2.0 SYNOPSIS

Name of Sponsor/Company: Elgan Pharma Ltd. **Name of Finished Product:** NTRA-2112 **Name of Active Ingredient:** Human insulin	**Individual Study Table**	**(For National Authority Use only)**

Title of Study:

A Multi-center, Double-blind, Randomized, Three-Arm, Parallel group, Placebo-Controlled Study to Assess the Efficacy and Safety of NTRA-2112 on Intestinal Malabsorption in Preterm Infants

Investigators:

46 Investigators

Study Centers:

Forty-six study centers in 11 countries (Belgium, Bulgaria, France, Germany, Hungary, Israel, Italy, the Netherlands, Spain, United Kingdom, and the United States of America)

Publication (Reference):

Mank E, Sáenz de Pipaón M, Lapillonne A, et al. Efficacy and Safety of Enteral Recombinant Human Insulin in Preterm Infants: A Randomized Clinical Trial. *JAMA Pediatr.* Published online February 28, 2022. doi:10.1001/jamapediatrics.2022.0020

Study Period:	**Phase of Development:**
First Subject Enrolled: 09 October 2016 Last Subject Completed: 25 April 2018	Phase 3

Primary Objective:

The primary objective of this study was to assess the efficacy of two doses of NTRA-2112 as compared to placebo on intestinal malabsorption in preterm infants as measured by the time to full enteral feeding (EN- defined as time to reach three (3) consecutive days of EN feeding ≥ 150 mL/kg/day).

Methodology:

The study was designed as a multicenter, double-blind, randomized, three-arm, parallel-group, placebo-controlled study to assess the efficacy and safety of NTRA-2112 in preterm infants.

Following screening procedures, eligible infants born between 26 and up to 32 weeks of pregnancy and weighing at least 500 g at birth were randomly assigned to one of the three treatment groups in a 1:1:1 ratio. Randomization took place as close as possible to treatment initiation and within 24 hours of confirmation of eligibility. Only single or twin births were eligible for enrollment.

Treatment commenced at postnatal age of at least six hours through and including 120 hours post birth. Commencement of the treatment period for infants who were fed solely on own mother's milk (OMM) was not to begin within the first 72 hours post birth.

The treatment period was defined as the first day of investigational product (IP) dosing (Day 1) for up to 28 days or until discharge from primary hospital, if prior to Day 28. During the treatment period, the study infants were to be under supervision and medical care at the Neonatal Intensive Care Unit (NICU). Study medication was administered through a nasogastric tube or orally, with planned feeds following a recommended feeding protocol.

A final visit was conducted on Day 28 or upon discharge from the primary hospital (if achieved before Day 28) with study treatment stopping at this point regardless of whether or not the subject remained in the hospital. For infants who were not discharged within the 28-day treatment period, additional evaluations were performed on the actual discharge day.

Follow-up visits were conducted at 3-, 12-, and 24-months corrected age.

Number of Subjects (Planned and Analyzed):

Approximately 450 evaluable infants (150 infants in each of the three treatment groups) were planned to be enrolled in the study. The study was stopped before full enrollment occurred as discussed in Section **Error! Reference source not found.**. Three hundred subjects were randomized into the 3 treatment groups as follows:

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- NTRA-2112, 0.04 U/g: 108 subjects
- NTRA-2112, 0.2 U/g: 94 subjects
- Placebo: 98 subjects

Gestation age distribution for 299 subjects (one subject did not report gestation age):

- 26 to 28 weeks: 146 subjects
- 29 to 32 weeks: 153 subjects

Subjects with data at follow-up visits:

- 3-month corrected age: 142
- 12-month corrected age: 12 at database lock

Diagnosis and Criteria for Inclusion:

Inclusion criteria:

1. Male or female pre-term infants 26 to 32 weeks gestation (32 weeks + 0 day maximum). Gestation age matching (±2 weeks) between maternal dates and/or early antenatal ultrasound*
   * If both exist and difference > 2 weeks, based on early antenatal ultrasound
2. Birth weight ≥ 500 g
3. Singleton, or twin birth
4. Postnatal age up through and including Day 5 (up to 120 hours post birth).
5. Fraction of inspired oxygen ≤ 0.60 at enrollment
6. Subjects must have demonstrated cardiovascular stability at time of enrollment and were considered unstable if they require > 40% oxygen with blood pressure support and the need for umbilical artery cauterization
7. Infant was able to tolerate enteral feed
8. Infant was expected to wean off parenteral nutrition (PN) at the primary hospital
9. Informed consent form (ICF) signed by parents or legal guardian
10. In the investigator's opinion, the infant was able to comply with the study procedures and sufficiently stable to partake in the trial as required until trial completion

Exclusion criteria:

1. Complete enteral feeding
2. Major congenital malformation (eg, infants with genetic, metabolic, and/or endocrine disorder diagnosed before enrollment)
3. High index of suspicion of infection before enrolment**
   **Defined as positive blood culture, Leukocytosis > 30,000 and Leukopenia < 4,000.
4. Intra-uterine growth retardation (IUGR) defined as either weight for gestation age less than the third percentile or less than the tenth percentile with Doppler abnormalities in utero***
   ***According to Fenton preterm growth chart. If no Doppler in utero was available for infants with intrauterine growth retardation between 3rd and 10th percentile of Fenton preterm growth charts, infant was eligible to participate in the study.
5. Confirmed necrotising enterocolitis (NEC)[1]

[1] NEC is characterized by the sudden onset of gastrointestinal distress that may include symptoms such as vomiting, abdominal distention, bloody stools, or dilated loops of bowel that leads to cessation of enteral feedings (Gregory et al., 2011).

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6. Maternal diabetes (Type I/II or gestational) requiring insulin during pregnancy or in mothers past medical history
7. Hyperinsulinemia requiring glucose administration of more than 12 mg/kg/min at randomization
8. Any systemic insulin administration at randomization
9. Nothing per os (NPO) for any reason at the study entry
10. Heart and chest compression or any resuscitation drugs given to the infant during delivery
11. Subject at risk for significant GI complications such as twin-to-twin transfusion syndrome or monochorionic monoamniotic twins
12. Participation in another interventional clinical study that may have interfered with the results of this trial

Test Product, Dose and Mode of Administration, Lot Number:

- NTRA-2112, 0.04 U/g to obtain 400 µU/mL daily enteral intake administered with preterm infants' nutrition
- NTRA-2112, 0.2 U/g to obtain 2000 µU/mL daily enteral intake administered with preterm infants' nutrition

Lot numbers are provided in Appendix 16.1.6.

Duration of Treatment:

Study drug was administered for up to 28 days.

Reference Therapy, Dose and Mode of Administration:

Placebo oral formulation was administered with preterm infants' nutrition.

Criteria for Evaluation:

Efficacy:

Primary Endpoint

Number of days to achieve full enteral feeding (NFE) is defined as number of days to the first day of achieving enteral feeding of at least 150 ml/kg/day, which must be sustained for at least three consecutive days.

For subjects who were marked as fluid restricted, the cut-off value for reaching FEF was 10% less, thus 135 mL/kg/day.

Key Secondary Endpoint*

- Number of days to discharge from the hospital or readiness for discharge from hospital, whichever occurred first. Readiness-for-discharge is defined as meeting all the following criteria:
  - Infant weight ≥ 1800 g
  - Stable body temperature
  - Capable of oral feeding (reached full enteral feeding either orally or NG and not dependent on PN)

Additional Secondary Endpoints:

- Growth velocity (g/kg/day)
- Change in Z-score at 6, 8, and 10 days from initiation of treatment
- Gain in body weight during the treatment and follow-up periods
- Number and percentage of infants reaching full enteral feeding within 6, 8, and 10 days from initiation of treatment.
- Total number of days receiving parenteral nutrition
- Number of days to 120 Kcal/kg/day
- Number of days to wean-off PN (defined as complete cessation of PN support)

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Exploratory Endpoints:

- Number of days to end gastric residuals over 2 mL/measurement according to the feeding protocol
- Gain in length during the treatment period and follow-up period (long-term follow-up period)
- Gain in head circumference during the treatment period and follow-up period (long-term follow-up period)

Safety:

Safety was assessed by reports of adverse events (AEs) (including episodes of NEC, sepsis, meningitis, hypoglycemia, and death), physical examinations, vital signs, and clinical laboratory (blood chemistry and hematology).

Statistical Methods:

Analysis Populations:

The safety analysis set consisted of all infants in whom any IP was initiated.

The full analysis set (FAS) (i.e., intention-to-treat [ITT]) consisted of all randomized infants.

Sample Size:

Presentation of sample size was based on the hypothesis that NFE (number of days needed to reach full enteral feeds) in either treatment group is shorter than in the placebo group. Based on limited historical data (Phase 2), the conservative mean is 8.0 days for the placebo group and 6.6 days for each of the 2 active dose groups. This assumes a standard deviation of 3.5 days for each group.

To calculate sample size, an independent groups t-test for 85% power to account for the reduced power of the non-parametric test was used (i.e., it was expected that a t-test with 85% power would have at least 80% when the Van Elteren test was applied). A 2-sided 5% test was used to compare each active dose to placebo. These calculations give N=115 infants per group. To account for the reduction in power caused by potential missing data, the sample size was increased to 130 per group or a total sample size of 390.

If both members of a pair of twins were eligible for the study, both twins were to receive the same randomization assignment, primary efficacy analysis of FAS is presented with all infants, sensitivity analysis presented with first born twin only with similar results.

Statistical Analysis:

The analysis is based on a nonparametric test. The NFEs for each infant in the FAS are ranked from shortest time (the best outcome) to longest time. Infants who did not reach the primary endpoint by day 28, but did so at a later time, were assigned administrative censor rank of 28 days. Infants who did not achieve FEF because they experienced NEC were assigned the next set of ranks; and were assigned ranks one greater than the rank of the longest time to achieve NFEs. All infants who died were assigned the next set of ranks. Thus, each infant who achieved FEF or who died or suffered NEC has a rank.

Study Infants were randomized by center and by gestation age group. The statistical analysis was planned to include stratification of the infants by gestation age group and region (United States, Europe, and Israel), but not by center. Due to the small sample size in United States and Israel, the analysis was only stratified by gestation age group. Because the analysis was based on ranks, a Van Elteren test was used to compare NTRA-2112 to placebo.

Treatment groups were compared to evaluate the balance achieved by randomization, which seemed balanced in entry criteria. Any observed differences between the groups were to be interpreted for their clinical significance and their potential use as covariates in sensitivity analyses of efficacy endpoints.

The safety analyses are mostly descriptive and narrative in nature, when possible, analyzed further. Serious adverse events (SAEs) and AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA) and tabulated by body system, preferred term, treatment group, severity, and relation to investigational product (IP). Descriptive statistics were provided by treatment group as appropriate.

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Efficacy Results:

Primary Endpoint:

- The number of days to FEF was significantly lower in the treatment arm NTRA-2112 0.2 U/g [Median=10 days, p=0.001**] and also in treatment arm NTRA-2112 0.04 U/g [Median=10 days, p=0.043*), compared to the placebo group [Median=15 days].
- In assessing the time to FEF by gestation age, in the younger gestation age group (26 -28 weeks) the number of days to FEF was lower in the treatment arm NTRA-2112 0.2 U/g [Median=12 days, p=0.037*], and treatment arm NTRA-2112 0.04 U/g [Median=15 days, p=NS] compared to placebo [Median=17.0 days].
- In assessing the time to FEF by gestation age, in the older gestation age group (29-32 weeks) the number of days to FEF was lower in the treatment arm NTRA-2112 0.2 U/g [Median=7, p=0.018*] and treatment arm NTRA-2112 0.04 U/g [Median=9 days, p=0.06], compared to placebo [Median= 11 days].
- When analyzing first-born twin only: Number of days to FEF was significantly lower in the treatment arm NTRA-2112 0.2 U/g [Median=10 days, p=0.011*) and treatment arm NTRA-2112 0.04 U/g was [Median=10 days, p=NS], compared to the placebo group [Median=14 days]
- In assessing the time to FEF with Kaplan-Meier survival estimators, a stratified log-rank test showed a statistically significant difference between the treatment arm NTRA-2112 0.2 U/g [p=0.004**] and the placebo curves.
- There were 42 infants in the study that did not reach FEF or had a full 28-day documentation, with similar distribution between the treatment arms (NTRA-2112 0.04 U/g=16, NTRA-2112 0.2 U/g=13, Placebo=13). The main reasons for missing data were discharge to secondary hospital (N=14), sponsor stop of study (N=14), consent withdrawal (N=4) and others (N=10).

Other Secondary Endpoints:

- There was a significant difference in percentage of infants reaching FEF within 6, 8, and 10 days from initiation of treatment
    - At Day 6 there was a statistically significant higher percentage of infants reaching FEF in both treatment groups; in the NTRA-2112 0.04 U/g group [p=0.03*, N=23 (24%)], and in the NTRA-2112 0.2 U/g group [p=0.005**, N=24 (29%)] compared to placebo [N=9 (11%)].
    - At Day 8 there was a statistically significant higher percentage of infants in the NTRA-2112 0.2 U/g group [N=35 (43%)] (p = 0.04*) than in the placebo group [N=22 (26%)] who reached FEF.
    - At Day 10 there was a statistically significant higher percentage of infants reaching FEF in both treatment groups; in the NTRA-2112 0.04 U/g group [p=0.025*, N=52 (55%)], and in the NTRA-2112 0.2 U/g group [p=0.02*, N=46 (56%)] compared to placebo [N=31 (36%)].
- The number of days to reach 120 ml/kg/day was significantly lower in the treatment arms- NTRA-2112 0.04 U/g [median=6.0 days, p=0.048*) and in treatment arm NTRA-2112 0.2 U/g [median=6.0 days, p=0.0098**), compared with the placebo group [median=8 days].
- The effect of NTRA-2112 was seen early and sustained throughout enteral nutrition progression compared to placebo, as observed in Kaplan-Meier survival curves.
- Number of days to complete wean-off parenteral nutrition was significantly lower in the treatment arm NTRA-2112 0.2 U/g [Median=6 days, p=0.037*] compared to the placebo group [Median=8 days].
- The Kaplan Meier survival curve shows a sustained effect of faster wean-off PN, with a meaningful difference in the higher dose group NTRA-2112 0.2 U/g compared to placebo (p=0.077 compared to placebo).

Safety Results:

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- Treatment-emergent AEs occurred with similar frequency in all 3 treatment groups.
- Treatment-emergent AEs occurred with similar frequency in all SOCs in both NTRA-2112 and placebo groups with the exception of gastrointestinal disorders, which was higher in the placebo group (32%) vs. the NTRA-2112 0.04 U/g (22.2%) group and the NTRA-2112 0.2 U/g (17.0%) group. Hence, the NTRA-2112 0.2 U/g group presented roughly 50% less AEs compared to placebo.
- The most frequently reported TEAEs (≥ 5%) were anemia neonatal, infantile apnea, and anemia.
- Most TEAEs were mild or moderate in severity. The frequency of CTCAE Grade 4 events (life-threatening) across treatment groups was 5.6%, 3.4%, and 8.2% in the NTRA-2112 0.04 U/g, NTRA-2112 0.2 U/g, and placebo groups, respectively. Grade 5 events (death) across the 3 treatment groups occurred in 3.7%, 1.1%, and 3.1% of subjects, respectively.
- Nine subjects had TEAEs that led to death, 4 in the NTRA-2112 0.04 U/g group, 1 in the NTRA-2112 0.2 U/g group, and 4 in the placebo group. An additional death (F1804-004) in the NTRA-2112 0.04 U/g group occurred during the follow-up period after last dose of treatment.
- Overall, there were a total of 23 events of NEC and NEC-like events in 21 infants in the FIT-04 Study. During the treatment period and the 28-day follow-up, there were 18 reported events of the term NEC. 7 events of NEC were reported with the NTRA-2112 0.04 U/g group, 3 events in the NTRA-2112 0.2 U/g group, and 8 events in the placebo group. Additionally, there were 4 events of necrotising colitis (1 in the NTRA-2112 0.2 U/g group and 3 in the placebo group) and 1 event of enterocolitis in the placebo group.
- When looking at the younger population alone (26-28 weeks GA) where NEC is most prevalent: NTRA-2112 0.04 U/g group- 6 (12.2%), NTRA-2112 0.2 U/g group – 2 (4.3%), Placebo – 10 (19.6%). In addition, an exploratory statistical analysis was performed with the hypothesis of reduction of severity of NEC, using a non-parametric Kruskal-Wallis method, and showed a note-worthy difference in the 26-28 GA group (p=0.077).
- Overall, 44 subjects had SAEs during the treatment period and 28-day follow-up period. Of these, 16 (5.5%) subjects reported SAEs that were considered related by the principal investigator.
- Most events were considered to be not related to IP. A total of 9.9% of subjects overall experienced TEAEs that were reported by the investigator as possibly or probably related to IP.
- Overall, 26 (8.9%) subjects had TEAEs during the treatment period that lead to discontinuation of IP. These events occurred in 7 (6.5%) subjects in the NTRA-2112 0.04 U/g group, 6 (6.8%) subjects in the NTRA-2112 0.02 U/g group, and 13 (13.4%) subjects in the placebo group
- An exploratory analysis for safety was performed, comparing the number of infants effected by adverse events of interest (infections, NEC, death) per group. Both treatment groups showed a smaller number of subjects experiencing one of the AEs - 38 (34.5%) in NTRA-2112 0.04 U/g dose group, 34 (35.8%) in the NTRA-2112 0.2 U/g dose group and 49 (50%) in the Placebo group. When performing pairwise comparison of proportions- difference between in NTRA-2112 0.04 U/g to Placebo was p=0.034, difference between NTRA-2112 0.2 U/g to placebo was p=0.065.
- No clinically relevant effect of NTRA-2112 and no clinically relevant treatment group differences were noted for any laboratory test, vital sign, or physical finding. Of note, there was no indication of hyperglycemia based on glucose levels monitored throughout the treatment period.
- No positive anti-insulin antibodies were present after final testing was complete.

Conclusion:

This is a patient population at risk for intestinal malabsorption, often dependent on prolonged parenteral nutrition thus have associated complications and adverse events. NTRA-2112 is an intervention that targets the intestine of the premature infants to address these needs through improved absorption of fluids and nutrients with enhanced gastrointestinal tolerance leading to improved full enteral feeds, earlier discontinuation of parenteral nutrition and faster time to discharge. Clinically important improvements are seen in this large controlled clinical trial in premature infants with malabsorption with both NTRA-2112 doses when assessing each gestation age group and the important outcomes captured.

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The results of the FIT-04 trial demonstrate significant and clinically meaningful reduction of 4 days to achieve full enteral feeding in both NTRA-2112 groups when compared to placebo. This is also accompanied by other important clinical endpoints; earlier wean-off parenteral nutrition, earlier discharge time, reduced NEC incidence and NEC severity, reduced CTCAE>grade 3 events, and reduced infection rates. All of these demonstrate a clinically meaningful effect on premature infants' intestinal malabsorption and general outcomes. Despite early termination of trial, the FIT-04 trial met its goals of demonstrating the safety and efficacy of NTRA-2112 for the treatment of intestinal malabsorption in preterm infants born prior to 32 weeks gestation.

Date of Report: 30 January 2022