DOSAGE REGIMES FOR THE ADMINISTRATION OF GLUCAGON-LIKE-PEPTIDE-2 (GLP-2) ANALOGUES

Field of the Invention

The present invention relates to dosage regimes for the administration of glucagon-like-peptide-2 (GLP-2) analogues and their medical use, for example in the treatment and/or prevention of stomach and bowel-related disorders and for ameliorating side effects of chemotherapy and radiation therapy. The present invention further relates to dosage regimes for the administration of glucagon-like-peptide-2 (GLP-2) analogues for inducing longitudinal growth of the intestines, for example for the treatment of patients with short bowel syndrome (SBS). The present invention further relates to medical uses for adjusting the volume of parenteral support (PS) provided to subjects receiving treatment with GLP-2 analogues in response to the treatment and to algorithms for determining PS volume changes.

Background of the Invention

Human GLP-2 is a 33-amino-acid peptide with the following sequence: Hy-His-Ala-Asp-Gly-Ser-Phe-Ser-Asp-Glu-Met-Asn-Thr-Ile-Leu-Asp-Asn-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Asn-Trp-Leu-Ile-Gln-Thr-Lys-Ile-Thr-Asp-OH. It is derived from specific post-translational processing of proglucagon in the enteroendocrine L cells of the intestine and in specific regions of the brainstem. GLP-2 binds to a single G-protein-coupled receptor belonging to the class II glucagon secretin family.

GLP-2 has been reported to induce significant growth of the small intestinal mucosal epithelium via the stimulation of stem cell proliferation in the crypts, and by inhibition of apoptosis in the villi (Drucker et al., 1996, Proc. Natl. Acad. Sci. USA 93: 7911-7916). GLP-2 also has growth effects on the colon. Furthermore, GLP-2 inhibits gastric emptying and gastric acid secretion (Wojdemann et al., 1999, J. Clin. Endocrinol. Metab. 84: 2513-2517), enhances intestinal barrier function (Benjamin et al., 2000, Gut 47: 112-119), stimulates intestinal hexose transport via the upregulation of glucose transporters (Cheeseman, 1997, Am. J. Physiol. R1965-71), and increases intestinal blood flow (Guan et al., 2003, Gastroenterology, 125: 136-147). A review of GLP-2 and its properties is available at:http://jn.nutrition.org/content/131/3/709.full.

It has been recognised in the art that glucagon-like peptide-2 receptor analogues have therapeutic potential for the treatment of intestinal diseases. However, the native hGLP-2, a 33 amino acid gastrointestinal peptide, is not a useful in a clinical setting due to its very

short half-life in humans of around 7 minutes for full length GLP-2 [1-33] and 27 minutes for truncated GLP-2 [3-33]. In large part, the short half-life is due to degradation by the enzyme dipeptidylpeptidase IV (DPP-IV). Accordingly, there have been attempts in the art to develop GLP-2 receptor agonists with better pharmacokinetic characteristics, in particular to improve the half-life of GLP-2 molecules. By way of example, GLP-2 analogues with substitutions have been suggested such as e.g. GLP-2 analogues containing Gly substitution at position 2 ([hGly2] GLP-2, teduglutide) which increases the half-life from seven minutes (native GLP-2) to about two hours. Acylation of peptide drugs with fatty acid chains has also proven beneficial for prolonging systemic circulation as well as increasing enzymatic stability without disrupting biological potency. However, while these attempts have improved the pharmacokinetics of GLP-2 analogues, and they are sometimes described in the art as "long acting", it must be kept in mind that this is in comparison to native hGLP-2 with half-lives of the order of several hours, rather than minutes. This in turn means that the GLP-2 analogues still need to be administered to patients one or more times per day.

There is considerable interest on the part of patients and healthcare providers in the development of low cost, long-acting, "user-friendly" therapeutic peptides. However, a major difficulty with the delivery of such therapeutic peptides is their short plasma half-life, mainly due to rapid serum clearance and proteolytic degradation via the action of plasma proteases, necessitating frequent, often daily, injections. Patients dislike injections, which leads to reduced compliance and reduced drug efficacy.

WO 2006/117565 (Zealand Pharma A/S) describes GLP-2 analogues which comprise one of more substitutions as compared to [hGly2]GLP-2 and which improved biological activity *in vivo* and/or improved chemical stability, e.g. as assessed in *in vitro* stability assays. In particular, GLP-2 analogues are described which have substitutions at one or more of positions 8, 16, 24 and/or 28 of the wild-type GLP-2 sequence, optionally in combination with further substitutions at position 2 and one or more of positions 3, 5, 7, 10 and 11, and/or a deletion of one or more of amino acids 31 to 33. These substiutions may also be combined with the addition of a N-terminal or C-terminal stabilizing peptide sequence. The daily or twice daily administration of these GLP-2 analogues is also described. Among the molecules disclosed in WO 2006/117565 is glepaglutide (ZP1848) which has been designed to be stable in liquid formulations, and is typically administered by daily dosing using an injection pen.

Summary of the Invention

Broadly, the present invention is based on the surprising finding that the pharmacokinetic (PK) and/or pharmacodynamic (PD) properties of GLP-2 analogues, e.g. of the type disclosed in WO 2006/117565 and WO 2008/056155, enables less frequent administration than has hitherto been proposed. Previous experiments had suggested that GLP-2 analogues, such as ZP1848, had a half-life of about 17 hours which, while being significantly longer than GLP-2 or other GLP-2 analogues, suggested that daily administration ought to be used in the treatment of patients. However, a Phase 2 clinical study in humans using the GLP-2 analogue ZP1848 has now found that the terminal plasma half-life of the molecule is in fact between 5 and 17 days. The terminal plasma half-life is the time required to divide the plasma concentration by two after reaching pseudo-equilibrium. This in turn surprisingly suggests that significantly less frequent dosing of the GLP-2 analogues of the present invention would be efficacious, including weekly and twice weekly dosing. The contents of WO 2006/117565 and WO 2008/056155 are expressly incorporated in their entirety for all purposes, and in particular in relation to the GLP-2 analogues disclosed in these documents.

The results from further studies have also confirmed that both metabolites of ZP1848, i.e. ZP2711 and ZP2469, were present following its administration *in vivo* and are pharmacologically active, with *in vitro* potency comparable to ZP1848. The plasma level of ZP2711 is in the same range as that of ZP1848, whereas the plasma level of ZP2469 is multiple times higher than that of ZP1848. Consequently, both metabolites can be included when evaluating systemic exposure as they boths contribute to *in vivo* efficacy. Due to the high plasma level of ZP2469 this compound has plasma levels above the bioanalytical detection limit (50 pM for ZP2469, 25 pM for ZP1848 and ZP2711), hence it is possible to determine the half-life of compound ZP2469. A terminal plasma half-life of between 5 and 17 days, as shown in the examples, suggests once or twice weekly dosing of ZP1848 would be efficacious.

This in turn enables the administration of the GLP-2 analogues according to the present invention, e.g. once or twice weekly adminstration. Alternatively or additionally, the dosing regime of the GLP-2 analogues of the present invention may comprise a plurality or course of doses separated in time by 2 days, 2.5 days, 3 days, 3.5 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days or 12 days. In a preferred embodiment, the doses are separated in time by 3 days, 3.5 days, 4 days, 5 days, 6 days, 7 days or 8 days. In a preferred embodiment, doses are separated in time by 3 days, 3.5 days, 4 days or 7 days. As will be appreciated in the art, the time between doses may be varied to some extent so

that each and every doses is not separated by precisely the same time. This will often be directed under the discretion of the physician. Thus, doses may be separated in time by a clinically acceptable range of times, e.g. from about 2 days to about 10 days, or from about 3 or 4 days to about 7 or 8 days. The results from the clinical trial that support these conclusions are included in the examples below.

Without being bound by theory, it is presently believed that the unexpected long half-life of the GLP-2 analogues, which enables once or twice weekly dosing, might be obtained due to the combination of the formation of a subcutaneous depot and the formation of metabolites which are slowly released from the subcutaneous depot and which are also agonistic on the GLP-2 receptor. The present inventors believe that the subcutaneous depot is formed on administration of GLP-2 analogues through a reaction between the analogues, i.e. the lysine tail, and with hyaluronic acid in the subcutaneous compartment. This type of depot has not been seen before for other compounds having a lysine tail, such as lixisenatide which has a half-life of 2-4 hours.

Accordingly, in first aspect, the present invention provides a glucagon-like peptide 2 (GLP-2) analogue for use in a method for the treatment and/or prevention of a stomach and bowel-related disorder in a human patient, wherein the GLP-2 analogue is represented by the formula:

R^1-Z^1-His-Gly-Glu-Gly-X5-Phe-Ser-Ser-Glu-Leu-X11-Thr-Ile-Leu-Asp-Ala-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Ala-Trp-Leu-Ile-Ala-Thr-Lys-Ile-Thr-Asp-Z^2-R^2

wherein:
R^1 is hydrogen, C_{1-4} alkyl (e.g. methyl), acetyl, formyl, benzoyl or trifluoroacetyl;
X5 is Ser or Thr;
X11 is Ala or Ser;
R^2 is NH_2 or OH; and
Z^1 and Z^2 are independently absent or a peptide sequence of 1-6 amino acid units of Lys;
or a pharmaceutically acceptable salt or derivative thereof; and

wherein the method comprises administering the GLP-2 analogue to the patient once or twice weekly.

In this aspect of the present invention, the glucagon-like peptide 2 (GLP-2) analogue may be used for the treatment and/or prevention of stomach and bowel-related disorders such

as ulcers, digestion disorders, malabsorption syndromes, short-gut syndrome, cul-de-sac syndrome, inflammatory bowel disease, celiac sprue (for example arising from gluten induced enteropathy or celiac disease), tropical sprue, hypogammaglobulinemic sprue, enteritis, regional enteritis (Crohn's disease), ulcerative colitis, small intestine damage or short bowel syndrome (SBS). Alternatively or additionally, the glucagon-like peptide 2 (GLP-2) analogue may be used for the treatment and/or prevention of stomach and bowel-related disorders such radiation enteritis, infectious or post-infectious enteritis, or small intestinal damage due to toxic or other chemotherapeutic agents. In this case, treatment with the GLP-2 analogue may optionally be combined with one or more anti-cancer therapies, and may therefore comprise adminstering one or more chemotherapeutic agent(s) to the patient or treating the patient with radiation therapy.

In a further aspect, the present invention provides a glucagon-like peptide 2 (GLP-2) analogue for use in a method for the treatment and/or prevention of a side effect of chemotherapy or radiation treatment in a human patient, wherein the GLP-2 analogue is represented by the formula:

R^1-Z^1-His-Gly-Glu-Gly-X5-Phe-Ser-Ser-Glu-Leu-X11-Thr-Ile-Leu-Asp-Ala-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Ala-Trp-Leu-Ile-Ala-Thr-Lys-Ile-Thr-Asp-Z^2-R^2

wherein:
R^1 is hydrogen, C_{1-4} alkyl (e.g. methyl), acetyl, formyl, benzoyl or trifluoroacetyl;
X5 is Ser or Thr;
X11 is Ala or Ser;
R^2 is NH_2 or OH; and
Z^1 and Z^2 are independently absent or a peptide sequence of 1-6 amino acid units of Lys;
or a pharmaceutically acceptable salt or derivative thereof; and

wherein the method comprises administering the GLP-2 analogue to the patient once or twice weekly.

In a further aspect, the present invention provides a glucagon-like peptide 2 (GLP-2) analogue for use in a method for increasing the longitudinal growth of the intestines in a human patient, wherein the GLP-2 analogue is represented by the formula:

R^1-Z^1-His-Gly-Glu-Gly-X5-Phe-Ser-Ser-Glu-Leu-X11-Thr-Ile-Leu-Asp-Ala-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Ala-Trp-Leu-Ile-Ala-Thr-Lys-Ile-Thr-Asp-Z^2-R^2

wherein:
R^1 is hydrogen, C_{1-4} alkyl (e.g. methyl), acetyl, formyl, benzoyl or trifluoroacetyl;
X5 is Ser or Thr;
X11 is Ala or Ser;
R^2 is NH_2 or OH; and
Z^1 and Z^2 are independently absent or a peptide sequence of 1-6 amino acid units of Lys;
or a pharmaceutically acceptable salt or derivative thereof; and

wherein the method comprises administering the GLP-2 analogue to the patient once or twice weekly.

In a further aspect, the present invention provides a glucagon-like peptide 2 (GLP-2) analogue for use in a method for the treatment of short bowel syndrome (SBS) in a human subject receiving a parenteral support (PS) regimen, the method comprising:

(i) administering to the subject a GLP-2 analogue represented by the formula:

R^1-Z^1-His-Gly-Glu-Gly-X5-Phe-Ser-Ser-Glu-Leu-X11-Thr-Ile-Leu-Asp-Ala-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Ala-Trp-Leu-Ile-Ala-Thr-Lys-Ile-Thr-Asp-Z^2-R^2

wherein:
R^1 is hydrogen, C_{1-4} alkyl (e.g. methyl), acetyl, formyl, benzoyl or trifluoroacetyl;
X5 is Ser or Thr;
X11 is Ala or Ser;
R^2 is NH_2 or OH; and
Z^1 and Z^2 are independently absent or a peptide sequence of 1-6 amino acid units of Lys;
or a pharmaceutically acceptable salt or derivative thereof; and

wherein the GLP-2 analogue is administered once or twice weekly for a time sufficient to lengthen and improve the function of the small intestine of the subject;

(ii) following step (i), reducing a frequency or volume of the parenteral support (PS) regimen in response to improved function of the small intestine; and

(iii) optionally repeating steps (i) and (ii) in response to the subject demonstrating improved function over time as a result of further longitudinal growth of the small intestine.

In a further aspect, the present invention provides a method for the treatment of short bowel syndrome (SBS) in a human subject receiving a parenteral support (PS) regimen, the method comprising:

(i) administering to the subject a GLP-2 analogue represented by the formula:

R^1-Z^1-His-Gly-Glu-Gly-X5-Phe-Ser-Ser-Glu-Leu-X11-Thr-Ile-Leu-Asp-Ala-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Ala-Trp-Leu-Ile-Ala-Thr-Lys-Ile-Thr-Asp-Z^2-R^2

wherein:
R^1 is hydrogen, C_{1-4} alkyl (e.g. methyl), acetyl, formyl, benzoyl or trifluoroacetyl;
X5 is Ser or Thr;
X11 is Ala or Ser;
R^2 is NH_2 or OH; and
Z^1 and Z^2 are independently absent or a peptide sequence of 1-6 amino acid units of Lys;
or a pharmaceutically acceptable salt or derivative thereof; and

wherein the GLP-2 analogue is administered once or twice weekly for a time sufficient to lengthen and further improve the function of the small intestine of the subject;

(ii) following step (i), reducing a frequency or volume of the parenteral support (PS) regimen in response to improved function of the small intestine; and

(iii) optionally repeating steps (i) and (ii) in response to the subject demonstrating continued improved function of the small intestine.

By way of example, the treatment period over which the GLP-2 analogue is administered to the subject may be from 18 to 150 weeks, for example from 18 to 100 weeks, or from 18 to 52 weeks. However, in other cases where the SBS is a chronic condition, patients may require treatment for the rest of their lives.

In the present context, the term “parenteral support" or "PS" includes the provision of nutrients and/or fluids to the subject receiving GLP-2 therapy as a means of providing the subject with the nutrients and/or fluids that they require, but are unable to absorb fully due to their condition. The detemination of the correct amount or volume of PS to provide to subjects with SBS who are receiving GLP-2 therapy is a challenge because if PS volume is

not adjusted in a timely and appropriate way, patients may experience fluid overload, are at risk of dehydration and may not achieve optimal clinical responses to the therapy. This is further complicated as the PS volume required by a subject will typically vary during the course of GLP-2 therapy depending on their response to the therapy. Typically, the assessment of the amount of PS volume required by the subject as GLP-2 therapy progresses is dependent on how long the therapy has continued and the responsiveness of individual patients to it. In view of this variation, an initial assessment of PS volume may be carried out within the first few days of GLP-2 therapy, and is typically then followed by a weekly assessment during the first month, a monthly assessment over the next 1-3 months, and thereafter an assessment every 3-6 months until the treatment is concluded. This is important as subjects may experience a rapid initial response to GLP-2 therapy, improving the function of the small intestine, for example even before any increase in the length of the intestine is observed. This in turn enables the PS volume to be reduced, thereby avoiding the risk of side effects, such as fluid overload.

Accordingly, in the above method, step (ii) may include the step of (a) determining the volume of PS required by the subject at that point in the treatment, (b) comparing it to a baseline PS volume determined at the start of therapy with the GLP-2 analogue and (c) reducing the frequency or volume of the PS where the subject demonstrates improved function of the small intestine. Optionally, the reduction of a frequency or volume of the parenteral support (PS) in step (ii) can be performed using the algorithm described below.

By way of illustration of the relationship between the amount of parenteral support required by patients and the degree of improvement in intestinal function, it is presently believed that a 40% increase in length of the small intestine would result in at least a further 10% improvement in function or absorptive capacity of the small intestine. Generally, the GLP-2 therapy according to the present invention leads to improved function or absorptive capacity of the small intestine of at least 10%, more preferably at least 20%, more preferably at least 30% more preferably at least 40%, and most preferably at least 50%. Additionally or alternatively, the amount of the reduction of parenteral support over the course of GLP-2 therapy is at least 10%, more preferably at least 20%, more preferably at least 30%, more preferably at least 40% and most preferably at least 50%. In a preferred embodiment, the reduction of parenteral support is at least 20%.

In a further related aspect, the present invention addresses one of the challenges facing patients and physicians when initiating GLP-2 therapy, namely the appropriate and individualized adjustment in volume of parenteral support (PS) provided to the patient.

This is important because if the PS volume is not adjusted in a timely and appropriate way, patients may experience fluid overload, risks of dehydration and may not achieve optimal clinical responses with the therapy.

For example, in a previous 24-week treatment phase 3 study with the GLP-2 analogue teduglutide, an attempt to reduce the parenteral nutrition volume by 10% per month was done at the earliest at week 4 after start of treatment, if the urine output increased by at least 10% from baseline. Many of the patients in this study suffered from fluid overload and stopped drinking (Jeppesen et al. 2011, Gut 2011;60:902-914). In a follow-up phase 3 study, a 24-week study of SBS patients who were given subcutaneous teduglutide (Jeppesen et al. 2012, Gastroenterology 2012;143:1473–1481), the protocol allowed a reduction of parenteral support volume to at least 10%, but not more than 30%, after 2 weeks of treatment, if the urine output increases by at least 10% from baseline. However, in this study, the patients also suffered from fluid overload, in particular at the beginning of the treatment.

Consequently, in this aspect, the present invention enables early assessment of altered PS fluid needs (for example, within a few days of initiating GLP-2 therapy) and provides algorithms for adjusting of PS volume during a course of GLP-2 therapy. It will be appreciated by those skilled in the art that this approach using the algorithm to adjust the PS volume provides a personalised adjustment of the PS volume for each patient. This aspect of the present invention is applicable to GLP-2 therapies using the GLP-2 analogues disclosed herein or using GLP-2 analogues known elsewhere in the art, such as teduglutide.

Accordingly, in a further aspect, the present invention provides a method for adjusting a parenteral support (PS) volume in a human subject receiving an amount or volume of parenteral support each week, the method comprising calculating a new PS volume using an algorithm in which a PS volume for a week is calculated by subtracting seven times the absolute increase in daily urine volume from a baseline volume, i.e. PS volume at the initiation of GLP-2 therapy, from the current weekly volume of parenteral support to provide the new PS volume for the subject. Thus, the new PS volume (weekly) for the subject equals the current PS volume (weekly) minus 7 × absolute increase in daily urine volume from the baseline volume.

In a further aspect, the present invention provides a method for determining a parenteral support (PS) volume for a human subject receiving GLP-2 therapy, the method comprising:

optionally determining a baseline daily urine volume at the start of GLP-2 therapy;

calculating a new PS volume (weekly) for the subject for a coming week based on a current PS volume (weekly) and a daily urine volume (e.g. from a physician visit) using an algorithm in which the new PS volume (weekly) for the subject equals the current PS volume (weekly) - 7 × absolute increase in daily urine volume from the baseline volume.

In some cases, the calculating step is carried out if the daily urine volume is at least 10% higher than baseline urine volume. This method may comprise the additional step of determining the current PS volume (weekly) for use in the calculation of the new PS volume using the algorithm.

It will be understood that, the method may further comprise the step of administering the PS volume to the subject. Furthermore, the method may be repeated, for example every week, month or at physician visits, enabling the PS volume to be reduced in response to the subject demonstrating continued improved function of the small intestine. In an embodiment, the adjustment of the PS volume results in an urine volume of least 800 ml/day (24 hours), such as at least 1000 ml/day (24 hours) or more. A particular advantage of this method is that the effects of treatment and the consequent adjustment of the PS volume may be carried out very early in the GLP-2 therapy, for example in the first few days of treatment, which is significantly quicker compared to prior art approaches. For example, according to the present invention, subjects may undergo a 0-4 week optimization, followed by a 2-4 weeks stabilization phase. The algorithm for PS volume reductions may be based on changes in urine volume measured during 48 hours.

In some embodiments of this aspect of the present invention, the GLP-2 therapy may comprise adminstration of a GLP-2 analogue represented by the formula disclosed herein, in particular using the examples of these GLP-2 analogues. In a preferred embodiment the GLP-2 analogue is ZP1848.

Preferably, the method includes the initial steps of determining the baseline volume and/or determining the daily urine volume. Some or all of the steps of the method may be repeated during the course of the GLP-2 therapy received by the subject. Preferably, the first assessment and the determination of changes in the PS volume adjustments is done early in the GLP-2 therapy, such as a few days after the start of the GLP-2 therapy, such as 2, 3, 4, 5, 6, 7 days after start of the GLP-2 therapy. Preferably, the time between

repeat testing to determine changes in the PS volume adjustments is initially weekly and later about every 1 to 3 months. For example, in one embodiment, the method may be used at weeks 1, 2, 4, 8 and 12 after commencement of treatment with the GLP-2 analogue, adjusting the PS volume in response to the effects of treament. The weekly PS volume administered should be monitored and adjusted throughout treatment with the GLP-2 analogue (e.g. glepaglutide) in order to avoid fluid overload. Generally, as the absorptive capacity of the intestines increases after treatment with the GLP-2 analogue, typically by elongation or thickening of the small intestine, the PS volume can be decreased to avoid fluid overload and to improve the treatment needs of the patient.

Other conditions that may be treated with the GLP-2 analogues of the invention, or for which the GLP-2 analogues may be useful prophylactically or therapeutically, include radiation enteritis, infectious or post-infectious enteritis, and small intestinal damage due to toxic or other chemotherapeutic agents. This may require administration of the GLP-2 analogue prior to, concurrently with or following a course of chemotherapy or radiation therapy in order to reduce side effects of chemotherapy such as diarrhoea, abdominal cramping and vomiting, and reduce the consequent structural and functional damage of the intestinal epithelium resulting from the chemotherapy or radiation therapy. Preferably, administration of the GLP-2 analogue according to the invention is initiated 1, 2, 3, 4, 5, 6 or 7 days prior to the initiation of the chemotherapy or radiation cycle. Preferably, administration of the GLP-2 analogue according to the invention is initiated the day before or same day as start of treatment with chemotherapy or radiation cycle and once or twice weekly thereafter.

In a further aspect, the present invention provides the use of a glucagon-like peptide 2 (GLP-2) analogue in the manufacture of a medicament for treating and/or preventing of a stomach and bowel-related disorder in a human patient, wherein the GLP-2 analogue is represented by the formula:

R^1-Z^1-His-Gly-Glu-Gly-X5-Phe-Ser-Ser-Glu-Leu-X11-Thr-Ile-Leu-Asp-Ala-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Ala-Trp-Leu-Ile-Ala-Thr-Lys-Ile-Thr-Asp-Z^2-R^2

wherein:

R^1 is hydrogen, C_{1-4} alkyl (e.g. methyl), acetyl, formyl, benzoyl or trifluoroacetyl;

X5 is Ser or Thr;

X11 is Ala or Ser;

R^2 is NH_2 or OH; and

Z^1 and Z^2 are independently absent or a peptide sequence of 1-6 amino acid units of Lys; or a pharmaceutically acceptable salt or derivative thereof; and

wherein the method comprises administering the GLP-2 analogue to the patient once or twice weekly.

In a further aspect, the present invention provides the use of a glucagon-like peptide 2 (GLP-2) analogue in the manufacture of a medicament for treating and/or preventing of a side effect of chemotherapy or radiation treatment in a human patient, wherein the GLP-2 analogue is represented by the formula:

R^1-Z^1-His-Gly-Glu-Gly-X5-Phe-Ser-Ser-Glu-Leu-X11-Thr-Ile-Leu-Asp-Ala-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Ala-Trp-Leu-Ile-Ala-Thr-Lys-Ile-Thr-Asp-Z^2-R^2

wherein:
R^1 is hydrogen, C_{1-4} alkyl (e.g. methyl), acetyl, formyl, benzoyl or trifluoroacetyl;
X5 is Ser or Thr;
X11 is Ala or Ser;
R^2 is NH_2 or OH; and
Z^1 and Z^2 are independently absent or a peptide sequence of 1-6 amino acid units of Lys; or a pharmaceutically acceptable salt or derivative thereof; and

wherein the method comprises administering the GLP-2 analogue to the patient once or twice weekly.

In a further aspect, the present invention provides a method of treating and/or preventing a stomach and bowel-related disorder in a human patient, the method comprising adminstering to the patient a therapeutically effective amout of glucagon-like peptide 2 (GLP-2) analogue represented by the formula:

R^1-Z^1-His-Gly-Glu-Gly-X5-Phe-Ser-Ser-Glu-Leu-X11-Thr-Ile-Leu-Asp-Ala-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Ala-Trp-Leu-Ile-Ala-Thr-Lys-Ile-Thr-Asp-Z^2-R^2

wherein:
R^1 is hydrogen, C_{1-4} alkyl (e.g. methyl), acetyl, formyl, benzoyl or trifluoroacetyl;
X5 is Ser or Thr;
X11 is Ala or Ser;

R^2 is NH_2 or OH; and
Z^1 and Z^2 are independently absent or a peptide sequence of 1-6 amino acid units of Lys; or a pharmaceutically acceptable salt or derivative thereof; and

wherein the method comprises administering the GLP-2 analogue to the patient once or twice weekly.

In a further aspect, the present invention provides a method of treating and/or preventing a side effect of chemotherapy or radiation treatment in a human patient, the method comprising adminstering to the patient a therapeutically effective amout of glucagon-like peptide 2 (GLP-2) analogue represented by the formula:

R^1-Z^1-His-Gly-Glu-Gly-X5-Phe-Ser-Ser-Glu-Leu-X11-Thr-Ile-Leu-Asp-Ala-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Ala-Trp-Leu-Ile-Ala-Thr-Lys-Ile-Thr-Asp-Z^2-R^2

wherein:
R^1 is hydrogen, C_{1-4} alkyl (e.g. methyl), acetyl, formyl, benzoyl or trifluoroacetyl;
X5 is Ser or Thr;
X11 is Ala or Ser;
R^2 is NH_2 or OH; and
Z^1 and Z^2 are independently absent or a peptide sequence of 1-6 amino acid units of Lys; or a pharmaceutically acceptable salt or derivative thereof; and

wherein the method comprises administering the GLP-2 analogue to the patient once or twice weekly.

In a further aspect, the present invention provides the use of a glucagon-like peptide 2 (GLP-2) analogue in the manufacture of a medicament for increasing the longitudinal growth of the intestines in a human patient, wherein the glucagon-like peptide 2 (GLP-2) analogue represented by the formula:

R^1-Z^1-His-Gly-Glu-Gly-X5-Phe-Ser-Ser-Glu-Leu-X11-Thr-Ile-Leu-Asp-Ala-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Ala-Trp-Leu-Ile-Ala-Thr-Lys-Ile-Thr-Asp-Z^2-R^2

wherein:
R^1 is hydrogen, C_{1-4} alkyl (e.g. methyl), acetyl, formyl, benzoyl or trifluoroacetyl;
X5 is Ser or Thr;

X11 is Ala or Ser;
R^2 is NH_2 or OH; and
Z^1 and Z^2 are independently absent or a peptide sequence of 1-6 amino acid units of Lys;
or a pharmaceutically acceptable salt or derivative thereof; and

wherein the method comprises administering the GLP-2 analogue to the patient once or twice weekly.

In a further aspect, the present invention provides a method for increasing the longitudinal growth of the intestines in a human patient, the method comprising adminstering to the patient a therapeutically effective amout of glucagon-like peptide 2 (GLP-2) analogue represented by the formula:

R^1-Z^1-His-Gly-Glu-Gly-X5-Phe-Ser-Ser-Glu-Leu-X11-Thr-Ile-Leu-Asp-Ala-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Ala-Trp-Leu-Ile-Ala-Thr-Lys-Ile-Thr-Asp-Z^2-R^2

wherein:
R^1 is hydrogen, C_{1-4} alkyl (e.g. methyl), acetyl, formyl, benzoyl or trifluoroacetyl;
X5 is Ser or Thr;
X11 is Ala or Ser;
R^2 is NH_2 or OH; and
Z^1 and Z^2 are independently absent or a peptide sequence of 1-6 amino acid units of Lys;
or a pharmaceutically acceptable salt or derivative thereof; and

wherein the method comprises administering the GLP-2 analogue to the patient once or twice weekly.

In all aspects of the present invention, the method of administering the glucagon-like peptide 2 (GLP-2) analogue optionally comprises administering a plurality of doses of the GLP-2 analogue to the patient, wherein the doses are separated in time by one week or half a week. In some cases, it may be desirable to divide a total dose into a plurality (e.g. two or three) separate doses, for example for administration at spaced apart injection sites, for example spacing the injection sites at least 5 cm apart.

Preferably, the doses of the GLP-2 analogues used in accordance with the present invention are in the range between 0.5 mg and 25 mg inclusive per patient once or twice weekly, optionally between 1 mg and 20 mg inclusive per patient once or twice weekly, optionally between 1 mg and 10 mg inclusive per patient once or twice weekly, optionally

between 2 mg and 7 mg inclusive per patient once or twice weekly, optionally between 5 mg and 7 mg inclusive per patient once or twice weekly, or optionally between 2 mg and 5 mg inclusive per patient once or twice weekly. In one embodiment, the dose of the GLP-2 analogues used in accordance with the present invention is 10 mg inclusive per patient once or twice weekly. In a course of treatment, the doses taken by the patient may either be the same or different in accordance to the instructions from the physician.

Preferably, the glucagon-like peptide 2 (GLP-2) analogue are administered to patients by injection, most typically by subcutaneous injection or intramuscular injection. In some preferred embodiments, the GLP-2 analogue may be administered using an injection pen, which allow patients to self-administer the analogue. In some aspects, administration of the GLP-2 analogue causes formation of a subcutaneous depot from which the GLP-2 analogue, or metabolites thereof, are released. Without wishing to be bound by any particular explanation, the subcutaneous depot may form through the interaction of the GLP-2 analogues administered in accordance with the present invention, in particular where the analogues comprise a lysine tail (i.e. a Z^1 group and/or a Z^2 group), through a reaction between the analogues and with hyaluronic acid in the subcutaneous compartment.

In some embodiments of the present invention, in the above formula, X5 is Thr and/or X11 is Ala. Examples of these glucagon-like peptide 2 (GLP-2) analogues include:

ZP1848 H-HGEGTFSSELATILDALAARDFIAWLIATKITDKKKKKK-NH_2 (SEQ ID NO: 1)
ZP2949 H-HGEGTFSSELATILDALAARDFIAWLIATKITDKKK-OH (SEQ ID NO: 2)
ZP2711 H-HGEGTFSSELATILDALAARDFIAWLIATKITDKK-OH (SEQ ID NO: 3)
ZP2469 H-HGEGTFSSELATILDALAARDFIAWLIATKITDK-OH (SEQ ID NO: 4)
ZP1857 H-HGEGTFSSELATILDALAARDFIAWLIATKITD-NH_2 (SEQ ID NO: 5)
ZP2530 H-HGEGTFSSELATILDALAARDFIAWLIATKITD-OH (SEQ ID NO: 6)

In some embodiments of the present invention, in the above formula X5 is Ser and/or X11 is Ser. Examples of these glucagon-like peptide 2 (GLP-2) analogues include:

ZP1846 H-HGEGSFSSELSTILDALAARDFIAWLIATKITDKKKKKK-NH_2 (SEQ ID NO: 7)
ZP1855 H-HGEGSFSSELSTILDALAARDFIAWLIATKITD-NH_2 (SEQ ID NO: 8)
ZP2242 H-HGEGSFSSELSTILDALAARDFIAWLIATKITDK-OH (SEQ ID NO: 9)

Embodiments of the present invention will now be described by way of example and not

limitation However, various further aspects and embodiments of the present invention will be apparent to those skilled in the art in view of the present disclosure.

"and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example "A and/or B" is to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as if each is set out individually herein.

Unless context dictates otherwise, the descriptions and definitions of the features set out above are not limited to any particular aspect or embodiment of the invention and apply equally to all aspects and embodiments which are described.

Detailed Description of the Invention

Definitions

Unless specified otherwise, the following definitions are provided for specific terms, which are used in the above written description.

Throughout the description and claims the conventional one-letter and three-letter codes for natural amino acids are used. All amino acid residues in peptides of the invention are preferably of the L-configuration, However, D-configuration amino acids may also be present.

Preferred compounds of the present invention have at least one GLP-2 biological activity, in particular in causing growth of the intestine. This can be assessed in *in vivo* assays, for example as described in the examples, in which the mass of the intestine, or a portion thereof is determined after a test animal has been treated or exposed to a GLP-2 analogue.

The GLP-2 analogues of the present invention have one or more amino acid substitutions, deletions, inversions, or additions compared with native GLP-2 and as defined above. This definition also includes the synonym terms GLP-2 mimetics and/or GLP-2 agonists. Further, the analogue of the present invention may additionally have chemical modification of one or more of its amino acid side groups, α-carbon atoms, terminal amino group, or terminal carboxylic acid group. A chemical modification includes, but is not limited to, adding chemical moieties, creating new bonds, and removing chemical moieties. Modifications at amino acid side groups include, without limitation, acylation of lysine ε-amino groups, N-alkylation of arginine, histidine, or lysine, alkylation of glutamic or aspartic

carboxylic acid groups, and deamidation of glutamine or asparagine. Modifications of the terminal amino include, without limitation, the des-amino, N-lower alkyl, N-di-lower alkyl, and N-acyl modifications. Modifications of the terminal carboxy group include, without limitation, the amide, lower alkyl amide, dialkyl amide, and lower alkyl ester modifications. Preferably herein lower alkyl is C_1-C_4 alkyl. Furthermore, one or more side groups, or terminal groups, may be protected by protective groups known to the ordinarily-skilled peptide chemist. The α-carbon of an amino acid may be mono- or di-methylated.

It should be understood that the peptides of the invention might also be provided in the form of a salt or other derivative. Salts include pharmaceutically acceptable salts such as acid addition salts and basic salts. Examples of acid addition salts include hydrochloride salts, citrate salts and acetate salts. Examples of basic salts include salts where the cation is selected from alkali metals, such as sodium and potassium, alkaline earth metals, such as calcium, and ammonium ions $^{+}N(R^3)_3(R^4)$, where R^3 and R^4 independently designates optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, optionally substituted aryl, or optionally substituted heteroaryl. Other examples of pharmaceutically acceptable salts are described in "Remington's Pharmaceutical Sciences",17th edition. Ed. Alfonso R. Gennaro (Ed.), Mark Publishing Company, Easton, PA, U.S.A., 1985 and more recent editions, and in the Encyclopaedia of Pharmaceutical Technology.

Other derivatives of the GLP-2 analogues of the invention include coordination complexes with metal ions such as Mn^{2+} and Zn^{2+}, esters such as *in vivo* hydrolysable esters, free acids or bases, hydrates, prodrugs or lipids. Esters can be formed between hydroxyl or carboxylic acid groups present in the compound and an appropriate carboxylic acid or alcohol reaction partner, using techniques well known in the art. Derivatives which as prodrugs of the compounds are convertible *in vivo* or *in vitro* into one of the parent compounds. Typically, at least one of the biological activities of compound will be reduced in the prodrug form of the compound, and can be activated by conversion of the prodrug to release the compound or a metabolite of it. Examples of prodrugs include the use of protecting groups which may be removed *in situ* releasing active compound or serve to inhibit clearance of the drug *in vivo*.

Z^1 and Z^2 are independently present and/or absent or a peptide sequence of 1-6 amino acid units of Lys, i.e. 1, 2, 3, 4, 5 or 6 Lys residues. The Lys residues may have either D- or L-configuration, but have an L-configuration. Particularly preferred sequences Z are sequences of four, five or six consecutive lysine residues, and particularly six consecutive

lysine residues. Exemplary sequences Z are shown in WO 01/04156. In certain embodiments, Z^1 is absent. In such cases, Z^2 may be either present or absent.

Pharmaceutical Compositions and Administration

The GLP-2 analogues of the present invention, or salts or derivatives thereof, may be formulated as pharmaceutical compositions prepared for storage or administration, and which comprise a therapeutically effective amount of a GLP-2 peptide of the present invention, or a salt or derivative thereof, in a pharmaceutically acceptable carrier. In accordance with the dosage regimes of the present invention, the GLP-2 analogues are admistered to patients, e.g. a human patient, once or twice weekly. Thus, a course of administration according to the present invention may entail administering to a patient a course of doses separated in time by 2 days, 2.5 days, 3 days, 3.5 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days,10 days, 11 days, 12 days, 13 days or 14 days, and more preferably separated in time by 3 days, 4 days, 5 days, 6 days, 7 days or 8 days. In some specific situations, the present invention may entail administering to a patient a fixed dose of e.g. 10 mg once or twice weekly.

The therapeutically effective amount of a compound of the present invention will depend on the route of administration, the type of mammal being treated, and the physical characteristics of the specific mammal under consideration. These factors and their relationship to determining this amount are well known to skilled practitioners in the medical arts. This amount and the method of administration can be tailored to achieve optimal efficacy so as to deliver the peptide to the large intestine, but will depend on such factors as weight, diet, concurrent medication and other factors, well known those skilled in the medical arts.

It is within the invention to provide a pharmaceutical composition, wherein the GLP-2 analogue, or a salt thereof is present in an amount effective to treat or prevent stomach and bowel-related disorders or to induce longitudinal growth of the intesines.

Pharmaceutically acceptable salts of the compounds of the invention having an acidic moiety can be formed using organic and inorganic bases. Suitable salts formed with bases include metal salts, such as alkali metal or alkaline earth metal salts, for example sodium, potassium, or magnesium salts; ammonia salts and organic amine salts, such as those formed with morpholine, thiomorpholine, piperidine, pyrrolidine, a mono-, di- or tri-lower alkylamine (*e.g.*, ethyl-tert-butyl-, diethyl-, diisopropyl-, triethyl-, tributyl- or dimethylpropylamine), or a mono-, di- or trihydroxy lower alkylamine (*e.g.*, mono-, di- or

triethanolamine). Internal salts also may be formed. Similarly, when a compound of the present invention contains a basic moiety, salts can be formed using organic and inorganic acids. For example, salts can be formed from the following acids: acetic, propionic, lactic, citric, tartaric, succinic, fumaric, maleic, malonic, mandelic, malic, phthalic, hydrochloric, hydrobromic, phosphoric, nitric, sulfuric, methanesulfonic, napthalenesulfonic, benzenesulfonic, toluenesulfonic, and camphorsulfonic as well as other known pharmaceutically acceptable acids. Amino acid addition salts can also be formed with amino acids such as lysine, glycine, or phenylalanine.

As is apparent to one skilled in the medical art, a "therapeutically effective amount" of the peptides or pharmaceutical compositions of the present invention will vary depending upon the age, weight and mammalian species treated, the particular compounds employed, the particular mode of administration and the desired effects and the therapeutic indication. Because these factors and their relationship to determining this amount are well known in the medical arts, the determination of therapeutically effective dosage levels, the amount necessary to achieve the desired result of preventing and/or treating the intestine and stomach related diseases described herein, as well as other medical indications disclosed herein, to inducing longitudinal growth of the intesines, will be within the ambit of the skilled person.

As used herein, “a therapeutically effective amount” is one which reduces symptoms of a given condition or pathology, and preferably which normalizes physiological responses in an individual with the condition or pathology. Reduction of symptoms or normalization of physiological responses can be determined using methods routine in the art and may vary with a given condition or pathology. In one aspect, a therapeutically effective amount of one or more GLP-2 analogues or pharmaceutical composition comprising the one or more GLP-2 analogues is an amount which restores a measurable physiological parameter to substantially the same value (preferably to within + 30%, more preferably to within + 20%, and still more preferably, to within 10% of the value) of the parameter in an individual without the condition or pathology.

In one embodiment of the invention administration of the compounds or pharmaceutical composition of the present invention is commenced at lower dosage levels, with dosage levels being increased until the desired effect of preventing/treating the relevant medical indication, such as intestine and stomach related diseases or increased longitudinal growth of the intestines, is achieved. This would define a therapeutically effective amount. Whether alone or as part of a pharmaceutical composition, the doses of the GLP-2

analogues used in accordance with the present invention are typically in the range between 0.5 mg and 25 mg inclusive per patient once or twice weekly, optionally between 1 mg and 20 mg inclusive per patient once or twice weekly, optionally between 1 mg and 10 mg inclusive per patient once or twice weekly, optionally between 2 mg and 7 mg inclusive per patient once or twice weekly, optionally between 5 mg and 7 mg inclusive per patient once or twice weekly, or optionallly between 2 mg and 5 mg inclusive per patient once or twice weekly. In one embodiment, a therapeutically effective amount of the GLP-2 analogues used in accordance with the present invention is about 10 mg per patient once or twice weekly. However, the skilled person will be able to adjust these doses in the event that an alternative dosing regime in accordance with the disclosure herein is selected.

In aspects of the invention relating to achieving increased longitudinal growth of the intestines, preferably treatment with the GLP-2 analogues of the present invention increases intestinal growth by at least 30% compared to the control, more preferably by at least 40% compared to the control, and most preferably by at least 50% compared to the control. It is generally preferred that the increase in longitudonal growth is accompanied by increased of absorptive capacity during and to some extent even after treatment has stopped. In the treatment of human patient at need to gaining increased longitudinal growth of the intestines, such as patients with short bowl syndrome (SBS), generally the treatment will last for at least 1 to 3 years and may optionally entail treatment once or twice weekly or in accordance with another of the treatment regimes of the present invention described herein.

The class of human patients with SBS includes patients having SBS-intestinal failure (SBS-IF) and patients who are on the border between having SBS-intestinal insufficiency (SBS-II) and SBS-intestinal failure (SBS-IF). In some cases, patients having SBS-intestinal failure (SBS-IF) are also called SBS-PS when they are dependent on parenteral support, and the patients having SBS-intestinal insufficiency (SBS-II) are also called SBS non-PS if they are not depending on parenteral support.

The spectrum of patient types with SBS is reviewed in Jeppensen, Journal of Parenteral and Enteral Nutrition, 38(1), 8S-13S, May 2014, doi: 10.1177/0148607114520994. A further division of SBS patient types can be made along the lines described in Schwartz et al., Clinical and Translational Gastroenterology (2016) 7, e142; doi:10.1038/ctg.2015.69. This separates SBS patients into early responders and late/slow responders. Without being bound by theory, the present inventors believe that the early responders are the ones who exhibit an early effect on treatment with a GLP-2 analogue caused by, among

other effects, an increase in the width/diameter of the small intestine, while the late or slow responders are the patients which mostly or first benefit to the treatment with a GLP-2 analogue caused by an increase in the length of the small intestine. The determination of whether a subject is an early or a late responder may be used to determine the duration of the treatment regime with the GLP-2 analogue, the timing of any clinical decision to reduce parenteral support and the interval between testing to determine whether a reduction in parenteral support is possible. Accordingly, in one embodiment, the patient is a late or slow responder. The length of the small intestines may for example be measured by CT scan (computed tomography scan), MRI (magnetic resonance imaging), histology, laparoscopic or other measurements or techniques known in the art.

In a further aspect, the present invention provides a glucagon-like peptide 2 (GLP-2) analogue for use in a method for the treatment and/or prevention of a patient having SBS-intestinal insufficiency (SBS-II), wherein the treatment prevents or delays the development of SBS-intestinal failure (SBS-IF), wherein the GLP-2 analogue is represented by the formula:

R^1-Z^1-His-Gly-Glu-Gly-X5-Phe-Ser-Ser-Glu-Leu-X11-Thr-Ile-Leu-Asp-Ala-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Ala-Trp-Leu-Ile-Ala-Thr-Lys-Ile-Thr-Asp-Z^2-R^2

wherein:
R^1 is hydrogen, C_{1-4} alkyl (e.g. methyl), acetyl, formyl, benzoyl or trifluoroacetyl; and
X5 is Ser or Thr;
X11 is Ala or Ser;
R^2 is NH_2 or OH; and
Z^1 and Z^2 are independently absent or a peptide sequence of 1-6 amino acid units of Lys;
or a pharmaceutically acceptable salt or derivative thereof.

In some embodiments, the method may comprise administering the GLP-2 analogue to the patient once or twice weekly and/or according to any of the other treatment regimes described herein.

In other related aspects, the present invention provides the use of a GLP-2 analogue is represented by the formula:

R^1-Z^1-His-Gly-Glu-Gly-X5-Phe-Ser-Ser-Glu-Leu-X11-Thr-Ile-Leu-Asp-Ala-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Ala-Trp-Leu-Ile-Ala-Thr-Lys-Ile-Thr-Asp-Z^2-R^2

wherein:
R^1 is hydrogen, C_{1-4} alkyl (e.g. methyl), acetyl, formyl, benzoyl or trifluoroacetyl;
X5 is Ser or Thr;
X11 is Ala or Ser;
R^2 is NH_2 or OH; and
Z^1 and Z^2 are independently absent or a peptide sequence of 1-6 amino acid units of Lys;
or a pharmaceutically acceptable salt or derivative thereof, for the manufacture of a medicament for the treatment and/or prevention of a patient having SBS-intestinal insufficiency (SBS-II), wherein the treatment prevents or delays the development of SBS-intestinal failure (SBS-IF).

In other related aspects, the present invention provides a method for treating and/or preventing of a patient having SBS-intestinal insufficiency, wherein the method prevents or delays the development of SBS-intestinal failure, wherein the GLP-2 analogue is represented by the formula:

R^1-Z^1-His-Gly-Glu-Gly-X5-Phe-Ser-Ser-Glu-Leu-X11-Thr-Ile-Leu-Asp-Ala-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Ala-Trp-Leu-Ile-Ala-Thr-Lys-Ile-Thr-Asp-Z^2-R^2

wherein:
R^1 is hydrogen, C_{1-4} alkyl (e.g. methyl), acetyl, formyl, benzoyl or trifluoroacetyl;
X5 is Ser or Thr;
X11 is Ala or Ser;
R^2 is NH_2 or OH; and
Z^1 and Z^2 are independently absent or a peptide sequence of 1-6 amino acid units of Lys;
or a pharmaceutically acceptable salt or derivative thereof.

For therapeutic use, the chosen GLP-2 analogue is formulated with a carrier that is pharmaceutically acceptable and is appropriate for delivering the peptide by the chosen route of administration. For the purpose of the present invention, peripheral parenteral routes include intravenous, intramuscular, subcutaneous, and intraperitoneal routes of administration. In one embodiment, the route of administration is the subcutaneous route or subcutaneous administration. The present pharmaceutical composition comprises a GLP-2 analogue of the invention, or a salt or derivative thereof and a pharmaceutically acceptable carrier. Suitable pharmaceutically acceptable carriers are those used conventionally with peptide-based drugs, such as diluents, excipients and the like.

Pharmaceutically acceptable carriers for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R. Gennaro edit. 1985). For example, sterile saline and phosphate-buffered saline at slightly acidic or physiological pH may be used. pH buffering agents may be phosphate, citrate, acetate, tris/hydroxymethyl)aminomethane (TRIS), N-Tris(hydroxymethyl)methyl –3- aminopropanesulphonic acid (TAPS), ammonium bicarbonate, diethanolamine, histidine, which is a preferred buffer, arginine, lysine, or acetate or mixtures thereof. Preferred buffer ranges are pH 4-8, pH 6.5-8, more preferably pH 7-7.5. Preservatives, such as para, meta, and ortho-cresol, methyl- and propylparaben, phenol, benzyl alcohol, sodium benzoate, benzoic acid, benzyl-benzoate, sorbic acid, propanoic acid, esters of p-hydroxybenzoic acid may be provided in the pharmaceutical composition. Stabilizers, preventing oxidation, deamidation, isomerisation, racemisation, cyclisation, peptide hydrolysis, such as e.g. ascorbic acid, methionine, tryptophane, EDTA, asparagine, lysine, arginine, glutamine and glycine may be provided in the pharmaceutical composition. Stabilizers, preventing aggregation, fibrillation and precipitation, such as Sodium dodecyl sulphate, polyethylene glycol, carboxymethyl cellulose, cyclodextrine may be provided in the pharmaceutical composition. Organic modifiers for solubilization or preventing aggregation, such as ethanol, acetic acid or acetate and salts thereof may be provided in the pharmaceutical composition. Isotonicity makers such as salts e.g. sodium chloride or most preferred carbohydrates e.g. dextrose, mannitol, lactose, trehalose, sucrose or mixtures thereof may be provided in the pharmaceutical composition.

Detergents, such as Tween 20, Tween 80, SDS, Poloxamers e.g. Pluronic F-68, Pluronic F-127, may be provided in the pharmaceutical composition. Dyes and even flavoring agents may be provided in the pharmaceutical composition. In another embodiment, a pharmaceutically acceptable acid addition salt of the GLP-2 peptide analogue is provided for. Suspending agents may be used.

Organic modifiers, such as ethanol, tertiary-buthanol, 2-propanol, ethanol, glycerol, Polyethylene glycol may be provided in the pharmaceutical formulation for lyophilization of a lyophilized product. Bulking agents and isotonicity makers such as salt e.g. sodium chloride, carbohydrates e.g. dextrose, mannitol, lactose, trehalose, sucrose or mixtures thereof, aminoacids e.g. glycine, glutamate, or excipients such as cystein, lecithin or human serum albumin, or mixtures thereof may be provided in the pharmaceutical composition for lyophilization.

The pharmaceutical compositions of the present invention may be formulated and used as tablets, capsules or elixirs for oral administration; suppositories for rectal administration; preferably sterile solutions or sterile powder or suspensions for injectable administration; and the like. The dose and method of administration can be tailored to achieve optimal efficacy but will depend on such factors as weight, diet, concurrent medication and other factors, which those skilled in the medical arts will recognize.

When administration is to be parenteral, such as intravenous, subcutaneous or intramuscular injectable pharmaceutical compositions can be prepared in conventional forms, either as aqueous solutions or suspensions; lyophilized, solid forms suitable for reconstitution immediately before use or suspension in liquid prior to injection, or as emulsions.

Diluents for reconstitution of the lyophilized product may be a suitable buffer from the list above, water, saline, dextrose, mannitol, lactose, trehalose, sucrose, lecithin, albumin, sodium glutamate, cysteine hydrochloride; or water for injection with addition of detergents, such as Tween 20, Tween 80, poloxamers e.g. pluronic F-68 or pluronic F-127, polyethylene glycol, and or with addition of preservatives such as para-, meta-, and ortho-cresol, methyl- and propylparaben, phenol, benzyl alcohol, sodium benzoate, benzoic acid, benzyl-benzoate, sorbic acid, propanoic acid, esters of p-hydroxybenzoic acid, and or with addition of an organic modifier such as ethanol, acetic acid, citric acid, lactic acid or salts thereof.

In addition, if desired, the injectable pharmaceutical compositions may contain minor amounts of non-toxic auxiliary substances, such as wetting agents, or pH buffering agents. Absorption enhancing preparations (e.g., liposomes, detergents and organic acids) may be utilized.

In one embodiment of the invention, the compounds are formulated for administration by infusion, e.g., when used as liquid nutritional supplements for patients on total parenteral nutrition therapy (for example neonatals, or patients suffering from cachexia or anorexia), or by injection, for example subcutaneously, intraperitoneal or intravenously, and are accordingly utilized as aqueous solutions in sterile and pyrogen-free form and optionally buffered to physiologically tolerable pH, e.g., a slightly acidic or physiological pH. Formulation for intramuscular administration may be based on solutions or suspensions in plant oil, e.g. canola oil, corn oil or soy bean oil. These oil based formulations may be

stabilized by antioxidants e.g. BHA (butylated hydroxianisole) and BHT (butylated hydroxytoluene).

Thus, the present peptide compounds may be administered in a vehicle, such as distilled water or in saline, phosphate buffered saline, 5% dextrose solutions or oils. The solubility of the GLP-2 analogue may be enhanced, if desired, by incorporating a solubility enhancer, such as detergents and emulsifiers.

The aqueous carrier or vehicle can be supplemented for use as injectables with an amount of gelatin that serves to depot the GLP-2 analogue at or near the site of injection, for its slow release to the desired site of action. Alternative gelling agents, such as hyaluronic acid, may also be useful as depot agents.

In one embodiment of the present invention the formulation comprises
a. L-histidine dissolved in water to obtain final concentrations of from 0.5 mM to 300 mM, preferably from 3 to 200mM, most preferably from 20 to100 mM;
b. mannitol to obtain up to 350 mM, preferably from 30 to 300 mM, most preferably from 100 mM to 230 mM; and
c. acetic acid to obtain up to 200 mM, preferably from 0.05 to 100 mM, most preferably from 0.5 to 50 mM into solution.
Appropriate amount of therapeutic compound is added to obtain concentrations of from 1 to 100 mg/mL, preferably from 5 to 50 mg/mL, most preferably from 10 to 30 mg/mL.
pH are adjusted to final pH at from 4 to 8, preferably from 6.5 to 7.5, most preferably from 6.7 to 7.3. The resulting solution is adjusted to target weight, sterile filtered and dispensed into appropriate aliquots in vials for pharmaceutical use. The formulation is further processed according to a liquid product or to a lyophilised product.

In another embodiment of the present invention the formulation comprises
a. L-histidine dissolved in water to obtain final concentrations of from 0.5 mM to 300 mM, preferably from 3 to 200mM, most preferably from 20 to100 mM L-histidine;
b. L-Arginine to obtain up to 200 mM, preferably from 0.5 to 100 mM, most preferably from 5 to 50 mM ;
c. mannitol to obtain up to 350 mM, preferably from 30 to 300 mM, most preferably from 100 mM to 230 mM; and
d. acetic acid to obtain up to 200 mM, preferably from 0.05 to 100 mM, most preferably from 0.5 to 50 mM into solution.

Appropriate amount of therapeutic compound is added to obtain concentrations of from 1 to 100 mg/mL, preferably from 5 to 50 mg/mL, most preferably from 10 to 30 mg/mL.
pH are adjusted to final pH at from 4 to 8, preferably from 6.5 to 7.5, most preferably from 6.7 to 7.3. The resulting solution is adjusted to target weight, sterile filtered and dispensed into appropriate aliquots in vials for pharmaceutical use. The formulation is further processed according to a liquid product or to a lyophilised product.

In still another embodiment of the present invention the formulation comprises
a. L-histidine dissolved in water to obtain final concentrations of up to 200 mM, preferably from 3 to 100 mM, most preferably from 5 to 50 mM L-histidine;
b. L-Arginine to obtain up to 200 mM, preferably from 0.5 to 100 mM, most preferably from 5 to 50 mM ;
c. mannitol to obtain up to 350 mM, preferably from 30 to 300 mM, most preferably from 100 mM to 230 mM; and
d. acetic acid to obtain up to 200 mM, preferably from 0.05 to 100 mM, most preferably from 0.5 to 50 mM into solution.
Appropriate amount of therapeutic compound is added to obtain concentrations of from 1 to 100 mg/mL, preferably from 5 to 50 mg/mL, most preferably from 10 to 30 mg/mL.
pH are adjusted to final pH at from 4 to 8, preferably from 6.5 to 7.5, most preferably from 6.7 to 7.3. The resulting solution is adjusted to target weight, sterile filtered and dispensed into appropriate aliquots in vials for pharmaceutical use. The formulation is further processed according to a liquid product or to a lyophilised product.

In yet another embodiment of the present invention the formulation comprises
a. N-acetate dissolved in water to obtain final concentrations of from up to 200 mM, preferably from 0.5 to 100 mM, most preferably from 5 to 50 mM L-histidine;
b. mannitol to obtain up to 350 mM, preferably from 30 to 300 mM, most preferably from 100 mM to 230 mM.
Appropriate amount of therapeutic compound is added to obtain concentrations of from 1 to 100mg/mL, preferably from 5 to 50 mg/mL, most preferably from 10 to 30 mg/mL.
pH are adjusted to final pH at from 4 to 8, preferably from 6.5 to 7.5, most preferably from 6.7 to 7.3. The resulting solution is adjusted to target weight, sterile filtered and dispensed into appropriate aliquots in vials for pharmaceutical use. The formulation is further processed according to a liquid product or to a lyophilised product

The GLP-2 analogues of the invention may also be formulated as a slow release implantation device for extended and sustained administration of the GLP-2 peptide

analogue. Such sustained release formulations may be in the form of a patch positioned externally on the body. Examples of sustained release formulations include composites of biocompatible polymers, such as poly(lactic acid), poly(lactic-co-glycolic acid), methylcellulose, hyaluronic acid, sialic acid, silicate, collagen, liposomes and the like. Sustained release formulations may be of particular interest when it is desirable to provide a high local concentration of a GLP-2 analogue of the invention.

The GLP-2 analogue may be utilized in the form of a sterile-filled vial or ampoule containing an intestinotrophic amount of the peptide, in either unit dose or multi-dose amounts. The vial or ampoule may contain the GLP-2 analogue and the desired carrier, as an administration ready formulation. Alternatively, the vial or ampoule may contain the GLP-2 peptide in a form, such as a lyophilized form, suitable for reconstitution in a suitable carrier, such as sterile water or phosphate-buffered saline.

The peptide compounds of the present invention may be used alone, or in combination with compounds having an anti-inflammatory effect. Without being bound by theory it is envisioned that such combination treatment may enforce the beneficial treatment effects of the present peptide analogues.

The therapeutic dosing and regimen most appropriate for patient treatment will of course vary with the disease or condition to be treated, and according to the patient parameters. Without wishing to be bound by any particular theory, it is expected that doses, between 0.1 and 25 mg per patient, and shorter or longer duration or frequency of treatment may produce therapeutically useful results, such as a statistically significant increase particularly in small bowel mass. In some instances, the therapeutic regimen may include the administration of maintenance doses appropriate for preventing tissue regression that occurs following cessation of initial treatment. The dosage sizes and dosing regimen most appropriate for human use may be guided by the results obtained by the present invention, and may be confirmed in further clinical trials.

An effective dosage and treatment protocol may be determined by conventional means, starting with a low dose in laboratory animals and then increasing the dosage while monitoring the effects, and systematically varying the dosage regimen as well. Numerous factors may be taken into consideration by a clinician when determining an optimal dosage for a given subject. Such considerations are known to the skilled person.

A human dose of a GLP-2 peptide according to the invention may be used in a dose of

between about 0.01 mg/kg and 100 mg/kg body weight, such as between about 0.01 mg/kg and 10 mg/kg body weight, for example between 10-100 μg/kg body weight. In further embodiments, a human dose (total dose) of a GLP-2 peptide according to the invention may be from about such as between and including 0.1 mg and 25 mg per patient between and including 0.5 mg and 20 mg per patient, such as between and including 1 mg and 15 mg per patient, such as between and including 1 mg and 10 mg per patient once or twice weekly or as a plurality of doses as defined herein separated in time by 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 days. In some instances, a fixed dose of the GLP-2 peptide according to the invention may be used in accordance with a dosing pattern disclosed herein, i.e. a dose which is the same regardless of the body weight of the patient, given once or twice weekly. By way of example, the fixed dose may be a dose of 5 mg, 6 mg, 7 mg, 8 mg, 9, mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg or 15 mg. Conveniently a fixed dose of 10 mg may be used. The use of fixed dosing has the advantage of increasing compliance and reducing the risk of patient dosing errors, including risks of miscalculating a weight based dose to be administered.

Medical Conditions

The peptides of the present invention are useful as a pharmaceutical agent for preventing or treating an individual suffering from gastro-intestinal disorders, including the upper gastrointestinal tract of the oesophagus by administering an effective amount of a GLP-2 analogue, or a salt thereof as described herein. The stomach and intestinal-related disorders include ulcers of any aetiology (e.g., peptid ulcers, drug-induced ulcers, ulcers related to infections or other pathogens), digestion disorders, malabsorption syndromes, short-bowel syndrome, cul-de-sac syndrome, inflammatory bowel disease, celiac sprue (for example arising from gluten induced enteropathy or celiac disease), tropical sprue, hypogammaglobulinemic sprue, enteritis, ulcerative colitis, small intestine damage, and chemotherapy induced diarrhea/mucositis (CID).

As mentioned above in general, individuals who would benefit from increased small intestinal mass and consequent and/or maintainance of normal small intestine mucosal structure and function are candidates for treatment with the present GLP-2 analogues. Particular conditions that may be treated with GLP-2 analogue include the various forms of sprue including celiac sprue which results from a toxic reaction to alpha-gliadin from heat and may be a result of gluten-induced enteropathy or celiac disease, and is marked by a significant loss of villae of the small bowel; tropical sprue which results from infection and is marked by partial flattening of the villae; hypogammaglobulinemic sprue which is observed commonly in patients with common variable immunodeficiency or hypogammaglobulinemia

and is marked by significant decrease in villus height. The therapeutic efficacy of the GLP-2 analogue treatment may be monitored by enteric biopsy to examine the villus morphology, by biochemical assessment of nutrient absorption, by patient weight gain, or by amelioration of the symptoms associated with these conditions.

Another particular condition which may be treated with the GLP-2 analogues of the invention, or for which the GLP-2 analogues may be useful therapeutically and/or prophylactically is short bowl syndrome (SBS), also known as short gut syndrome or simply short gut) which results from surgical resection, congenital defect or disease-associated loss of absorption in the bowel in which patients are subsequently unable to maintain fluid, electrolyte, and nutrient balances on a conventional diet. Despite an adaptation that occurs generally in the two years after resection, SBS patients have reduced dietary uptake and fluid loss.

Other conditions that may be treated with the GLP-2 analogues of the invention, or for which the GLP-2 analogues may be useful prophylactically, include in addition to the above mentioned radiation enteritis, infectious or post-infectious enteritis, and small intestinal damage due to cancer-chemotherapeutic or toxic agents.

The GLP-2 analogues may also be used for the treatment of malnutrition, for example cachexia and anorexia.

A particular embodiment of the invention is concerned with using the present peptides for the prevention and/or treatment of intestinal damage and dysfunction. Such damage and dysfunction is a well-known side effect of cancer-chemotherapy treatment. Chemotherapy administration is frequently associated with unwanted side effects related to the gastronintestinal system such as mucositis, diarrhoea, bacterial translocation, malabsorption, abdominal cramping, gastrointestinal bleeding and vomiting. These side effects are clinical consequences of the structural and functional damage of the intestinal epithelium and frequently make it necessary to decrease the dose and frequency of chemotherapy. Administration of the present GLP-2 peptide analogues may enhance trophic effect in the intestinal crypts and rapidly provide new cells to replace the damaged intestinal epithelium following chemotherapy. The ultimate goal achieved by administering the present peptides is to reduce the morbidity related to gastrointestinal damage of patients undergoing chemotherapy treatment while creating the most optimal chemotherapy regime for the treatment of cancer. Concomitant prophylactic or therapeutic

treatment may be provided in accordance with the present invention to patients undergoing or about to undergo radiation therapy.

The stem cells of the small intestinal mucosa are particularly susceptible to the cytotoxic effects of chemotherapy due to their rapid rate of proliferation (Keefe et al., Gut 2000; 47: 632–7). Chemotherapy-induced damage to the small intestinal mucosa is clinically often referred to as gastrointestinal mucositis and is characterized by absorptive and barrier impairments of the small intestine. For example, it has been shown that, the broadly used chemotherapeutic agents, 5-FU, irinotecan and methothrexate increase apoptosis leading to villus atrophy and crypt hypoplasia in the small intestine of rodents (Keefe et al., Gut 47: 632–7, 2000; Gibson et al., J Gastroenterol Hepatol. Sep;18(9):1095-1100, 2003; Tamaki et al., J Int Med Res. 31(1):6-16, 2003). Chemotherapeutic agents have been shown to increase apoptosis in intestinal crypts at 24 hours after administration and subsequently to decrease villus area, crypt length, mitotic count per crypt, and enterocyte height three days after chemotherapy in humans (Keefe et al., Gut 2000; 47: 632–7). Thus, structural changes within the small intestine directly lead to intestinal dysfunction and in some cases diarrhea.

Gastrointestinal mucositis after cancer chemotherapy is an increasing problem that is essentially untreatable once established, although it gradually remits. Studies conducted with the commonly used cytostatic cancer drugs 5-FU and irinotecan have demonstrated that effective chemotherapy with these drugs predominantly affects structural integrity and function of the small intestine while the colon is less sensitive and mainly responds with increased mucus formation (Gibson et al., J Gastroenterol Hepatol. Sep;18(9):1095-1100, 2003; Tamaki et al., J Int Med Res. 31(1):6-16, 2003).

The novel GLP-2 analogues of the present invention may be useful in the prevention and/or treatment of gastrointestinal injury and side effects of chemotherapeutic agents. This potentially important therapeutic application may apply to currently used chemotherapeutic agents such as but not limited to: 5-FU, Altretamine, Bleomycin, Busulfan, Capecitabine, Carboplatin, Carmustine, Chlorambucil, Cisplatin, Cladribine, Crisantaspase, Cyclophosphamide, Cytarabine, Dacarbazine, Dactinomycin, Daunorubicin, Docetaxel, Doxorubicin, Epirubicin, Etoposide, Fludarabine, Fluorouracil, Gemcitabine, Hydroxycarbamide, Idarubicin, Ifosfamide, Irinotecan, Liposomal doxorubicin, Leucovorin, Lomustine, Melphalan, Mercaptopurine, Mesna, Methotrexate, Mitomycin, Mitoxantrone, Oxaliplatin, Paclitaxel, Pemetrexed, Pentostatin, Procarbazine, Raltitrexed, Streptozocin, Tegafur-uracil, Temozolomide, Thiotepa, Tioguanine/Thioguanine, Topotecan, Treosulfan,

Vinblastine, Vincristine, Vindesine, Vinorelbine, Bleomycin, Busulfan, Capecitabine, Carboplatin, Carmustine, Chlorambucil, Cisplatin, Cladribine, Crisantaspase, Cyclophosphamide, Cytarabine, Dacarbazine, Dactinomycin, Daunorubicin, Docetaxel, Doxorubicin, Epirubicin, Etoposide, Fludarabine, Fluorouracil, Gemcitabine, Hydroxycarbamide, Idarubicin, Ifosfamide, Irinotecan, Liposomal doxorubicin, Leucovorin, Lomustine, Melphalan, Mercaptopurine, Methotrexate, Mitomycin, Mitoxantrone, Oxaliplatin, Paclitaxel, Pemetrexed, Pentostatin, Procarbazine, Raltitrexed, Streptozocin, Tegafur-uracil, Temozolomide, Thiotepa, Tioguanine/Thioguanine, Topotecan, Treosulfan, Vinblastine, Vincristine, Vindesine, and Vinorelbine.

A further embodiment of the invention is concerned with using the present peptides for increasing the longitudinal growth of the intestines in a patient, e.g. in a human patient. As shown in the Examples below, the GLP-2 analogues of the invention are capable of increasing the longitudinal growth of the intestines relative to the control. Without being bound by theory, it is presently believed that the longer half-life leads to a more potent and prolonged stimulation of the GLP-2 receptors which results in an increased longitudinal growth relative to that of the control. It is also possible that the longer terminal plasma half-life of the GLP-2 analogues used in accordance with the present invention leads to more potent stimulation of longitudinal growth of the intestines as compared to other GLP-2 analogues of the prior art known to have relatively shorter half-lives.

This capability is of particular value in patients with SBS as this will lead to increased absorptive capacity also after treatment is stopped. Such patient would be treated for at least 1 to 3 years, such as at least 1 to 4 years, such as 1 to 10 years, such as 1 to 20 years, such as 1 to 35 years with the objective of inducing longitudinal growth of the intestines.

As already described herein, SBS patients who are on the border between intestinal insufficiency (SBS-II) or SBS-PS patients and intestinal failure (SBS-IF) or SBS non-PS may therefore have particular value from having their intestines lengthened over a 1 to 3 year treatment course, whereafter their risk if intestinal failure is decreased, for example involving weekly or twice weekly dosing over the period of treatment. This involves less risk for central catheter needs and the risk of sepsis associated with its use.

The dosages regimes disclosed herein may be applied to the treatment of stomach and bowel-related disorders, such as SBS, in which the subject of the treatment is provided with parenteral support (PS) while receiving treatment with the GLP-2 analogues according

to the present invention. Parenteral support involves the administration of nutrition and liquid to patients whose gastrintestinal tract is impaired or compromised, while they are being treated with GLP-2 analogues. However, one of the challenges facing patients and physicians in the course of GLP-2 therapy concerns the determination of the amount or the volume of PS provided to the treated subject and the appropriate and individualized adjustment of the amount or the volume of PS provided to the patient. In particular, this is important because if the amount or the volume PS is not adjusted in an appropriate way, the treated subjects may experience fluid overload, risks of dehydration and may not achieve optimal clinical responses with the therapy. Consequently, the present invention provides algorithms for adjusting of the amount or the volume PS during a course of treatment using a GLP-2 analogue as disclosed herein.

Accordingly, in a further treatment related aspect, the present invention provides a glucagon-like peptide 2 (GLP-2) analogue for use in a method for the treatment of short bowel syndrome (SBS) in a human subject receiving a parenteral support (PS) regimen, the method comprising:

(i) administering to the subject a GLP-2 analogue as defined herein, wherein the GLP-2 analogue is administered once or twice weekly for a period of time sufficient to lengthen and improve the function of the small intestine of the subject; and

(ii) following step (i), reducing a frequency or amount of the parenteral support (PS) regimen in response to improved function of the small intestine.

(iii) optionally repeating steps (i) and (ii) in response to the subject demonstrating continued improved function of the small intestine.

By way of example, the time interval over which the GLP-2 analogue is administered in step (i) of the method is from 18 to 150 weeks, for 18 to 100 weeks, or for 18 to 52 weeks. Generally, the amount of improved function or absorptive capacity of the small intestine is at least 10%, such as at least 20%, such as at least 30% such as at least 40%, such as at least 50%. Additionally or alternatively, the amount of the reduction of parenteral support is at least 10%, at least 20%, at least 30%, at least 40% or at least 50%. In a preferred embodiment, the reduction of parenteral support is at least 20%.

In order to assist with the determination of the amount or the volume of PS provided to subjects receiving GLP-2 therapy, in a further aspect, the present invention provides a

method for adjusting a parenteral support (PS) in a human subject receiving an amount or volume of parenteral support each week, the method comprising calculating a new amount or volume of PS using an algorithm in which an amount or volume of PS for a week is calculated by subtracting seven times the absolute increase in daily urine volume from a baseline from the current weekly volume of parenteral support to provide the new amoutn or volume of PS for the subject. Preferably, the method includes the initial steps of determining the baseline volume and/or determining the daily urine volume. Some or all of the steps of the method may be repeated during the course of the GLP-2 therapy received by the subject. Preferably, the time between repeat testing to determine changes in the PS volume adjustments is about every 1 to 3 months. For example, in one embodiment, the method may be used at weeks 1, 2, 4, 8 and 12 after commencement of treatment with the GLP-2 analogue, adjusting the amount or volume of PS in response to the effects of treament. The weekly amount or volume of PS administered should be monitored and adjusted throughout treatment with the GLP-2 analogue (e.g. glepaglutide) in order to avoid fluid overload. Generally, as the absorptive capacity of the intestines increases after treatment with the GLP-2 analogue, typically by elongation or thickening of the small intestine, the amount or volume of PS can be decreased to avoid fluid overload and to improve the treatment needs of the patient.

Examples

The following examples are provided to illustrate preferred aspects of the invention and are not intended to limit the scope of the invention. The GLP-2 analogues administered according to the dosage regimes described herein can be made according to the methods such as solid phase peptide synthesis described in WO 2006/117565, the content of which is expressly incorporated by reference in its entirety.

Example 1. Synthesis of ZP1848 metabolites

Solid phase peptide synthesis was performed on a CEM Liberty Peptide Synthesizer using standard Fmoc chemistry. TentaGel S Ram S resin (1,33 g; 0.25 mmol/g) was swelled in DMF (10 ml) prior to use and transferred between tube and reaction vessel using DCM and DMF.

Coupling

An Fmoc-amino acid in DMF/DCM (2:1; 0.2 M; 5 ml) was added to the resin in a CEM Discover microwave unit together with COMU/DMF (0.5 M; 2 ml) and DIPEA&DMF (2.0 M; 1 ml). The coupling mixture was heated to 75°C for 5 min while nitrogen was bubbled through the mixture. The resin was then washed with DMF (4 x 10 ml). Fmoc-Phe-Ser(Psi

Me,Me,Pro)-OH pseudoproline was used for amino acid number six and seven.

Deprotection

Piperidine/DMF (20%; 10 ml) was added to the resin for initial deprotection and the mixture was heated by microwaves (30 sec; 40°C). The reaction vessel was drained and a second portion of piperidine/DMF (20%; 10 ml) was added and heated (75°C; 3 min.) again. The resin was then washed with DMF (6 x 10 ml).

The resin was washed with EtOH (3 x 10 ml) and Et2O (3 x 10 ml) and dried to constant weight at room temperature (r.t.). The crude peptide was cleaved from the resin by treatment with TFA/DODT (95/5; 60 ml, 2 h; r.t.). Most of the TFA was removed at reduced pressure and the crude peptide was precipitated and washed three times with diethylether and dried to constant weight at room temperature.

HPLC purification of the crude peptide

The crude peptide was first purified to 45% by preparative reverse phase HPLC using a PerSeptive Biosystems VISION Workstation equipped with a Gemini NX 5µ C-18 110A, 10x250 mm column and a fraction collector and run at 35 ml/min with a gradient of buffer A (0.1% TFA, aq.) and buffer B (0.1% TFA, 90% MeCN, aq.). Fractions were analyzed by analytical HPLC and MS and relevant fractions were pooled and lyophilized. The product (143mg) was purified a second time with a C4 Jupiter 2, 12x25cm column, to yield 27mg, with a purity of 89% as characterized by HPLC and MS. Calculated monoisotopic MW = 3377.61, found 3377,57.

Table 1: Synthesized compounds

Compound	Sequence
Teduglutide	H-HGDGSFSDEMNTILDNLAARDFINWLIQTKITD-OH
ZP1848	H-HGEGTFSSELATILDALAARDFIAWLIATKITDKKKKKK-NH_2
ZP2949	H-HGEGTFSSELATILDALAARDFIAWLIATKITDKKK-OH
ZP2711	H-HGEGTFSSELATILDALAARDFIAWLIATKITDKK-OH
ZP2469	H-HGEGTFSSELATILDALAARDFIAWLIATKITDK-OH

Example 2. GLP-2R EC50 measurements

Generation of cell line expressing human GLP-2 receptors

The hGLP2-R was purchased from MRC-geneservice, Babraham, Cambridge as an Image clone: 5363415 (11924-I17). For subcloning into a mammalian expression vector, primers

for subcloning were obtained from DNA-Technology, Risskov, Denmark. The 5' and 3' primers used for the PCR reaction include terminal restriction sites for cloning and the context of the 5' primer is modified to a Kozak consensus without changing the sequence of the product encoded by the ORF. A standard PCR reaction was run using Image clone 5363415 (11924-I17) as a template with the above mentioned primers and Polymerase Herculase II Fusion in a total vol. of 50µl. The generated PCR product was purified using GFX PCR and Gel band purification kit, digested with restriction enzymes and cloned into the mammalian expression vector using Rapid DNA Ligation Kit. Ligation reaction was transformed to XL10 Gold Ultracompetent cells and colonies were picked for DNA production using Endofree Plasmid maxi kit. Subsequent sequence analysis was conducted by MWG Eurofins, Germany. The clone was confirmed to be the hGLP-2 receptor, splice variant rs17681684.

HEK293 cells were transfected using the Lipofectamine PLUS transfection method. The day before transfection, HEK293 cells were seeded in two T75 flasks at a density of 2 x 10^6 cells / T75 flask in cell culturing medium without antibiotics. On the day of transfection, cells were washed with 1x DPBS and medium was replaced with Optimem to a volume of 5 mL / T75 flask before addition of Lipofectamine-plasmid complexes were added gently and drop wise to the cells in T75 flasks and replaced with growth medium after 3 hours and again to growth medium supplemented with 500µg/mL G418 after 24 hours. After 4 weeks in G418 selection, clones were picked and tested in a functional assay. One clone was selected for use in compound profiling.

GLP-2 receptor efficacy assay

The cAMP AlphaScreen® assay from Perkin Elmer was used to quantitate the cAMP response to activation of the GLP2 receptor. Teduglutide was used as a reference compound for GLP2 receptor activation. Data from test compounds eliciting an increase in the intracellular level of cAMP were normalized relative to the positive (Teduglutide) and negative control (vehicle) to calculate the EC50 and maximal response from the concentration response curve. The results are listed in Table 2.

Table 2: GLP-2R EC50 measurements

ZP No.	EC50_Avg (nM)	EC50_SD (nM)
Teduglutide	0.03	0.019
ZP1848	0.3	0.14
ZP2949	0.11	0.013
ZP2711	0.072	0.0076
ZP2469	0.052	0.0057
ZP1846	0.26	

Example 3: Phamacokinetic and Pharmacodynamic Profiling of GLP-2 analogues

A phase 2 clinical trial was conducted to i.a. determine the PK profile of ZP1848 and metabolites in human SBS patients.

Method

The study was designed as a randomized, cross-over and double-blinded with three different doses of ZP1848 (10 mg, 1 mg, 0.1 mg) in 16 SBS patients.

The study protocol was approved by the Danish Medical Agency and The Danish Committee on Health Research Ethics.

The 18 SBS patients (16 patients completed the study) were randomized evenly and in a double-blinded fashion to each of the six cross-over dose level combinations: 10 mg/1 mg, 10 mg/0.1 mg, 1 mg/10 mg, 1 mg/0.1 mg, 0.1 mg/10 mg, 0.1 mg/1 mg of ZP1848. The patients were injected once daily (QD) subcutaneously with the above doses of ZP1848.

Blood samples for PK analysis were collected on several study visits throughout the study period: the study comprised a 1st baseline balance study (4 days), a 1st treatment period (21 days) including a 1st treatment balance study (last 4 days of 1st treatment period), followed by a wash-out period (at least 4 weeks); and a 2nd baseline balance study (4 days), a 2nd treatment period (21 days) including a 2nd treatment balance study (last 4 days of 2nd treatment period) and a follow-up period (at least 4 weeks).

PK sampling was performed at visit 11 (1st treatment, balance study) and visit 17 (second day of 2nd baseline study - after a wash out period), and again at visit 25 (2nd treatment balance study) and at visit 29 (last day of follow-up period).

At day visit 11 and visit 25 a full PK profile in steady state was investigated. PK samples were taken prior to injection of ZP1848, and at the following time points (+/- 10 minutes) after trial product administration: 1h, 2h, 4h, 6h, 8h, 10h, 12h and 24h. Each PK sample contained 2 mL of blood, resulting in a total of 46 mL blood drawn for PK purposes. The plasma sample was immediately stored at -80°C after sampling.

Plasma samples were analyzed after immunoaffinity extraction followed by liquid chromatography mass spectrometry (LC-MS/MS). For each patient plasma concentrations were used for calculation of the pharmacokinetic parameters using the non-compartmental approach in Excel. Plasma terminal elimination half-life (T½) was determined as $\ln(2)/k_e$ where k_e is the magnitude of the slope of the log linear regression of the log concentration versus time profile during the terminal phase.

Results

When ZP1848 is injected into the SC (subcutaneous) compartment, two functionally active metabolites are formed (ZP2469 and ZP2711, both C-terminal truncated analogs of glepaglutide, ZP1848). The overall PK profile of ZP1848 therefore comprises the effect of ZP1848 and its two main metabolites.

Both metabolites are pharmacologically active, as shown above, with *in vitro* potency comparable to ZP1848. The plasma level of ZP2711 is in the same range as that of ZP1848, whereas the plasma level of ZP2469 is multiple times higher than that of ZP1848. Consequently, it is necessary to include both metabolites when evaluating systemic exposure as they boths contribute to *in vivo* efficacy.

Due to the high plasma level of ZP2469 this compound has plasma level above the bioanalytical detection limit (50 pM for ZP2469, 25 pM for ZP1848 and ZP2711), hence it is possible to determined the half-life of compound ZP2469. A terminal plasma half-life of between 5 and 17 days, as shown in the table, suggests once weekly dosing of ZP1848 would be efficacious.

Table 3: PK results from Phase 2 clinical trial

Subject	Compound	Day	Visit no	Conc. pM	Half-life (days)
1003	ZP2469	0	25	4490	7.2
1003	ZP2469	32	29	209	
1007	ZP2469	0	25	14700	7.2
1007	ZP2469	36	29	460	
1008	ZP2469	0	11	6770	9.5
1008	ZP2469	56	17	116	
1010	ZP2469	0	11	4340	11
1010	ZP2469	56	17	112	
1013	ZP2469	0	25	4940	9.6
1013	ZP2469	35	29	394	
1014	ZP2469	0	11	2220	5.2
1014	ZP2469	28	17	52.8	
1015	ZP2469	0	11	556	7.9
1015	ZP2469	28	17	56.4	
1015	ZP2469	0	25	3640	8.7
1015	ZP2469	32	29	288	
1016	ZP2469	0	11	4750	11
1016	ZP2469	49	17	236	
1018	ZP2469	0	25	4660	8.7
1018	ZP2469	40	29	195	
1020	ZP2469	0	11	1040	12
1020	ZP2469	35	17	133	
1013	ZP2711	0	11	131	14
1013	ZP2711	28	17	32.6	
1015	ZP2711	0	25	543	10
1015	ZP2711	32	29	61.4	

Example 4: Longitudinal growth by Subcutaneous Administration of ZP1848 to Beagle Dogs for 39 Weeks Followed by a 6 Week Recovery Period

Method

ZP1848 (0.25, 1 or 5 mg/kg/day) was given for 39 weeks subcutaneously to beagle dogs (22 to 24 weeks). Vehicle was used as the control. Recovery from any effect was evaluated during a 6 week recovery period. The study was conducted in accordance with the applicable sections of the United Kingdom Animals (Scientific Procedures) Act 1986, Amendment Regulations 2012 (the Act).

After 39 weeks the dogs were sacrificed and all animals were subject to a detailed necropsy. The length of the small and large intestines were measured separately and recorded in centimeters. The entire length of the gastrointestinal tract (starting at the duodenum and ending at the rectum) was removed and trimmed of any excessive mesentery, connective or adipose tissue and the measurements for the small and large intestine recorded by laying the small intestine and large intestine on a clean surface and measured with a tape measure. The sections required for histopathology were left unopened and flushed through with water. The remainder were opened, cleaned with water, carefully blotted dry prior to weighing. On completion of length and weight measurements, the gastrointestinal tract was processed and fixed in 10% Neutral Buffered Formalin, as per standard necropsy practice for this species.

Results

There was an increase in small intestine length after 39 weeks of treatment at all doses and in both sexes, with the extent of the increase being greatest in males and females given 5 mg/kg/day where there was a 46% and 37% increase in males and females, respectively. This finding had partially recovered by the end of the six-week recovery period, with 16 and 19% increases from controls in males and females, respectively. In the same study, an increase in small intestine weight after 39 weeks of treatment at all doses and in both sexes, with the extent of the increase being greatest in males and females given 5 mg/kg/day where there was a 103% and 80% increase in males and females, respectively. This finding had partially recovered by the end of the six-week recovery period, with 78 and 34% increases from controls in males and females, respectively.

Example 5: A Phase 1, Open-Label, Partially Randomized, 3-Part, Parallel Group Trial to Evaluate the Pharmacokinetic Profile of glepaglutide (ZP1848) After a Single Intravenous Injection and After Multiple Subcutaneous Injections in Healthy Human Subjects

A Phase 1 clinical trial to characterize the pharmacokinetic (PK) profiles of glepaglutide (ZP1848) and its primary active metabolites following once-weekly and once daily

subcutaneous (SC) injections and after a single intravenous (IV) infusion in healthy subjects was conducted. This included evaluating the pharmacodynamic effects on plasma citrulline levels following SC dosing in healthy subjects. The trial confirmed the feasibility of dosage regimes in which GLP-2 analogues are administered to patients in weekly dosage regimes and compare weekly dosage regimes with daily dosing.

Trial design:

The trial design was a single-center, open-label, partially randomized, 3-part, parallel group trial to characterize the PK profile of glepaglutide administered by SC injection once daily for 7 days (Part 1), SC injection once weekly for 6 weeks (Part 2), and as a single IV infusion (Part 3) in healthy subjects.

In Part 1, eligible subjects were randomized 1:1 to Group A or B; in Part 2, eligible subjects were randomized 1:1 to Group C or D; all subjects in Part 3 (Group E) were assigned to the same dose level.

Number of subjects:

Approximately 75 male and female subjects were enrolled, with 15 subjects in each of 5 dose groups, to ensure that at least 12 subjects complete each group.

Diagnosis and main criteria for inclusion:

Healthy male and female subjects aged between 18 and 60 years, inclusive, with a body mass index (BMI) between 18.0 and 30.0 kg/m^2, inclusive, and preferably subjects have a BMI of ≤ 25.0 kg/m^2.

Test products, dose, and mode of administration:

Glepaglutide was supplied as an aqueous solution at concentrations of 2 and 10 mg/mL.

Group A: 1 mg glepaglutide once daily, given as single SC injections on Days 1 to 7.

Group B: 5 mg glepaglutide once daily, given as single SC injections on Days 1 to 7.

Group C: 5 mg glepaglutide once weekly, given as single SC injections on Days 1, 8, 15, 22, 29, and 36.

Group D: 10 mg glepaglutide once weekly, given as single SC injections on Days 1, 8, 15, 22, 29, and 36.

Group E: 1 mg glepaglutide, given as an IV infusion at a rate of 4 mg/h for 15 minutes on Day 1.

Duration of treatment

Planned Screening duration: approximately 28 days

Total Duration (Screening to End of Trial):

Groups A and B: 71 days

Groups C and D: 100 days

Group E: 51 days

Criteria for evaluation:

Pharmacokinetics:

Blood samples were collected for the analysis of plasma concentrations of glepaglutide and its primary active metabolites, ZP2469 ($ZP1848_{1-34}$) and ZP2711 ($ZP1848_{1-35}$); samples were assayed using validated analytical methods. The following PK parameter estimates were calculated using standard non-compartmental methods: terminal elimination half-life ($t_{1/2}$), total body clearance of drug after IV administration (CL; IV dose, parent drug only), apparent total clearance (CL/F; SC doses, parent drug only), volume of distribution at steady state (V_{ss}; IV dose, parent drug only), apparent volume of distribution at steady state (V_{ss}/F; SC doses, parent drug only), volume of distribution (V_z; IV dose, parent drug only), apparent volume of distribution (V_z/F; SC doses, parent drug only), maximum observed plasma concentration (C_{max}), area under the plasma concentration-time curve (AUC) over a dosing interval (AUC_τ), AUC from time zero to infinity (AUC_{inf}), AUC from time zero to the time of the last measurable concentration (AUC_{last}), and time of the maximum observed plasma concentration (t_{max}). Metabolite exposure (based on C_{max}, AUC_τ, and AUC_{inf}) were determined for each group as a ratio of the 2 primary active metabolites, ZP2469 ($ZP1848_{1-34}$) and ZP2711 ($ZP1848_{1-35}$), relative to the glepaglutide parent drug.

Pharmacodynamics:

Blood samples were collected for the measurement of plasma citrulline (for SC doses only).

Statistical methods:

Pharmacokinetics:

The PK parameters estimates for glepaglutide, ZP2469 ($ZP1848_{1-34}$), and ZP2711 ($ZP1848_{1-35}$) were listed and summarized using standard descriptive statistics. The primary PK parameters are $t_{1/2}$ for glepaglutide, ZP2469 ($ZP1848_{1-34}$), and ZP2711 ($ZP1848_{1-35}$); and CL (IV dose), CL/F (SC doses), V_{ss} (IV dose), V_{ss}/F (SC dose), V_z (IV dose), and V_z/F (SC doses) for glepaglutide. The half-life is calculated as LN(2)/k_e where ÷k_e is estimate from the slope of the terminal part of the LN(concentration) versus time curve, with repeats

regressions of LN(concentration) values using the last three points with non-zero concentrations, then the last four points, last five points, etc. Points with a concentration value of zero are not included. Points prior to C_{max} are not used. For each regression, an *Adjusted-R^2* is computed: *Adjusted-R^2=1- ((1-R^2)×(n-1))/(n-2)*; where n is the number of data points in the regression and R^2 is the square of the correlation coefficient. k_e using the regression with the largest adjusted R^2 and, 1) If the adjusted R^2 does not improve, but is within 0.0001 of the largest adjusted R^2 value, the regression with the larger number of points is used. 2) k_e must be calculated from at least three data points. 3) The estimated slope must be negative, so that its negative k_e is positive. The results of the calculations carried out with preliminary data are shown in Table 4, while the results of the calculations with the complete final data set are shonw in Table 5.

Pharmacodynamics:

Plasma citrulline concentrations and changes from baseline were listed and summarized using descriptive statistics.

Results:

Initial results and analysis for a selection of subjects enrolled in Groups A, B, C and D found that ZP2469 had a half-life in hours as shown in the Table 4 below. The half-life found using once weekly dosing supports the feasibility of using dosing of the GLP-2 peptides of the present invention once weekly or twice weekly.

Table 4: Half-life preliminary results

		No Doses		
		1	6	7
Group	Subject No.	Half-life ZP2469 (hr)		
A	101			20
Once Daily	104			95
	105			76
	109			36
	110			33
	112			87
B	102			94
Once Daily	103			75
	106			108
	107			209
	108			59
	111			139
C	201	38	11	
Once weekly	203	100	17	
	204	17	16	
	208	38	32	
	209	48	40	
	212	20	22	
D	202	56	60	
Once weekly	205	52	55	
	206	38	74	
	207	43	34	
	211	34	58	

Once the study was completed, a final more complete data set became available and was also used to calculate the half-life. The half-life calculated in the specific time period from 72 to 168 hours after dosing (regardless of the *Adjusted-R^2* value and number data points) for the metabolite ZP2469 is shown in the Table 5 below.

Table 5: Half-life final results

		Number of doses		
		1	6	7
Group	Subject	Half-life of ZP2469 in the interval from 72 to 168 hours post dose (hr)		
A	105			75
1 mg	112			87
once daily	115			94
	119			80

	125			54
	126			57
B	102			42
5 mg	103			168
once daily	106			77
	107			101
	108			53
	111			101
	114			34
	116			46
	122			100
	123			94
	127			40
	128			84
	129			50
C	203	329	26	
5 mg	204	41	35	
once weekly	208	38	59	
	209	48	94	
	212	59	75	
	213	48	57	
	214	61	43	
	216	NA	52	
	219	217	107	
	221	45	33	
	222	39	68	
	225	NA	47	
	226	91	65	
	229	75	452	
D	202	66	60	
10 mg	205	54	55	
once weekly	206	120	74	
	207	36	58	
	210	46	NA	
	211	35	70	
	215	60	59	
	217	26	30	
	218	158	102	
	220	53	53	
	223	43	60	
	224	33	42	
	227	45	48	
	228	43	56	
	230	38	17	

Example 6: Intestinotrophic effects of glepaglutide following chronic exposure in rats

Methods:

Wistar rats were dosed subcutaneously (SC) with vehicle, 1, 3 and 10 mg/kg ZP1848 on a daily basis for 26 weeks. Further sub-groups of control and high-dose animals were allowed a 6-week recovery period following completion of the dosing period. Sub-groups of control and high-dose animals were allowed a 6-week recovery period following completion of the dosing period. The length and the weight of the small and large intestines were measured at necropsy as indicators of intestinotrophic effects and the intestinal tract was evaluated histologically.

Results:

ZP1848 induced dose-related significant ($p<0.01$) increases in length and weight of the small intestines in rats (see Table 5). Furthermore, the length and weight of the large intestine was also slightly increased. ZP1848 produced a dose-related increase in mucosal hyperplasia of the duodenum, jejunum and ileum. Interestingly, at the end of the recovery period significant intestinotrophic effects were still present in the high-dose groups although partial recovery was seen.

Table 6: Percentage (%) increase relative to control group (males/females)

	Main Study animals	Recovery animals
Dose (mg/kg)	10	10
SI length	38/47	39/45
SI weight	190/238	85/101

SI = small intestine

Conclusion:

A significant dose-related intestinotrophic effect was seen following 26 weeks of ZP1848 exposure in rats. A similar effect was also seen after 7 days of dosing in rats. At all doses in the 26 week study, increased length and weight, as well as macroscopic thickening and villous hypertrophy, were noted in all segments of the small intestine. These findings were still present following a 6-week recovery period, indicating prolonged intestinotrophic effects of glepaglutide.

Example 7: Absolute changes in absolute fecal wet weight output in SBS-IF/SBS-II patients

Method

The study was designed as a randomized, cross-over and double-blinded with three different doses of ZP1848 (10 mg, 1 mg, 0.1 mg) in 16 SBS patients. The study protocol was approved by the Danish Medical Agency and The Danish Committee on Health Research Ethics.

The 18 SBS patients were randomized evenly and in a double-blinded fashion to each of the six cross-over dose level combinations: 10 mg/1 mg, 10 mg/0.1 mg, 1 mg/10 mg, 1 mg/0.1 mg, 0.1 mg/10 mg, 0.1 mg/1 mg of ZP1848. The SBS patient group comprised 9 females, 9 males; hereof 13 SBS-IF patients and 5 SBS-II patients. Mean age was 62 years, mean short bowel length was 110 cm, and two SBS-IF patients had > 50% colon in continuity. The patients were injected once daily subcutaneously with the above doses of ZP1848.

The aim of this study was to evaluate efficacy of ZP1848 on fecal wet weight output in patients with SBS-II and SBS-IF and thus the absolute change from baseline in wet weight of fecal output as measured by metabolic balance studies prior to and on the last 3 days of each treatment period.

Results:

Of the 18 patients randomized and treated with ZP1848, 16 completed the trial. The results changes in absolute fecal wet weight output (g/day) are shown in the below Table 7. The improvements were of the same magnitude in all patient groups, i.e. SBS-II, SBS-IF and patients with colon in continuity. This demonstrates that ZP1848 is effective for the treatment of both classes of SBS patients. Furthermore, the present inventors believe that these results support that GLP-2 analogues therapy using ZP1848 is effective in preventing or treating SBS-II patients becoming SBS-IF.

Table 7: Changes in absolute fecal wet weight output, SBS-II vs SBS-IF

Changes in Absolute Fecal Wet Weight Output (g/day) (Means [95% CI])	**0.1 mg**	**1 mg**	**10 mg**	**1 mg + 10 mg**
II+IF (n=16)	173 [-160, 506] p=0.274	-592 [-913, -272] p=0.002	-833 [-1152, -515] p=0.0002	-713 [-935, -490] p<0.0001
IF (n=11)	196 [-301, 693] p=0.3575	-587 [-1080, -95] p=0.0280	-894 [-1420, -368] p=0.0072	-741 [-1079, -402] p=0.0025
II (n=5)	-37 [-873, 800] p=0.9094	-303 [-1310, 704] p=0.4503	-837 [-1495, -178] p=0.0243	-570 [-1459, -45] p=0.0393

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While the invention has been described in conjunction with the exemplary embodiments described above, many equivalent modifications and variations will be apparent to those skilled in the art when given this disclosure. Accordingly, the exemplary embodiments of the invention set forth are considered to be illustrative and not limiting. Various changes to the described embodiments may be made without departing from the spirit and scope of the invention. All documents cited herein are expressly incorporated by reference.