

**A screening method for diagnosing growth hormone deficiency
in pediatric patients by using macimorelin**

Background of the Invention

5 Growth hormone (GH) is a major body system-wide metabolic hormone that regulates protein, lipid, and carbohydrate homeostasis and is required for growth, development, and maintenance of the body and mind. GH is produced in the anterior lobe of the pituitary gland upon stimulation by growth hormone releasing hormone (GHRH) from the hypothalamus gland. GH is secreted from the pituitary
10 in a pulsatile fashion of approximately 6-10 random bursts during a 24-hour period.

Growth hormone deficiency (GHD) may be classified broadly into four categories based on the source of the GH deficiency: 1) pituitary or "classic" GHD, 2) hypothalamic GHD, 3) functional GHD and 4) idiopathic GHD. GHD may become
15 clinically overt in childhood or in the adult. In the USA, it is estimated that the incidence of GHD in children is between 1 in 4,000 and 1 in 10,000. More than 50,000 adults in the US are GHD and 6,000 new cases are reported each year, including GHD children who transition to GHD as an adult (Human Growth Foundation www.hgfound.org).

20 GHD is a disease which in children is characterized by a reduction in auxological parameters, like growth failure and short stature. GHD in children can be congenital or acquired and either isolated or combined with other pituitary hormone defects. There are many well-defined causes of GHD in children, but the cause is often unknown (idiopathic GHD). The definitions of the various types of
25 GHD and other causes of short stature are given in the International Classification of Pediatric Endocrine Diagnoses (ICPED). If untreated, childhood-onset GHD leads to permanent short stature. In children, the diagnosis of GHD rests on a detailed medical history, clinical features, growth (auxological) analysis, biochemical tests of components of the GH-IGF axis (GH = growth hormone; IGF

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= Insulin-like growth factor) and radiological assessment of skeletal maturation and of pituitary anatomy using MRI (GHRS, 2000).

Similar to the situation in adults, the diagnosis of GHD in children depends on biochemical tests which are based on growth hormone stimulation tests (GHSTs) determining the GH level that can be induced with agents known to stimulate the release of GH. GHSTs currently in use, like the insulin tolerance test (ITT) or the glucagon stimulation test (GST), have not been developed and approved specifically for this purpose, but were adapted from other indications, and thus have limitations with regard to performance characteristic such as sensitivity of specificity, safety or feasibility (Molitch et al., 2011; Cook et al., 2009).

The ITT has been considered the gold standard for evaluation of GHD. An intravenous administration of insulin is used to induce hypoglycaemia, which in turn leads to GH release. However, this test is labor intensive as due to the potential risks associated with hypoglycaemia, which is associated with symptoms like tremor, somnolence, and tachycardia, intensive medical monitoring of the subject is required. The side effects are often reported as dangerous. Furthermore, the ITT is contraindicated in subjects with seizure disorders and ischemic heart disease (Yuen 2011; Yuen 2013). Therefore, ITT is not widely used because of its inconvenience and safety concerns.

The GST is an alternative that has grown in usage (Molitch 2011; Yuen 2011; Yuen 2013). The common side effects of the GST include nausea, vomiting and headaches. There are the limitations of the length of the test (3-4 hours) and the need for an intramuscular injection. There remains a real unmet medical need for alternative tests that are safe and reliable.

A test to diagnose GHD based on macimorelin, an orally-available peptidomimetic ghrelin receptor agonist with growth hormone secretagogue (GHS) activity, has been disclosed by Larsen in WO 2007/093820 A1.

Ghrelin potently stimulates GH release (Kojima 1999). The GH-releasing effects of ghrelin are thought to be mediated by specific receptors mainly present at the

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pituitary and hypothalamic level (Nogueiras 2006). In membrane preparations containing the GHS-receptor derived from human hypothalamus and pituitary gland, it was demonstrated that macimorelin shows binding potency to the human GHS receptor comparable to that of its natural ligand, ghrelin (Broglio 2002).
5 Macimorelin is readily absorbed from the gastrointestinal tract and is assumed to exert its action in the same way as ghrelin.

Based on the ability of macimorelin to exert the release of a GH pulse shortly after oral administration in healthy subjects, macimorelin has been developed as an oral diagnostic agent for GH deficiency in adults.

10 Macimorelin as a compound and its use in treatment of GHD has been disclosed by Martinez et al. in WO 01/96300 A1.

A Macimorelin GHST in adult GHD (AGHD) has been disclosed by Garcia et al. (J Clin Endocrinol Metab. 2013-1157, in J Clin Endocrinol Metab. 2018-00665 and a poster titled "Validation of Macimorelin As a Diagnostic Test for Adult Growth
15 Hormone Deficiency (AGHD): A Phase 3 Study in Comparison with the Insulin Tolerance test (ITT)" presented on the 99th Annual Meeting of the Endocrine Society in 2017).

In WO 2019/121762 A1 a method for measuring growth hormone level in a human or animal subject has been disclosed. The method comprises oral administration
20 of a macimorelin containing composition to a subject, collecting one, two or three post-administration samples within a range of 25 to 95 minutes after administration from said subject, and comparing the level of growth hormone in the one, the two or the three samples to a single threshold value, wherein the single threshold value is 2.8 ng/mL, and wherein "single threshold value" refers to
25 a threshold growth hormone level to indicate adequate response to macimorelin stimulation.

There is considerable controversy about the role of GH stimulation testing in pediatric patients, since low GH level in provocation tests frequently occur and there are concerns about the validity and reproducibility of GHSTs.

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Accordingly, guidelines for the diagnosis of GHD in children commonly require the outcome of two GHSTs to conclude on the diagnosis of GHD, unless there are typical brain defects which would require the use of one GHST only (GHRS, 2000, GHRS 2019).

5 The consensus guidelines of the GH Research Society published in 2000 (GHRS, 2000), as well as the American Association of Clinical Endocrinologists guidelines published in 2003 (Gharib, 2003) and national guidelines (Binder, 2014) recommend that a limited number of GHST agents should be used after an overnight fast in a well standardized GHST protocol. These include arginine
10 (ARG), clonidine, glucagon, insulin, and L-dopa.

The consensus guidelines of the GH Research Society state that the combination of GHRH and ARG as GHST was considered of value in the diagnosis of GHD in childhood and adulthood, provided appropriate cut-off points were applied (GHRS, 2000). This combination was shown to have high sensitivity and high
15 specificity in children and adolescents (Maghnie, 2002), in late adolescents and young adults (Corneli, 2007). In this latter trial, the cut-off points were established in lean patients only. Considering that GH secretion is a function of weight and adiposity (Colao, 2009), cut-off points appropriate for overweight and obese pediatric patients (taking into account age, BMI and waist circumference) remain
20 to be defined.

In a child with clinical criteria for GHD, a peak GH concentration below 10 ng/mL has traditionally been used as cut-off point to support the diagnosis. Sensitivity, specificity and GH cut-off points used for different GHSTs in different studies have recently been reviewed (van Vught, 2009).

25 On this background, various GHSTs are being used in clinical practice, and the requirement for two GHSTs as part of the standard diagnostic procedures is variably being met, either by repetition of the same GHST or by serial performance of two different GHSTs.

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These two GHSTs are conducted either on the same day or on two consecutive days, and they require 4 to 6 blood samples per test. Especially in small children, the amount of blood taken is considered a safety concern, and safe volume limits are to be respected as e.g. recommended by the WHO (Howie 2011). Apart from
5 this safety topic, it is to be noted that the conduct of two tests is time- and resource consuming, and the associated burden for the children and their parents as well as for the pediatric endocrinologist is high.

Therefore, there is the proven need for a single test instead of two tests, with proven safety, tolerability, easy application, strong test characteristics on
10 sensitivity and specificity, and reliable repeatability. A brief press release concerning the present invention was published in 2020 to announce positive results in a dose-finding pediatric study of macimorelin.

Summary of the Invention

In one aspect, the present invention provides a screening method for diagnosing
15 growth hormone deficiency in pediatric patients by using macimorelin comprising:

- (a) providing one to five blood samples, taken from a subject within a range from about 15 to about 100 minutes following an administration of an amount of macimorelin effective for inducing growth hormone secretion;
- (b) measuring the growth hormone level of each blood sample provided in step
20 (a);
- (c) comparing the measured growth hormone level obtained in step (b) with a single threshold value, wherein the single threshold value is 10.0 ng/mL or higher;
- (d) determining the subject, whose highest growth hormone level in the blood
25 samples obtained in step (b) is lower than the single threshold value, as having growth hormone deficiency, and determining the subject, whose highest growth hormone level in the blood samples obtained in step (b) is no

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lower than the single threshold value, as having no growth hormone deficiency.

In another aspect, the present invention relates to the substance macimorelin for use in diagnosing growth hormone deficiency in pediatric patients, wherein

- 5 (a) one to five blood samples are provided, taken from a subject within a range from about 15 to about 100 minutes following an administration of an amount of macimorelin effective for inducing growth hormone secretion;
- (b) the growth hormone level of each blood sample provided in step (a) is measured;
- 10 (c) the measured growth hormone level obtained in step (b) is compared with a single threshold value, wherein the single threshold value is 10.0 ng/mL or higher;
- (d) the subject, whose highest growth hormone level in the blood samples obtained in step (b) is lower than the single threshold value, is determined
15 as having growth hormone deficiency, and the subject, whose highest growth hormone level in the blood samples obtained in step (b) is no lower than the single threshold value, is determined as having no growth hormone deficiency.

20 In a further aspect, the present invention relates to a screening method for diagnosing growth hormone deficiency in pediatric patients by using macimorelin comprising:

- (a) providing at least one blood sample, taken from a subject within a range from about 15 to about 100 minutes following an administration of an amount of macimorelin effective for inducing growth hormone secretion;
- 25 (b) measuring the growth hormone level of each blood sample;
- (c) comparing each of the measured growth hormone level with a single threshold value;

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- (d) diagnosing whether the subject suffers from growth hormone deficiency or not based on the comparison of growth hormone level measured in step (b) with said single threshold value in said at least one blood sample; wherein determining the subject as having or not having growth hormone deficiency is exclusively based on the growth hormone level induction by a single macimorelin administration.

Definitions

The terms indicated for explanation of the methods of the invention always, unless otherwise indicated in the description or the claims, have the following meaning.

As used herein, a “subject” or “pediatric patient” is a human child of either gender (a male or a female) between the age of about 2 to less than 18 years. For example, the subject is between the age of about 3 to less than 18, about 4 to less than 18, about 5 to less than 18, about 6 to less than 18, about 7 to less than 18, about 8 to less than 18, about 9 to less than 18, about 10 to less than 18, about 11 to less than 18, about 12 to less than 18, about 2 to less than 17, about 2 to less than 16, about 2 to less than 15, about 2 to less than 14, about 2 to less than 13, about 2 to less than 12, about 2 to less than 10, about 2 to less than 9 or about 2 to less than 8 years.

As used herein, “macimorelin” refers to a peptidomimetic compound acting as the ghrelin receptor agonist with growth hormone secretagogue (GHS) activity. Its chemical structure and use in the treatment of GHD are disclosed in U.S. Patent No. 6,861,409, WO 01/96300, and WO 2007/093820.

As used herein, the term “effective amount” refers to an amount of a given substance that is sufficient in quantity to produce a desired effect. For example, an effective amount of macimorelin for inducing growth hormone secretion in a recipient is an amount of the compound capable to achieve a detectable increase in the secretion of growth hormone upon its administration to the subject.

The term “test” and “testing”, as used in this application, describes to an act that leads to the clarification of a suspect of presence of a specific condition based on

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a subject's symptoms and to the identification of the condition as being present or absent. In other words, "testing" a condition encompasses the confirmation or exclusion of the condition.

5 The term "treat" or "treating" as used in this application, describes to an act that leads to the elimination, reduction, alleviation, reversal, or prevention or delay of onset or recurrence of any symptom of a relevant condition.

As used herein, the term "blood sample" encompasses a whole blood sample as well as a fraction of whole blood such as serum or plasma sample. Whenever two or more blood samples are used for testing in the same method scheme, these
10 blood samples are of the same type. For example, if the first sample is serum, then the second and any subsequent samples are also serum. In addition, if more than one blood sample is provided, the term "blood samples" refers to blood samples taken at different time points following administration of an amount of macimorelin effective for inducing hormone secretion. Two blood samples may
15 refer to blood samples taken, for example, at about 30 ± 10 minutes and about 45 ± 10 minutes or at about 30 ± 10 minutes and about 60 ± 10 minutes following administration of an amount of macimorelin effective for inducing hormone secretion. Three blood samples may refer to blood samples taken, for example, at about 30 ± 10 minutes, at about 45 ± 10 minutes and about 60 ± 10 minutes or
20 preferably about 30 ± 10 minutes, at about 45 ± 10 minutes and about 90 ± 10 minutes or, alternatively, at about 30 ± 10 minutes, at about 60 ± 10 minutes and about 90 ± 10 minutes following administration of an amount of macimorelin effective for inducing hormone secretion.

The term "about" as used herein denotes a range of $\pm 10\%$ of a reference value.
25 For examples, "about 10" defines a range of 9 to 11.

The term "single threshold value" relates to a threshold growth hormone level to indicate adequate response to macimorelin stimulation: instead of the 2.8 ng/mL threshold value commonly used in adult tests, the threshold value used in the methods of the present invention is in a higher range of about 10.0 ng/mL or
30 higher, for example about 10.0-25.0 ng/mL, about 10.0-20.0 ng/mL, about 10.1-

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19.5 ng/mL, about 10.2-19.0 ng/mL, about 10.3-18.5 ng/mL, about 10.4-18.0 ng/mL, about 10.5-17.5 ng/mL, about 10.6-17.0 ng/mL, about 11.0-16.5 ng/mL, about 12.0-16.0 ng/mL, about 13.0-15.5 ng/mL, about 14.0-15.0 ng/mL, about 15.0-16.0 ng/mL, about 15.5-18.0 ng/mL, about 16.0-18.0 ng/mL, about 16.5-18.0 ng/mL, about 17.0-18.0 ng/mL, about 17.5-18.5 ng/mL, about 18.0-19.0 ng/mL, about 18.5-19.5 ng/mL, about 19.0-20.0 ng/mL, about 20.0-21.0 ng/mL or about 25.0-30.0 ng/mL. The single threshold value used in the methods of the present invention can also be a single value, for example 10.5 ng/mL, 11.0 ng/mL, 11.5 ng/mL, 12.0 ng/mL, 12.5 ng/mL, 13.0 ng/mL, 13.5 ng/mL, 14.0 ng/mL, 14.5 ng/mL, 15.0 ng/mL, 15.5 ng/mL, 16.0 ng/mL, 16.5 ng/mL, 17.0 ng/mL, 17.5 ng/mL, 18.0 ng/mL, 18.5 ng/mL, 19.0 ng/mL, 20.0 ng/mL or 25.0 ng/mL. In addition, the single threshold value refers to ng per mL in whole blood or serum/plasma. Most preferably, the single threshold value refers to ng per mL in serum. The terms "single threshold value" and "cut-off point" are used interchangeably.

Weight percent, percent by weight, % by weight, % w/w and the like are synonyms that refer to the concentration of a substance as the weight of that substance divided by the weight of the composition and multiplied by 100.

All patents, patent applications, and other publications cited in this application are incorporated by reference in the entirety for all purposes.

Figures

Figure 1 shows the individual macimorelin concentration versus time for Cohort 1, i.e. 0.25 mg/kg body weight, linear scale (Pharmacokinetic Analysis Set (PKS), Number of Patients (N) = 24).

Figure 2 shows the individual macimorelin concentration versus time for Cohort 2, i.e. 0.5 mg/kg body weight, linear scale (PKS, N=24).

Figure 3 shows the individual macimorelin concentration versus time for Cohort 3, i.e. 1.0 mg/kg body weight, linear scale (PKS, N=24).

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Figure 4 shows the individual GH concentration after macimorelin GHST versus time for Cohort 1, i.e. 0.25 mg/kg body weight, linear scale, (Pharmacodynamic Analysis Set (PDS), N=24).

5 Figure 5 shows the individual GH concentration after macimorelin GHST versus time for Cohort 2, i.e. 0.5 mg/kg body weight, linear scale, (PDS, N=24).

Figure 6 shows the individual GH concentration after macimorelin GHST versus time for Cohort 3, i.e. 1.0 mg/kg body weight, linear scale, (PDS, N=24).

Figure 7 shows the Receiver Operating Characteristic (ROC) Analysis of Macimorelin GHST for Cohort 1, i.e. 0.25 mg/kg body weight, (PDS, N=24).

10 Figure 8 shows the Receiver Operating Characteristic (ROC) Analysis of Macimorelin GHST for Cohort 2, i.e. 0.5 mg/kg body weight, (PDS, N=24).

Figure 9 shows the Receiver Operating Characteristic (ROC) Analysis of Macimorelin GHST for Cohort 3, i.e. 1.0 mg/kg body weight, (PDS, N=24).

Detailed Description of the Invention

15 An object of this invention is to provide a stand-alone method for measuring growth hormone level in pediatric patients and for detecting GHD in pediatric patients. The aim is to develop a new method which not only reduces the burden on test administrators and test subjects by reducing the test time duration and number of blood draws but also should provide safe, reliable, and superior
20 diagnostic performance.

The object of the invention has surprisingly been solved in one aspect by providing a screening method for diagnosing growth hormone deficiency in pediatric patients by using macimorelin comprising:

(a) providing one to five blood samples, taken from a subject within a range from
25 about 15 to about 100 minutes following an administration of an amount of macimorelin effective for inducing growth hormone secretion;

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- (b) measuring the growth hormone level of each blood sample provided in step (a);
- (c) comparing the measured growth hormone level obtained in step (b) with a single threshold value, wherein the single threshold value is 10.0 ng/mL or higher;
- (d) determining the subject, whose highest growth hormone level in the blood samples obtained in step (b) is lower than the single threshold value, as having growth hormone deficiency, and determining the subject, whose highest growth hormone level in the blood samples obtained in step (b) is no lower than the single threshold value, as having no growth hormone deficiency.

Thus, the one to five blood samples are taken from a subject not earlier than about 15 minutes following an administration of an amount of macimorelin effective for inducing growth hormone secretion and not later than about 100 minutes following an administration of an amount of macimorelin effective for inducing growth hormone secretion.

For example, if a single growth hormone level in the blood samples is no lower than the single threshold value, the subject is determined as having no growth hormone deficiency. In the alternative, if the growth hormone level in all blood samples are lower than the single threshold value, the subject is determined as having growth hormone deficiency.

In a preferred embodiment, the screening method for diagnosing growth hormone deficiency in pediatric patients by using macimorelin is an *in vitro* screening method for diagnosing growth hormone deficiency in pediatric patients by using macimorelin.

In a preferred embodiment, the single threshold value for growth hormone is within a range from about 10.0 to about 25.0 ng/mL, preferably from about 10.2 to about 20.0 ng/mL, further preferably from about 12.0 to about 19.0 ng/mL, further

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preferably from about 14.0 to about 18.0 ng/mL, more preferably from about 16.0 to about 18.0 ng/mL and most preferably from about 17.0 to about 18.0 ng/mL.

In a preferred embodiment, in step (a) one to four blood samples are provided, preferably wherein in step (a) one to three blood samples are provided, more preferably wherein in step (a) two or three blood samples are provided. As explained above, if more than one blood sample is provided, these blood samples are taken at different time points following administration of an amount of macimorelin effective for inducing hormone secretion.

In another preferred embodiment, in step (a) the blood samples are taken from a subject within a range from about 20 to about 100 minutes, preferably within a range from about 25 to about 100 minutes, more preferably within a range from about 25 to about 95 minutes and most preferably within a range from about 30 to about 90 minutes, following an administration of an amount of macimorelin effective for inducing growth hormone secretion. Thus the blood samples are, for example, taken not earlier than about 20 minutes but not later than about 100 minutes, preferably not earlier than about 25 minutes but not later than about 100 minutes, more preferably not earlier than about 25 minutes but not later than about 95 minutes, and most preferably not earlier than about 30 minutes but not later than about 90 minutes following an administration of an amount of macimorelin effective for inducing growth hormone secretion.

In yet another preferred embodiment, the blood samples are taken from the subject at about 10 to about 60 minute intervals, preferably about 15 to about 30 minute intervals, if more than one blood sample is provided. Alternatively, the blood samples can be taken at any time interval deemed appropriate by the attending physician. For example, the blood samples can be taken in about 5, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, about 50, about 45 or about 60 minute intervals.

In a further preferred embodiment, in step (a) the blood samples are whole blood samples, serum samples or plasma samples. Preferably, in step (a) the blood samples are serum samples or plasma samples. If more than one blood sample is

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taken, the two or more blood samples are from the same type, and these blood samples are therefore either whole blood samples, serum samples or plasma samples. Most preferably, the blood samples are serum samples.

In a further preferred embodiment, in step (a) about 0.8 mg to about 1.2 mg per kg subject body weight of macimorelin is administered, preferably wherein in step (a) about 1.0 mg per kg subject body weight of macimorelin is administered. Typically, an effective amount of macimorelin can be in the range of from about 0.8 to about 0.9 mg per kg body weight of the subject at the low-end, and from about 1.0, about 1.1 to about 1.2 mg/kg body weight of the subject at the high-end, or within a range defined by any one of the low-end amounts and any one of the high-end amounts, for instance, from about 0.9 to about 1.1 mg/kg body weight. An effective amount of macimorelin can also be a single value, for example, of about 0.8, about 0.9, about 1.0, about 1.1 or about 1.2 mg/kg body weight. Body weight (recorded in kg) may preferably be rounded to the closest integer. Most preferred, in step (a) about 1.0 mg per kg subject body weight of macimorelin is administered.

For example, one macimorelin unit dose consists of 1817.2 mg containing composition for preparation of an oral suspension in water. Typically, the prepared suspension contains 0.5 mg macimorelin per mL suspension. A body weight adjusted aliquot of the reconstituted suspension is administered to the pediatric subject, resulting in a dose of 1.0 mg/kg body weight in children. Said unit dose is defined for a macimorelin calculated as a free base with a content of 100%. The mass of the macimorelin free base or its free base equivalent within said unit dose is adjusted according to the content.

In a preferred embodiment, in step (a) the macimorelin is administered in a composition comprising macimorelin as a suitable pharmaceutical salt, wherein preferably the suitable pharmaceutical salt is selected from the acetate salt of macimorelin, the trifluoro acetate salt of macimorelin or a combination thereof.

In a further preferred embodiment, in step (a) the administration of macimorelin is oral administration.

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If the administration of macimorelin is oral administration, the macimorelin may be prepared as an oral suspension. The suspension may be administered within about 90 minutes, preferably within about 60 minutes, more preferably within about 30 minutes after preparation of the oral suspension. Preferably, the oral
5 suspension is drunk over a time period of not more than about 1 minute, preferably over a time period of not more than about 30 seconds.

In a further preferred embodiment, the subject has fasted for about 10 hours, preferably about 9 hours, more preferably about 8 hours, prior administration of macimorelin. Further preferably, in step (a) the subject fasts for about 100 minutes
10 following macimorelin administration, meaning in step (a) the subject may fast throughout the about 100 minutes, about 95 minutes or about 90 minutes following administration of macimorelin.

In a further preferred embodiment, in step (a) one blood sample is provided, which is taken from the subject at about 60 ± 30 minutes after administration of macimorelin. The one blood sample may alternatively be taken from the subject at
15 about 30 ± 10 , about 40 ± 10 , about 45 ± 10 , about 50 ± 10 , about 60 ± 10 , about 70 ± 10 , about 80 ± 10 , about 90 ± 10 or about 100 ± 10 minutes after administration of macimorelin. The one blood sample may be taken at any time point within a range from about 15 to about 100 minutes following administration of macimorelin that is
20 deemed appropriate by the attending physician.

In another further preferred embodiment, in step (a) two blood samples are provided, which are taken from the subject at about 30 ± 10 minutes and at about 45 ± 10 minutes after administration of macimorelin. The two blood samples may alternatively be taken from the subject at about 20 ± 10 , about 30 ± 10 , about 40 ± 10 ,
25 about 45 ± 10 , about 50 ± 10 , about 60 ± 10 about 70 ± 10 , about 80 ± 10 , about 90 ± 10 or about 100 ± 10 minutes after administration of macimorelin. The two blood samples may be taken at any time points within a range from about 15 to about 100 minutes following administration of macimorelin that are deemed appropriate by the attending physician.

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In another preferred embodiment, in step (a) two blood samples are provided, which are taken from the subject at about 30 ± 10 minutes and at about 60 ± 10 minutes after administration of macimorelin.

In another preferred embodiment, wherein in step (a) three blood samples are provided, which are taken from the subject at about 30 ± 10 minutes, at about 45 ± 10 minutes and at about 60 ± 10 minutes after administration of macimorelin or wherein in step (a) three blood samples are provided, which are taken from the subject at about 30 ± 10 minutes, at about 45 ± 10 minutes and at about 90 ± 10 minutes after administration of macimorelin or wherein in step (a) three blood samples are provided, which are taken from the subject at about 30 ± 10 minutes, at about 60 ± 10 minutes and at about 90 ± 10 minutes after administration of macimorelin. The three blood samples may alternatively be taken from the subject at about 20 ± 10 , about 30 ± 10 , about 40 ± 10 , about 45 ± 10 , about 50 ± 10 , about 60 ± 10 , about 70 ± 10 , about 80 ± 10 , about 90 ± 10 or about 100 ± 10 , minutes after administration of macimorelin. The three blood samples may be taken at any time points within a range from about 15 to about 100 minutes following administration of macimorelin that are deemed appropriate by the attending physician.

If in step (a) more than three blood samples are provided, these blood samples may be taken at any time point within a range from about 15 to about 100 minutes following administration of macimorelin deemed appropriate by the attending physician. For example, suitable time points are about 20 ± 10 , about 30 ± 10 , about 40 ± 10 , about 45 ± 10 , about 50 ± 10 , about 60 ± 10 , about 70 ± 10 , about 80 ± 10 , about 90 ± 10 or about 100 ± 10 minutes after administration of macimorelin.

In a particularly preferred method in step (a) one to four, further preferred one to three, more preferred two or three blood samples are provided, which are taken from the subject at the times selected from the group consisting of at about 30 ± 10 minutes, at about 45 ± 10 minutes, at about 60 ± 10 minutes and at about 90 ± 10 minutes after administration of macimorelin.

In a further preferred embodiment, in step (a) the macimorelin is administered in a composition comprising macimorelin and optionally further pharmaceutically

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acceptable excipients, such as carrier substances. Preferably, the macimorelin is administered in a composition comprising macimorelin and sweetener. A suitable sweetener is, for example, saccharin. Advantageously, saccharin was found to be a suitable taste masking agent for macimorelin.

5 In a further preferred embodiment, in step (a) the macimorelin is administered in a composition comprising about 3.5% (w/w) macimorelin (calculated as free base), about 93.1% (w/w) spray-dried lactose monohydrate, about 2.0% (w/w) crospovidone Type A, about 0.1% (w/w) colloidal silicon dioxide, about 1.0% (w/w) sodium stearyl fumarate, and about 0.3% (w/w) saccharin sodium dihydrate.

10 In a further preferred embodiment, the subject is a human child from the age of 2 to less than 18 years, preferably the subject is a human child from the age of 2 to less than 17 years, more preferred the subject is a human child from the age of 2 to less than 16 years.

In a further preferred embodiment, the method is a stand-alone test and does not
15 need to be repeated and no alternative growth hormone stimulation test is required to reliably diagnose growth hormone deficiency in pediatric patients.

In a further preferred embodiment, determining the subject as having or not having growth hormone deficiency according to step (d) is exclusively based on the growth hormone level induction by a single macimorelin administration.

20 Surprisingly, it has been found that the method of the present invention is suitable as stand-alone test since no further GHST is required to reliably diagnose GHD in pediatric patients.

The object of the invention has surprisingly been solved in another aspect by providing the substance macimorelin for use in diagnosing growth hormone
25 deficiency in pediatric patients, wherein

(a) one to five blood samples are provided, taken from a subject within a range from about 15 to about 100 minutes following an administration of an amount of macimorelin effective for inducing growth hormone secretion;

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- (b) the growth hormone level of each blood sample provided in step (a) is measured;
- (c) the measured growth hormone level obtained in step (b) is compared with a single threshold value, wherein the single threshold value is 10.0 ng/mL or higher;
- (d) the subject, whose highest growth hormone level in the blood samples obtained in step (b) is lower than the single threshold value, is determined as having growth hormone deficiency, and the subject, whose highest growth hormone level in the blood samples obtained in step (b) is no lower than the single threshold value, is determined as having no growth hormone deficiency.

The one to five blood samples are taken from a subject not earlier than about 15 minutes following an administration of an amount of macimorelin effective for inducing growth hormone secretion and not later than about 100 minutes following an administration of an amount of macimorelin effective for inducing growth hormone secretion.

In a preferred embodiment, the substance macimorelin for use in diagnosing growth hormone deficiency in pediatric patients is a substance macimorelin for use in in vitro diagnosing growth hormone deficiency in pediatric patients.

In a preferred embodiment, the single threshold value for growth hormone is within a range from about 10.0 to about 25.0 ng/mL, preferably from about 10.2 to about 20.0 ng/mL, further preferably from about 12.0 to about 19.0 ng/mL, further preferably from about 14.0 to about 18.0 ng/mL, more preferably from about 16.0 to about 18.0 ng/mL and most preferably from about 17.0 to about 18.0 ng/mL.

In a preferred embodiment, in step (a) one to four blood samples are provided, preferably wherein in step (a) one to three blood samples are provided, more preferably wherein in step (a) two or three blood samples are provided. If more than one blood sample is provided, these blood samples are taken at different

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time points following administration of an amount of macimorelin effective for inducing hormone secretion.

In another preferred embodiment, in step (a) the blood samples are taken from a subject within a range from about 20 to about 100 minutes, preferably within a
5 range from about 25 to about 100 minutes, more preferably within a range from about 25 to about 95 minutes and most preferably within a range from about 30 to about 90 minutes, following an administration of an amount of macimorelin effective for inducing growth hormone secretion. Consequently, the blood samples are, for example, taken not earlier than about 20 minutes but not later than about
10 100 minutes, preferably not earlier than about 25 minutes but not later than about 100 minutes, more preferably not earlier than about 25 minutes but not later than about 95 minutes, most preferably not earlier than about 30 minutes but not later than about 90 minutes following an administration of an amount of macimorelin effective for inducing growth hormone secretion.

15 In yet another preferred embodiment, the blood samples are taken from the subject at about 10 to about 60 minute intervals, preferably about 15 to about 30 minute intervals, if more than one blood sample is provided. Alternatively, the blood samples can be taken at any time interval deemed appropriate by the attending physician. For example, the blood samples can be taken in about 5,
20 about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, about 50, about 45 or about 60 minute intervals.

In a further preferred embodiment, in step (a) the blood samples are whole blood samples, serum samples or plasma samples. Preferably, in step (a) the blood samples are serum samples or plasma samples. If more than one blood sample is
25 taken, the two or more blood samples are from the same type, and these blood samples are therefore either whole blood samples, serum samples or plasma samples. Most preferably, the blood samples are serum samples.

In a further preferred embodiment, in step (a) about 0.8 mg to about 1.2 mg per kg subject body weight of macimorelin is administered, preferably wherein in step (a)
30 about 1.0 mg per kg subject body weight of macimorelin is administered.

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Typically, an effective amount of macimorelin can be in the range of from about 0.8 to about 0.9 mg per kg body weight of the subject at the low-end, and from about 1.0, about 1.1 to about 1.2 mg/kg body weight of the subject at the high-end, or within a range defined by any one of the low-end amounts and any one of the high-end amounts, for instance, from about 0.9 to about 1.1 mg/kg body weight. An effective amount of macimorelin can also be a single value, for example, of about 0.8, about 0.9, about 1.0, about 1.1 or about 1.2 mg/kg body weight. Body weight (recorded in kg) may preferably be rounded to the closest integer.

For example, one macimorelin unit dose consists of 1817.2 mg containing composition for preparation of an oral suspension in water. Typically, the prepared suspension contains 0.5 mg macimorelin per mL suspension. A body weight adjusted aliquot of the reconstituted suspension is administered to the pediatric subject, resulting in a dose of 1.0 mg/kg body weight in children. Said unit dose is defined for a macimorelin calculated as a free base with a content of 100%. The mass of the macimorelin free base or its free base equivalent within said unit dose is adjusted according to the content.

In a preferred embodiment, in step (a) the macimorelin is administered in a composition comprising macimorelin as a suitable pharmaceutical salt thereof, wherein preferably the suitable pharmaceutical salt is selected from the acetate salt of macimorelin, the trifluoro acetate salt of macimorelin or a combination thereof.

In a further preferred embodiment, in step (a) the administration of macimorelin is oral administration.

If the administration of macimorelin is oral administration, the macimorelin may be prepared as an oral suspension. The suspension may be administered within about 90 minutes, preferably within about 60 minutes, more preferably within about 30 minutes after preparation of the oral suspension. Preferably, the oral suspension is drunk over a time period of not more than about 1 minute, preferably over a time period of not more than about 30 seconds.

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In a further preferred embodiment, the subject has fasted for about 10 hours, preferably about 9 hours, more preferably about 8 hours, prior administration of macimorelin. Further preferably, in step (a) the subject fasts for about 100 minutes following macimorelin administration, meaning in step (a) the subject may fast
5 throughout the about 100 minutes, about 95 minutes or about 90 minutes following administration of macimorelin.

In further preferred embodiment, in step (a) one blood sample is provided, which is taken from the subject at about 60 ± 30 minutes after administration of macimorelin. The one blood sample may alternatively be taken from the subject at
10 about 30 ± 10 , about 40 ± 10 , about 45 ± 10 , about 50 ± 10 , about 60 ± 10 , about 70 ± 10 , about 80 ± 10 , about 90 ± 10 , about 100 ± 10 minutes after administration of macimorelin. The one blood sample may be taken at any time point within a range from about 15 to about 100 minutes following administration of macimorelin that is deemed appropriate by the attending physician.

In another further preferred embodiment, in step (a) two blood samples are provided, which are taken from the subject at about 30 ± 10 minutes and at about 45 ± 10 minutes after administration of macimorelin. The two blood samples may alternatively be taken from the subject at about 20 ± 10 , about 30 ± 10 , about 40 ± 10 , about 45 ± 10 , about 50 ± 10 , about 60 ± 10 , about 70 ± 10 , about 80 ± 10 , about 90 ± 10
20 or about 100 ± 10 , minutes after administration of macimorelin. The two blood samples may be taken at any time points within a range from about 15 to about 100 minutes following administration of macimorelin that are deemed appropriate by the attending physician.

In another preferred embodiment, in step (a) two blood samples are provided,
25 which are taken from the subject at about 30 ± 10 minutes and at about 60 ± 10 minutes after administration of macimorelin.

In another preferred embodiment, in step (a) three blood samples are provided, which are taken from the subject at about 30 ± 10 minutes, at about 45 ± 10 minutes and at about 60 ± 10 minutes after administration of macimorelin or wherein in step
30 (a) three blood samples are provided, which are taken from the subject at about

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30±10 minutes, at about 45±10 minutes and at about 90±10 minutes after administration of macimorelin or wherein in step (a) three blood samples are provided, which are taken from the subject at about 30±10 minutes, at about 60±10 minutes and at about 90±10 minutes after administration of macimorelin.

5 The three blood samples may alternatively be taken from the subject at about 20±10, about 30±10, about 40±10, about 45±10, about 50±10, about 60±10, about 70±10, about 80±10, about 90±10, or about 100±10 minutes after administration of macimorelin. The three blood samples may be taken at any time points within a range from about 15 to about 100 minutes following administration of macimorelin

10 deemed appropriate by the attending physician.

If more than three blood samples are provided, these blood samples may be taken at any time points within a range from about 15 to about 100 minutes following administration of macimorelin deemed appropriate by the attending physician. For example suitable time points are about 20±10, about 30±10, about

15 40±10, about 45±10, about 50±10, about 60±10, about 70±10, about 80±10, about 90±10 or about 100±10 minutes after administration of macimorelin.

In a particularly preferred embodiment in step (a) one to four, further preferred one to three, more preferred two or three blood samples are provided, which are taken from the subject at the times selected from the group consisting of at about

20 30±10 minutes, at about 45±10 minutes, at about 60±10 minutes and at about 90±10 minutes after administration of macimorelin.

In a further preferred embodiment, in step (a) the macimorelin is administered in a composition comprising macimorelin and optionally further pharmaceutically acceptable excipients, such as carrier substances. Preferably, the macimorelin is

25 administered in a composition comprising macimorelin and sweetener. A suitable sweetener is, for example, saccharin. Saccharin was found to be a suitable taste masking agent for macimorelin.

In a further preferred embodiment, in step (a) the macimorelin is administered in a composition comprising about 3.5% (w/w) macimorelin (calculated as free base),

30 about 93.1% (w/w) spray-dried lactose monohydrate, about 2.0% (w/w)

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crospovidone Type A, about 0.1% (w/w) colloidal silicon dioxide, about 1.0% (w/w) sodium stearyl fumarate, and about 0.3% (w/w) saccharin sodium dihydrate.

In a further preferred embodiment, the subject is a human child from the age of 2 to less than 18 years, preferably the subject is a human child from the age of 2 to less than 17 years, more preferred the subject is a human child from the age of 2 to less than 16 years.

In a further preferred embodiment, the substance is used in a stand-alone test and does not need to be repeated and no alternative growth hormone stimulation test is required to reliably diagnose growth hormone deficiency in pediatric patients.

In a further preferred embodiment, determining the subject as having or not having growth hormone deficiency according to step (d) is exclusively based on the growth hormone level induction by a single macimorelin administration.

Surprisingly, it has been found that this method of the present invention is suitable as stand-alone test since no further GHST is required to reliably diagnose GHD in pediatric patients.

The object of the invention has surprisingly been solved in a further aspect by providing a screening method for diagnosing growth hormone deficiency in pediatric patients by using macimorelin comprising:

- (a) providing at least one blood sample, taken from a subject within a range from about 15 to about 100 minutes following an administration of an amount of macimorelin effective for inducing growth hormone secretion;
- (b) measuring the growth hormone level of each blood sample;
- (c) comparing each of the measured growth hormone level with a single threshold value;
- (d) diagnosing whether the subject suffers from growth hormone deficiency or not based on the comparison of growth hormone level measured in step (b)

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with said single threshold value in said at least one blood sample;
wherein determining the subject as having or not having growth hormone deficiency is exclusively based on the growth hormone level induction by a single macimorelin administration.

5 In a preferred embodiment, the screening method for diagnosing growth hormone deficiency in pediatric patients by using macimorelin is an *in vitro* screening method for diagnosing growth hormone deficiency in pediatric patients by using macimorelin.

10 In a preferred method in step (a) one to four, further preferred one to three, more preferred two or three blood samples are provided, which are taken from the subject at the times selected from the group consisting of at about 30 ± 10 minutes, at about 45 ± 10 minutes, at about 60 ± 10 minutes and at about 90 ± 10 minutes after administration of macimorelin.

15 In a preferred embodiment, in step (d) the subject, whose highest growth hormone level measured in step (b) is lower than the single threshold value, is determined as having growth hormone deficiency, and the subject, whose highest growth hormone level measured in step (b) is no lower than the single threshold value, is determined as having no growth hormone deficiency

20 In another preferred embodiment, the single threshold value is within a range from about 10.0 to about 25.0 ng/mL, preferably from about 10.2 to about 20.0 ng/mL, further preferably from about 12.0 to about 19.0 ng/mL, further preferably from about 14.0 to about 18.0 ng/mL, more preferably from about 16.0 to about 18.0 ng/mL and most preferably from about 17.0 to about 18.0 ng/mL.

25 In yet another preferred embodiment, about 0.8 mg to about 1.2 mg per kg subject body weight of macimorelin is administered, preferably about 1.0 mg per kg subject body weight of macimorelin is administered.

In a further preferred embodiment, the subject is a human child from the age of 2 to less than 18 years, preferably the subject is a human child from the age of 2 to

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less than 17 years, more preferred the subject is a human child from the age of 2 to less than 16 years.

One main feature of the methods of this invention is the single stimulation test format in contrast to the standard two-test format currently in use by medical professionals. Rather than having two separate tests, which are performed at least one day apart and involve as many as 8-12 blood draws, the new methods of this invention require only one test and as few as only 1 to 5, preferably 2 to 4 blood draws in order to achieve reliable diagnostic performance in accuracy, specificity, and sensitivity for detecting growth hormone deficiency, thus greatly reducing the testing burden and potential harm to the children being tested. Advantageously, these blood samples can be collected within a short period of time, such as in a time period of a total of about 90 minutes after administration of macimorelin, in intervals of about 15 up to about 30 minutes.

Surprisingly, the methods of the present invention have achieved significant improvement by using a higher threshold value for diagnosing growth hormone deficiency. Conventionally, when adult patients are tested for GHD, a single threshold value of about 2 to 3 ng/mL is used and when pediatric patients are tested for GHD, a single threshold value of below 10 ng/mL is used. The present inventors have unexpectedly discovered that better diagnostic performance can be achieved for pediatric patients when a higher single threshold value of 10.0 ng/mL or higher is used. For example, a single threshold value of about 16.0 to 19.0 ng/mL, preferably of about 17.0 to 18.0 ng/mL has been found to very effectively indicate GHD in the stand-alone methods of this invention.

In addition, the methods of the present invention have achieved significant improvement by using a higher macimorelin dose for diagnosing growth hormone deficiency. While the conventional dose of macimorelin used in current practice, especially when adult patients are tested for GHD, is 0.5 mg/kg patient body weight, the present inventors have unexpectedly discovered that better diagnostic performance can be achieved when a higher dose of macimorelin is used in the growth hormone stimulation test for pediatric patients. For example, a macimorelin

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dose of about 0.8 to about 1.2 mg/kg, preferably about 1.0 mg/kg body weight has been found to very effectively indicate GHD in the one-test method of this invention.

The invention provides methods for measuring growth hormone level in a human child, including methods of assessing pituitary-related GH deficiency in a human child, after single oral administration of macimorelin to the child:

- as a stand-alone test (one test method and a single GH stimulation are required only),
- with two to four blood samples collected in a time period of, for example, a total of about 90 minutes after administration of macimorelin, in intervals of about 15 up to about 30 minutes
- with a GH cut-off point in a range of about 10.0 ng/mL or higher, preferably about 10.0 ng/mL to about 25.0 ng/mL, more preferably about 10.2 ng/mL to about 20 ng/mL, and most preferably about 17.0 to about 18.0 ng/mL.

The following examples are provided by way of illustration only and not by way of limitation. Those of skill in the art will readily recognize a variety of non-critical parameters that could be changed or modified to yield essentially the same or similar results.

EXAMPLES

Growth hormone deficiency (GHD) in children is a rare, etiologically diverse condition that results in growth failure and short stature. Inadequate response to two different growth hormone stimulation tests (GHST) is required for the diagnosis of GHD. Macimorelin acetate, a potent, orally administered growth hormone (GH) secretagogue, is approved by the FDA and EMA for the diagnosis of GHD in adults (AGHD). This study (AEZS-130-P01) was designed to investigate macimorelin acetate as a diagnostic test in children with suspected GHD.

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This was an open-label, group comparison, dose escalation trial to investigate the safety, tolerability, pharmacokinetics and pharmacodynamics of single-dose 0.25, 0.5, and 1.0 mg/kg oral macimorelin acetate in pediatric subjects with suspected GHD. The macimorelin GHST was administered between two standard GHST, conducted as per local clinical practice, with a recovery period of 7–28 days between tests. Blood samples were collected pre-dose (± 15 min) and 15, 30, 45, 60, 90, 120, and 360 minutes after macimorelin acetate intake.

Overall, 24 pediatric subjects (8 per cohort [C1, C2, C3]) were included in the pharmacokinetic/pharmacodynamic (PK/PD) analysis. Five males and 3 females were observed in C1 and C2, 7 males and 1 female in C3. In all three cohorts, at least 3 subjects represented Tanner stages I or II. All 24 subjects (100%) were white, with a median age of 9.8, 9.0 and 10.5 years (range 4–15 years) and a median body-mass index of 16.1 kg/m² (12.4–21.4 kg/m²) at screening. Overall, 88 adverse events were reported, many related to the standard GHST; none were considered related to the macimorelin test. Maximum plasma concentrations for macimorelin were mainly observed between 30–45 min. The mean $C_{\max}$ values were 3.46, 8.13 and 12.87 ng/mL for C1, C2, and C3, respectively. The AUCs increased with dose; the mean AUC₀₋₆ values were 6.69, 18.02 and 30.92 h*ng/mL. The mean elimination half-lives were 1.22, 1.61 and 1.71 h, respectively. PK and PD profiles for all three cohorts were comparable, with peak GH levels mainly observed within 30–60 min following macimorelin intake.

Macimorelin acetate was safe and well tolerated in all dosing cohorts. A dose-dependent increase in macimorelin $C_{\max}$ and AUC in children and adolescents correlated well with data from adult subjects. A robust dose-proportional GH response was also achieved. PD results showed that GH response was comparable in all dose groups, with a slight shift to earlier $t_{\max}$ at higher macimorelin doses.

Furthermore, the outcome of the macimorelin GHST showed a surprisingly high agreement with the outcome of the two standard GHSTs as well as the final diagnose as assessed by the Principal Investigator. In C3, GH secretion was

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stimulated evidently in all 8 patients. Finally, the outcome of the macimorelin GHST applied as a single test showed agreement with the outcome of the combination of the two sGHSTs as well as with the PI assessment in 7 of 8 subjects.

5 **Example 1: A macimorelin containing composition for diagnosing CGHD**

The macimorelin containing composition comprises the following ingredients as listed in Table 1.

Table 1: Composition per unit dose

Composition	Unit quantity	Percentage Quantity
Macimorelin (content = 100%)	63.6 mg	3.5%
Lactose monohydrate, spray-dried	1691.8 mg	93.1%
Crospovidone, Type A	36.3 mg	2.0%
Colloidal silicon dioxide	1.8 mg	0.1%
Sodium stearyl fumarate	18.2 mg	1.0%
Saccharin sodium, dihydrate	5.5 mg	0.3%
Total	1817.2mg	100.0%

10 One macimorelin dose unit consists of 1817.2 mg containing composition for preparation of an oral suspension in water. Typically, the prepared suspension contains 0.5 mg macimorelin per mL suspension.

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A body weight adjusted aliquot of the reconstituted suspension is administered to the pediatric subject, resulting in a dose of 1.0 mg/kg body weight in children. It is to be noted that in adults a body weight adjusted aliquot of the reconstituted suspension is administered, resulting in a dose of 0.5 mg/kg body weight of the adult subject.

Said unit dose is defined for a macimorelin calculated as a free base with a content of 100%. The mass of the macimorelin free base or its free base equivalent within said unit dose is adjusted according to the content.

Macimorelin can be included in said unit dose as a suitable pharmaceutical salt. Examples for suitable pharmaceutical salts are the acetate salt and the trifluoroacetate salt.

Said unit dose might be filled into a suitable container for easy availability of the GHD test. Examples for a suitable container are a sachet or containers of suitable size made of glass or plastic.

For a subject of 60 kg, the container is an sachet made of polyethylene laminated aluminum foil with macimorelin containing composition comprises 63.6 mg macimorelin, 1691.8 mg spray-dried lactose monohydrate, 36.3 mg crospovidone Type A, 1.8 mg colloidal silicon dioxide, 18.2 mg sodium stearyl fumarate, and 5.5 mg saccharin sodium dihydrate. When reconstituted in 120 mL of water, 2 mL provides 1.0 mg macimorelin.

Example 2: Use of Saccharin in the macimorelin containing composition to mask bad taste

In a multicenter, randomized, two-way, crossover study, 100 subjects with confirmed adult growth hormone deficiency (AGHD) received GHRH + L-Arg and macimorelin as GHSTs to determine the diagnostic efficacy of macimorelin for AGHD.

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This study was conducted in two parts. In the first part, the macimorelin containing composition described in Example 1, and in a dosing of 0.5 mg/kg, but without saccharin was used and mild to moderate bad taste was reported by 12 out of 52 (21%) subjects.

5 During a study halt, saccharin was found to be a suitable taste masking agent, although the bitter taste was still not fully masked. In the second part of the study, the macimorelin containing composition described in Example 1 (with saccharin) was used, and only 1 out of 48 (2%) test subjects reported bad taste in mild intensity.

10 **Example 3: Pharmacokinetic, Pharmacodynamic as well as explorative test characteristics of macimorelin as a diagnostic test**

Study AEZS-130-P01 was an open label, group comparison, dose escalation trial to investigate safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of macimorelin acetate after single oral dosing of 0.25 mg/kg, 0.5 mg/kg, and
15 1 mg/kg in pediatric patients with suspected GHD.

The objectives were defined as following:

Primary:

- To investigate the safety and tolerability of macimorelin acetate after ascending single oral doses of macimorelin in pediatric patients with
20 suspected GHD.

Secondary:

- To investigate the PK of macimorelin acetate in pediatric patients with suspected GHD,
- To investigate the PD of macimorelin acetate as measured by growth
25 hormone (GH) release in pediatric patients with suspected GHD,

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- To explore the PK/PD relationship following single oral dose administration of macimorelin acetate in pediatric patients with suspected GHD.

Methodology:

5 Plasma concentrations of macimorelin and serum concentrations of GH and were analyzed in central laboratories.

Macimorelin plasma concentrations: the analysis of plasma samples for macimorelin concentration was carried out at a central laboratory, Prolytic GmbH, Germany, using a validated liquid chromatography-mass spectrometry (LCMS/MS) method with a detection limit of 0.2 ng/mL (Franz, 2005).

10 Preliminary pharmacokinetics (PK) were determined by: time of maximum measured concentration ($t_{\max}$) and maximum concentration ($C_{\max}$) of macimorelin plasma concentrations in the sampling period.

GH serum concentration: the analysis of serum samples for GH concentration was carried out at a central laboratory by a validated immunochemiluminometric assay
15 (IDS-iSYS Human Growth Hormone (hGH), Immunodiagnostic Systems Ltd [UK]) (Manolopoulou et al., 2012). This assay is standardized to the recombinant growth hormone calibration standard WHO 98/574, and complies with recommendations on assay standardization as outlined by Clemmons (Clemmons et al., 2011).

20 The analytical laboratory applied for GH was: Central Laboratory Synevo Łódź, Krakusa Str. 28, 93-515 Łódź. Poland. The lower limit of quantification was < 0.05 ng/mL.

Number of Patients

Overall, macimorelin was administered in a single oral dose to 24 pediatric
25 patients with suspected GHD, with 8 patients per dose group. Of these 8 patients, at least 3 patients per dose group were pre-pubertal (Tanner Stage I) and pubertal (Tanner Stage II-IV), respectively.

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Main criteria for patient inclusion:

Subjects had to meet all of the following criteria to be eligible for admission to the trial:

1. Male or female pediatric subjects from 2 to less than 18 years of age;
- 5 2. Suspected GHD based on auxological and clinical criteria;
3. Indication for the performance of provocative growth hormone stimulation test (GHST).

10 A subject with sex steroid priming prior to GHSTs that are part of the standard diagnostic procedures must also have sex steroid priming for the macimorelin GHST.

Investigational Medicinal Product (IMP) Macimorelin

A single-use aluminum pouch (synonymous: sachet) contained 63.6 mg macimorelin, which provides 0.5 mg/mL of macimorelin when dissolved in 120 mL of water.

15 Sequential cohorts of trial participants received macimorelin at ascending single oral doses: i.e. 0.25 mg/kg body weight in Cohort 1 (C1), 0.5 mg/kg body weight in Cohort 2 (C2), and 1 mg/kg body weight in Cohort 3 (C3).

20 For determination of macimorelin PK / PD, blood samples were taken at pre-dose, then 15, 30, 45, 60, 90, 120 and 360 minutes after the administration of macimorelin.

As a test, a single oral dose of macimorelin acetate was administered to a patient on the day of the macimorelin GHST.

Macimorelin GHST Preparation

Macimorelin oral suspension was prepared by trial personnel and dosed according to the following instructions (Step #1- Step #5), considering here as example the dose of cohort C3 (i.e.; 1.0 mg/kg):

- 5 1. Weigh the patient and determine the number of pouches/sachets needed (one pouch will be required for a patient). Body weight (recorded in kg) will be rounded to the closest integer;
2. Dissolve the entire contents of the pouch in 120 mL of water (i.e. one pouch in 120 mL, two pouches in 240 mL, as applicable) in a suitable transparent
10 glass or polypropylene container and stir gently for at least 3 minutes (a small amount of undissolved particles will remain);
3. Based on the macimorelin dose of 1.0 mg/kg, determine the required volume of the suspension which corresponds to the patient body weight, i.e. required volume of suspension is 2 mL/kg (for example, a 30 kg patient
15 requiring macimorelin dose of 1.0 mg/kg will require 60 mL of the prepared suspension);
4. Measure the required volume for the patient by using a graduated syringe and transfer it to a drinking glass (volumes below 20 mL should be administered with an oral syringe to minimize the risk of losses from
20 incomplete emptying; see below for instruction);
5. The suspension must be used within 30 minutes after preparation.

Fasting prior to the GHST

Patients had to be fasting for 8 hours prior to the start and throughout the sampling period of the macimorelin stimulation test.

25 Administration

The administration of the macimorelin oral suspension was done under the supervision of trial personnel. The patient was advised to drink - over a period of

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no more than 30 seconds - the entire content of the glass container prepared in Step #4 of the cohort specific dosing instructions as given above.

Blood sampling

5 Blood samples were collected at the following time-points: pre-dose (sampling time window: +/- 15 minutes), then 15, 30, 45, 60, 90, 120 minutes (+/- 5 min window) and 360 minutes (+/- 10 minutes window) after administration of macimorelin. Serum concentrations of GH and plasma concentrations of macimorelin were analyzed in central laboratories.

10 The investigational macimorelin GHST was performed after the first sGHST had been completed. A recovery period of at least 1 week and a maximum of 4 weeks was introduced between GHSTs to avoid carry-over effects or interference between subsequent GHSTs and to provide an adequate follow-up period for observation of possibly drug-related adverse events to the previously used provocative agents.

15 Standard GHSTs used in Study P01

Two standard growth hormone stimulation tests (sGHSTs) had to be performed in a patient according to local practice. The sGHST agents were considered as 'background' and not as IMPs.

20 The following pharmacological agents were accepted for sGHSTs: insulin (insulin tolerance test (ITT)), arginine, arginine/growth hormone releasing hormone (GHRH), clonidine, glucagon, L-dopa.

Single dose of a sGHST agent was administered intramuscular (i.m.), intravenous (i.v.), subcutaneous (s.c.) or peroral (depending on formulation) on the day of the sGHST. Batch numbers were recorded on-site in Patient Records and 'standard
25 GHST Patients Accountability Logs'.

Criteria for Evaluation

Trial Endpoints

SAFETY AND TOLERABILITY

- 5 • Patientive tolerability (including acceptability of taste and impact on sleep, appetite, and gastrointestinal symptoms), Adverse events (AEs);
- Determination of changes in laboratory parameters which are relevant to safety;
- Influence on vital parameters (pulse rate, blood pressure, ECG).

10 PHARMACOKINETICS

- Concentration-time profiles of macimorelin;
- Target parameters: AUC, C_{max} , T_{max} , $T_{1/2}$.

PHARMACODYNAMICS

- Concentration-time profiles of GH;
- 15 • Target parameters: C_{max} , T_{max} ;
- Preliminary PK/PD: T_{max} for macimorelin vs. T_{max} for GH; C_{max} for macimorelin vs. C_{max} for GH.

OTHER

- 20 • Establishment of a recommended dose for diagnostic purposes in pediatric patients with suspected GHD;
- Exploration of a suitable GH cut-off point for a subsequent testing to establish the diagnosis of GHD in pediatric patients.

Statistical methods:

25 All statistical analyses were considered exploratory in nature. Datasets were analyzed using SAS version 9.3 or above.

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In general, summary statistics (n, arithmetic mean, standard deviation, median, minimum, and maximum) for quantitative variables and frequency tables for qualitative data were presented by dose group.

5 Macimorelin PK: PK parameters were analyzed for the PK Analysis Set (PKS) and are summarized by n (number of measurements), arithmetic mean, standard deviation and coefficient of variation (CV), median, minimum, maximum value and, in addition (T_{max} excluded) by geometric mean, geometric standard deviation, and geometric CV. For T_{max} additionally frequency counts as well as median, minimum, and maximum are presented.

10 Macimorelin PD: irrespective of the availability of PK data, GH concentration data were analyzed for the PD Analysis Set (PDS). GH peak concentrations were correlated with the outcome of the clinical diagnostic procedure (diagnosis of GHD confirmed or not confirmed).

15 PK/PD Analysis: Plasma concentrations of macimorelin of individual patients were correlated with the respective GH concentrations at the same time points, as well as the outcome of the clinical diagnostic procedure (diagnosis of GHD confirmed or not confirmed).

Results and Conclusions:

20 Out of a total of 27 patients screened, 24 patients were administered the macimorelin test, with 8 patients in each of the three dosing cohorts (C1, C2, and C3).

Thus, the safety population (SAF) as well as the PK analysis set (PKS), PD analysis set (PDS), and PK/PD set consisted of 24 patients.

Baseline characteristics:

25 In total, 17 (70.8%) of patients were male, 7 (29.2%) female, and 100% were of white origin. At screening, the median parameters for all three dosing cohorts were for age 10.5 years (range: 4 - 15 years), height 123.35 cm (range:

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46.0 - 152.5 cm), weight 25.5 kg (range: 12 - 43 kg) and body mass index (BMI) 16.1 kg/m² (range: 12.4 - 21.4 kg/m²).

The Tanner status was distributed as following: in C1 as well as C3, 4 patients showed Tanner I and 4 patients Tanner II, and in C2 5 patients showed Tanner I and 3 patients Tanner II. Sex steroid priming was applied in two male patients in C3 by i.m. administration of a testosterone depot preparation.

Concerning the baseline medical history, only for two patients in C2 a deficiency of other pituitary axes (i.e., thyroidal deficiency) was reported. As part of the standard 'diagnostic work-up', IGF-1 and IGF-BP3 values were captured in the electronic case report form (eCRF) as collected according to the local diagnostic standard.

IGF-1 values were presented for 7 patients in C1, and for 8 patients each in C2 and C3, with a median of 88.00 µg/L (SD 68.72) in C1, 100.00 µg/L (SD 97.90) in C2, and 119.50 µg/L (SD 68.88) in C3. IGF-BP3 values became available for one patient in C3.

The bone age showed in median a value of 102.2 months (range: 24 – 156 months). As part of auxology parameters, height SDS was in median -2.35 (range -3.2 – 1.7), BMI SDS -0.60 (range -2.1 – 2.0), and annualized height velocity SDS -1.50 (range: -3.3 – 0.5).

SGHSTs: Overall, the ITT was administered to 22 patients (i.e. to 5 patients (20.8%) at visit 1 (V1) and 17 patients (70.8%) at visit 3 (V3)), arginine to 8 patients (33.3%) at V1, and clonidine to 16 patients (i.e., 11 patients (45.8%) at V1 and 5 patients (20.8%) at V3). Glucagon was administered to one patient only, and L- dopa was not administered at all.

Treatment compliance for macimorelin was 100% in all three cohorts.

Pharmacokinetic and Pharmacodynamic results:Plasma concentration data

5 In general, macimorelin plasma concentrations showed a dose-dependent increase (Figure 1) with high inter-individual variability. Following administration of macimorelin the plasma concentrations showed a rapid increase with maximum levels observed between 0.25 and 2 h. At the last sampling point 6 h after administration, plasma concentrations had strongly declined.

Pharmacokinetic:

10 In general, macimorelin plasma concentrations showed a dose-dependent increase (Figure 1) with high inter-individual variability. Following administration of macimorelin the plasma concentrations showed a rapid increase with maximum levels observed between 0.25 to 2 h. At the last sampling point 6 h after administration, plasma concentrations had strongly declined.

15 The AUCs and $C_{\max}$ of macimorelin show a dose-dependent increase with an arithmetic mean AUC₀₋₆ of 6.69 h*ng/mL in C1, 18.02 h*ng/mL in C2, 30.92 h*ng/mL in C3, and an arithmetic mean $C_{\max}$ of 3.46 ng/mL in C1, 8.13 ng/mL in C2, and 12.87 ng/mL in C3 (Table 2).

20 Mean $T_{\max}$ is comparable between all three groups with an arithmetic mean of 45.5 min in C1, 40.6 min in C2, and 31.9 min in C3. Mean $T_{1/2}$ shows a slight increase with higher doses, i.e. 73.18 min in C1, 96.31 min in C2, and 102.85 min in C3.

Table 2: Summary of main Pharmacokinetic Parameters (PK Set, N=24)

Cohort	Statistics	AUC 0-6 (h*ng/mL)	C _{max} (ng/mL)	T _{max} (min)	T _{1/2} (min)
Cohort 1	n	8	8	8	4
	Arithmetic Mean (SD)	6.685 (3.093)	3.460 (1.783)	45.5 (32.8)	73.183 (29.437)
	Arithmetic CV (%)	46.273	51.543	72.1	40.225
	Min - Max	3.35 - 12.49	1.51 - 7.44	15 - 120	39.45 - 105.96
Cohort 2	n	8	8	8	7
	Arithmetic Mean (SD)	18.015 (9.800)	8.126 (4.176)	40.6 (22.3)	96.307 (41.031)
	Arithmetic CV (%)	54.399	51.393	54.8	42.604
	Min - Max	3.28 - 35.98	2.62 - 16.1	15 - 90	41.39 - 151.01
Cohort 3	n	8	8	8	8
	Arithmetic Mean (SD)	30.920 (11.510)	12.868 (3.011)	31.9 (5.3)	102.851 (19.938)
	Arithmetic CV (%)	37.227	23.402	16.6	19.385
	Min - Max	16.31 - 49.73	8.5 - 17.79	30 - 45	77.66 - 133.36

Pharmacodynamic:

- 5 As shown in Figure 2, GH concentration is increasing following macimorelin administration with a tendency to higher values with ascending dose. The large inter-patient variability is to be expected in the observed population with suspect of having GHD.

10 Following macimorelin administration, peak GH levels were observed in C1 within 0.5 – 1 h (mean T_{max} at 52.5 min (SD 11.3)), in C2 within 0.25 – 1 h (mean T_{max} 37.5 min (SD 13.9)) , and in C3 within 0.5 – 0.75 h (mean T_{max} 37.5 min (SD 8.0)) (cf. Table 3).

Table 3: Summary of Pharmacodynamic Parameters (PK/PD Set, N=24)

Cohort	Statistics	C _{max} (ng/mL)	T _{max} (min)
Cohort 1	n	8	8
	Arithmetic Mean (SD)	9.791 (6.226)	52.5 (11.3)
	Arithmetic CV (%)	63.585	21.6
	Median	9.195	60.0
	Min - Max	0.51 - 21.73	30 - 60
Cohort 2	n	8	8
	Arithmetic Mean (SD)	14.590 (8.046)	37.5 (13.9)
	Arithmetic CV (%)	55.149	37.0
	Median	13.040	37.5
	Min - Max	5.06 - 27.42	15 - 60
Cohort 3	n	8	8
	Arithmetic Mean (SD)	29.533 (18.829)	37.5 (8.0)
	Arithmetic CV (%)	63.757	21.4
	Median	21.100	37.5
	Min - Max	11.35 - 59.73	30 - 45

Exploratory Analyses for the GH cut-off point:

- 5 Peak GH values by GHD diagnosis were compared based on GHST results and investigator's assessment. Diagnostic characteristics of GH values (i.e., sensitivity, specificity, and Youden indices (non-weighted, weighted)) tested as cut-off points were listed with the most solid expression of diagnostic characteristics to be noted for C1 at a peak GH of 10.03 ng/mL, for C2 at a peak
- 10 GH of 10.43 ng/mL, and for C3 at 17.13 ng/mL.

The diagnostic outcome of the GHSTs is presented in Table 4. In this table, the diagnostic outcome of the sGHST is considered as 'confirmed', if both sGHSTs are available and both resulted in a peak GH $\leq$ 7 ng/mL or 'not confirmed', if at least one of the peaks is above 7 ng/mL. The outcome 'not confirmed' is

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categorized to 'excluded', if both sGHST results are available and the GH peaks are above 7 ng/ml, and to 'equivocal', if the case does not fit to any of the above described. The investigator's assessment is based on local diagnostic standard practice. The macimorelin GHST was tested against a cut-off point calculated from the individual peak GH values.

Table 4: Diagnostic Outcome of GHSTs (Number of Subjects = 24)

Cohort	Cut-off GH for Macimorelin GHST (ng/mL)	Patient	Peak GH (ng/mL) after Macimorelin GHST	Macimorelin GHST	Investigator's assessment	sGHSTs
C1	10.030	HU01-01	8.38	Confirmed	Not Confirmed	Not Confirmed (Equivocal)
		HU01-02	8.61	Confirmed	Confirmed	Not Confirmed (Equivocal)
		HU02-01	0.51	Confirmed	Not Confirmed	Not Confirmed (Equivocal)
		HU02-02	21.73	Not Confirmed	Not Confirmed	Not Confirmed (Excluded)
		HU02-03	5.22	Confirmed	Confirmed	Not Confirmed (Equivocal)
		HU02-04	10.03	Confirmed	Confirmed	Confirmed
		HU03-01	14.07	Not Confirmed	Not Confirmed	Not Confirmed (Excluded)
		HU03-02	9.78	Confirmed	Not Confirmed	Not Confirmed (Equivocal)
C2	10.430	BY01-01	5.06	Confirmed	Confirmed	Not Confirmed (Excluded)
		PL03-01	20.73	Not Confirmed	Not Confirmed	Not Confirmed (Excluded)
		RS01-01	7.41	Confirmed	Confirmed	Confirmed
		UA02-01	10.43	Confirmed	Confirmed	Confirmed
		UA02-02	21.36	Not Confirmed	Not Confirmed	Not Confirmed (Excluded)

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Cohort	Cut-off GH for Macimorelin GHST (ng/mL)	Patient	Peak GH (ng/mL) after Macimorelin GHST	Macimorelin GHST	Investigator's assessment	sGHSTs
		UA03-01	16.41	Not Confirmed	Not Confirmed	Not Confirmed (Excluded)
		UA03-02	9.67	Confirmed	Confirmed	Confirmed
		UA03-03	27.42	Not Confirmed	Confirmed	Confirmed
C3	17.130	PL03-02	17.13	Confirmed	Confirmed	Confirmed
		RS01-04	14.46	Confirmed	Confirmed	Confirmed
		RU01-01	59.73	Not Confirmed	Not Confirmed	Not Confirmed (Excluded)
		UA04-01	49.16	Not Confirmed	Not Confirmed	Not Confirmed (Equivocal)
		UA04-02	25.07	Not Confirmed	Not Confirmed	Not Confirmed (Excluded)
		UA04-03	14.63	Confirmed	Not Confirmed	Not Confirmed (Equivocal)
		UA06-01	11.35	Confirmed	Confirmed	Confirmed
		UA06-02	44.73	Not Confirmed	Not Confirmed	Not Confirmed (Equivocal)

Based on the considerations outlined above, Table 4 is presenting agreement between principal investigator's (PI's) assessment and outcome of both sGHSTs: in 21 (87.5%) patients (*i.e.*, 8 confirmed and 13 not confirmed) there is an agreement between investigators assessment and sGHST outcomes. In 3 (12.5%) patients investigator concluded GHD, while sGHSTs excluded (in 1 patient) the diagnosis or were equivocal (in 2 patients).

Furthermore, the diagnostic results can be summarized as following (Table 5): the macimorelin GHST shows 'GHD not confirmed' only in 1 (9.09%) patient in C2 from overall 11 patients assessed as 'GHD' by the investigator in all three cohorts.

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From a total of 13 patients assessed by the investigator as 'not confirmed' of having GHD, the macimorelin GHST confirmed GHD in 3 (23.08%) patients in C1 and in 1 (7.69%) patient in C3, respectively.

Table 5: Summary of Diagnostic Results of Macimorelin GHST vs. sGHST and vs. Investigator's Assessment

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		PI's Assessment		sGHST	
	Macimorelin GHST	GHD (N=11)	Non GHD (N=13)	Confirmed (N=8)	Not Confirmed (N=16)
Cohort 1	Confirmed, n (%)	3 (27.27 %)	3 (23.08 %)	1 (12.50 %)	5 (31.25 %)
	Not Confirmed, n (%)	0	2 (15.38 %)	0	2 (12.50 %)
	Total, n (%)	3 (27.27 %)	5 (38.46 %)	1 (12.50 %)	7 (43.75 %)
Cohort 2	Confirmed, n (%)	4 (36.36 %)	0	3 (37.50 %)	1 (6.25 %)
	Not Confirmed, n (%)	1 (9.09 %)	3 (23.08 %)	1 (12.50 %)	3 (18.75 %)
	Total, n (%)	5 (45.45 %)	3 (23.08 %)	4 (50 %)	4 (25 %)
Cohort 3	Confirmed, n (%)	3 (27.27 %)	1 (7.69 %)	3 (37.50 %)	1 (6.25 %)
	Not Confirmed, n (%)	0	4 (30.77 %)	0	4 (25 %)
	Total, n (%)	3 (27.27 %)	5 (38.46 %)	3 (37.50 %)	5 (31.25 %)

Considering the data presented above, strongest test characteristics for the macimorelin test is observed in C3: GH secretion was stimulated evidently in all 8 patients. Finally, the outcome of the macimorelin GHST applied as a single test showed agreement with the outcome of the combination of the two sGHSTs as well as with the PI assessment in 7 of 8 subjects.

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Receiver Operating Characteristic (ROC) Analysis

For all GH cut-off points tested, the ROC curve for C1 shows the lowest sensitivity and specificity if being compared with C2 and C3 (Figure 3). The related area under the curve (AUC) is increasing with ascending dose.

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When comparing the characteristics of GH cut-off points between the three cohorts, the cut-off point of 17.130 ng/mL GH for C3 shows the strongest test characteristics with a sensitivity of 1.0, specificity of 0.8, Youden indices ≥ 0.80 , and a ROC AUC of 0.93 (cf. Table 6).

- 5 A sensitivity analysis was performed observing the ROC AUC development based on the test outcome of the sGHSTs categorized as 'confirmed' vs. 'not-confirmed'. Again, strongest test characteristics are expressed for C3, with e.g. a sensitivity of 1.00, specificity of 0.80, and a ROC AUC of 0.933, in comparison with C2 (sensitivity 0.75, specificity 0.75, ROC AUC 0.563) and C1 (sensitivity 1.0, specificity 0.71, ROC AUC 0.714).
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Table 6: Summary of ROC Analysis: AUC and Characteristics of Cut-off Points by Cohort (PDS, N=24)

Statistics	Cohort 1 (N=8)	Cohort 2 (N=8)	Cohort 3 (N=8)
Peak GH cut-off point (ng/mL)	10.030	10.430	17.130
Specificity	0.40	1.00	0.80
Sensitivity	1.00	0.80	1.00
Youden-index	0.40	0.80	0.80
Weighted Youden-index (w=0.6)	0.52	0.76	0.84
Weighted Youden-index (w=0.7)	0.64	0.72	0.88
NPV	1.00	0.75	1.00
PPV	0.50	1.00	0.75
ROC AUC	0.60	0.80	0.93

NPV = negative predictive value, PPV = positive predictive value

PK and PD Summary

Overall, the macimorelin PK and PD of C1, C2, and C3 show comparable profiles:

- the macimorelin T_{max} is comparable for all three groups, with mean T_{max} values of about 0.5 to 0.75 h;
- the mean macimorelin C_{max} shows a dose-proportional increase;
- the AUC increases with the macimorelin dose;
- maximum GH release is observed at 0.25 to 2 h after macimorelin administration, with mean T_{max} values of about 0.5 to 1 h.

Maximum values for AUC and C_{max} are observed in C3 at a macimorelin dosing of 1.0 mg/kg body weight. Furthermore, the sensitivity analysis supports the dosing in C3 with strongest test characteristics expressed at a cut-off point of approximately 17 ng/mL GH, with a specificity of 0.80, a sensitivity of 1.00, a Youden-index of 0.80 and an ROC AUC of 0.933.

Safety results:

Overall, 88 AEs were recorded in 23 patients of the SAF, i.e. 27 events in 8 patients of C1, 28 events in 8 patients of C2, and 33 events in 7 patients of C3.

A total of 70 treatment emergent adverse events (TEAEs) was recorded in 21 patients of the SAF, i.e. 22 events in 8 patients of C1, 24 events in 6 patients of C2, and 24 events in 7 patients of C3.

None of the TEAEs was reported as related to the macimorelin test.

No SAEs or serious TEAEs were reported in the course of this trial. None of the AEs or TEAEs reported led to a withdrawal of a patient.

Majority of AEs was related to the ITT, i.e. 62 (70.5%) events in 21 (91.3%) of the patients. Clonidine related AEs (13 (14.8%)) were observed in 7 (30.4%) of the patients, and an arginine related AE was reported in 1 (4.3%) patient. It is to be noted that the ITT was administered to 22 patients, arginine to 8 patients (33.3%),

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clonidine to 16 patients, and Glucagon to one patient only.

As AEs were reported infectious diseases, a single case of anal fissures, and side effects of the test agents used for the sGHSTs.

5 AEs were mostly of mild to moderate intensity. ITT related AEs comprised symptoms of hypoglycemia (e.g. tremor, sweating), which is a clinical endpoint of this sGHST. Related to clonidine, hypotension related symptoms were reported, which are known side effects of this sGHST agent.

10 Severe intensity was reported for TEAEs of one patient in Cohort 1: 5 AEs (i.e., palpitations, tachycardia, hunger, hyperhidrosis, and tremor) as part of the expected hypoglycemia occurred in patient HU01-01 during an ITT.

Clinical laboratory, vital signs, physical findings, ECG:

No clinically significant changes are observed for the safety clinical laboratory parameters, vital signs and physical examination. No clinically significant abnormalities and no significant changes in ECG parameters are described.

15 Tolerability Questionnaire:

The GHST Tolerability Questionnaire was to be completed by the patient or the parent/legal guardian.

Overall, in majority agreement or strong agreement to pre-defined statements was to be noted for macimorelin in all three dosing groups.

20 In C1, disagreement was described in one case related to 'acceptable taste' and in one case related to 'stomach felt well on the following day'. In one case strong disagreement was ticked for 'bowel movement on the following day'.

No strong disagreement or disagreement was stated on any of the macimorelin test related questionnaires of C2.

25 One case of disagreement to 'acceptable taste' was described in C3.

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As a handwritten comment, 'bitter taste' was reported by two patients following macimorelin GHST. None of these comments were assessed as AE by the investigator.

5 Overall, the feedback provided by patients or parents/legal guardians following the macimorelin test demonstrates the good tolerability and safety of the test.

Conclusions:

10 This trial was performed to investigate the safety, tolerability, PK and PD of macimorelin acetate after single oral dosing of 0.25 mg/kg, 0.5 mg/kg, and 1.0 mg/kg in pediatric patients with suspected GHD. Furthermore, it served to identify a suitable macimorelin dose for further testing in a test validation trial, and to explore a GH cut-off point for testing.

For all three dosing cohorts, C_{max} and T_{max} for macimorelin plasma levels were found to be in the range expected from the adult development program.

15 In the pediatric population observed, a dose-dependent increase of macimorelin C_{max} (3.46 vs. 8.13 vs. 12.87 ng/mL) as well as of the mean AUC₀₋₆ (6.69 vs. 18.02 vs. 30.92 h*ng/mL) was observed. The elimination half-lives $t_{1/2}$ were in the range of 1.25 – 1.75 h.

In general, the PK parameters in the pediatric population were in a similar range to those in adults.

20 A macimorelin dose of 0.25 mg/kg (C1) has not resulted in a maximum stimulation of GH secretion in children, which becomes evident in the review of PK/PD data as well as comparison with the agreement between an explored GH cut-off point versus PI assessment and results of sGHSTs.

25 A dose of 0.5 mg/kg (C2) showed strong GH release, a high level of agreement between the principal investigator (PI) assessment based on the macimorelin GHST vs. sGHST as well as a ROC AUC of 0.80. However, a dose of 1.0 mg/kg (C3) appears to lead to a more consistent, strong GH stimulation, most probably

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due to sufficiently high macimorelin exposure in all subjects.

Finally, the sensitivity analysis supports the dosing in C3 with strongest test characteristics expressed at a cut-off point of approximately 17 ng/mL GH, with a specificity of 0.80, a sensitivity of 1.00, a Youden-index of 0.80 and a ROC AUC of 0.93.

Furthermore, the outcome of the macimorelin GHST showed a surprisingly high agreement with the outcome of the two standard GHSTs as well as the final diagnose as assessed by the Principal Investigator. In C3, GH secretion was stimulated evidently in all 8 patients. Finally, the outcome of the macimorelin GHST applied as a single test showed agreement with the outcome of the combination of the two sGHSTs as well as with the PI assessment in 7 of 8 subjects.

In conclusion, in all three dosing cohorts, macimorelin has shown good safety and tolerability without any AEs reported in the population observed. PK and PD profile are in a range expected from the adult development program. The overall characterization of macimorelin in this first pediatric trial supports the choice of a macimorelin dose of 1.0 mg/kg for the investigation in a Phase 3 trial on test validity.

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