Correlating Sustained Feeding Tolerance (SFT) to adverse outcomes during randomized administration of *L. reuteri* for the prevention of necrotizing enterocolitis (NEC) and improved feeding tolerance in preterm infants: The CONNECTION TRIAL

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IBP-9414, a pharmaceutical grade probiotic. IBP-9414 is of pharmaceutical grade with quality standards equivalent to all drug products. Manufacturing requires full pharmaceutical GMP (21 CFR Part 210/211) throughout the entire process from cell banking to final drug product, including rigorous controls of raw materials, ingredients and excipients. The quality requirements are particularly high considering its intended use in neonatal intensive care units (NICUs) with testing for absence of a range of specified organisms as potential contaminants as well as batch control, shelf-life, and validated procedures for correct dosing of $1x10^9$ CFU.

INTRODUCTION

Evaluation of the efficacy and safety of the pharmaceutical grade probiotic *L. reuteri* for preventing necrotizing enterocolitis (NEC) and reaching sustained feeding tolerance (SFT) are the primary goals of the Connection Trial (Box 1). This is the first phase 3 study under an US IND (CTX in EU) powered to evaluate these goals. However, clinically validated endpoints are lacking for SFT.

There are also other adverse outcomes such as late onset sepsis and bronchopulmonary dysplasia in premature infants that have been related to intestinal dysbiosis, which besides NEC and SFT may also respond to *L. reuteri* administration.

OBJECTIVE

Gather and analyze data on clinical outcomes from premature infants enrolled in the Connection Trial with respect to correlations to the time to SFT.

METHODS

Clinical outcomes were gathered from investigator Adverse Event (AE) reports but for 'Confirmed NEC Events', which represent NEC confirmed by independent adjudication of plain abdominal X-rays taken for clinical signs of NEC, and/or by laparotomy or autopsy. Weight gain was based on daily recordings as was treatment with parenteral antibiotics. The duration of hospitalization was calculated until follow-up at 40 weeks ± 7 days gestational age.

Regression models were used to evaluate the association with clinical outcomes of a 1-day difference in time to SFT in 248 preterm neonates ≤ 32 weeks gestation (birth weight 750-1,000 gram) randomized to a daily enteral dose of *L. reuteri* ($1x10^9$ CFU) or placebo. Continuous variables were analyzed using linear regression models, dichotomous variables were analyzed using logistic regression models, and count variables were analyzed using negative binomial models. The time to SFT was calculated from the first dose of the investigational product till the first day when SFT had been achieved. The definition of SFT is shown in Box 2. All data was analyzed without knowledge of the treatment arm allocation of the infants.

In addition to the regression models, a score was derived based on the number of event groups registered in each infant for the following 7 outcomes: Confirmed NEC events, relevant gastrointestinal AEs, late onset sepsis, clinically suspected sepsis, bronchopulmonary dysplasia, retinopathy of prematurity, and concurrent respiratory and cardiac AEs, where each event was assigned 1 point for a total of a maximum of 7 points. The time to SFT by score (0, 1, 2 and 3+) is presented in the box plots in Figure.

RESULTS

SFT was reached at mean 17.2 days (range 3-52 days). All clinical outcomes correlated significantly to a 1-day difference in time to SFT but signs of feeding intolerance (as defined by each investigator) and the combination of concurrent respiratory and cardiac serious adverse events (Table). The number of days to SFT was directly related to the number of select clinical outcomes in the infants (Figure).

CONCLUSION

The robust relationship of time to the SFT to multiple significant clinical events supports that the combination of at least 120 ml/kg of daily enteral feeding, absence of parenteral nutrients and a modest body weight gain is clinically relevant and could become a surrogate marker for the risk of adverse outcomes in prospective trials of preterm infants.

BOX 1. The 'Connection trial' at a brief (ClinicalTrials.gov Identifier: NCT03978000).

- Primary Endpoints: Prevention of NEC and time to SFT.
- Secondary endpoints: Medical NEC, Surgical/autopsy NEC, all-cause mortality, weight gain, duration of hospitalization, feeding intolerance.
- Eligible patients: Infants with a birth weight of 500-1,500 gram and gestational age of 23-32 weeks meeting the inclusion and exclusion criteria of the study. Currently infants of 500-1,000 gram can be entered into the trial.
- Contribution centers: NICUs in Bulgaria, France, Hungary, Israel, Poland, Romania, Serbia, Spain, UK, and US.
- Study drug: IBP-9414 containing pharmaceutical grade *L. reuteri* given orally or by enteral tube at a single daily dose of $1x10^9$ CFU or placebo from within 48 hours of birth to 34 weeks +6 days GA, with follow-up at 40 weeks ± 7 days.
- Study design: The study is designed to interfere as little as possible with the clinical routine at participating units.
- Currently 850 infants have been entered into the study and independent safety evaluations have been conducted according to the protocol for the first 300+ and 600+ patients.

TABLE. Clinical outcome groups evaluated for statistical correlations to a 1-day difference in the time to SFT. The parameter estimates are presented with 95 % confidence intervals (CI) and p-values.

	Mean	N events	Estimate*	95 % CI	p-value
Confirmed NEC Events		20	1.0765	(1.0357-1.1189)	0.0002
Days with Clinical Signs of Feeding Intolerance	5.2		1.0029	(0.9791-1.0272)	0.8155
Relevant Gastrointestinal AEs		55	1.0586	(1.0293-1.0887)	0.0001
Late Onset Sepsis		29	1.0671	(1.0326-1.1028)	0.0001
Weight Gain (g/day)	21.2		-0.0831	(-0.1333--0.0328)	0.0014
Clinically Suspected Sepsis		18	1.0531	(1.0108-1.0972)	0.0133
Bronchopulmonary Dysplasia		85	1.0275	(1.0018-1.0539)	0.0360
Retinopathy of Prematurity		65	1.0500	(1.0221-1.0786)	0.0004
Number of Respiratory AEs	1.2		1.0331	(1.0214-1.0449)	<0.0001
Number of Days of Hospitalization	75.8		0.7710	(0.5086-1.0335)	<0.0001
Number of SAEs	0.4		1.0420	(1.0076-1.0776)	0.0162
Days with Antibiotic Use	15.4		1.0585	(1.0353-1.0821)	<0.0001
Concurrent Respiratory and Cardiac AEs		9	1.0392	(0.9848-1.0967)	0.1611

*For linear regression the estimate is the linear slope, for logistic regression the estimate is the odds ratio, and for negative binomial regression the estimate is the incidence rate ratio, all associated with a one day increase of time to sustained feeding tolerance.

BOX 2. Definition of Sustained Feeding Tolerance (SFT) and time to SFT.

SFT was defined as 10 consecutive days with enteral feeding at ≥120 ml/kg/day in combination with no use of parenteral nutrition (i.e. intravenous amino acids and/or lipids) and an average body weight gain ≥10g/kg body weight/day during the 10-day period.

The time to SFT was calculated as the number of days from the first dose of investigational product until the first day when SFT was reached.

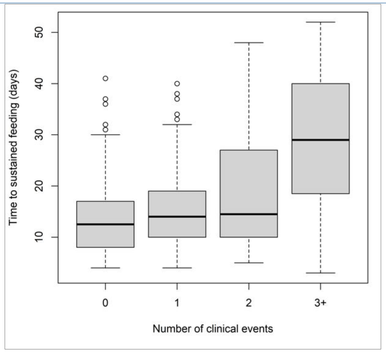

FIGURE. Box plots showing the time to SFT for infants with 0, 1, 2 or 3+ adverse clinical outcomes. The box plots illustrate median values with 25th and 75th percentiles, extreme values within 1.5 times of the interquartile range and values further away as dots.

ABSTRACT

Background. Validated endpoints are lacking for outcomes relating to sustained feeding tolerance in controlled studies of premature infants.

Introduction. The Connection trial is a phase 3 study of the pharmaceutical grade probiotic IBP-9414 (*Lactobacillus reuteri*) under US and EU INDs. In addition to NEC, the primary endpoint is the time to sustained feeding tolerance (SFT) in infants of a birth weight (BW) of 500-1500 grams randomized 1:1 to a daily enteral dose of IBP-9414 ($1x10^9$ CFU) or placebo from within 48 hours of birth until 34 weeks + 6 days GA.

Objective. Determining a correlation between SFT and adverse outcomes may provide a valuable analytic tool in the efficacy analysis of enteral feeding regimens.

Material. A blinded analysis of how clinical outcomes correlate to SFT was performed for the first 248 infants with a BW of 750-1000 gr (mean 890 gr). SFT was defined as the first day of enteral feeds ≥120ml/kg body weight maintained for ≥10 consecutive days during which no parenteral nutrition was required and the mean body weight increased with mean ≥10 g/kg/day.

Results. SFT was reached at mean 17.2 days (sd 10.4 days). Clinical outcomes associated with SFT were evaluated with linear regression models. They included events of NEC (p=0.0002), relevant gastrointestinal adverse events (AEs, p=0.0001), clinically suspected sepsis (p=0.0133), late onset sepsis (p=0.0001), bronchopulmonary dysplasia (p=0.036), retinopathy of prematurity (=0.0004), daily weight gain (p=0.0014), respiratory AEs (p<0.0001), duration of hospitalization (p<0.0001) and days with antibiotic medication (p<0.0001). SFT increased in direct proportion to the number of events occuring in individual infants.

Conclusion. This blinded evaluation of very low BW infants underlines that several adverse outcomes strongly correlate to SFT. We infer that Sustained Feeding Tolerance could be used as a surrogate marker for adverse outcomes in prospective randomized trials in preterm infants.