Name of Active Ingredient

NLY01 (PEGylated [Cys_{40}] exenatide)

Title of Trial, Trial ID, and Eudra CT No.

A Phase 2a Dose-Finding Study to Assess the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of NLY01, a PEGylated Exenatide, when Administered as a Single Dose in Subjects with Type 2 Diabetes, Trial ID: NLY01-D1, Eudra CT no.: 2019-000398-21.

Trial Center

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Publication (Reference) Unpublished

Trial Period Oct 2019 – Dec 2020	**Phase of Development** 2a

Background for the Trial

NLY01 is a long-acting, PEGylated analog of exenatide. The purpose of this trial was to assess safety and tolerability and a preliminary dose-response for NLY01 in subjects with type 2 diabetes. NLY01 has a longer half-life than any other marketed GLP-1R agonist (12 days in healthy volunteers). This provides for sustained exposure when given once weekly and the potential for less frequent administration following an appropriate titration phase. This was a single-dose trial designed to measure pharmacokinetics and pharmacodynamic (PD) effect on glucose and other metabolic parameters such as appetite and body weight. The duration of an effect on PD parameters was assessed over a one-month period. Safety and tolerability was also assessed. Only 2 of 3 planned cohorts completed the study limiting evaluable data for dose-finding.

Objectives

Primary Objectives

To investigate the safety and tolerability of a single subcutaneous injection of NLY01 at different dose levels in subjects with T2DM

To investigate the effect of a single subcutaneous injection of NLY01 at different dose levels on pharmacodynamic (PD) parameters (24 h-insulin, glucagon, glucose profiles) in subjects with T2DM

Secondary Objectives

To investigate the PK profile of a single subcutaneous injection of NLY01 at different dose levels in subjects with T2DM

To investigate the effect of a single subcutaneous injection of NLY01 at different dose levels on PD parameters (gastric emptying, weight, appetite) in subjects with T2DM

To investigate the PK/PD correlation and concentration/effect of a single subcutaneous injection of NLY01 at different dose levels in subjects with T2DM

Methodology

This was a randomized, parallel, double-blind, placebo-controlled, single-center trial designed to assess the effect of NLY01 when administered as a single dose on glycemic control in subjects with T2DM. All subjects were on background metformin monotherapy during participation in the study. Subjects were randomized in groups of 6 to receive a single subcutaneous dose of 2.5, 5, or 10 mg NLY01 or placebo.

The 10 mg group was discontinued due to a serious generalized hypersensitivity reaction in one subject. For study subjects, the visit schedule consisted of pre-screening and screening events to establish eligibility, then dosing on Day 1 followed by assessment visits beginning days 3-6, then days 4, 5, 8, 15, 16, 29 and 30. PD assessments included fasting plasma glucose, serum insulin and plasma glucagon, assessments of gastric emptying following paracetamol intake, and appetite assessment at pre-defined timepoints. Safety assessments included local tolerability at the injection site, vital signs recording, electrocardiogram (ECG), laboratory safety parameters, physical examination, immunogenicity assessments (anti-drug antibodies [ADA]) and questioning about adverse events (AEs). During all in-house stays, subjects received standardized meals. During outpatient periods, subjects were encouraged to maintain their usual diet and exercise habits and to report fasting glucose levels exceeding 220 mg/dL on 3 consecutive days.

Number of Subjects

In this trial, 38 subjects were screened, 20 were randomized, and 20 completed the whole trial period.

Diagnosis and Main Criteria for Inclusion

Male or female subjects from age 18 to 65 years (both inclusive) with T2DM for at least 1 year, treated with metformin for at least 3 months either alone or in combination with a second oral anti-diabetic drug. HbA1c between 7.0 % and 9.0 % for subjects on metformin or between 6.5 % and 8.5 % for subjects with metformin + a second OAD. Subjects also needed to have acceptable clinical laboratory test results, be considered generally healthy apart from T2DM and sign the informed consent.

Duration of Treatment

The treatment consisted of a single dose administration. Safety, PD, and PK were monitored for 35 days following dose administration.

Primary Endpoints

Primary Safety Endpoints

AEs (including nausea, vomiting, diarrhea, hypoglycemia, injection site reactions)

Primary Pharmacodynamic Endpoints

- Plasma glucose: change of fasting plasma glucose, postprandial plasma glucose (PPPG), PPPG excursions, and 24 h-plasma glucose
- Serum insulin and plasma glucagon: change in 24 h-serum insulin and plasma glucagon concentrations

Secondary Endpoints

Key Pharmacodynamic

- Body weight: change in body weight
- Appetite: change in appetite assessed by eVAS
- Gastric emptying: change in maximum concentration of paracetamol (Cmax.par)
- Exploration of the time course of the PD response of NLY01 in terms of plasma glucose area under the curve (AUC) on study days following dosing compared to baseline (Day -1) and in comparison to the placebo group.

Key Pharmacokinetic

- Cmax, tmax, AUCt (to the last quantifiable point), AUC0-168h, t1/2

Key Secondary Safety Endpoints

- Safety laboratory parameters
- Physical examination
- Vital signs
- ECG

Statistical Methods

WinNonlin was employed for all PK calculations. PD endpoints were analyzed with a linear mixed model with change from baseline endpoint as dependent value, dose group, day and their interaction as fixed effects, baseline as covariate and subject as random factor and presentation of the estimated mean differences from baseline or between treatments together with two-sided 90 and 95% CIs. PK endpoints were analyzed with a linear mixed model with log-transformed data and dose group as fixed effect and presentation of estimated mean ratios of NLY01 dose groups together with two-sided 90 and 95% CIs.

RESULTS

Overall Summary of Trial Results

This initial dose-finding and safety trial showed that NLY01 had the expected effects of a GLP-1R agonist. Dose related reductions in both fasting and post-prandial plasma glucose concentrations were observed when compared to baseline and placebo both 2 to 5 days after a single dose of 2.5 and 5 mg NLY01. For the 5 mg dose group, the reductions were apparent on Day 4 and 5 when compared to placebo. When assessed later, on Day 15/16 and 29/30, plasma glucose concentrations in the 5 mg group remained numerically lower than placebo. Although 24 h-insulin concentrations did not differ significantly over the evaluation period, 24 h-glucagon concentrations were lower with 5 mg NLY01 at most measured timepoints (Day 1 to 29). Additionally, sensations of fullness and satiety were more pronounced, and appetite and hunger less pronounced in the 5 mg NLY01 group than placebo, and this effect persisted throughout the assessment period. The AE profile included an expected dose-dependent increase in gastrointestinal symptoms. The half-life of NLY01 in this study was similar to that determined in healthy volunteers, however, C_{max} and AUC were 2-3x lower in this study population. Following accumulation, similar doses may be more effective when administered weekly. It is likely that higher doses would be more effective.

Pharmacodynamics Results
Primary Endpoints

Fasting glucose: Results were obtained for the 2.5 and 5 mg cohorts. Data for the 10 mg cohort could not be obtained. Following treatment with NLY01 at doses of 2.5 and 5 mg, there was a dose-related decrease from baseline in FPG of 1.2 to 1.5 mmol/L on Days 2-5 ($p \leq 0.0401$). In placebo-treated subjects, FPG was unchanged or trended upward over the 1-month assessment period when compared to baseline ($p \geq 0.3357$). NLY01 treatment resulted in a dose-related decrease in FPG on Day 2 following dosing with both 2.5 mg and 5 mg NLY01 ($p \leq 0.0401$). This trend remained evident on Day 4 and 5 at both doses. With the 5 mg dose, FPG trended lower (~0.6 to 1.2 mmol/L) than placebo on Day 16, 29 and 30.

Post Prandial Plasma Glucose (PPPG): Plasma glucose concentrations after meals were decreased after dosing with 2.5 mg and 5 mg NLY01 when compared to baseline. In the 5 mg NLY01 group, decreases in PPPG were larger and evident after all meals (breakfast, lunch, and dinner) on Day 1 and 4 when compared to baseline (reductions up to -2.9 mmol/L; $p \leq 0.0646$). To a lesser extent, PPPG concentrations decreased in the 2.5 mg NLY01 group on Day 1 and 4 after some meals (reductions up

to 1.3 mmol/L; p≤0.0468). With 5 mg NLY01, a clear reduction from baseline PPPG was still apparent after lunch on Day 29 (~ -1.0 mmol/L, p=0.0638). In the 2.5 mg NLY01 group, PPPG concentrations on Day 15 and 29 showed either minimal decreases or increased after breakfast by about 1.2 to 1.5 mmol/L compared to baseline (p≤0.0214). In contrast to the reductions observed after NLY01 dosing, in the placebo group, PPPG increased compared to baseline on all assessment days after breakfast and additionally after dinner on Day 4 and 29 (increase of ~ 1.1 to 2.3 mmol/L p≤0.0357). Compared to placebo, consistent beneficial reductions in PPPG concentrations (~ -1.2 to -2.1 mmol/L) were seen in the 2.5 mg NLY01 group on Day 1 (dinner) and Day 4 (all meals, p≤0.0870). In the 5 mg NLY01 group, clear reductions relative to placebo (~ -1.7 to -3.4 mmol/L) were evident at all meals of Day 1 and 4, and additionally single meals on Day 15 (breakfast) and 29 (breakfast and dinner; p≤0.0255).

24-hour Glucose, Insulin, Glucagon: Compared to placebo, 24 h plasma glucose concentrations in the 5 mg NLY01 dose group were about -1.6 to -2.2 mmol/L lower on Day 1/2, Day 4/5 and Day 29/30 (p≤0.0087) and in the 2.5 mg NLY01 dose group about 1.3 mmol/L lower on Day 4/5 (p=0.0393). The 24 h-serum insulin profile did not change relative to baseline in any NLY01 dose group. The 24 h-plasma glucagon profile in the 5 mg NLY01 group was lower on Day 4 and Day 29 compared to baseline (~ -7.0 pmol/L; p≤0.0606). Reductions from baseline on Day 1 and Day 15 were less pronounced (~ -3.8 to -5.1 pmol/L; p≥0.1731). In the 2.5 mg NLY01 group, 24 h-plasma glucagon was not different from baseline (p≥0.6908). In the placebo group, the 24 h-plasma glucagon profile was higher than baseline on Day 1 and Day 15 (~ 6.7 and 7.4 pmol/L; p≤0.0769) and to a lesser extent on Day 4 (~ 6.0 pmol/L; p=0.1138). The 24 h-plasma glucagon profile in the 5 mg NLY01 group was -11.3 to 13.0 pmol/L lower than placebo on Day 1, Day 4 and Day 15; the difference on Day 29 was less (~ -8.2 pmol/L; p=0.1265). The 24 h-plasma glucagon profile in the 2.5 mg NLY01 group did not differ meaningfully from placebo (p≥0.1575).

Secondary Endpoints

Appetite and Body Weight: A consistent treatment-related trend toward increased fullness and reduced appetite were apparent in the 5 mg group. Feelings of fullness in the 5 mg NLY01 group were higher after dosing on Day 1, 4 and 29 (changes of ~14 to 23 mm; p<0.1). Subjects in the 5 mg NLY01 group also reported being able to eat less compared to baseline (change of ~13 mm). Reduced appetite in the 5 mg NLY01 group were most pronounced on Day 1 and 4 (p≤0.0777) when compared to baseline. In the placebo group, subjects reported increased hunger over time. When compared to placebo, subjects in the 5 mg NLY01 group felt less hungry and rather full on Day 29 (p≤0.0930; changes in sensations of ~22 mm) and also overall appetite was reduced on Day 29 (p=0.0900). Otherwise, sensations in any of the dose groups did not markedly differ from those in the placebo group. Consistent differences in body weight between placebo and treated groups were not evident.

Gastric Emptying: Delayed gastric emptying was apparent following NLY01 treatment. Maximum paracetamol concentration ($C_{max.par}$) were unchanged by placebo treatment but increased compared to baseline in both NLY01 dose groups on Day 4 (by ~ 5.7 mg/L [2.5 mg NLY01] and 3.7 mg/L [5 mg NLY01]).

Safety Results

All AEs were treatment emergent. The incidence of treatment-emergent adverse events (TEAEs) was based on the number of exposed subjects in each dose group which was lower for the 10 mg NLY01 dose groups compared to the remaining groups.

Two TEAEs in 1 subject were considered serious. In this subject, a generalize hypersensitivity reaction occurred 8 days after dosing in the 10 mg group and this was followed, in the same subject, by right facial nerve paresis 32 days after treatment. Both events in this subject resolved following corticosteroid treatment. No deaths occurred and none of the TEAEs in the study led to subject discontinuation. Most TEAEs in this study were mild. The most frequently reported TEAEs were injection site reactions (4

events in 4 subjects [28.6%] with NLY01 and none with placebo). Next most frequent were diarrhea (3 in 2 subjects with NLY01 [14.3%]), nausea (2 events in 2 subjects with NLY01 [14.3%]) and decreased appetite (2 events in 2 subjects with NLY01 [14.3%]). Vomiting was reported once in 1 subject with NLY01 (7.1%). Gastrointestinal disorders in form of nausea or diarrhea showed dose-dependent increases in incidence. No relevant mean changes in safety laboratory test results and ECG recordings occurred during the trial.

CONCLUSIONS

Pharmacodynamics

- A single s.c. dose of NLY01 (on Day 1) led to a reduction of FPG concentrations relative to baseline on Day 2 in the 2.5 mg dose group and on Day 2, 4 and 5 in the 5 mg dose group. For the 5 mg dose group, FPG was numerically lower than placebo through Day 30.
- Compared to placebo, consistent reductions in PPPG concentrations were seen in the 2.5 mg NLY01 group on Day 1 (dinner) and Day 4 (all meals). In the 5 mg NLY01 group, clear reductions from baseline and relative to placebo were evident at all meals on Day 1 and 4, and relative to placebo additionally for single meals on Day 15 and 29. Changes in PPPG excursions were less pronounced.
- In the 5 mg NLY01 group, reductions in 24 h-plasma glucose concentrations on Day 1/2 and 4/5 were evident from baseline, and relative to placebo on Day 1/2, 4/5 and on 29/30. Reductions in the 2.5 mg NLY01 group were lower and most pronounced on Day 4/5 when compared to placebo.
- The 24 h-serum insulin concentrations in both NLY01 dose groups did not change relevantly over time and relative to placebo.
- The 24 h-plasma glucagon profile in the 5 mg NLY01 group was lower on Day 4 and 29 compared to baseline. The reductions from baseline on Day 1 and 15 were apparent, but less pronounced (i.e. numerically different). Compared to placebo, reductions in glucagon in the 5 mg NLY01 group were evident through the entire assessment period (with $p<0.1$ until Day 15). In the 2.5 mg NLY01 group, the 24 h-plasma glucagon profile did not differ relevantly from baseline or placebo.
- Gastric emptying based on serum paracetamol concentrations did not change consistently after NLY01 dosing.
- Body weight decreases (up to 1 kg) in the 5 mg NLY01 group were evident on Day 4, 15 and 29 when compared to baseline. Weight loss was numerically greater in the 5 mg NLY01 group than in placebo on Day 15 and 29.
- Subjects in the placebo group reported being hungrier over time, and subjects in the 2.5 mg NLY01 group did not report consistent changes in appetite/satiety scores, but subjects in the 5 mg NLY01 group consistently reported a greater sensation of fullness or satiety and had reduced appetite and hunger compared to the placebo group ($p<0.1$ vs. placebo for fullness, hunger and overall appetite on Day 29).

Pharmacokinetics

Single s.c. doses of 2.5 and 5 mg NLY01 led to dose-dependent increases in NLY01 concentrations and exposure (AUC and Cmax) over time. Tmax was about 7 days and $t_{1/2}$ was 8 to 12 days.

Safety

A single s.c. dose of 2.5 or 5 mg NLY01 in subjects with T2DM as add-on to metformin appeared safe and was well tolerated. Two SAEs were occurred in one subject. An event of generalized hypersensitivity began 8 days after administration of a 10 mg dose of NLY01 and this was followed by right facial nerve paresis. There were no hypersensitivity events in other subjects. The most frequently

reported AEs were mild injection site reactions and gastrointestinal symptoms mostly in form of mild nausea and loose stool, which increased in incidence with rising doses and were expected for a GLP-1R agonist.

There were no vital signs or ECG recordings, physical examination findings, or clinical laboratory test results that raised any safety or tolerability concerns.

Overall Conclusion

Single s.c. doses of 2.5 or 5 mg NLY01 showed a dose dependent NLY01 exposure pattern and appeared safe and well tolerated in subjects with T2DM as add-on to metformin. In the 5 mg dose group, plasma glucose and glucagon decreased from baseline particularly during the first days after dosing and given the continuous increases in the placebo group values over the study period, were often relatively lower than placebo up to Day 29/30. Appetite appeared dampened relative to placebo on Day 29, while body weight was only slightly reduced compared to baseline and placebo by Day 29.