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Glepaglutide, a Long-Acting Glucagon-like Peptide-2 Analogue, Reduces Parenteral Support in Patients With Short Bowel Syndrome: A Phase 3 Randomized Controlled Trial

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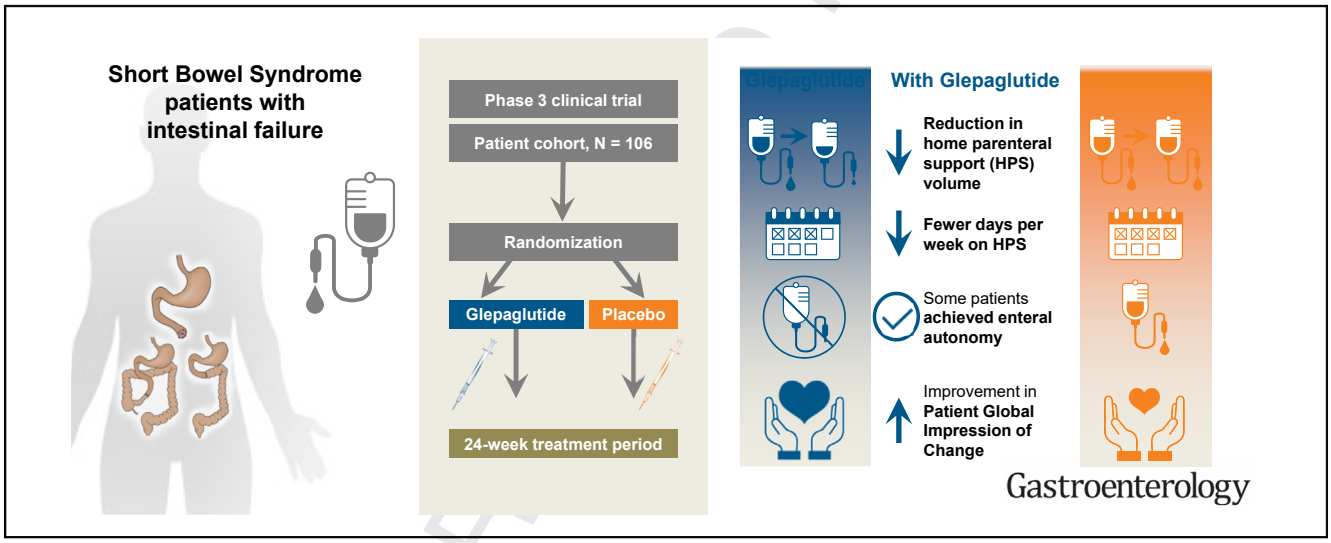

BACKGROUND & AIMS: Glepaglutide is a long-acting glucagon-like peptide (GLP)-2 analogue developed to improve intestinal absorption in patients with short bowel syndrome (SBS). The authors conducted a trial to establish the efficacy and safety of glepaglutide in reducing parenteral support (PS) needs in patients with SBS with intestinal failure. **METHODS:** In an international, placebo-controlled, randomized, parallel-group, double-blind, phase 3 trial, patients with SBS with intestinal failure requiring PS ≥3 d/wk were randomized 1:1:1 to 24 weeks of glepaglutide 10 mg twice weekly or once weekly or placebo. PS volume was equivalently reduced if mean urine volume of a 48-hour balance period exceeded baseline values by >10%. **RESULTS:** One hundred six patients were randomized and dosed. Glepaglutide twice weekly significantly reduced weekly PS volumes from baseline to week 24 vs placebo (mean change, −5.13 vs −2.85 L/wk; $P = .0039$; primary end point). Results were similar across major anatomic subgroups. Glepaglutide twice weekly was also superior to placebo for key secondary end points of proportion of patients achieving clinical response, defined as ≥20% PS volume reduction from baseline to weeks 20 and 24 (65.7% vs 38.9%; $P = .0243$) and patients achieving a reduction in days on PS ≥1 d/wk from baseline to week 24 (51.4% vs 19.4%; $P = .0043$). Complete PS weaning ("enteral autonomy") was achieved for 5 patients (14%) receiving glepaglutide twice weekly vs 0 for patients receiving placebo. No statistically significant differences were found for glepaglutide once weekly vs placebo for primary or key secondary end points. Significant glepaglutide benefits on patient-reported outcome (Patient Global Impression of Change) were found. Glepaglutide was assessed to be safe and well tolerated. **CONCLUSIONS:** Glepaglutide treatment in patients with SBS with intestinal failure resulted in clinically relevant reductions in PS requirements and was well tolerated. (ClinicalTrials.gov, Number: NCT03690206; ClinicalTrialsRegister.eu, Number: 2017-004394-14).

Keywords: Glepaglutide; Short Bowel Syndrome; Clinical Trial.

Short bowel syndrome (SBS; defined as <200 cm of functional small intestine[1–3]) is characterized by reduced intestinal absorptive capacity due to extensive surgical bowel resection or congenital diseases.[1,4–6] This impairs the ability of affected individuals to maintain fluid and metabolic balances when receiving a conventional diet and, if left untreated, can lead to dehydration; malnutrition; metabolic diseases; and weight loss.[7,8] Many patients with SBS also have severe diarrhea and large stomal losses as a consequence of their impaired absorption, which has negative impact on their social interaction, emotional well-being, and quality of life due to fear of fecal incontinence and stoma bag leakage.[9–12]

SBS is a highly heterogenous disease. Some patients with SBS are able to adapt physiologically and may further compensate for their malabsorption by hyperphagia, a condition also referred to as intestinal insufficiency or non–parenteral support (PS)-dependent SBS.[13] More severely affected individuals depend on the safe and well-adjusted provision of PS consisting of nutrients, fluids, electrolytes, vitamins, and trace elements to maintain body function, homeostasis, and health, a condition also referred to as intestinal failure (IF) or PS-dependent SBS.[2] For those dependent on PS it is life-sustaining, but at the same time can potentially be associated with life-threatening complications when used as a long-term treatment. Among these complications are sepsis (mainly due to catheter-related bloodstream infections),[14] central vein thrombosis, IF-associated liver disease,[15] and renal impairment.[16] In addition, PS administration is very time-consuming, and the treatment burden of PS is substantial and includes physical restrictions as well as social and emotional impacts.

Conventional pharmacologic treatment options for patients with SBS include antisecretory and gastrointestinal (GI) motility–modulating drugs. These drugs reduce loss of water and electrolytes, especially in patients with a high fecal output to minimize the symptoms and consequences of diarrhea.[17]

Over the past several decades, the GI tract has been recognized as the largest endocrine organ system in the body. The well-coordinated neuroendocrine gut–interorgan axis communication enables dynamic modulation of intestinal mucosal growth and function, regulation of GI secretions, motility, splanchnic blood, and lymph flow, as well as epithelial barrier function to ensure an optimal intestinal absorptive function. Among patients with SBS, those with distal bowel resections exhibit intestinal endocrine deficiencies due to reduced intestinal length and mucosal mass. This includes reduced numbers of specific mucosal sensory cells and associated low levels of circulating intestinal hormones, especially the glucagon-like peptide (GLP)-1, GLP-2, and polypeptide YY.[18] By studying both the pathophysiological consequences of endocrine dysregulation and the dynamic, progressive, adaptive recovery after diverse intestinal resections, key regulators of the multiple intestinal functions have been identified. In fact, such studies in patients with SBS have paved the way for the developments of pro-adaptive hormonal treatments in the form of GLP-2 analogues.[18,19]

GLP-2 is a specific, endogenous, intestinal, pro-adaptive factor that plays a key role in enhancing intestinal mucosal morphology, function, and integrity under normal and pathophysiological conditions. The introduction of GLP-2 analogue treatment has been a paradigm shift in the treatment of SBS, targeting the pathophysiology of SBS by aiming to reinforce the structural and functional integrity of the remaining intestine. Exogenous GLP-2 induces significant hyperplasia of the small intestinal mucosal epithelium

WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

Glepaglutide is a novel, long-acting, ready-to-use glucagon-like peptide-2 analogue in development for treatment of patients with short bowel syndrome.

NEW FINDINGS

In this randomized, placebo-controlled trial in patients with short bowel syndrome dependent on parenteral support, twice weekly dosing with glepaglutide significantly reduced the need for parenteral support (primary end point) and improved patient-reported outcome.

LIMITATIONS

The number of trial participants was limited by the relative rarity of the condition. The trial treatment period was limited to 24 weeks, but with an option to continue in an extension trial.

CLINICAL RESEARCH RELEVANCE

This pivotal phase 3 trial demonstrated the efficacy of twice weekly glepaglutide dosing in reducing or eliminating the need for parenteral support in the investigated patient population. In addition to primary outcomes, this is the first trial to demonstrate a significant treatment benefit of glucagon-like peptide-2 analogue treatment on patient-reported outcomes in patients with short bowel syndrome in a placebo-controlled phase 3 trial.

BASIC RESEARCH RELEVANCE

The efficacy and safety profile of glepaglutide was at least on par with that of shorter-acting members of the drug class, and the results add to the body of evidence supporting a positive benefit–risk balance for the use of glucagon-like peptide-2 analogue treatment to improve intestinal absorption in patients with short bowel syndrome.

Abbreviations used in this paper: **CiC, colon-in-continuity; GI, gastrointestinal; GLP, glucagon-like peptide; IF, intestinal failure; OW, once weekly; PGIC, Patient Global Impression of Change; PS, parenteral support; SAE, serious adverse event; SBS, short bowel syndrome; TW, twice weekly.**

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via stimulation of stem cell proliferation in the crypts and via inhibition of apoptosis in the villi.[20] Additional reported effects of GLP-2 include inhibition of accelerated GI motility and gastric acid hypersecretion, stimulation of nutrient absorption, enhancement of intestinal barrier function, and increased intestinal blood flow.[21–29] Degradation of GLP-2 by the enzyme dipeptidyl peptidase 4 occurs rapidly, with subsequent clearance via the kidney.[30] The short half-life of 5–7 minutes for circulating native GLP-2[31] is a significant practical limitation for its use in a therapeutic setting. This is improved for the currently marketed GLP-2 analogue teduglutide, which has a half-life in circulation of approximately 2 hours.[32] However, treatment is time-consuming due to the requirement for daily drug product reconstitution and dosing.

Glepaglutide is a novel, long-acting GLP-2 analogue in a stable, aqueous formulation for subcutaneous administration to treat patients with SBS. The stability in aqueous solution allows for dosing of glepaglutide as a ready-to-use liquid formulation. The mean effective half-life is 88 hours,[33] which enables extension of the dosing interval beyond daily dosing. This is the first phase 3 trial investigating the efficacy and safety of once weekly (OW) and twice weekly (TW) dosing of glepaglutide in reducing the need for PS in patients with SBS with IF.

Materials and Methods

All authors had access to the trial data and have reviewed and approved the final manuscript.

Trial Design

This was a multinational, placebo-controlled, randomized, parallel-group, double-blind, phase 3 trial to demonstrate the superiority of OW and TW subcutaneous injections of 10 mg glepaglutide vs placebo in stable patients with SBS with chronic IF. The trial (Clinicaltrials.gov, Number: NCT03690206) was conducted at 29 hospital centers across the United States (n = 7), the United Kingdom (n = 5), Belgium (n = 1), Canada (n = 3), Denmark (n = 2), France (n = 2), Germany (n = 5), the Netherlands (n = 1), and Poland (n = 3). Patients were randomized 1:1:1 via an interactive web response system to 24 weeks of treatment with either glepaglutide TW, glepaglutide OW, or placebo, with trial drug administered subcutaneously in either the abdomen or the thigh. Randomization was performed using a block randomization scheme stratified by the patient's weekly PS volume requirement at baseline (<12 vs ≥12 L/wk). To maintain blinding, all 3 treatment groups involved TW dosing (glepaglutide and/or placebo). The primary objective of the trial was to confirm the efficacy of glepaglutide in reducing or eliminating the need for PS.

The trial was carried out in accordance with the Declaration of Helsinki,[34] International Conference on Harmonisation guidelines,[35] and Good Clinical Practice.[36] An institutional review board or independent ethics committee approved the trial at each center, and all participants provided written informed consent before undergoing any trial-related procedures or assessments. The full trial protocol is included in the Supplementary Material.

Participants

Key inclusion criteria comprised diagnosis of SBS, defined as remaining small bowel in continuity of estimated length of <200 cm or 79 in (documented by either intraoperative notes or imaging)[1]; latest intestinal resection at least 6 months before screening and considered stable with regard to PS need; requirement for PS at least 3 d/wk; presence of a stoma or a colon-in-continuity (CiC); and aged 18–90 years. Thus, all patients were type III according to the European Society for Clinical Nutrition and Metabolism functional classification of chronic IF,[17] that is, metabolically stable patients with a chronic condition requiring PS over months or years. In addition, a number of exclusion criteria were defined (of which several were rechecked at time of randomization) to ensure the validity of efficacy assessments and to exclude patients with significant comorbidities that could bias the safety evaluation. These included having more than 2 SBS-related or PS-related hospitalizations within 6 months before screening; poorly controlled inflammatory bowel disease that was moderately or severely active, or fistula interfering with measurements or examinations required in the trial; bowel obstruction; known radiation enteritis or significant villous atrophy; cardiac disease within the last 6 months before screening; clinically significant abnormal electrocardiogram (ECG); acute or unstable chronic liver disease; history of colorectal cancer; severe hepatic impairment; use of GLP-1, GLP-2, dipeptidyl peptidase 4 inhibitors, human growth hormone, somatostatin, or analogues thereof within 3 months before screening; and unstable biological therapy within 6 months before screening. Patients with severe renal impairment were excluded, although no difference in glepaglutide pharmacokinetic properties has been reported between renally impaired subjects and subjects with normal renal function.[37] The full list of patient selection and randomization criteria is included in the Supplementary Material.

Procedures

After informed consent and initial confirmation of eligibility, patients entered a run-in phase consisting of PS optimization and stabilization phases spanning a total period of 1–2 months and taking place before randomization and dosing. Aiming at achieving predefined stabilization criteria for oral fluid intake and urine volume (for details see Supplementary Material), this was to ensure a reliable baseline for assessing the efficacy of glepaglutide treatment in reducing PS requirements.

During the subsequent 24-week treatment phase, patients were randomized 1:1:1 in a double-blind fashion to either glepaglutide 10 mg OW, glepaglutide 10 mg TW, or placebo. Investigational products were delivered as ready-to-use solutions in vials, from which the patients were to withdraw 0.5 mL using a single-use syringe and inject it subcutaneously. Dosing on visit days was done at the trial site after laboratory sampling and assessments. PS requirements were evaluated through the use of 48-hour balance periods pertaining to the 48 hours leading up to the treatment initiation visit, as well as leading up to the site visits at weeks 1, 2, 4, 8, 12, 16, 20, and 24 after treatment initiation. The balance periods involved a fixed drinking menu (individually predefined during the optimization phase) and measurements of urine volume. Based on the

balance periods, the PS volume could be adjusted according to the following predefined algorithm:

- If: daily urine volume of the current visit is at least 10% higher than baseline urine volume
- Then: new PS volume (weekly) = current PS volume (weekly) – 7 × absolute increase in daily urine volume from baseline.

The volume and content of PS used were recorded by the patient on an ongoing basis in an eDiary. The completeness and accuracy of eDiary data entry were reviewed by the investigator in dialogue with the patient at each trial visit. Urine production was to stay >1 L/d for all patients in accordance with treatment guidelines.[2] Once investigational product treatment had been initiated, PS volume could be adjusted at weeks 1, 2, 4, 8, 12, 16, 20, and 24, according to the aforementioned predefined algorithm. The composition of the PS was at the discretion of the investigator, and changes in PS volume outside of the algorithm were permitted if patient safety was at risk, for example, due to fluid overload or dehydration. The rationale for deviating from the algorithm was to be documented in the electronic case report form. Unscheduled visits (preceded by a 48-hour measurement period) could be considered by the investigator for adjustment of PS.

Other efficacy parameters included body weight and "Patient Global Impression of Change" (PGIC), which is a self-rated, 7-point, patient-reported outcome scale.[38,39] Other patient-reported outcome measures comprised the SBS Impact Scale (an SBS disease-specific, patient-reported outcome questionnaire in development by Zealand Pharma) and the EQ-5D-5L, which is an established, self-assessment, health-related, quality-of-life questionnaire.

Safety was assessed throughout the trial period. For immunogenicity, serum samples were analyzed for antibody development using a tiered approach (ie, screening, confirmation, and titration of confirmed anti-glepaglutide antibody–positive samples), followed by characterization of anti-glepaglutide antibody–positive samples for in vitro glepaglutide-neutralizing potential, for binding to the predominant active metabolite (M2),[33] and for cross-reactivity to GLP-2. In case of a positive result in the characterization assays, a titer was estimated. The binding antibody assays were DELFIA-based immunoassays using plates coated with drug for capturing potential anti-glepaglutide antibodies, which were subsequently detected using Europium-labeled protein A/G. "Screened positive" results were confirmed by means of immunodepletion with an excess amount of drug. For characterization of the antibodies with respect to binding to the predominant metabolite (M2) or cross-reaction with GLP-2, coating of plates and immunodepletion were conducted with M2 and GLP-2, respectively, instead of glepaglutide. The in vitro glepaglutide-neutralizing potential of the detected anti-drug antibodies was investigated in an activity assay based on cells expressing the GLP-2 receptor and using glepaglutide as a stimulatory agent.

Primary and Key Secondary End Points

The primary efficacy end point was the change in weekly PS volume from baseline to week 24. The following 4 key secondary end points were defined for the trial: (1) clinical response, defined as achieving at least a 20% reduction in weekly PS volume from baseline to both weeks 20 and 24; (2) reduction in days on PS ≥1 d/wk from baseline to week 24; (3) change in weekly PS volume from baseline to week 12; and (4) reduction in weekly PS volume of 100% (weaned off) at week 24.

Statistical Analysis

A parallel gatekeeping testing procedure (testing hierarchy) was applied to protect the overall type I error rate of α when testing the aforementioned primary and key secondary end points across the 2 glepaglutide treatment groups vs placebo.

The primary end point analysis applied a restricted maximum likelihood-based repeated-measures approach to compare treatment groups with respect to the mean change from baseline in weekly PS volume at week 24. The model used weekly PS volume assessments at weeks 1, 2, 4, 8, 12, 16, 20, and 24 (derived as the weekly PS volume received during a valid 7-day period) as a dependent variable, and included the covariates of treatment group, baseline weekly PS volume, visit (categorical variable), stratification factor (weekly PS volume requirements <12 vs ≥12 L/wk), and visit by treatment group interaction. Variance estimation was based on an unstructured covariance matrix within each treatment group. The primary comparisons were the contrasts (differences in least squares means) between the glepaglutide treatment groups and the placebo group at the week 24 visit in a linear normal model for repeated measures. The change over the entire 24-week treatment period was modeled, whereas the treatment effect was reported for the prespecified time points at 12 and 24 weeks after initiation of treatment. Missing values were imputed using a copy-reference multiple imputation method. Of the 4 key secondary end points, the 3 responder end points were analyzed using the Cochran-Mantel-Haenszel test adjusted for the stratification factor (baseline weekly PS volume requirement <12 vs ≥12 L/wk). Missing values for these end points were imputed as no response. For the remaining key secondary end point of change in weekly PS volume from baseline to week 12, the contrasts at week 12 were derived from the same statistical model as described for the primary end point. For PGIC, the difference in percentage of responders between each glepaglutide-treated group and placebo was tested using the Cochran-Mantel-Haenszel approach, adjusted for the stratification factor. Missing PGIC data were imputed with a nonresponse.

The trial was originally designed with a fixed sample size of 129 patients with SBS (43 patients planned for each of the 3 treatment groups). Because of recruitment slow-down and delays caused by the COVID-19 pandemic, the trial design was changed in March 2021 to a group sequential design, with 1 interim analysis to provide the possibility to stop early for efficacy or futility. With the continued uncertainty about the global prognosis for COVID-19 in 2022 and the possible consequences for trial conduct and completion, in January 2022, it was decided to revoke the planned interim analysis and finalize the trial including all patients screened at that time who would meet the randomization criteria. With the resulting final trial population of 106 patients, the power to show superiority on the primary end point for either OW or TW glepaglutide relative to placebo was approximately 95%.

Results

Patients

In total, 154 patients were screened and 141 patients were enrolled in the optimization and stabilization phase. Of these, 106 were randomized during the period from October 2018 to July 2022: 35 in each of the glepaglutide groups and 36 in the placebo group. In the glepaglutide TW group, 31 of 35 patients (89%) completed the 24-week treatment period, whereas 4 patients withdrew from the trial and did not attend the week 24 visit (2 because of respective adverse events of subileus and generalized hypersensitivity reaction and 2 were the patients' decisions). Of these 4 patients, 2 attended a follow-up visit. In the glepaglutide OW group, 34 of 35 patients (97%) completed treatment, whereas 1 patient discontinued treatment because of an adverse event of cholecystitis, but remained in the trial and attended the week 24 visit. All patients in the placebo group completed treatment. A patient disposition flowchart is presented in Figure 1.

Baseline characteristics were similar overall across treatment groups, with minor and clinically nonsignificant imbalances between groups observed for age and PS volume requirement at baseline (Table 1). Mean (SD) age at time of consent was 55.0 (12.4) years, and the gender distribution was approximately even; 54% were female. A total of 49% of patients had a jejunostomy (SBS anatomic group 1), 45% had a jejunocolonic anastomosis (SBS anatomic group 2), and the remaining 6% had a jejuno-ileo-colonic anastomosis (SBS anatomic group 3). Patients with a stoma comprised 55% of the trial population. The distribution of patients with a stoma was marginally uneven across treatment groups, with percentages of 49%, 57%, and 58% in the glepaglutide TW group, glepaglutide OW group, and placebo group, respectively. Length of remnant small bowel was similar across treatment groups. Mean (SD) weekly PS volume requirement at baseline was 14.4 (7.8) L/wk, distributed across a mean (SD) of 5.9 (1.5) d/wk.

Efficacy

Primary end point. For the primary end point of change in weekly PS volume from baseline to week 24, glepaglutide TW treatment significantly reduced PS requirements vs placebo (−5.13 L/wk; 95% CI, −6.24 to −4.02 L/wk vs −2.85 L/wk; 95% CI, −3.93 to −1.77 L/wk; estimated difference of −2.28 L/wk; 95% CI, −3.83 to −0.73 L/wk; $P = .0039$). The estimated mean relative PS volume reduction with glepaglutide TW was 45% vs 22% with placebo. No statistically significant difference was found for glepaglutide OW vs placebo (estimated difference of −0.91 L/wk; 95% CI, −2.52 to 0.71 L/wk; $P = .2700$). However, a clear dose–response relationship of increasing PS volume reduction with increasing glepaglutide dose was indicated (Figure 2). The conclusion of superiority for glepaglutide TW relative to placebo with respect to the primary end point was supported by the applied sensitivity analyses (Supplementary Table 2).

For subgroups defined by presence of CiC, the change in PS volume requirement with glepaglutide TW vs placebo appeared slightly more pronounced in patients without CiC (estimated difference of −2.65 L/wk; 95% CI, −4.86 to −0.43 L/wk) than in patients with CiC (estimated difference of −2.08 L/wk; 95% CI, −4.21 to 0.04 L/wk) within the trial period, but the overall treatment effect appeared similar between the 2 subgroups (Figure 3). In addition, estimated relative volume reductions were similar at 45%

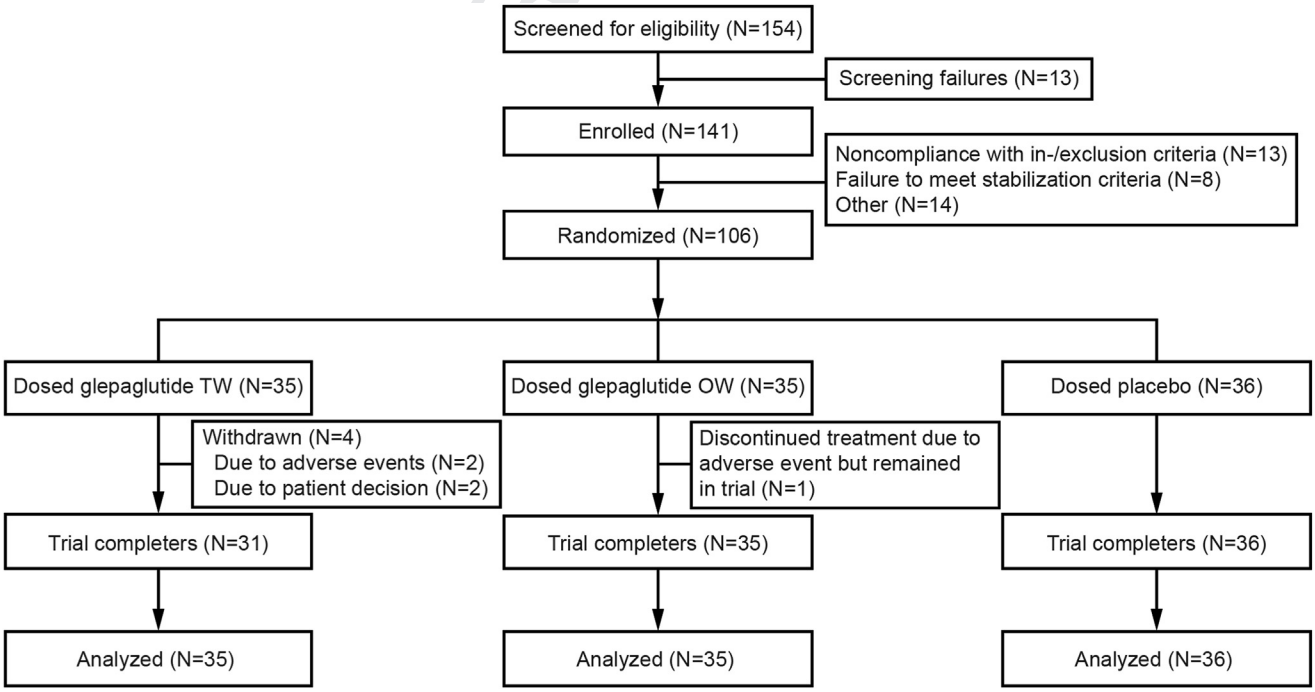


Figure 1. Trial flowchart.

Table 1. Baseline Characteristics

Characteristic	Glepaglutide 10 mg TW (n = 35)	Glepaglutide 10 mg OW (n = 35)	Placebo (n = 36)	Total (n = 106)
Age, *y*				
Mean (SD)	56.9 (13.4)	54.0 (12.0)	54.0 (11.8)	55.0 (12.4)
Range	20–82	22–74	34–81	20–82
Body mass index, kg/m^2				
Mean (SD)	23.6 (3.4)	22.7 (3.2)	23.5 (3.6)	23.3 (3.4)
Range	19.8–32.5	18.3–32.5	15.1–33.9	15.1–33.9
Women, n (%)	19 (54)	18 (51)	20 (56)	57 (54)
Underlying cause of SBS, n (%)				
Crohn's disease	14 (40)	12 (34)	16 (44)	42 (40)
Mesenteric vascular disease	10 (29)	7 (20)	4 (11)	21 (20)
Surgical complications	3 (9)	13 (37)	9 (25)	25 (24)
Intestinal volvulus	4 (11)	1 (3)	1 (3)	6 (6)
Abdominal trauma	3 (9)	1 (3)	2 (6)	6 (6)
Other	1 (3)	1 (3)	4 (11)	6 (6)
SBS anatomic classification, n (%)				
Group 1 (jejunostomy)	14 (40)	18 (51)	20 (56)	52 (49)
Group 2 (jejunocolonic anastomosis)	19 (54)	15 (43)	14 (39)	48 (45)
Group 3 (jejuno-ileo-colonic anastomosis)	2 (6)	2 (6)	2 (6)	6 (6)
Length of remnant small bowel, *cm*				
Mean (SD)	84.6 (44.8)	76.5 (45.7)	97.3 (53.7)	86.2 (48.6)
Range	0–180	10–190	5–199	0–199
Remnant colon (%) in patients with CiC				
Mean (SD)	59.0 (28.6)	63.5 (23.8)	64.3 (21.5)	62.0 (24.8)
Range	7–100	30–100	14–100	7–100
Stoma, n (%)				
Yes	17 (49)	20 (57)	21 (58)	58 (55)
End jejunostomy	6 (35)	9 (45)	11 (52)	26 (45)
End ileostomy	7 (41)	8 (40)	8 (38)	23 (40)
End colostomy	3 (18)	2 (10)	1 (5)	6 (10)
Loop ileostomy	—	1 (5)	—	1 (2)
Loop jejunostomy	1 (6)	—	—	1 (2)
Other	—	—	1 (5)	1 (2)
No	18 (51)	15 (43)	15 (42)	48 (45)
Time since most recent bowel resection, *y*				
Mean (SD)	7.0 (7.9)	6.3 (7.0)	5.2 (5.8)	6.2 (6.9)
Range	0.7–31.0	0.6–24.2	0.7–20.7	0.6–31.0
Weekly PS volume requirements, *L/wk*				
Mean (SD)	13.8 (8.1)	14.5 (7.5)	14.8 (7.9)	14.4 (7.8)
Range	3.0–31.3	3.0–28.9	4.3–31.0	3.0–31.3
Weekly no. of days with PS, n (%)				
3–5 d	12 (34)	7 (20)	13 (36)	32 (30)
6–7 d	23 (66)	28 (80)	23 (64)	74 (70)
Time since start of PS, *y*				
Mean (SD)	7.6 (8.3)	7.0 (6.9)	5.0 (5.2)	6.5 (6.9)
Range	0.7–31.0	0.4–26.2	0.8–20.7	0.4–31.0
Liver function,[a] n (%)				
Normal	23 (66)	21 (60)	27 (75)	71 (67)
Mild impairment	11 (31)	12 (34)	9 (25)	32 (30)
Moderate impairment	1 (3)	2 (6)	—	3 (3)

Table 1. Continued

Characteristic	Glepaglutide 10 mg TW (n = 35)	Glepaglutide 10 mg OW (n = 35)	Placebo (n = 36)	Total (n = 106)
Renal function,[b] n (%)				
Normal	9 (26)	16 (46)	19 (53)	44 (42)
Mild impairment	19 (54)	13 (37)	9 (25)	41 (39)
Moderate impairment	7 (20)	6 (17)	8 (22)	21 (20)

[a]Based on criteria of the National Cancer Institute Organ Dysfunction Working Group.[40] Normal: bilirubin upper limit of normal (ULN) or below and alanine aminotransferase (AST) ULN; mild impairment: bilirubin ULN or below and AST above ULN or bilirubin >1.0× to 1.5× ULN and AST any; moderate impairment: bilirubin >1.5× to 3× ULN and AST any; severe impairment = bilirubin >3× ULN and AST any.
[b]Based on estimated glomerular filtration rate (mL/min/1.73 m^2), calculated using the Chronic Kidney Disease Epidemiology Collaboration refit formula.[41] Normal: ≥90; mild impairment: ≥60 to <90; moderate impairment: ≥30 to <60; severe impairment: ≥15 to <30.

and 47%, respectively. No subgroup trend was found for glepaglutide OW vs placebo.

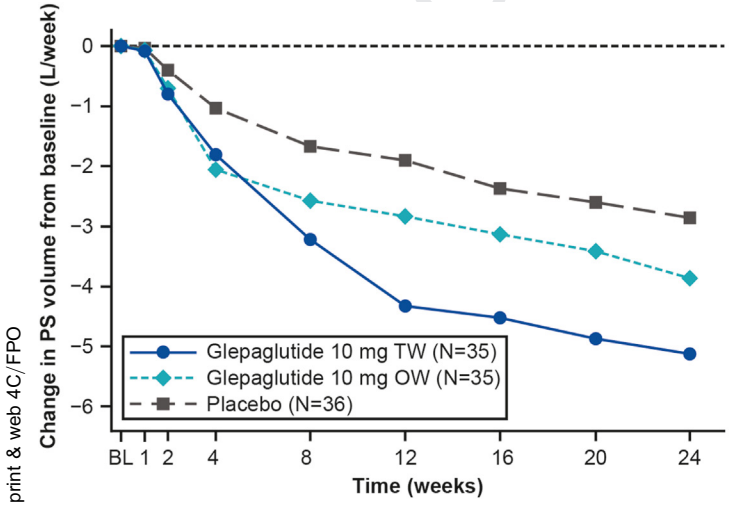
Key Secondary End Points

Glepaglutide TW was also superior to placebo for the first key secondary end point of proportion of patients achieving clinical response, defined as ≥20% PS volume reduction from baseline to weeks 20 and 24 (65.7% vs 38.9%; estimated difference of 26.6%; 95% CI, 4.3% to 48.9%; $P = .0243$), as well as for the second key secondary end point of percentage of patients achieving a reduction in days on PS ≥1 d/wk from baseline to week 24 (51.4% vs 19.4%; estimated difference of 31.7%; 95% CI, 11.4% to 51.9%; $P = .0043$). With respect to the clinical relevance of the first key secondary end point, the clinical meaningfulness of the chosen ≥20% reduction threshold was supported by an anchor-based analysis assessing the association between PGIC and percentage change in PS volume from baseline to weeks 12 and 24.

The results for change in weekly PS volume from baseline to week 12 align with those of the primary end point (Figure 2), indicating an early onset of treatment effect of glepaglutide.

Finally, the ultimate treatment goal of complete weaning off from PS (achieving enteral autonomy) at week 24 was achieved for 5 patients (14%) receiving glepaglutide TW and for 4 patients (11%) receiving glepaglutide OW vs for 0 patients receiving placebo (Figure 4). The corresponding nominal P values ($P = .0160$ for glepaglutide TW vs placebo and $P = .0424$ for glepaglutide OW vs placebo) did not lead to a formal conclusion of superiority on this end point for either of the glepaglutide treatment arms relative to placebo according to the predefined statistical testing hierarchy. The mean baseline PS volume requirement in the patients who achieved enteral autonomy was 6.8 L/wk (range, 3 to 14 L/wk). Of the 9 patients who achieved enteral autonomy, 2 had undergone a jejunostomy.

Response rates for glepaglutide OW were higher than for placebo for the binary key secondary end points, but the differences were not statistically significant. Results for the primary and key secondary end points are summarized in Supplementary Table 3.



	Glepaglutide 10 mg TW (N=35)	Glepaglutide 10 mg OW (N=35)	Placebo (N=36)
Least-squares mean [95% CI]	−5.13 [−6.24; −4.02]	−3.76 [−4.96; −2.56]	−2.85 [−3.93; −1.77]
Difference vs. placebo [95% CI]	−2.28 [−3.83; −0.73]	−0.91 [−2.52; 0.71]	–
P value	.0039	.2700	–

Figure 2. PS volume (liters per week) change from baseline (BL) by visit.

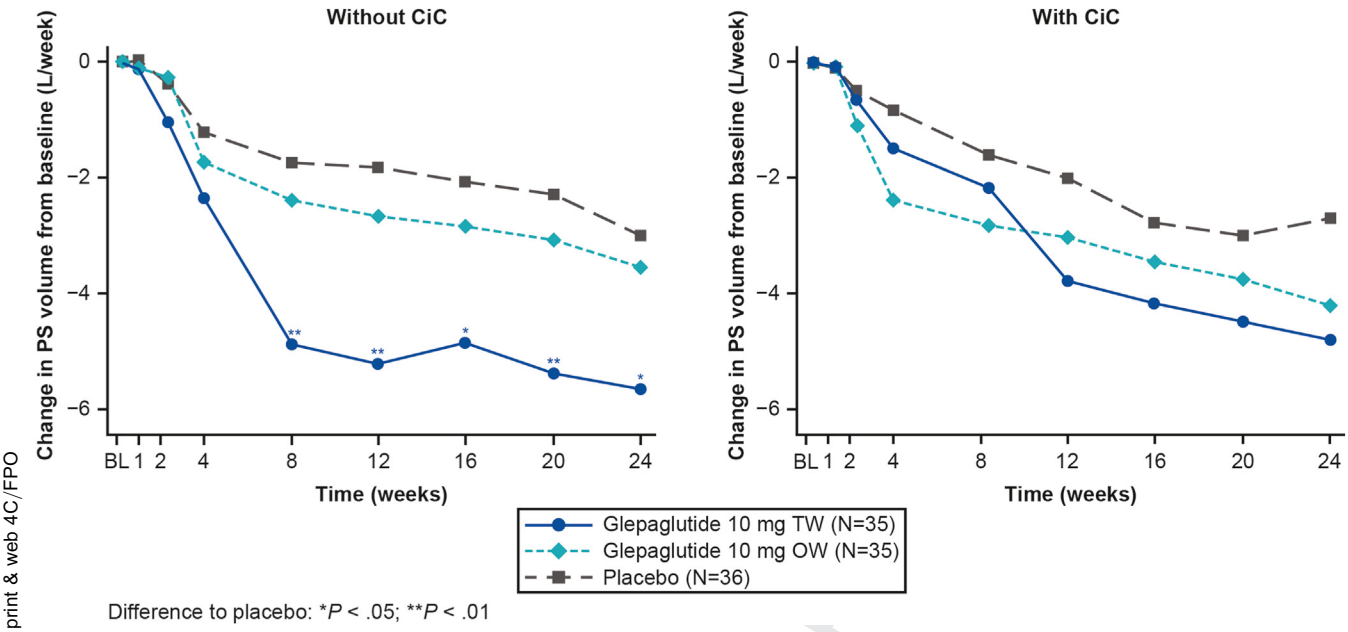


Figure 3. PS volume (liters per week) change from baseline (BL) by visit and major anatomical subgroup.

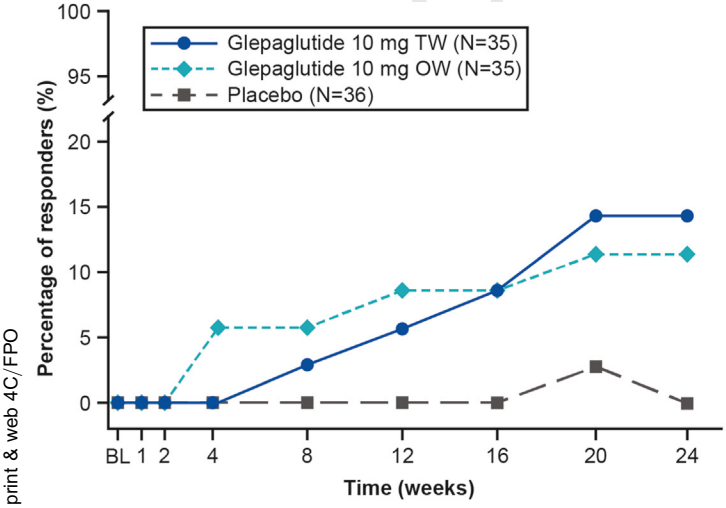
Selected Secondary End Points

Consistent with the results for PS volume and reduction in days on PS, the most notable reduction in duration of PS infusion was observed for patients receiving glepaglutide TW, who achieved a mean (SD) change from baseline to week 24 of −24.4 (23.3) h/wk compared with −9.4 (16.1) h/wk for placebo. Only PS volume (not energy content) was adequately collected in the trial.

For patient-reported outcomes, more actively treated patients than placebo-treated patients reported improvement on the PGIC scale, particularly within the categories of "much improved" and "very much improved" (Table 2). A significantly greater proportion of patients in the glepaglutide TW and OW groups scored within the collapsed category of "much/very much improved" at week 24 relative to placebo (48.6% and 31.4% on glepaglutide TW and OW vs 5.6% on placebo; $P < .0001$ and $P = .0058$, respectively). For the SBS Impact Scale, trends toward a treatment benefit of glepaglutide were noted for domains of pain, exhausted/tired, sleep, and affected mood, whereas no noteworthy differences between treatment groups were noted for EQ-5D-5L (data not shown).

The mean percentage change in body weight from baseline to week 24 was essentially unchanged across treatment groups: −0.27% for glepaglutide TW, −0.41% for glepaglutide OW, and −0.46% for placebo.

Mean concentrations of citrulline, a biomarker for intestinal mucosal/enterocyte mass,[42] increased from baseline in both glepaglutide dose groups, with estimated relative increases from baseline of approximately 47% and 19% for glepaglutide TW and OW, respectively, vs 4% for placebo ($P = .0025$ for TW vs placebo).



	Glepaglutide 10 mg TW (N=35)	Glepaglutide 10 mg OW (N=35)	Placebo (N=36)
n/N (%)	5/35 (14.3)	4/35 (11.4)	0/36 (0)
Difference vs. placebo [95% CI]	14.1 [2.5; 25.6]	11.2 [0.7; 21.6]	–
Nominal *P* value	.0160	.0424	–

Figure 4. Proportion of patients achieving enteral autonomy. BL, baseline.

Table 2. Patient Global Impression of Change at Week 24

Variable	Glepaglutide 10 mg TW (n = 35)	Glepaglutide 10 mg OW (n = 35)	Placebo (n = 36)
Wk 24, n (%)			
Very much improved	4 (11.4)	1 (2.9)	0
Much improved	13 (37.1)	10 (28.6)	2 (5.6)
Minimally improved	5 (14.3)	16 (45.7)	11 (30.6)
No change	6 (17.1)	5 (14.3)	18 (50.0)
Minimally worse	2 (5.7)	0	1 (2.8)
Much worse	0	0	0
Very much worse	0	0	0
Missing	5 (14.3)	3 (8.6)	4 (11.1)
Difference to placebo,[a] n (%); *P* value			
Improved	22 (62.9); .0259	27 (77.1); .0006	13 (36.1)
Much/very much improved	17 (48.6); <.0001	11 (31.4); .0058	2 (5.6)

[a]The difference in percentage of responders between each glepaglutide-treated group and placebo was tested using the Cochran-Mantel-Haenszel approach, adjusted for the stratification factor.

Safety and Tolerability

Patient-years of observation for safety amounted to 16.6, 17.6, and 17.8 in the glepaglutide TW, glepaglutide OW, and placebo group, respectively. More adverse events were reported in the glepaglutide TW and OW groups (407 and 364 events, respectively) than in the placebo group (95 events), which was primarily attributable to mild injection site reactions reported for glepaglutide. In the glepaglutide groups, the most frequent types of adverse events (reported for ≥10% of the patients) were injection site reactions, stoma complications (primarily swelling or enlargement of the stoma nipple), GI events (primarily nausea, vomiting, and abdominal pain), pyrexia, and fatigue.

Overall, the majority of adverse events were nonserious and mild and resolved during the trial. Of note, more patients treated with glepaglutide TW had severe adverse events (28.6%) compared with those treated with glepaglutide OW (11.4%) or placebo (5.6%), but with no apparent clustering with respect to types of events. Similarly, in the subgroup of patients with a stoma, more patients experienced stoma complications with glepaglutide TW (41.2%) than with glepaglutide OW (20.0%) or placebo (0%).

Serious adverse events (SAEs) were reported for 25.7% of patients in both glepaglutide groups and for 19.4% of patients receiving placebo; these events generally reflected the patients' underlying disease and comorbidities. Of the 34 SAEs reported across treatment groups (Supplementary Table 4), 13 were related to administration of PS (eg, device-related sepsis, vascular device infection, catheter site necrosis, device breakage, and device malfunction). Other SBS-related SAEs (all reported in the glepaglutide TW group) included 1 event of stoma site hemorrhage that progressed to another SAE of anemic blood loss; 1 event of metabolic acidosis (D-lactic acidosis); and 2 events of anemic iron deficiency in 1 patient, which represented worsening of a pre-existing condition.

One patient reported experiencing generalized erythema, flushing, dyspnea, and fear after administration of the 19th dose of glepaglutide TW. The event was classified as an SAE of generalized hypersensitivity. Limited information was available, as the patient did not seek medical attention for the event and only informed the investigator by phone the following day. The patient subsequently chose to withdraw consent. No deaths occurred during the trial.

In total, 5 adverse events of special interest were reported during the treatment period: 1 event of cholecystitis in a patient receiving active treatment and 4 events related to suspicion of liver injury in 4 patients distributed across all 3 treatment groups; all 4 patients were asymptomatic, and the transient elevations in liver enzymes had resolved at the next visit. No adverse events of special interest of neoplasm development or pancreatitis were reported for the trial during or after treatment with trial drug.

In accordance with the physiological effect of the drug, adverse events related to fluid retention/fluid overload were reported by more patients in the glepaglutide TW group (20.0%) than in the glepaglutide OW and placebo groups (2.9% and 5.6%, respectively), and most events were reported during treatment initiation. None of the adverse events related to fluid retention and fluid overload were serious or severe, and none led to temporary or permanent discontinuation of the investigational product.

No clinically relevant changes over time or differences between treatment groups were noted for biochemistry or hematology parameters, except for a trend toward a decrease in liver parameter values in the glepaglutide groups. For alkaline phosphatase and gamma-glutamyl transferase, there was an indication of a dose–response, with the largest decrease observed in the glepaglutide 10 mg TW group, a smaller decrease in the glepaglutide 10 mg OW group, and no or a small decrease in the placebo group. In all treatment groups (including placebo), a small numerical decline in renal function markers over the 24 weeks

of treatment was observed. No clinically relevant findings were noted for vital signs or electrocardiogram. Based on electrocardiogram data, an increase from baseline heart rate of 2–3 beats/min was seen in the glepaglutide groups but not in the placebo group.

A total of 61 of 70 patients (87%) dosed with glepaglutide developed treatment-induced anti-glepaglutide antibodies during the course of trial. The corresponding incidences were 57 (81%) for antibodies binding to the predominant active metabolite[33]; 24 (34%) for in vitro glepaglutide-neutralizing antibodies; and 17 (24%) for antibodies cross-reacting with endogenous GLP-2. The development of anti-drug antibodies was similar for TW and OW dosing, except that the antibodies appeared slightly earlier with TW dosing than with OW dosing.

With respect to antibody levels, titers for anti-glepaglutide antibodies and for antibodies binding to the predominant active 34-amino-acid glepaglutide metabolite showed a tendency for a plateauing effect from week 12, whereas titers for in vitro glepaglutide-neutralizing antibodies and antibodies cross-reacting with endogenous GLP-2 still seemed to be increasing by the end of the 24-week trial period. There were no apparent differences in titer levels between the 2 glepaglutide treatment groups. The trial showed no apparent associations between anti-drug antibody development and glepaglutide pharmacokinetic properties, efficacy, or safety, except for injection site reactions, where a tendency for higher incidences of injection site reactions was seen in patients who became anti-drug antibody–positive during the trial, compared with patients who remained negative. Injection site reactions were often seen before onset of anti-drug antibodies, whereas other patients developed antibodies in apparent absence of injection site reactions. Therefore, no firm conclusions regarding the causal relationship between injection site reactions and anti-glepaglutide antibody development could be drawn.

Discussion

In this pivotal, randomized, placebo-controlled trial testing the efficacy and safety of the long-acting GLP-2 analogue glepaglutide in moderately to severely PS-dependent patients with SBS, the estimated mean PS volume reduction after 24 weeks of glepaglutide TW dosing was 5.1 L/wk, corresponding to a 45% volume reduction. This corresponded to a significant and clinically relevant PS volume reduction of 2.28 L/wk relative to placebo. In addition, 66% of patients receiving glepaglutide TW achieved clinical response (≥20% PS volume reduction, the clinical meaningfulness of which was supported by a separate anchor-based analysis), and 51% of patients achieved a reduction in days on PS of ≥1 d/wk. Of note, the mean PS volume reduction of almost 3 L/wk in the placebo group attests to the trial effect, as well as the intrinsic benefit of strict adherence to a PS-weaning procedure and algorithm.

The obtained PS volume reductions with glepaglutide TW are comparable with those obtained with the currently marketed GLP-2 analogue teduglutide.[43,44] Moreover, particularly encouraging and not previously demonstrated with GLP-2 analogue treatment in a similar trial setting,[44] complete weaning off PS (enteral autonomy) was achieved for 14% of patients receiving glepaglutide TW, whereas no patients in the placebo group were completely weaned off PS. This end point was also achieved for 11% of patients receiving glepaglutide OW. Compared with the overall trial population, patients who completely weaned off PS at week 24 were characterized by a lower mean PS volume need at baseline (6.8 vs 14.4 L/wk) and a higher prevalence of having a CiC (78% vs 51% of patients). However, there were no patients characteristics that could serve as a definite prospective identification of patients who would obtain a complete response.

This trial builds on the positive outcome of a phase 2 trial testing several dose regimens of glepaglutide.[45] Given the significantly protracted pharmacokinetic properties of glepaglutide,[33] for the current trial, it was hypothesized that an appropriate pharmacodynamic effect would be ensured by dosing 10 mg glepaglutide either OW or TW. This was unequivocally demonstrated for glepaglutide TW. In addition, the fact that a proportion of patients receiving glepaglutide OW was completely weaned off PS suggests that glepaglutide OW may be an adequate dose for some patients, despite the trial not being able to demonstrate a statistically significant benefit of glepaglutide OW relative to placebo for the primary and key secondary end points. This is further supported by the result for the biomarker citrulline, which suggests a dose-dependent intestinotrophic effect of glepaglutide on the intestinal epithelium.

Baseline characteristics were similar overall across treatment groups, although notably with a lower proportion of patients with a stoma in the glepaglutide TW group than in the placebo group (49% vs 58%). As a generalization, patients without CiC (stoma) exhibit absorption deficits for both fluids and nutrients, whereas the absorption deficit in patients with CiC is mainly for nutrients. Patients with a stoma would therefore be expected to have a higher PS volume requirement than patients with CiC and to exhibit a relatively more robust response to glepaglutide treatment with respect to absolute PS volume reduction, as also suggested from this trial for the comparison of glepaglutide TW vs placebo in the presence or absence of CiC. For PS volume end points, the minor imbalance between treatment groups in proportions of patients with a stoma would, therefore, if anything, tend to bias results in favor of placebo.

The trial completion rate of 96% was high considering the trial setting (including conduct during the COVID-19 pandemic), the trial duration, and the complex patient population. Because missing values were imputed using a copy-reference multiple imputation approach, the fact that all withdrawals occurred in patients actively treated would again tend to bias PS volume end point results in favor of placebo.

In addition to primary outcomes, this is the first trial to demonstrate a significant treatment benefit of GLP-2 analogue treatment on patient-reported outcomes in patients with SBS in a placebo-controlled phase 3 trial. A substantial benefit of glepaglutide treatment relative to placebo, as reported on the PGIC scale, was demonstrated. As confirmed from a separately conducted analysis of qualitative patient interviews, this was

at least partly attributable to glepaglutide-treated patients spending less time connected to a central line for PS infusion, thereby enabling better sleeping patterns and increased autonomy in everyday activities. In line with this, of the 9 patients who achieved enteral autonomy, 8 had replied "much improved" or "very much improved" when asked about their overall change in status since the start of the trial—only 1 replied "no change."

Glepaglutide was assessed to be safe and well tolerated. The safety profile was generally similar between the TW and OW treatment groups and consistent with known GLP-2 class effects. Apart from injection site reactions, GI events were the most frequent adverse events with glepaglutide, in line with the physiological actions of the drug and previous findings for GLP-2 analogue treatment.[44,46–48]

Stoma complications were among the most frequent adverse events for glepaglutide in the subgroup of patients with a stoma. The majority of the events were mild, nonserious swelling of the stoma nipple, and a few events pertained to mild stoma enlargement or stoma irritation. Stoma complications are likely to be ascribed to increases in mesenteric blood flow,[49] but hypertrophic effects may also contribute. This is supported by the dose–response relationship for stoma complications observed in this study, as well as by literature reports of stoma complications with the GLP-2 analogue teduglutide.[44,46,50] Anti-drug antibody development was seen in the majority of patients receiving glepaglutide. Of these, some developed in vitro glepaglutide-neutralizing antibodies and GLP-2 cross-reacting antibodies, but with no discernible clinical impact.

Of note for the safety of initiating glepaglutide treatment, resultant fluid overload due to insufficient PS weaning could be a potential safety concern, particularly in patients with cardiac decompensation. In this respect, it is reassuring that none of the adverse events of the present trial that were related to fluid retention and fluid overload were serious or severe. Another potential concern could be that reductions in PS were achieved at the expense of loss in body weight. In this trial, mean body weight overall remained essentially unchanged from baseline to week 24 in all 3 treatment groups.

In conclusion, 24 weeks of TW treatment with 10 mg glepaglutide was effective in reducing—and in some cases eliminating—the requirement for PS in patients with SBS with chronic IF. A significant improvement in patient-reported outcomes was found for patients receiving glepaglutide relative to those receiving placebo. Glepaglutide appeared to be safe and well tolerated, and the safety profile was generally consistent with known GLP-2 class effects. The long-term efficacy and safety of OW and TW glepaglutide treatment is being investigated in extension trials involving up to 4.5 years of cumulative exposure to glepaglutide.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at https://doi.org/10.1053/j.gastro.2024.11.023.

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Conflicts of interest
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Data Availability
Restrictions apply to the availability of individual patient data generated in this trial. The corresponding author may be contacted for further details, including any conditions under which access to some data may be provided.

Supplementary Material

Trial Participant Selection Criteria

Inclusion criteria. Patients who met all of the following criteria were eligible to participate in the trial:

1. Provided informed consent obtained before any trial-related activity.
2. Was 18 years or older and 90 years or younger at screening.
3. Had a diagnosis of SBS, defined as remaining small bowel in continuity of estimated <200 cm (equal to 79 in) and with the latest intestinal resection being at least 6 months before screening and considered stable with regard to PS need. No restorative surgery planned in the trial period.
4. Required PS at least 3 d/wk.
5. Was willing to adhere to an individual predefined drinking menu during 48-hour measurement periods.
6. Was willing to maintain a stable weight (±5%) for the duration of the trial (24 weeks).
7. Had:
   a. A stoma or
   b. CiC with documented colonoscopy performed during screening and that did not give rise to any safety concerns.

NOTE. A colonoscopy performed within 6 months before screening and not giving rise to any safety concerns was accepted. For patients with a remnant colon, which was not connected to the passage of foods and was thereby dormant, a computed tomography scan or magnetic resonance imaging (if standard of care at site) sufficed at the discretion of the investigator.

8. Had (a) a stoma or (b) CiC and was able to separate stool and urine during the 48-hour measurement periods

Exclusion criteria. Patients who met any of the following criteria were not eligible to participate in the trial:

1. Had more than 2 SBS-related or PS-related hospitalizations (eg, catheter-related bacteremia or sepsis, bowel obstruction, and severe water-electrolytes disturbances) within 6 months before screening
2. Had poorly controlled inflammatory bowel disease that was moderately or severely active or fistula interfering with measurements or examinations required in the trial.
3. Had bowel obstruction.
4. Had known radiation enteritis or significant villous atrophy, for example, due to active celiac disease.
5. Had cardiac disease, defined as decompensated heart failure (New York Heart Association class III–IV), unstable angina pectoris, and/or myocardial infarction within the last 6 months before screening.
6. Had clinically significant abnormal electrocardiogram as judged by the investigator.
7. Had repeated (2 or more consecutive measurements separated by at least 15 minutes) systolic blood pressure measurements >180 mm Hg.
8. Was diagnosed with HIV, acute liver disease, or unstable chronic liver disease.
9. Had any history of colon cancer. History of any other cancers (except margin-free resected cutaneous basal or squamous cell carcinoma or adequately treated in situ cervical cancer) unless disease-free state for at least 5 years.
10. Had an estimated creatinine clearance (by the Cockcroft-Gault formula) <30 mL/min.
11. Had hepatic impairment defined as:
    a. Total bilirubin ≥2× the upper limit of normal, or
    b. Aspartate aminotransferase ≥5× upper limit of normal, or
    c. Alanine aminotransferase ≥5× upper limit of normal.
12. Had used GLP-1, GLP-2, human growth hormone, somatostatin, or analogues thereof within 3 months before screening.
13. Had used dipeptidyl peptidase 4 inhibitors within 3 months before screening.
14. Had systemic immunosuppressive therapy that was introduced or had been unstable within 3 months before screening.
15. Had unstable biological therapy (eg, anti–tumor necrosis factor-α or natalizumab) within 6 months before screening, including significant changes in doses or switch of drug.
16. Female patients of childbearing potential, who were pregnant, breastfeeding, intended to become pregnant, or were not using highly effective contraceptive methods.
17. Had a known or suspected hypersensitivity to glepaglutide or related products.
18. Had previous exposure to glepaglutide.
19. Had previous participation (randomization) in this trial.
20. Current, or within 30 days before screening, participation in another interventional clinical trial that included administration of an active compound.

21. Had mental incapacity or language barriers that precluded adequate understanding or cooperation or unwillingness to comply with trial requirements.
22. Had any condition or disease or circumstance that, in the investigator's opinion, put the patient at any undue risk, prevented completion of the trial, or interfered with the analysis of the trial results.
23. Was committed to an institution by virtue of an order issued either by the judicial or administrative authorities.
24. Was an employee of the sponsor or investigator or otherwise dependent on them.

Randomization criteria. The patient had to meet all of the following criteria at the time of randomization.

1. Required PS at least 3 d/wk and maintains a stable PS volume for at least 2 weeks. PS volume was considered stable if all of the criteria below were fulfilled:
   - Actual PS use (volume and content) matched prescribed PS (±10% deviation in volume was acceptable) and
   - 48-hour urine volumes at 2 consecutive visits within a 2-week interval (±4 days, ie, visits were to be 10–18 days apart) were similar (a maximum of ±25% deviation was acceptable), while the oral fluid intake was constant (the two 48-hour oral intakes differed $<10\%$) and maximum 3.5 L/d and
   - Urine volume was on average $\geq$1 L/d and $\leq$2.5 L/d.
2. Had no SBS-related hospitalizations within 30 days before randomization. NOTE. Hospitalizations related to trial procedures were allowed.
3. Since screening, did not have poorly controlled inflammatory bowel disease that was moderately or severely active, or fistula interfering with measurements or examinations required in the trial.
4. Had no bowel obstruction since screening.
5. Had no cardiac disease, defined as decompensated heart failure (New York Heart Association class III–IV), unstable angina pectoris, and/or myocardial infarction since screening
6. Had no clinically significant abnormal electrocardiogram as judged by the investigator.
7. Had repeated (2 or more consecutive measurements separated by at least 15 minutes) systolic blood pressure measurements $\leq$180 mm Hg.
8. Had not used GLP-1, GLP-2, human growth hormone, somatostatin, or analogues thereof since screening.
9. Had not used dipeptidyl peptidase 4 inhibitors since screening.
10. Had no systemic immunosuppressive therapy that has been introduced or had been unstable since screening.
11. Had no unstable biological therapy (eg, anti–tumor necrosis factor-α or natalizumab) since screening, including significant changes in doses or switch of drug.
12. Had no unstable doses (including as needed use) within 2 weeks before randomization:
    - Antimotility drugs, for example, loperamide, diphenoxylate, codeine, or other opiates
    - H2 antagonists
    - Antidiarrheal agents
    - Bile acid sequestering agents
    - Oral glutamine
    - Proton pump inhibitors
    - Diuretics
    - Systemic antibiotics or antibiotics affecting the gastrointestinal tract
    - Oral rehydration fluids
13. Female patients of childbearing potential used highly effective contraceptive methods and were not pregnant, breastfeeding, or intended to become pregnant

Optimization and Stabilization Phases

During the optimization phase, the investigator could change the PS volume and content according to institutional standard practice if the patient was considered unstable or not optimized, while aiming at a urine volume of 1–2.5 L/d. Before each optimization visit, the patient was to measure his/her urine volume over 48 hours while adhering to a predefined 48-hour drinking menu. During this period, the patient was to record urine volume and oral fluid intake in an eDiary. The effect of PS optimizations was investigated after 2 weeks. With no more than 2 rounds of PS optimization being allowed, this limited the optimization phase to a maximum duration of 4 weeks. During the optimization phase, the investigator and the patient were allowed to redefine and optimize the individual drinking menu to best fit the patient's needs. Once the drinking menu had been set at the end of the optimization phase, the patient was required to adhere to this drinking menu during the 48-hour balance periods throughout the remainder of the trial.

A stabilization phase of 2–4 weeks of duration immediately followed the optimization phase (the last optimization phase visit could serve as the first stabilization phase visit). No changes in the prescribed weekly PS volume or schedule were allowed during this phase. Before each stabilization phase visit, the patient was to measure his/her urine volume over 48 hours while adhering to the set drinking menu and report their urine volume and oral fluid

intake in the eDiary. Stabilization phase visits occurred every 2 weeks until fulfilling the following PS stability criteria that qualified for randomization to investigational product treatment:

- PS use (volume and content) matched prescribed PS (±10% deviation in total volume is acceptable), and
- 48-hour urine volumes at 2 consecutive visits within a 2-week interval (±4 days) were similar (up to ±25% deviation was considered acceptable), while the oral fluid intake was constant (the two 48-hour oral intakes differed by <10%) and not exceeding 3.5 L/d, and
- Urine volume was a mean of ≥1 L/d and ≤2.5 L/d.

If stability could not be obtained during the 4-week period due to unforeseen events, such as infections, illness, or similar, a second stabilization phase of up to 4 weeks was allowed.

Assumptions Regarding Statistical Power Calculation

The sample size calculations for this trial were based on the effect observed in the teduglutide phase 3 trial.[44] The PS volume changes from baseline after 24 weeks of treatment (primary end point) were expected to be −4.5 L/wk and −4.3 L/wk with TW and OW dosing, respectively, and −2.3 L/wk for placebo. The SD of the treatment effect (OW or TW vs placebo) was assumed to be 2.62. A total of 101–112 patients with SBS were planned for inclusion, with 33–37 patients planned for each of the 3 treatment groups. This trial size would result in 93%–95% power for detecting the assumed difference for the primary end point with either OW TW glepaglutide treatment. The assumed effects include imputed effects for patients with missing data. The power calculations are shown in Supplementary Table 1, including the scenario where OW and TW dosing are assumed to be slightly worse.

Supplementary Reference

e1. MedDRA. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. MedDRA, version 24.1. Available at: https://alt.meddra.org/files_acrobat/intguide_24_1_English.pdf. Accessed December 21, 2024.

Supplementary Table 1. Power (%) to Show Superiority of Either Once Weekly or Twice Weekly Compared With Placebo on Primary End Point (Change in Parenteral Support Volume From Baseline to Week 24 (L/wk)

Effect assumptions			No of patients (total)			
TW	OW	PBO	99	108	117	129
−4.5	−4.3	−2.3	93	95	96	98
−4.3	−4.1	−2.3	88	91	93	95

NOTE. The 2 comparisons, OW vs placebo and TW vs placebo (PBO), were tested 2-sided in parallel at $\alpha = .025$ to control the overall type 1 error at 5% level.

Supplementary Table 2. Parenteral Support Volume (L/wk) Change From Baseline to Week 24: Treatment Policy Estimand–Summary of Statistical Analysis

Treatment group	n	Delta[a]	Least squares mean (95% CI)	Difference to placebo	
				95% CI	*P* Value
Primary analysis, MI CR					
Glepaglutide					
10 mg TW	35	—	−5.13 (−6.24 to −4.02)	−2.28 (−3.83 to −0.73)	.0039
10 mg OW	35	—	−3.76 (−4.96 to −2.56)	−0.91 (−2.52 to 0.71)	.2700
Placebo	36	—	−2.85 (−3.93 to −1.77)	—	—
Sensitivity analysis 1,[b] MI J2R					
Glepaglutide					
10 mg TW	35	—	−4.86 (−5.97 to −3.75)	−2.01 (−3.55 to −0.47)	.0107
10 mg OW	35	—	−3.76 (−4.96 to −2.56)	−0.91 (−2.52 to 0.71)	.2698
Placebo	36	—	−2.85 (−3.93 to −1.77)	—	—
Sensitivity analysis 2,[c] MI CIR					
Glepaglutide					
10 mg TW	35	—	−5.12 (−6.17 to −4.07)	−2.27 (−3.78 to −0.76)	.0032
10 mg OW	35	—	−3.76 (−4.96 to −2.56)	−0.91 (−2.52 to 0.71)	.2698
Placebo	36	—	−2.85 (−3.93 to −1.77)	—	—
Sensitivity analysis 3,[d] 14-d period					
Glepaglutide					
10 mg TW	35	—	−5.28 (−6.49 to −4.07)	−2.53 (−4.23 to −0.84)	.0034
10 mg OW	35	—	−2.16 (−4.47 to 0.14)	0.59 (−1.99 to 3.17)	.6534
Placebo	36	—	−2.75 (−3.98 to −1.52)	—	—
Sensitivity analysis 4,[e] MI CR tipping point					
Glepaglutide					
10 mg TW	35	3.9	−4.68 (−5.88 to −3.49)	−1.83 (−3.44 to −0.22)	.0258
10 mg OW	35	0	−3.76 (−4.96 to −2.56)	−0.91 (−2.52 to 0.71)	.2700
Placebo	36	—	−2.85 (−3.93 to −1.77)	—	—
Sensitivity analysis 5,[f] observed					
Glepaglutide					
10 mg TW	35	—	−5.16 (−6.20 to −4.11)	−2.31 (−3.81 to −0.81)	.0031
10 mg OW	35	—	−3.76 (−5.00 to −2.51)	−0.91 (−2.55 to 0.73)	.2737
Placebo	36	—	−2.85 (−3.97 to −1.73)	—	—

CIR, copy increment from reference; CR, copy reference; J2R, jump to reference; MI, multiple imputation.
NOTE. The mixed model for repeated measures includes treatment group, visit, stratification factor, and treatment-by-visit interaction as factors and baseline PS volume (L/wk) as covariate. Variance estimation is based on an unstructured covariance matrix within treatment group. Stratification factor: weekly PS volume requirements <12 L/wk and ≥12 L/wk.
[a]Delta: Minimum amount added to CR-imputed values for glepaglutide 10-mg groups in order to get insignificant *P* value for the comparison with placebo (nothing is added/subtracted).
[b]Sensitivity analysis 1: Patient's conditional outcomes are assumed to "jump" to those of placebo after treatment discontinuation.
[c]Sensitivity analysis 2: Patient's conditional outcomes are assumed to mimic the gradient from placebo after treatment discontinuation.
[d]Sensitivity analysis 3: Patient's PS volumes were derived from a retrospective 14-day period instead of the 7-day period.
[e]Sensitivity analysis 4: Patient's CR-imputed values were varied independently in each treatment arm by adding an appropriate delta PS volume until conclusions changed.
[f]Sensitivity analysis 5: Patient's observed data used only, regardless of treatment discontinuation.

Supplementary Table 3. Primary and Key Secondary End Points: Testing Hierarchy With Gatekeeping[a] Procedure–Statistical Analysis

Variable	Glepaglutide 10 mg TW (n = 35)				Glepaglutide 10 mg OW (n = 35)			
	Difference to placebo (95% CI)	Significance level	Nominal *P* value	Evaluation[b]	Difference to placebo (95% CI)	Significance level	Nominal *P* value	Evaluation[b]
Test hierarchy (primary end point, first and second key secondary end points)								
Primary end point: Change in actual PS volume from baseline to wk 24 (MI CR)	−2.28 (−3.83 to −0.73)	.025	.0039	Pass	−0.91 (−2.52 to 0.71)	.050	.2700	Fail
Key secondary end point 1: Clinical response, defined as at least 20% reduction in actual weekly PS volume from baseline to both wk 20 and 24 (NR)	26.6 (4.3 to 48.9)	.025	.0243	Pass	5.5 (−16.2 to 27.1)	NA	.6255	NA
Key secondary end point 2: Reduction in days on PS ≥1 d/wk from baseline to wk 24 (NR)	31.7 (11.4 to 51.9)	.025	.0043	Pass	13.3 (−5.4 to 32.0)	NA	.1675	NA
Test hierarchy (third and fourth key secondary end points)								
Key secondary end point 3: Change actual PS volume from baseline to wk 12 (MI CR)	−2.42 (−3.95 to −0.90)	NA	.0019	N/A	−0.87 (−2.37 to 0.63)	NA	.2547	NA
Key secondary end point 4: Reduction in weekly PS volume of 100% (weaned off) at wk 24 (NR)	14.1 (2.5 to 25.6)	NA	.0160	N/A	11.2 (0.7 to 21.6)	NA	.0424	NA

CR, copy reference; MI, multiple imputation; NA, not applicable; NR, nonresponse imputation; PS, parenteral support.

[a]Gatekeeping: Within each glepaglutide group, the primary end point and the first and second key secondary end points are hierarchically evaluated at a significance level of .025 (2-sided), only continuing to the next end point if there is a statistically significant difference to placebo. If there is a statistically significant difference to placebo for all 3 end points within a glepaglutide group, testing in the other glepaglutide group will be evaluated at a significance level of .05 (2-sided). Otherwise, this treatment group is evaluated at significance level of .025. If there is a statistically significant difference to placebo for all 6 hypothesis tests (3 end points in 2 treatment groups), the last 2 key secondary end points are evaluated hierarchically at .05 significance level (2-sided), starting with glepaglutide 10 mg TW, only continuing to the next end point if there is a statistically significant difference to placebo in the preceding level.

[b]Evaluation: Pass: statistically significant. Fail: Not statistically significant. N/A: Test hierarchy stopped at a previous level because a statistically significant difference to placebo was not found.

Supplementary Table 4. Serious Adverse Events

System organ class/preferred term	Glepaglutide 10 mg TW (n[a] = 35, PYO = 16.60) n[b] (%[c])	Glepaglutide 10 mg TW (n[a] = 35, PYO = 16.60) Events, n	Glepaglutide 10 mg OW (n[a] = 35, PYO = 17.58) n[b] (%[c])	Glepaglutide 10 mg OW (n[a] = 35, PYO = 17.58) Events, n	Placebo (n[a] = 36, PYO = 17.80) n[b] (%[c])	Placebo (n[a] = 36, PYO = 17.80) Events, n
All adverse events	9 (25.7)	15	9 (25.7)	11	7 (19.4)	8
Infections and infestations	3 (8.6)	5	5 (14.3)	6	3 (8.3)	3
Device-related sepsis	2 (5.7)	3	2 (5.7)	2	1 (2.8)	1
Infection	1 (2.9)	1	—	—	—	—
Large intestine infection	1 (2.9)	1	—	—	—	—
Vascular device infection	—	—	2 (5.7)	2	2 (5.6)	2
COVID-19	—	—	1 (2.9)	1	—	—
Gastrointestinal viral infection	—	—	1 (2.9)	1	—	—
Injury, poisoning, and procedural complications	2 (5.7)	2	—	—	2 (5.6)	2
Stoma site hemorrhage[d]	1 (5.9)	1	—	—	—	—
Acetabulum fracture	1 (2.9)	1	—	—	—	—
Alcohol poisoning	—	—	—	—	1 (2.8)	1
Procedural pneumothorax	—	—	—	—	1 (2.8)	1
Blood and lymphatic system disorders	2 (5.7)	3	—	—	—	—
Blood loss anemia	1 (2.9)	1	—	—	—	—
Iron deficiency anemia	1 (2.9)	2	—	—	—	—
General disorders and administration site conditions	1 (2.9)	1	3 (8.6)	3	—	—
Pyrexia	1 (2.9)	1	2 (5.7)	2	—	—
Catheter site necrosis	—	—	1 (2.9)	1	—	—
Gastrointestinal disorders	1 (2.9)	1	—	—	1 (2.8)	1
Hemorrhoids	1 (2.9)	1	—	—	—	—
Rectal hemorrhage	—	—	—	—	1 (2.8)	1
Immune system disorders	1 (2.9)	1	—	—	—	—
Hypersensitivity	1 (2.9)	1	—	—	—	—
Metabolism and nutrition disorders	1 (2.9)	1	—	—	—	—
Metabolic acidosis	1 (2.9)	1	—	—	—	—
Nervous system disorders	1 (2.9)	1	—	—	—	—
Dizziness	1 (2.9)	1	—	—	—	—
Hepatobiliary disorders	—	—	1 (2.9)	1	—	—
Cholecystitis	—	—	1 (2.9)	1	—	—
Vascular disorders	—	—	1 (2.9)	1	—	—
Peripheral arterial occlusive disease	—	—	1 (2.9)	1	—	—
Product issues	—	—	—	—	2 (5.6)	2
Device breakage	—	—	—	—	1 (2.8)	1
Device malfunction	—	—	—	—	1 (2.8)	1

PYO, patient-years of observation.
[a]Number of patients in safety analysis set.
[b]Number of patients experiencing at least 1 event.
[c]Percentage of patients experiencing at least 1 event.
[d]For the high-level term "Stoma complications" and related preferred terms, the denominator used in the calculation of percentage is based on patients with stoma (glepaglutide 10 mg TW: n = 17; glepaglutide 10 mg OW: n = 20; placebo: n = 21).
MedDRA, version 24.1.[e1]