Re-analysis of a randomized placebo (PBO) controlled trial of intravenous (IV) choline chloride for IFALD using state-of-the-art analytic and imaging methods and contemporary definition of IFALD

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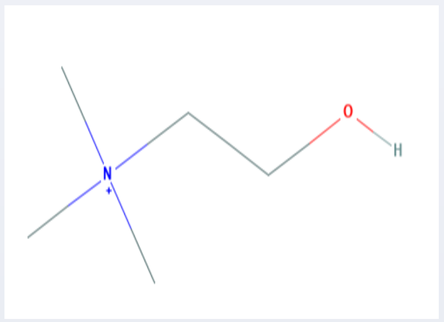
INTRODUCTION

Intestinal Failure Associated Liver Disease (IFALD) A Contemporary Definition of Long-Observed Disease:

1. Requires long-term PN: Has chronic IF; and
2. Cholestasis: Elevated alkaline phosphatase/ and/or elevated bilirubin, or histology
3. Steatosis: Imaging techniques, or biopsy
4. May also have: other signs of liver injury (elevated LFTs / fibrosis / cirrhosis / End Stage Liver Disease [ESLD])

Choline– A Key Essential Nutrient:

- An essential nutrient recognized in 1998, ubiquitous in the normal diet, mostly as phosphatidylcholine (interchangeably known as lecithin) in eggs, meat, nuts, and vegetables [(1)]
- Quaternary amine, methyl donor in many metabolic reactions, similar to B-vitamins and folate
- Necessary for cell membrane structure (phospholipids), triglyceride transport via VLDL synthesis, cholesterol transport in bile, intracellular messaging, brain development and function (acetylcholine). 95% stored in tissues as phospholipids
- Synthesis from the potential precursor amino acid methionine is impaired by parenteral (vs enteral) delivery route to the liver [(3,4)]
- Not included in PN products in sufficient amounts; recognized by ASPEN as needed but unavailable as a commercial PN product [(2)]
- Essential for cell health and survival: hepatocytes die from apoptosis in choline-deprived medium;[(5)] Increased DNA damage and apoptosis in lymphocytes in choline-deficient vs normal humans,[(6)] consistent with increased liver cancer rates in rodents after long-term choline depletion



OBJECTIVE

Table 1. Review, transform and analyze data from Buchman et al. 2001 using state of the art methods to inform a confirmatory phase 3 trial and meet regulatory requirements

Buchman et al 2001	
Randomized Double-blind Ph2 Trial [(1)]	
N = 15	>16 years old requiring >80% PN
Randomization	Usual PN or PN + 2g IV choline/Day
Duration of Treatment	24 Weeks
Visits	Weeks 2,4,6,12,16, 20, 24 weeks. Follow-up wk 34
Dose	2 g choline chloride qd in PN solution
Results	Steatosis resolved in all IV choline treated patients by CT scan (Hounsfield Units)

METHODS

Table 2. Transformation of Buchman et al 2001 Methods and Data Format: New Analytic Approaches

Format, Variable, or Method	Original	New	Rationale
Database	Patient and research charts (paper), miscellaneous tables and spreadsheets	Electronic Common Technical Document format	Required for FDA and EMA submissions and review
Steatosis (liver fat quantification)	Unenhanced CT scan calibrated to a standardized quantity (Hounsfield units)	MRI-Proton Density Fat Fraction	New gold standard, large validation database, Improved sensitivity, reliability across machines
Cholestasis	Heterogeneity of ALP baselines	Subgroup with abnormal ALP (>ULN; >1.5ULN)	Contemporary definition of IFALD (steatosis + cholestasis)
Statistical Model	Intent-to-treat, observed cases, Wilcoxon Rank-Sum Test	MMRM with baseline as a covariate, and treatment group, visit, and their interaction as fixed effects	Current standard for clinical trials analysis

- Data were imported from original source documents into an electronic Common Technical Document (eCTD) format.
- Because MRI-Proton Density Fat Fraction (PDFF) has become the gold standard method for non-invasive quantification of hepatic steatosis, CT data were transformed to MRI-PDFF using an established linear equation [(7)].
- The trial was then re-analyzed using a mixed model repeated measures (MMRM) statistical approach, and in a subgroup of patients meeting contemporary definitions of IFALD. Defined as steatosis and cholestasis: (ALP≥1.5 ULN).
- Missing data were reasonably assumed to be missing at random.

RESULTS

Table 3. Baseline Demographic and Clinical Characteristics

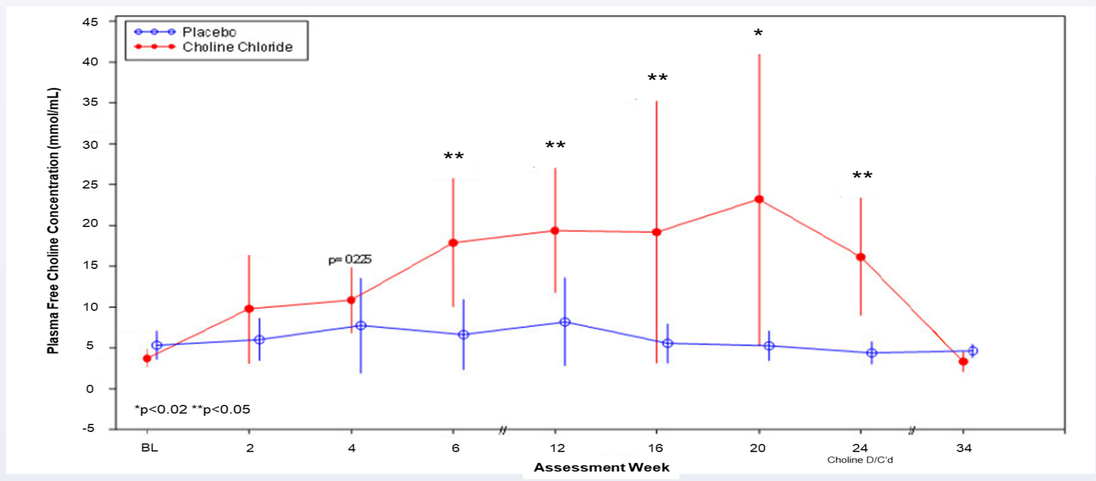
	Choline chloride group (N=7)	Placebo group (N=8)	Total Sample (N=15)
Age	33.6 (9.81)	38.8 (15.91)	36.3 (13.23)
Gender (M/F)	4/3	6/2	10/5
Body weight (kg) Mean(SD)	58.07 (6.42)	68.23 (17.3)	63.61 (13.94)
Ideal body weight (kg) Mean(SD)	63.62 (2.94)	65.51 (14.00)	64.75 (10.62)
Duration of TPN (years) Mean(SD)	12.3 (6.06)	11.3 (7.25)	11.8 (6.47)
Underlying disease			
Short bowel syndrome	1	2	3
Crohn's Disease	4	3	7
Mesenteric Vein Thrombosis	0	2	2
Pseudo-obstruction	2	1	3
Other medical history	3	6	9

- There were no significant differences between groups in gender distribution, age, ideal body weight, and duration of PN.
- Subjects in the Choline Chloride for Injection group had lower mean body weight (58.07 kg) compared to those in the placebo group (68.23 kg).
- The baseline plasma free choline concentration for both the Choline Chloride for Injection and placebo groups was ≤ 5 nmol/mL.
- Baseline values of laboratory parameters such as complete blood count (CBC), ALP, ALT, AST, GGT, and total bilirubin were similar between treatment groups.

Dr. Zummo is an employee and shareholder of ArTara Therapeutics. Dr. Buchman is a shareholder of ArTara Therapeutics. Dr. Jeppesen and Mr. Lin are consultants to ArTara Therapeutics.

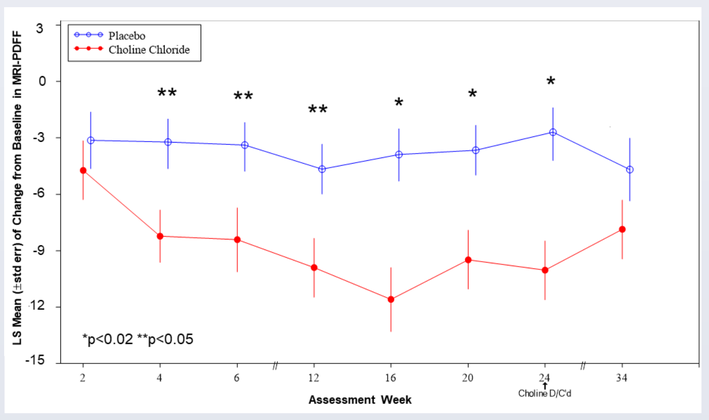
RESULTS

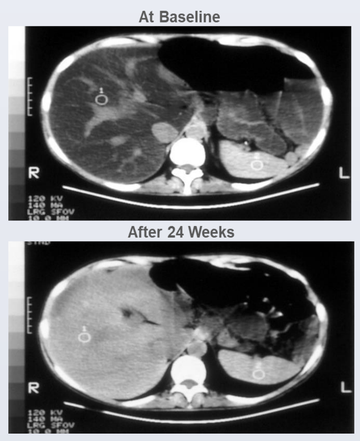
Figure 1. Normal Choline Levels Reached and Sustained



- At baseline, the mean plasma free choline (3.7 ± 1.08 [choline chloride] versus 5.3 ± 1.73 [placebo], p = 0.0504) concentrations were comparable between the choline chloride and placebo groups.
- Treatment with choline chloride increased the mean plasma free choline levels reaching a peak at Week 20 (23.2 ± 17.89 nmol/mL).
- In the Choline Chloride group, values were within or greater than the estimated normal range of choline (i.e., 6.7 to 26.9 nmol/mL) throughout the 24-week study and quickly returned to baseline levels when treatment was discontinued.

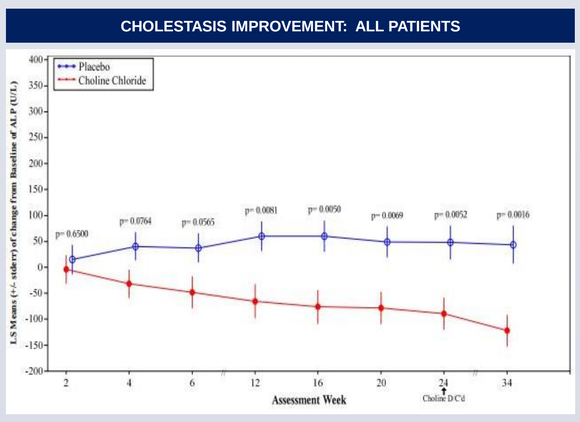
Figure 2. Reversal of Steatosis

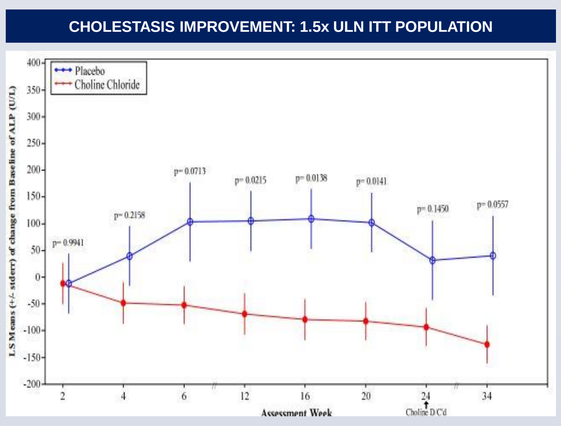




- CT converted to MRI-PDFF: *MRI-PDFF (%) = -0.572*Liver CT(HU) + 37.264(1)
- MRI-PDFF (%) values at baseline were 14.98 ± 3.729 [choline chloride] and 23.72 ± 9.126 [placebo]).
- Upon conversion of CT values to MRI-PDFF, significant differences in the LS mean change from baseline in MRI-PDFF were observed in the Choline Chloride for Injection group in comparison to placebo group at Week 4 through Week 24, demonstrating a clinically meaningful and statistically significant reduction in steatosis.
- When LS mean percent changes from baseline in MRI-PDFF were compared between treatment groups, significant differences in LS mean changes (range, 31.7% to 53.6%) were observed from Weeks 4 to 24 with p values of 0.0009 to 0.0297 favoring the Choline Chloride for Injection group.
- Comparing groups on the relative (%) change of MRI-PDFF, drug-PBO differences from Weeks 4-24 were large and clinically meaningful (range 31%-54%).

Figure 3. Cholestasis - Sustained improvement throughout study in IFALD-defining pathology





- At baseline, LS mean ALP concentration was 239.3 ± 118.93 in the choline chloride group and 148.1 ± 100.16 in the placebo group.
- In the subgroup of subjects with ALP concentration > 1.5x ULN at baseline, mean values at baseline were also comparable between the choline chloride and placebo groups (294.20 ± 87.947 versus 277.00 ± 128.693, respectively).
- MRMM analyses demonstrated statistically significant decreases in ALP concentrations at Week 12 (p = 0.008), Week 16 (p = 0.005), Week 20 (p = 0.007), and Week 24 (p = 0.005) for the Choline Chloride for Injection group, demonstrating a reduction in cholestasis.
- A trend towards significance was observed at Week 4 (p = 0.076) and Week 6 (p = 0.056). At Week 34, 10 weeks after discontinuation of Choline Chloride for Injection treatment, LS mean change from baseline in ALP concentrations still demonstrated statistically significant decreases (p = 0.002), demonstrating a significant improvement in cholestasis with treatment with Choline Chloride for Injection.
- In the sub-group analyses, improvement in ALP was consistent and substantial, with 20-30% improvement over 12-24 weeks of treatment.

Safety Summary

- A total of five serious adverse events (SAEs) were reported in four subjects.
  - Choline chloride Group: hospitalization for dehydration and fever (n=1)
  - Placebo Group: catheter sepsis (n=2); hepatic failure resulting in death (n=1), and peroneal pain due to recurrent desmoid tumor (n=1)
- None of the SAEs were considered related to study drug administration as assessed by the investigator.

CONCLUSIONS

- Using state of the art imaging (MRI-PDFF), a contemporary definition of IFALD, modern analytic approaches (MMRM), and source-verified data consistent with modern regulatory requirements, the original findings from Buchman et al 2001 were replicated and extended.
- Treatment with 2 g/day of IV Choline Chloride restored choline levels to physiologic levels and resulted in resolution of steatosis and a clinically meaningful improvement in cholestasis and was safe and well-tolerated.
- These robust results in a rare, but serious condition affecting PN patients informed the design of the Phase 3 IV Choline Chloride study

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