or incidences of AE were observed between the groups of different races, or between men and women. Safety and efficacy of palifermin has not been evaluated in patients with hepatic impairment (see section 4.2 and 5.2 of the SPC). Safety data from the use of palifermin in paediatric patients or in pregnant women have not been provided. Palifermin should not be used in children or adolescents until further data become available, and the use during pregnancy is

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not recommended unless clearly necessary (see SPC, sections 4.2 and 4.6, respectively). As it is not known whether palifermin is excreted in human milk, it should not be administered to women who are breast-feeding (see SPC, section 4.6). No studies on the effects on the ability to drive and use machines have been performed (see SPC, section 4.7). The SPC contraindicates the use of palifermin in patients with hypersensitivity to palifermin or to any of the excipients of the medicinal product, or to *Escherichia coli*-derived proteins (see sections 4.3).

The maximum amount of palifermin that can be safely administered in a single dose has not been determined. A dose of 250 µg/kg has been administered intravenously to 8 healthy volunteers without serious adverse effects (see SPC section 4.9).

To date, no significant effect of palifermin on long-term disease outcomes in this patient population has been observed. However, the long-term safety of palifermin has not been fully evaluated with respect to overall survival, progression free survival and secondary malignancies and the safety of the product has not been established in patients with KGF receptors expressing non-haematological malignancies (see SPC, section 4.4). Secondary malignancies after ionising radiation and antineoplastic drugs usually show a peak between 5-10 years and long-term follow-up is essential since in the haematology transplant indications, development of non-haematological, secondary tumours are a major concern. Although there were no major concerns regarding immunogenicity, there were however, no data beyond 3 months. Annual safety data updates on the ongoing long-term follow-up studies 960226 and 990123 will be provided to the CHMP. Moreover, the Center for International Blood and Marrow Transplant Research (CIBMTR) databases will be used to identify cases of suspected secondary malignancies and cancer relapse. The proposed risk management plan, including the use of the CIBMTR, has been considered satisfactory.

5.   Overall conclusions and benefit/risk assessment

Quality

During development of the manufacturing process, different process development stages in different manufacturing sites have been documented. The applicant has provided adequate data to demonstrate comparability of commercial and clinical trial batches.

Non-clinical pharmacology and toxicology

Palifermin is a human keratinocyte growth factor (KGF), produced by recombinant DNA technology in *Escherichia coli*. KGF is a protein that targets epithelial cells by binding to specific cell-surface receptors thereby stimulating proliferation, differentiation, and upregulation of cytoprotective mechanisms (eg, induction of antioxidant enzymes). Palifermin differs from endogenous human KGF in that the first 23 N-terminal amino acids have been deleted to improve protein stability.
Epithelial protection by exogenously administered rHuKGF has been demonstrated in animal models of gastrointestinal injury induced by radiation or chemotherapy, oral mucosal toxicity induced by radiation or chemoradiotherapy, and acute and chronic salivary gland toxicity (xerostomia) induced by radiation. In addition, murine and non-human primate models of lethal irradiation and transplantation with bone marrow were studied. These models were appropriate for the intended clinical indication.
Pharmacokinetic studies demonstrated approximately dose proportional increases in systemic exposure following single i.v. administration to rats, monkeys and mice. The effective half-life ($t_{1/2}$) ranged from approximately 1 to 4 hours in these species. Elimination of palifermin-related material occurs mainly by the renal route and exposure doubled in bilaterally-nephrectomised rats. Similar increases in exposure may also occur in renally-impaired patients.
There were no major concerns arising from the results of the non-clinical toxicological studies. The findings were generally evidence of the pharmacological activity of rHuKGF, and mostly reversible. Safety margins could not always be calculated, partly because pharmacological effects occurred at all doses, or because rHuKGF was not measurable at the NOEL. Margins that could be calculated, based on NOAELs, were low but acceptable.

Efficacy

A phase III, multicenter, randomized, double-blind, placebo-controlled trial, has shown that, in patients with hematologic malignancies (non-Hodgkin's lymphoma, Hodgkin's disease, acute myelogenous leukaemia, acute lymphoblastic leukaemia, chronic myelogenous leukaemia, chronic lymphocytic leukaemia or multiple myeloma) who received TBI plus high-dose chemotherapy followed by autologous PBPC transplantation, the mean (SD) duration of WHO grade 3 or 4 oral mucositis in the palifermin group (3.7 [4.1] days) was 64% lower than in the placebo group (10.4 [6.2] days) for the mITT population. The reduction was statistically significant ($p < 0.001$) and was reproducible across study centers, underlying disease, and number of radiotherapy fractions used in the TBI conditioning regimen. The secondary assessment of mouth and throat soreness showed a lower score (indicating less soreness) in the palifermin group *versus* the placebo group, with a reduction of 38% of the mean (SD) AUC. The incidence of WHO grade 4 oral mucositis was 62% of patients in the placebo group and 20% of patients in the palifermin group ($p < 0.001$). The median cumulative dose of opioid analgesic used for oral mucositis was 211.6 mg in the palifermin group compared with 534.9 mg in the placebo group ($p < 0.001$). The incidence of opioid use was 78% for the palifermin group and 97% for the placebo group ($p < 0.001$).

For regimens used in the treatment of hematologic malignancies that are not associated with a high incidence of severe mucositis, no evidence has been provided to confirm that the risk of treatment is still outweighed by the benefit when the symptoms experienced by the patient are much milder than those seen in the pivotal trial.

Safety

Overall, the safety results analysed showed that palifermin was well tolerated. Adverse events that occurred $\geq 5\%$ more often in palifermin than placebo were rash, fever, pruritus, erythema, edema, oral lesion, pain, and granulocytopenia. These adverse events were reversible, usually mild in severity, and infrequently led to discontinuation of dosing. Skin (e.g., rash, pruritus, erythema, and oedema) and oral (e.g., mouth/tongue discoloration or thickness, and taste disorders) adverse events were considered possibly related to the pharmacologic action of palifermin on KGFR-expressing tissues. However, these adverse effects have been consistently notable among all oncology patient groups including haematological, head and neck and colorectal malignancies, despite differences in dosage and treatment schedules. The association of these events with palifermin treatment was more apparent before administration of cytotoxic therapy. However, after cytotoxic therapy, a high level of background toxicities has complicated accurate interpretation of the data.

In all settings studied, palifermin was associated with transient, asymptomatic elevations of amylase (62% of patients receiving palifermin *versus* 54% receiving placebo) and lipase (27% of patients receiving palifermin *versus* 23% receiving placebo). Clinical signs of overt pancreatitis were not associated with these increases. In the fractionated chemoradiotherapy setting in patients with advanced head and neck cancer, grade 3 decreases in ANC occurred in a higher proportion of patients in the palifermin group (22%) than in the placebo group (2%). The potential occurrence of visual adverse events after palifermin administration is of theoretical concern since KGF receptors have been shown to be present on the lens of the eye. Based on the available data, the risk associated with the administration of palifermin and the development of cataracts or worsening of pre-existing cataracts was low.

The long-term safety of palifermin has not been fully evaluated with respect to overall survival, progression free survival and secondary malignancies, and the safety of the product has not been established in patients with KGF receptors expressing non-haematological malignancies. Annual safety data updates on the ongoing long-term follow-up studies will be provided to the CHMP. Moreover, the Center for International Blood and Marrow Transplant Research (CIBMTR) databases, which are part of the risk management plan, will be used to identify cases of suspected secondary malignancies and cancer relapse.

Benefit/risk assessment

The indication was supported by one pivotal phase III and one phase II study. Based on the results of the clinical studies described in this application, the efficacy of palifermin has been demonstrated for the prevention and treatment of mucositis following TBI and myeloablative chemotherapy. In the pivotal phase III study, assessing a pre-post 6-dose regimen of palifermin, the statistically significant reduction seen in the median duration of WHO grade 3 and 4 oral mucositis (9 days in the placebo group *versus* 3 days in the palifermin group), the primary endpoint, was supported by statistically significant reductions in duration of oral mucositis as assessed by other validated oral mucositis rating scales. Consistent results were observed in terms of secondary endpoints, i.e., the incidence, the severity of oral mucositis and the requirements for opioid analgesia.

The incidence and severity of oral mucositis varies significantly across different conditioning regimens, ranging from approximately 20% incidence of grade 3 or 4 oral mucositis observed with the BEAM (BCNU, etoposide, Ara-C, and melphalan) regimen to approximately 50% incidence reported with melphalan-based regimens [52], and up to approximately 95% incidence for TBI-based regimens. The choice of the myelotoxic regimen used in the pivotal trial (TBI followed by high-dose chemotherapy), a standard treatment regimen used in North America, associated with a high rate of grade 3/4 oral mucositis, is not used as routinely in Europe, where less mucotoxic chemotherapy-only regimens (such as BEAM) are usually administered. Nevertheless, some chemotherapy-only regimens do give rise to a comparatively high incidence of mucositis. Thus, the question of appropriateness of results extrapolation from TBI-based regimens to other chemotherapy-based conditioning regimens has been debated. The CHMP concluded that the benefit-risk observed in patients undergoing myeloablative regimens could not be extrapolated to less mucotoxic regimens.

However, concerning non-TBI regimens, given that the underlying pathophysiology of oral mucositis is essentially the same regardless of the type of chemo-radiotherapy regimen that caused it, it was considered that the clinical benefit seen in the phase III study could be extrapolated to myeloablative chemotherapy conditioning regimens associated with high incidence of severe oral mucositis. Furthermore, considering the limited clinical data in the allogeneic transplant setting and the safety concerns in allogeneic transplants as regards the development of graft *versus* host disease, the indication has been restricted to patients with haematological malignancies receiving myeloablative therapy requiring autologous haemopoietic stem cell support (see SPC section 4.1).

In terms of safety, palifermin was in general well tolerated. The most frequent adverse events, i.e. gastrointestinal disorders, skin and subcutaneous tissue disorders, were consistent with the pharmacologic action on KGFR-expressing tissues. To date, no significant effect of palifermin on long-term disease outcomes in this patient population has been observed. However, the long-term safety of palifermin has not been fully evaluated with respect to overall survival, progression free survival and secondary malignancy and the safety of the product has not been established in patients with KGF receptors expressing non-haematological malignancies.

Overall, the benefit/risk profile for palifermin is considered favourable to decrease the incidence, duration and severity of oral mucositis in patients with haematological malignancies receiving myeloablative therapy associated with a high incidence of severe mucositis and requiring autologous haemopoietic stem cell support.

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the benefit/risk ratio of Kepivance (palifermin) indicated to decrease the incidence, the duration and severity of oral mucositis in patients with haematological malignancies receiving myeloablative therapy associated with a high incidence of severe mucositis and requiring autologous haemopoietic stem cell support was favourable.

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