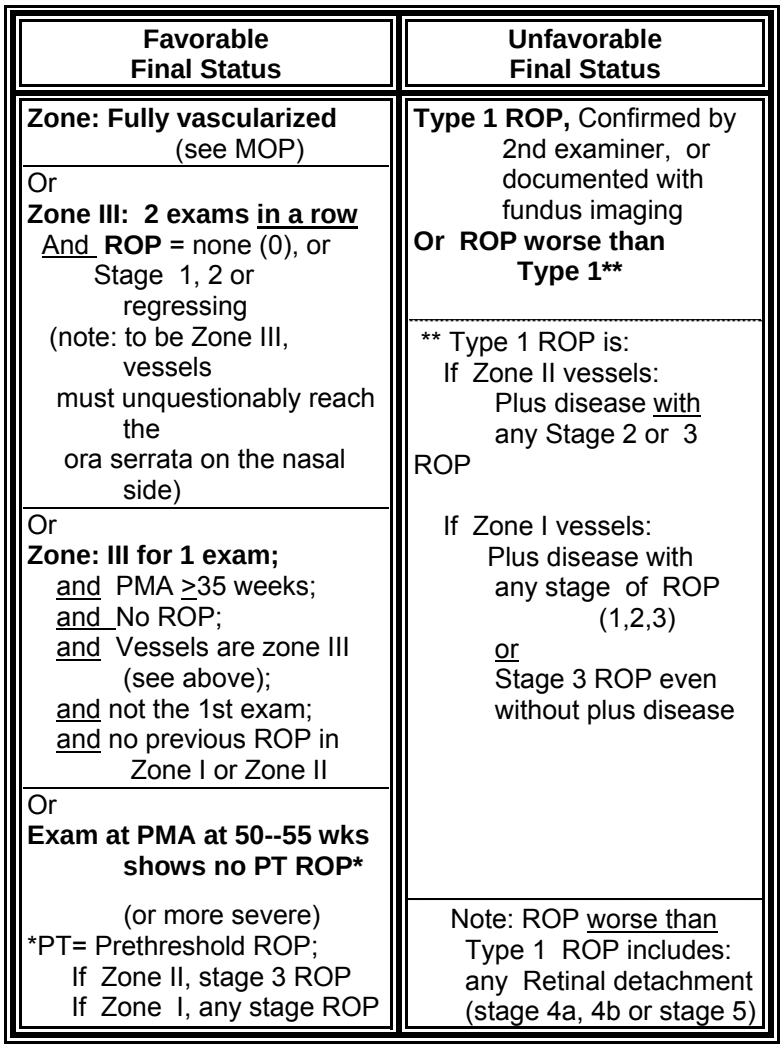
Supplementary Online Content

Phelps DL, Watterberg KL, Nolen TL, et al; for the Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Effects of *myo*-inositol on type 1 retinopathy of prematurity among preterm infants <28 weeks' gestational age: a randomized clinical trial. *JAMA*. doi:10.1001/jama.2018.14996

This supplementary material has been provided by the authors to give readers additional information about their work.

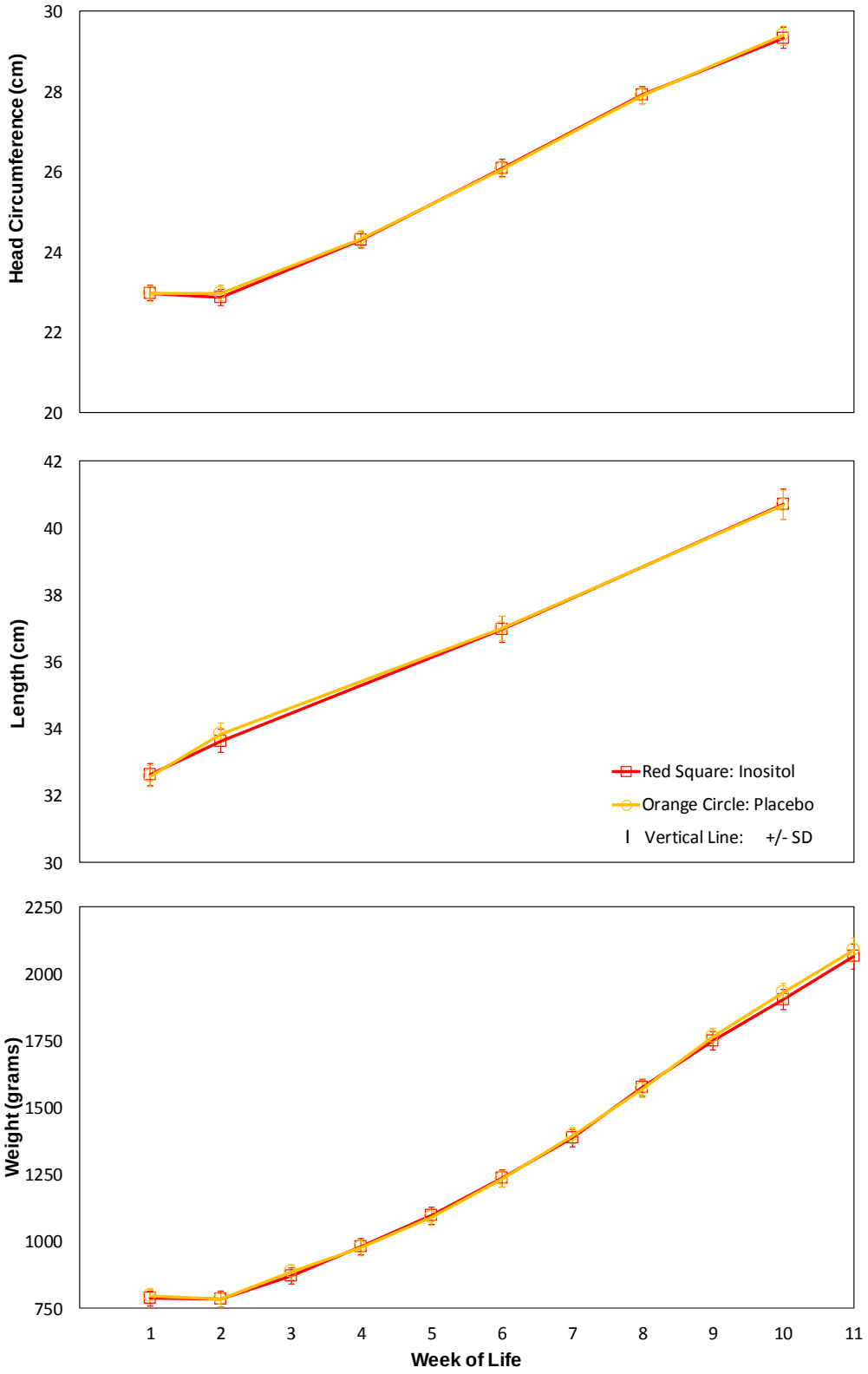
eFigure 1. ROP endpoints: *myo*-inositol to reduce ROP



Refs: ETROP outcomes and recommendations: Early Treatment For Retinopathy Of Prematurity Cooperative G. Revised indications for the treatment of retinopathy of prematurity: results of the early treatment for retinopathy of prematurity randomized trial. Arch Ophthalmol. 2003;121(12):1684-1694.

AAP/AAO joint recommendations: Fierson WM, American Academy of Pediatrics Section on O, American Academy of O, American Association for Pediatric O, Strabismus, American Association of Certified O. Screening examination of premature infants for retinopathy of prematurity. Pediatrics. 2013;131(1):189-195

eFigure 2. Growth charts



Growth was measured prospectively throughout study drug administration. Weight was recorded weekly, length at 1, 2, 6 and 10 weeks, and head circumference every 2 weeks. The figures show the estimated mean growth measure at each time point based on repeated measure model controlling for site and gestational age strata. There were no significant growth differences between treated and placebo infants.

eText. Evaluation of drug lot effects

Two *myo*-inositol drug lots were used during this trial. The first lot (Lot 1) was used from the beginning of the study in April 2014 until April 2015. The second drug lot (Lot 2) was used beginning in March 2015. Consistent with the requirements of CFR 211.166, each of the two lots was subject to standard drug stability testing. The *myo-i*nositol stability program included testing of drug under proposed storage conditions (25^{o}C, 60% relative humidity) and accelerated storage conditions (40^{o}C, 75% relative humidity) in both inverted and upright storage configurations.

Drug Lot 1 successfully completed release testing at the time of manufacture, and its stability testing program. Stability testing was done on sets of samples stored upright and inverted at 25^{o}C, 60% relative humidity at time points 0, 3, 6, 9, 12, 18, 24, and 30 months, and on samples stored upright and inverted at 40^{o}C, 75% relative humidity at time points 0, 1, 3, and 6 months (24 total data points). Lot 1 met all quality specifications (including particulate counts according to USP 788 and a standardized visual inspection to detect extraneous particulate matter) at all time points and no product quality concerns were identified.

At approximately 17 months of storage, particulate material was identified by the standardized visual inspection in 17 of approximately 900 vials tested from Lot 2.

To address concerns that the results from the trial might be confounded by the particulate material identified in the Lot 2 *myo-i*nositol material, additional analyses were conducted to evaluate potential effects of Lot on key outcome measures. Additional models, consistent with those used for the primary analyses, were generated to evaluate the effect of Lot 1 and Lot 2 on both primary and secondary outcomes. The results are presented in eTable 1 (Primary and Secondary Outcomes) and eTable 2 (Other Clinical Outcomes). To maximize the potential to observe a difference between the two treatment lots, only treated participants who were administered treatments from exclusively Lot 1 or Lot 2 were used in the analyses (81 participants received treatment from both lots). As shown in eTables 1 and 2, these analyses provide no evidence that treatment with Lot 2 *myo-i*nositol increased the risk of poor outcomes in comparison with Lot 1. Analyses were also conducted comparing infants that received all their *myo-i*nositol doses from Lot 1 to those who received any of their *myo-i*nositol doses from Lot 2 with similar results (not shown).

eTable 1. Post-hoc analysis of primary and secondary efficacy outcomes for lot 1 use only vs. lot 2 use only[a]: on-treatment population

	Lot 1 Use Only				Lot 2 Use Only				*myo*-Inositol Lot	
On-Treatment Population[b]	***Myo*-Inositol (N=124)**	**Placebo (N=124)**	**RR [95% CI][c]**	**P-value[c]**	***Myo*-Inositol (N=145)**	**Placebo (N=158)**	**RR [95% CI][c]**	**P-value[c]**	**RR [95% CI][d]**	**P-value[d]**
Primary Outcome:										
Type 1 ROP[e] or death before ROP endpoint, including adjudicated endpoints[f]	40 (33%)	29 (23%)	1.43 [0.98,2.08]	0.06	41 (29%)	31 (20%)	1.46 [0.98,2.18]	0.06	0.90 [0.64,1.28]	0.57
Prespecified Exploratory Analysis of Primary Outcome Components:										
Death before ROP endpoint	21 (17%)	15 (12%)	1.36 [0.75,2.45]	0.31	24 (17%)	16 (10%)	1.64 [0.91,2.95]	0.10	1.00 [0.59,1.68]	0.99
Type 1 ROP[e]	18 (18%)	14 (14%)	1.28 [0.68,2.40]	0.44	14 (13%)	11 (9%)	1.54 [0.73,3.27]	0.26	0.72 [0.39,1.35]	0.31
Type 1 ROP[e] including adjudication[f]	19 (19%)	14 (13%)	1.41 [0.75,2.61]	0.28	17 (14%)	15 (11%)	1.40 [0.74,2.67]	0.31	0.78 [0.44,1.40]	0.41
Secondary Outcomes:										
Any ROP	70 (68%)	64 (59%)	1.14 [0.93,1.38]	0.20	71 (59%)	90 (63%)	0.98 [0.82,1.17]	0.81	0.80 [0.64,1.00]	0.05
Type 2 or more severe ROP	51 (50%)	52 (48%)	1.03 [0.79,1.34]	0.81	53 (45%)	68 (49%)	0.95 [0.75,1.21]	0.68	0.78 [0.58,1.05]	0.10
All-cause mortality up to 55 weeks PMA	27 (22%)	16 (13%)	1.64 (0.95,2.84)	0.07	24 (17%)	16 (10%)	1.64 (0.91,2.95)	0.10	0.78 (0.48,1.26)	0.31

NOTE: Analyses for each outcome include all randomized individuals with available data for that outcome; as such, percentages are based for each outcome on the number of individuals with corresponding outcome data. Missing outcomes occur when neither a confirmed favorable or unfavorable outcome can be determined (e.g. lost to follow-up prior to ROP status and/or insufficient data available for adjudication). See figure 1 for more details on availability of outcome data. More infants are included for Any ROP than Type 1 ROP as some infants experienced some level of ROP even though final ROP status was never met (i.e. favorable or unfavorable for Type 1 ROP).

[a] Outcomes summarized in this table were collected up to 55 weeks PMA

[b] The On-Treatment population includes all randomized infants who started treatment. This excludes 6 infants (4 *myo*-Inositol 2 Placebo) from the intention to treat population (ITT) who were randomized to treatment, but never treated.

[c] Robust Poisson regression adjusting for center where possible and GA strata were used to obtain p-values and estimate adjusted relative risks and 95% CI for *myo*-Inositol compared to Placebo for efficacy outcomes.

[d] Robust Poisson regression adjusting for center where possible and GA strata were used to obtain p-values and estimate adjusted relative risks and 95% CI for *myo*-Inositol in Lot 1

use only compared to *myo*-Inositol in Lot 2 use only for efficacy outcomes.
[e] Type 1 ROP is the severity meeting criteria for surgical intervention.
[f] Type 1 ROP outcome was assigned by adjudication (Lot 1 use only: 2 *myo*-Inositol, 6 Placebo; Lot 2 use only: 10 *myo*-Inositol, 14 Placebo) when the final ROP endpoint was not available (see text).

eTable 2. Post-hoc analysis of secondary efficacy and safety clinical outcomes[a] for lot 1 use only vs. lot 2 use only: on-treatment population

	Lot 1 Use Only			Lot 2 Use Only			*Myo*-Inositol Lot
On-Treatment Population[b]	***Myo*-Inositol (N=124)**	**Placebo (N=124)**	**P-value[c]**	***Myo*-Inositol (N=145)**	**Placebo (N=158)**	**P-value[c]**	**P-value[d]**
Secondary Efficacy Outcomes							
All-cause mortality	21 (17%)	15 (12%)	0.21	24 (17%)	16 (10%)	0.08	0.81
BPD[e]	66 (63%)	62 (57%)	0.37	70 (56%)	79 (55%)	0.24	0.29
BPD or Death from BPD prior to 37 Weeks	66 (63%)	62 (57%)	0.37	70 (56%)	79 (55%)	0.24	0.29
Severe IVH Grade III or IV[f]	26 (21%)	23 (19%)	0.60	17 (12%)	21 (14%)	0.67	0.007
Safety Outcomes							
Late onset Sepsis (after 72 hours of age)	30 (24%)	28 (23%)	0.69	40 (28%)	30 (19%)	0.06	0.10
NEC[g] suspected or proven	15 (12%)	12 (10%)	0.62	11 (8%)	14 (9%)	0.84	0.63
Surgical NEC	8 (6%)	3 (2%)	0.12	4 (3%)	6 (4%)	0.69	0.55
Spontaneous GI[h] perforation without NEC	9 (7%)	9 (7%)	0.89	5 (3%)	12 (8%)	0.14	0.12
Days on ventilator through 28 days, Mean (SD)	15.9 (1.1)	16.1 (1.1)	0.83	14.7 (1.0)	14.8 (0.9)	0.67	0.46
Days on Oxygen through 28 days, Mean (SD)	22.8 (0.8)	23.7 (0.9)	0.37	22.7 (0.7)	23.2 (0.6)	0.65	0.42
Days on parenteral nutrition	29.6 (2.2)	27.0 (1.7)	0.38	29.3 (1.9)	25.9 (1.5)	0.17	0.94
Pulmonary Hemorrhage	11 (9%)	13 (10%)	0.86	10 (7%)	9 (6%)	0.76	0.29
PDA[i]	64 (52%)	66 (53%)	0.52	74 (51%)	77 (49%)	0.75	0.62
PDA requiring indomethacin	33 (27%)	34 (27%)	0.55	23 (16%)	26 (16%)	0.66	0.14
PDA requiring surgery	19 (15%)	18 (15%)	0.93	14 (10%)	17 (11%)	0.70	0.08
Seizure treatment 2 or more days	4 (3%)	2 (2%)	0.38	3 (2%)	3 (2%)	0.83	0.50
Hearing screen at discharge failed in either ear	13 (16%)	10 (12%)	0.74	17 (18%)	14 (13%)	0.22	0.96
Pre-Specified Exploratory Outcomes							
Cystic areas in parenchyma within 28 days	4 (9%)	1 (3%)	0.05	6 (13%)	4 (9%)	0.67	0.58

NOTE: Analyses for each outcome include all randomized individuals with available data for that outcome; as such, percentages are based for each outcome on the number of individuals with corresponding outcome data.

[a] Clinical outcomes summarized in this table were collected through the first of death, discharge, transfer, or 120 days after birth.

[b] The On-Treatment population includes all infants started treatment. This excludes the 6 infants (4 Inositol 2 Placebo) from the intention to treat population (ITT) who were randomized to treatment, but never treated.

[c] For binary outcomes, Cochran Mantel Haenszel tests adjusting for center where possible and GA strata were used to obtain p-values for *myo*-Inositol compared to placebo. For continuous measure of days on ventilator and supplemental oxygen, ANOVA adjusting for center where possible and GA strata were used to obtain the p-values for *myo*-Inositol compared to placebo.

[d] For binary outcomes, Cochran Mantel Haenszel tests adjusting for center where possible and GA strata were used to obtain p-values for *myo*-Inositol in Lot 1 only to Lot 2 only. For continuous measure of days on ventilator and supplemental oxygen, ANOVA adjusting for center where possible and GA strata were used to obtain the p-values for *myo*-Inositol in Lot 1 only to Lot 2 only.

[e] BPD = Bronchopulmonary dysplasia defined as requiring oxygen at 36wks PMA to maintain an oxygen saturation ≥ 90%.

[f] IVH = Intraventricular hemorrhage

[g] NEC = Necrotizing enterocolitis

[h] GI = Gastrointestinal

[i] PDA = Patent ductus arteriosus