PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	A Multicenter, Randomized Trial of Preterm Infants receiving Caffeine and Less Invasive Surfactant Administration Compared to Caffeine and Early Continuous Positive Airway Pressure (CaLI Trial): Study Protocol
AUTHORS	Ines, Felix; Hutson, Shandee; Coughlin, Katherine; Hopper, Andrew; Banerji, Anamika; Uy, Cherry; Finer, Neil; Rich, Wade; Morales, Ana; Steen, Jane; Katheria, Anup

VERSION 1 – REVIEW

REVIEWER	Christian Wieg Dpt. of Neonatology, Klinikum Aschaffenburg, Germany
REVIEW RETURNED	30-Mar-2020

GENERAL COMMENTS	Title ff: As it is a two center trial, the term "multicenter trial" should be avoided. Introduction: p4/l20 and p5/l49: In contrast to several European countries LISA is not accepted worldwide Methods: it is crucial for the success of LISA to establish the aerization of the lung before surfactant is administrated. So the PEEP level during the first minutes of transition should be determined to be 5 to at least 8 mbar. In the protocol the time of surfactant therapy sholud be set to min.15 to max. 45 minutes after birth. If LISA is performed to early during transition it may cause hemodynamic imbalances comparable to early tracheal intubation. Intubation criteria in the NICU: The PEEP level (CPAP) and MAP (NIV) have to be defined, the FiO2 alone is not sufficient to describe an oxygenation failure.

REVIEWER	Norbert Teig University Children's Hospital Bochum Dpt.of Neonatology and Paediatric Intensive Care Ruhr-University Bochum Germany
REVIEW RETURNED	16-Apr-2020

GENERAL COMMENTS	The authors are presenting a protocol outline of a study on less invasive surfactant administration compared to a conventional mode of surfactant instillation in very preterm infants less than 30 completed weeks of gestation. Several randomized have been published on this topic in recent years. The protocol presents no real new study aims with the exception that it will be performed in the US

	which is one of the countries where LISA is still not widely adopted. If the hypothesis of this study became true, however, it could evoke important changes in the practice of the US neonatal community. Major comments: Intervention: The infants are planned to be placed on CPAP in the delivery room with a pressure of 5-6 cm of water which seems rather low for infants in the early transition period. The European consensus recommends at least 6 cm water pressure when using CPAP in this setting. The infants are to be randomized in infants on CPAP who shall receive surfactant by LISA and those who get surfactant after intubation and ventilation. The protocol states that the LISA group will receive surfactant as soon as they are randomized (between 5 minutes and 2 hours of age) regardless of the severity of lung disease . A within the trial also relatively mature preterm (28-29 weekers) are randomized, which often do need no surfactant at all and can be safely managed on CPAP, this approach exposes some infant to an unnecessary risk (of surfactant administration) and needs further explanation . The conventional group, however, is scheduled to be intubated only after having reached a defined grade of disease severity, which includes high oxygen demands or hypercapnia/acidosis for at least 2 hours. Surfactant this will be given to all these infants under no circumstances prior to 2 hours after birth. This is not in line with current evidence. The European guidelines of surfactant administration recommend giving surfactant if infants needed more than 30% oxygen on noninvasive support. By this trial design one does not compare two different methods of surfactant administration but two completely different philosophies (actually early less invasive vs. late invasive surfactant administration). No criteria are stated when to give surfactant to the conventional group. I presume it will be given to all intubated babies, but this does not appear from the paper. Uniform criteria should be given when to extubate intubated babies to obviate bias between both groups. Caffeine is planned to be given differently in both groups. The LISA group will receive it prior to the LISA procedure, the conventional group as soon as possible after birth. What is the reason for this difference (and bias)? Criteria for intubation are not specific enough. What does “prolonged hypoxia” and “prolonged PPV” mean in this setting. A clear cut definition is important as it will determine how many infants at all will still be not intubated after 5 minutes and will be available for randomization. Outcomes:

	The primary outcome should be combined with the mortality rate within the first 72 hrs of life because this is a competing risk. As important secondary outcomes should be included: - FIP or NEC > stage I (the papper von Kribs et al in JAMA Pediatr has seen more babies to be operated on with perforated bowels in the LISA group) - ROP with need for treatment (because non-invasive support may increase the risk for fluctuating oxygen saturations) - Death until discharge No definition is given for “requirement of supplemental oxygen at 36 weeks”. Is this tested by room air challenge? What is the threshold Oxygen saturation for supplementing O2? How to proceed with infants on CPAP or HHHFNC without additional oxygen need? No definiton is given for “neurodevelopemntal impairment” and its grading. Randomization procedure: It is always challenging to perform ethical sound research in the delivery room setting. The proposed trial plans to get parental consent prior to birth, which certainly will be the best way. However, the plan also allows for getting consent within the first two hours after birth. This postpones potentially beneficial treatment for the infants (surfactant) or increases the drop-out rate (and makes the study not representative for widespread use) as the infants already had to be intubated prior to consent. Statistics: As multiples are randomized to the same intervention: was sample size calculation correct for this procedure? Publication: As the primary study outcome is ventilation within the first 3 days of life it becomes not quite clear why the authors plan to analyze their data only after 24 months. This would considerably delay the dissemination of the primary study result. Certainly, safety concerns are an important reason why many neonatologists hesitate to perform LISA and the assessment of neurodevelopment at 24 months of age should be undertaken, but wouldn’t it be wiser to split the analysis into two publications? Minor comments: It is stated that LISA is not widely accepted. At least it should be conceded that it is not universally accepted but has found wide acceptance in some areas (e.g. mainland Europe (Klotz D et al, Eur J Ped 2017)). The authors want to document esophageal surfactant administration by using a gastric tube. In my experience there is often a spillover of surfactant from the trachea into the pharynx (and consecutively into the stomach) even if your tracheal catheter is placed correctly. So surfactant in the gastric aspirate does not necessarily proof that your catheter was in the wrong position.

	The introduction of the abstract is a copy and paste version of the actual introduction and thus should be shortened (including the referral to reference 4). No initial dose for caffeine is given in the paper. The first author of reference 2 is “Göpel” not “Gopel” Reference 3 is a retrospective study. The same group has published a large randomized trial in extremely immature infants, which should be cited instead (Kribs et al, JAMA Pediatr 2015). Reference 4 is incompletely cited Reference 7 cites an older version of the European consensus on surfactant administration. The most recent consensus has been published 2019.

REVIEWER	Claudia Roll Department of neonatology, pediatric intensive care, sleep medicine Vest Children's Hospital Datteln University Witten-Herdecke 45711 Datteln Germany
REVIEW RETURNED	19-Apr-2020

GENERAL COMMENTS	This trial actually addresses the merits of prophylactic surfactant administered to all infants via LISA regardless of oxygen requirements, as opposed to a wait-and-see strategy until the requirement for supplemental oxygen exceeds 40%. As the protocol does not allow for secondary surfactant administration given by LISA as well, need for surfactant arising after the randomization will inevitably start endotracheal mechanical ventilation and scored as treatment failure. Major points: 1) I have some concerns about the primary endpoint “need for intubation within the first 72 hours of life” because I think this is not of upmost clinical importance. 2) The criteria for treatment failure (intubation) in the delivery room are very vague (“ineffective ventilation”, “prolonged PPV”, “prolonged hypoxia”). Moreover, an infant may meet inclusion criteria (heart rate > 100 ppm on CPAP) but treatment failure criteria (FiO2 > 40%) at the same time. 3) The clinical situation of infants with prenatal consent who undergo the intervention at 5 min of age in the delivery room is quite different from that of infants with postnatal consent who will undergo the intervention in the NICU. In my view, this would have required stratification. 4) The authors should compare the design of the trial to that of previous trials randomizing preterm infants to LISA or control (Göpel et al, Lancet 2011; Kribs et al, JAMA Pediatr 2015) to describe the added value of the new trial. 5) The authors declare the trial to run from January 2020 to January 2025. The trial is already recruiting patients (www.clinicaltrials.gov) prior to publication of the protocol. Minor points: Page 1: The title should be more precisely describe the study topic. It should be stated explicitly, that the LISA group receives early continuous positive airway pressure as well and that the only

	difference is that one group gets early prophylactic surfactant by LISA, while the other group gets CPAP only. Page 2: The first reference given (this is in the introduction part of the abstract) is number 4. If the authors give references in the abstract, the first sentence should be referenced as well (I suppose this would be reference number 1). The merits of avoiding endotracheal intubation in the delivery room have been addressed as well in two sequential meta-analyses (Fischer HS et al, Pediatrics 2013; Fischer HS et al, Eur Respir Rev 2018). The last sentence of the introduction in the abstract states the aim of the study. As in the title, it does not become clear that the LISA group will receive early CPAP in the same way as the control group. Page 3: Article summary: The fact that this is the first LISA study in the US is in my opinion not of importance for the summary. The small sample size should be overcome by including more centers. Introduction: References 2, 3. Reference 3 refers to a retrospective observational cohort study on LISA administration. LISA was not compared to CPAP alone, but was considered within the wider range of complications of prematurity. I think it is mandatory for this paper to cite the NINSAPP study as well (Kribs A et al, JAMA Pediatr 2015). In this randomized controlled study in extremely preterm infants, LISA was compared to surfactant administration during mechanical ventilation. The authors call their study “multicenter” (line 53), however, they plan to include just 2 centers. They should make that clear at this point, and they should consider to make it a real multicenter study by including more centers to get timely and reliable results. There is some redundancy in the manuscript. In the last sentence, page 3, the authors statement on publishing is the same they made before. Page 4: See last sentence page 3. The paragraph starting line 7 gives again information that has been given before. I think the manuscript needs some reorganization to present information in a more consistent and less redundant fashion. Specific aims: Here, the fact that both groups will be managed with early CPAP is given for the first time (“compared to CPAP alone”). This should be done from the beginning (title, abstract), see above. Page 5: Methods and analysis: multicenter and “2 neonatal intensive care units” - see comment above. Page 6 Inclusion criteria: It should be clarified that “spontaneously breathing” is with CPAP. Randomization: Again, there is redundancy in this part of the manuscript. Reorganization is necessary.

	It remains unclear to me whether CPAP only or CPAP or NIMV is allowed before randomization. Page 9 NICU: Abbreviations SMB and LLU are not introduced.

REVIEWER	Peter Dargaville Royal Hobart Hospital, Hobart, Australia I am the Chief Investigator of the OPTIMIST-A trial, a multicentre collaborative trial of minimally-invasive surfactant therapy in preterm infants managed on CPAP.
REVIEW RETURNED	23-Apr-2020

GENERAL COMMENTS	Ines and coworkers present a protocol for a randomized controlled trial of surfactant administration via LISA compared with continuation of CPAP in preterm infants <30 weeks gestation at age 5-120 minutes. Both groups will have received caffeine. The intervention is not blinded. Post-intervention management includes criteria for intubation, but does not protocolise other aspects of management. The primary outcome measure is need for intubation (or reaching treatment failure criteria, even if not intubated) in the first 72 h. A total of 75 subjects will be enrolled in each group, giving 80% power to detect a 22% absolute risk reduction in the intubation rate, from the current estimated rate of 49%. This proposed study pursues an important line of enquiry regarding early respiratory management for the preterm infant. The design has elements that are similar to one completed and one ongoing RCT of surfactant delivery via thin catheter, but as the authors state there is not yet wide acceptance of the LISA approach (although see comments below). The protocol as written has some major shortcomings, including a) some important flaws in trial design that could seriously limit its impact, b) lack of detail in many aspects, and c) lack of a logical structure. MAJOR COMMENTS 1. The trial as currently designed is something between a study of delivery room care and a trial of early respiratory management in the NICU. I urge the authors to either focus on either delivery room care, or, preferably, to make this a trial of early respiratory management, with eligibility extending beyond 2 hours. 2. It seems difficult to justify a trial design where there is no FiO2 threshold, or any other measure of RDS severity, to identify infants who actually have RDS and may benefit from exogenous surfactant. We already have seen in the VON-DRM study (Dunn Pediatrics 2011) that non-selective surfactant delivery soon after birth (albeit via INSURE) has no advantage over application and continuation of CPAP. Particularly not for the 26-29 week infants, who will be numerically the bulk of participants in the CaLi study. I strongly urge the authors to reconsider this aspect of design. Why not make this a trial of selective early LISA versus continuation of CPAP, imposing as an inclusion criterion some measure of RDS severity so as to leave out infants with no or very mild RDS, for whom CPAP alone would be expected to be sufficient. 3. If such a trial design is adopted, a contraction of the gestation range should be considered. One imagines that at the two participating centres, the majority of infants born at 24 and 25 weeks gestation would be intubated in the DR as part of resuscitation (despite the best efforts of care providers to transition them on CPAP). Why not limit the gestation range to 26-29 weeks,

	a group for which we expect non-invasive support (including mask PPV) to suffice during the delivery room transition in most cases? 4. It is difficult to understand why this is being proposed as a trial of caffeine as well as LISA. As the authors state (page 10, line 41), caffeine is already given immediately after birth in both centres. I strongly recommend that the study design (and the study name) be simplified by removal of caffeine as one of the interventions under study. All that then is required is to state that the receipt of caffeine is one of the inclusion criteria. Whilst other studies have not mandated the use of caffeine prior to LISA, it is widely practiced and strongly recommended in recent guidelines (e.g. Kribs Clin Perinatol 2016, Vento Neonatology 2019). 5. The introduction to the study is not adequate. 5a. A three-quarter page introduction with a handful of citations is not sufficient to describe the landscape in which the trial is being conducted, and state the case for conducting it. 5b. The introduction starts with a statement about delivery room resuscitation, but the CaLi study is about early respiratory management. 5c. The Isayama meta-analysis (JAMA 2016) does indeed find an advantage of LISA over other early respiratory therapies for death/BPD, but of course includes a mixture of DR trials (e.g. COIN/SUPPORT/VON-DRM etc) done in unselected populations, and non-DR trials (almost all of the LISA studies) which are in selected populations of preterm infants. On this basis, it is a leap of faith to propose that the Isayama paper provides support for a DR study of LISA. 5d. The landscape in which this trial will conducted is very poorly described. Even the details of current management in the infants at the two participating centres would be useful. What proportion of infants 24-29 weeks gestation at each centre are currently intubated immediately after birth. What proportion are thus likely to be eligible? 5e. Further to 5d, there is also a selective quoting on what is an emerging literature on surveys of current practice in relation to the use of thin catheter surfactant techniques (e.g. Klotz 2017, Heiring 2017, Jeffreys 2019, Beltempo 2018, Kurepa 2019 – done in the US). It needs to be clear that LISA/MIST is an emerging therapy around which there is a fair deal of experience (as the surveys attest), and but perhaps not certainty with regard to its benefit, especially not compared with continuation of CPAP. The latest version of the European Consensus Guidelines should be referenced (rather than the 2017 version). 5f. It would be useful at this juncture also to document what the local experience of the proposed thin catheter method has been (numbers, safety, success). 5g. The last paragraph (page 6, lines 7-18) contains text that largely belongs in the Protocol itself. 6. The structure of the protocol requires a great deal of work. 6a. I urge the authors to draw on the structure of protocols of other studies, including those previously conducted by senior members of the study team. Examples of issues to be addressed: 6b. Under the major heading Specific aims we find primary and secondary outcomes, study timeframe and costs 6c. Information about screening and consent is found in several locations, including on page 13, lines 12-27 under the heading Human subjects protection.

	6d. The section headed Study design should be a major heading with subheadings for all the usual elements that go with a clinical trial. 6e. The section on Data collection should move before Statistical Analysis. 6f. Other sections contain text on a mixture of topics. Each sub-section should be reviewed to ensure that the text in that section belongs there. 7. The protocol lacks detail and requires clarification in numerous places 7a. Some more detail of important aspects of the LISA technique is required. i) Will the proceduralists be trained in the technique, and how? ii) Why use intubation tape to mark the catheter insertion depth (instead of a wax pencil?). Have the authors trialled this method of marking the catheter? Tape generally does not stick well to these catheters, which are made of fluorinated polymer. iii) Will premedication or sedation be given? What other measures of comfort will be used? iv) Is videolaryngoscopy an option? v) How will CPAP be maintained during the procedure? 7b. Why give caffeine at birth in the CPAP group, and prior to the procedure in the LISA group (page 10, line 27-29)? This is in contradiction to what is stated later (line 40-42). 7c. It seems odd to include the criteria for intubation in the delivery room (page 11, lines 3-9), because these would or should apply to infants not yet being considered for the study. 7d. The criteria for intubation in the NICU (page 11, lines 14-22) require clarification. i) Will it really be necessary to have FiO2 >0.40 for two hours before qualifying for intubation (FiO2 might have got to 0.60 or 0.80 in that time). ii) There must be an upper limit for SpO2 as well as a lower limit. iii) Why do the blood gases need to be 2 hours after randomisation? 7e. Under Human Subject Protection it is stated (page 12, line 28-33) that 'both groups will receive the same surfactant…". But this is not necessarily true – some infants in the CPAP group will not qualify for intubation and thus will not receive surfactant. 7f. The statistical analysis plan is rudimentary. It should focus first and foremost on the handling of the primary outcome, and then other outcomes, documenting how alpha error is to be avoided. What are the 'various hemodynamic parameters' and why are they specifically mentioned? 7g. Why has a 22% absolute risk reduction been chosen? 7h. The information on handling of adverse event reporting is detailed, but suggests that there may be a rather burdensome amount of reporting to the DSMC /Ethics committee. The collection of data on secondary outcomes will take care of many of the standard AEs that occur in preterm infants. 7i. Is it really correct to expect that the DSMB will 'protect all study patients'? (page 14, line 37). 8. The authors are somewhat dismissive of the possibility of blinding the intervention (page 9, lines 5-6), especially given that the trial is being done in only two centres, both of which one presumes are reasonably well-resourced. Blinding of such an intervention has been achieved previously in an INSURE study (Reininger J Perinatol 2005) and has been written into the design of the OPTIMIST-A trial (Dargaville BMC Pediatrics 2014).

	OTHER COMMENTS Abstract The abstract at present recapitulates most of what can be found in the introduction (or vice versa), and gives no detail on many aspects of the trial design (PICOT, plus numbers of subjects etc). Figure 1. The flowchart should be a visual representation of all aspects of the trial processes, including screening/consent/eligibility (inclusions and exclusions)/ randomisation/intervention/post-intervention management. It is lacking several of these elements at present.

VERSION 1 – AUTHOR RESPONSE

Please leave your comments for the authors below Title ff: As it is a two center trial, the term "multicenter trial" should be avoided.

- Since the submission of the protocol we have added a third center and now will fit the definition of a multicenter trial.

Introduction: p4/l20 and p5/l49: In contrast to several European countries LISA is not accepted worldwide

- Sentence has been clarified to indicate that the LISA procedure is widely accepted in Europe, however, it is still not accepted widely in our country.

Methods: it is crucial for the success of LISA to establish the aerization of the lung before surfactant is administrated. So the PEEP level during the first minutes of transition should be determined to be 5 to at least 8 mbar.

-In the United States, PEEP/CPAP levels that are accepted and used in NICU's are 5-7 cmH2O.

In the protocol the time of surfactant therapy should be set to min.15 to max. 45 minutes after birth. If LISA is performed to early during transition it may cause hemodynamic imbalances comparable to early tracheal intubation.

- In order to make this trial pragmatic we set the assessment time for randomization at 5 minutes. None of our infants have had LISA before 15 minutes life. We have added this to the protocol. However due to the co-intervention of caffeine we are unable to set an upper limit to 45 minutes. We strongly believe the success of LISA is dependent on caffeine despite it not be written in previous trials.

Intubation criteria in the NICU: The PEEP level (CPAP) and MAP (NIV) have to be defined, the FiO2 alone is not sufficient to describe an oxygenation failure.

- We will add a minimum PEEP of 6 and MAP of 8 cm of H20 as criterion with FiO2 before infants can be intubated.

This trial actually addresses the merits of prophylactic surfactant administered to all infants via LISA regardless of oxygen requirements, as opposed to a wait-and-see strategy until the requirement for supplemental oxygen exceeds 40%.

- Yes, this trial is meant to evaluate prophylactic surfactant so using an FiO2 approach is not indicated.

As the protocol does not allow for secondary surfactant administration given by LISA as well, need for surfactant arising after the randomization will inevitably start endotracheal mechanical ventilation and scored as treatment failure.

- Yes we decided that if a child needed additional surfactant that they should be given it via endotracheal intubation. We also wanted to avoid the dilemma of an older infant (as opposed to right after birth) requiring premedication to intubate, a practice for which we do not have experience using the LISA technique. While this will increase the failure rate it also ensures infants will “safely” get our standard of care which is endotracheal intubation and surfactant use.

1) I have some concerns about the primary endpoint “need for intubation within the first 72 hours of life” because I think this is not of upmost clinical importance.

- This primary outcome has been used in a number of respiratory trials particularly for small studies. While collection of other data including BPD or neurodevelopmental outcomes will be reported this is still meant to be a pilot study.

2) The criteria for treatment failure (intubation) in the delivery room are very vague (“ineffective ventilation”, “prolonged PPV”, “prolonged hypoxia”). Moreover, an infant may meet inclusion criteria (heart rate > 100 ppm on CPAP) but treatment failure criteria (FiO2 > 40%) at the same time.

- Our criteria should not be compared to rescue surfactant studies that give surfactant when an infant has a high or low FiO2 requirement. The infants that are felt to be able to transition on CPAP are included. So an infant that is on 40 percent oxygen may actually wean down in the delivery room, but an infant who is bradycardic by 5 minutes may need intubation. The goal for the inclusion criteria is to avoid randomizing infants that would need intubation shortly after birth.

3) The clinical situation of infants with prenatal consent who undergo the intervention at 5 min of age in the delivery room is quite different from that of infants with postnatal consent who will undergo the intervention in the NICU. In my view, this would have required stratification.

- To date only 1 of 18 infants enrolled have been consented after birth. The infant that is consented after birth (parents had been approached prior to delivery but were undecided) was still randomized and given LISA within 30 minutes. So there does not seem to be an effect of consent after birth. Again no infants are actually getting LISA at 5 minutes. They just cannot get randomized before 5 minutes. Caffeine must also be administered prior to LISA which delays the procedure.

4) The authors should compare the design of the trial to that of previous trials randomizing preterm infants to LISA or control (Göpel et al, Lancet 2011; Kribs et al, JAMA Pediatr 2015) to describe the added value of the new trial.

- We have added additional text comparing our trial to the Göpel and Kribs trial. The major difference is the use of the angiocatheter and caffeine. Otherwise the trial is similar to the Göpel trial with a similar primary outcome.

5) The authors declare the trial to run from January 2020 to January 2025. The trial is already recruiting patients

- Yes this is correct. Recruitment started in January 2020, prior to the submission of this protocol for consideration in the BMJ Open.

Page 1:
The title should be more precisely describe the study topic. It should be stated explicitly, that the LISA group receives early continuous positive airway pressure as well and that the only difference is that one group gets early prophylactic surfactant by LISA, while the other group gets CPAP only.

- To our knowledge all of the infants in the Göpel and Kribs study received continuous pressure as well (via a Benevista valve). I don't think they added this to their title. Nor did they use the word only CPAP in the control arm (which is what the Göpel trial did).

Page 2:
The first reference given (this is in the introduction part of the abstract) is number 4. If the authors give references in the abstract, the first sentence should be referenced as well (I suppose this would be reference number 1). The merits of avoiding endotracheal intubation in the delivery room have been addressed as well in two sequential meta-analyses (Fischer HS et al, Pediatrics 2013; Fischer HS et al, Eur Respir Rev 2018).

- This has been revised

The last sentence of the introduction in the abstract states the aim of the study. As in the title, it does not become clear that the LISA group will receive early CPAP in the same way as the control group.

- Both groups (LISA vs CPAP) will be stabilized within the first 5 minutes of life. When the infant is assessed to be stable on CPAP, infant will get randomized to either continue on CPAP or receive surfactant via the LISA method while continuing on CPAP.
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Page 3:
The fact that this is the first LISA study in the US is in my opinion not of importance for the summary. The small sample size should be overcome by including more centers.

- As mentioned above, since the submission to BMJ Open, a third center has been added to the CaLI trial in order to allow diverse enrollment of subjects

Introduction:
References 2, 3. Reference 3 refers to a retrospective observational cohort study on LISA administration. LISA was not compared to CPAP alone, but was considered within the wider range of complications of prematurity. I think it is mandatory for this paper to cite the NINSAPP study as well (Kribs A et al, JAMA Pediatr 2015). In this randomized controlled study in extremely preterm infants, LISA was compared to surfactant administration during mechanical ventilation.

- The introduction has been revised to be more specific in the aims and goals of this trial. References may have been revised as well.

The authors call their study "multicenter" (line 53), however, they plan to include just 2 centers. They should make that clear at this point, and they should consider to make it a real multicenter study by including more centers to get timely and reliable results.

- Since the submission of this protocol to BMJ Open, a third center has been added to the CaLI trial, University of California Irvine Medical Center

There is some redundancy in the manuscript. In the last sentence, page 3, the authors statement on publishing is the same they made before.

- Revisions have been made appropriately

Page 4:
See last sentence page 3. The paragraph starting line 7 gives again information that has been given before. I think the manuscript needs some reorganization to present information in a more consistent and less redundant fashion.

- Manuscript has been reorganized as suggested

Specific aims: Here, the fact that both groups will be managed with early CPAP is given for the first time ("compared to CPAP alone"). This should be done from the beginning (title, abstract), see above.

- Abstract has been revised to show a clearer indication of the use of CPAP

Page 5:
Methods and analysis: multicenter and "2 neonatal intensive care units" - see comment above.

- Since the submission of this protocol to BMJ Open, a third center has been added to the CaLI trial, University of California Irvine Medical Center

Page 6
Inclusion criteria: It should be clarified that "spontaneously breathing" is with CPAP. Randomization: Again, there is redundancy in this part of the manuscript. Reorganization is necessary.
It remains unclear to me whether CPAP only or CPAP or NIMV is allowed before randomization.

- Both groups (LISA vs CPAP) will be stabilized within the first 5 minutes of life. When the infant is assessed to be stable on CPAP, infant will get randomized to either continue on CPAP or receive surfactant via the LISA method while continuing on CPAP. All centers are using CPAP as the initial mode of stabilization (in the first 2 hours of life). Some centers that transition infants to NIMV will be allowed to do so and we will perform subgroup analysis to determine if there was an effect. In addition the mode of support prior to intubation in both arms will be recorded. For example we would be able to determine if more infants were intubated from being CPAP versus NIMV in either arm.

Page 9
NICU: Abbreviations SMB and LLU are not introduced.

- Abbreviations have been updated, as well as content and use of abbreviations.

Please leave your comments for the authors below Ines and coworkers present a protocol for a randomized controlled trial of surfactant administration via LISA compared with continuation of CPAP in preterm infants <30 weeks gestation at age 5-120 minutes. Both groups will have received caffeine. The intervention is not blinded. Post-intervention management includes criteria for intubation, but does not protocolise other aspects of management.

- Since this trial is meant to evaluate the use of prophylactic surfactant, all aspects of management and protocols were emphasized on the LISA procedure. Management post the LISA procedure will be unit-based standard of care for infants of gestational age in study.

The primary outcome measure is need for intubation (or reaching treatment failure criteria, even if not intubated) in the first 72 h.

- The intent of the study is to evaluate if prophylactic surfactant reduces the need of invasive mechanical ventilation within 72 hours. As indicated in the protocol, approximately 49% of infants between 24+0 and 29+6 weeks gestational age were intubated and mechanically ventilated between 5 minutes of life and 72 hours of life.

A total of 75 subjects will be enrolled in each group, giving 80% power to detect a 22% absolute risk reduction in the intubation rate, from the current estimated rate of 49%.

1. The trial as currently designed is something between a study of delivery room care and a trial of early respiratory management in the NICU. I urge the authors to either focus on either delivery room care, or, preferably, to make this a trial of early respiratory management, with eligibility extending beyond 2 hours.

- Since the trial is designed to evaluate the use of prophylactic surfactant, the idea is to initiate the procedure soon after birth after allowing the infant to transition and stabilize. However, some neonatologists prefer giving surfactant in the delivery room while some prefer to give it early in the NICU with umbilical line placement and CXR confirmation.

- Since caffeine is a co-intervention we chose a pragmatic design that allows for caffeine administration in the first 2 hours regardless of location. This method still fits within the definition of early surfactant but making the location of where it is given less relevant. We also believe this will make our results more generalizable than making this an “only delivery room or early respiratory management study.”

2. It seems difficult to justify a trial design where there is no FiO2 threshold, or any other measure of RDS severity, to identify infants who actually have RDS and may benefit from exogenous surfactant. We already have seen in the VON-DRM study (Dunn Pediatrics 2011) that non-selective surfactant delivery soon after birth (albeit via INSURE) has no advantage over application and continuation of CPAP. Particularly not for the 26-29 week infants, who will be numerically the bulk of participants in the CaLi study. I strongly urge the authors to reconsider this aspect of design. Why not make this a trial of selective early LISA versus continuation of CPAP, imposing as an inclusion criterion some measure of RDS severity so as to leave out infants with no or very mild RDS, for whom CPAP alone would be expected to be sufficient.

- The Dunn et al trial was included in a Cochrane meta-anlaysis that did not show a difference between early prophyalxis vs rescue in infants <30 weeks (ref, Rojas-Reyes et al Cochrane Mar 2012). There is still an important distinction between these data and our study. The meta-analysis by Isayama et al (JAMA. 2016 Sep 13;316(10):1116) demonstrated that performing the LISA approach (which included prophylaxis use as in the trial by Gopel et al) was at least as good or better than CPAP and had the lowest odd ratio (OR, 0.49; 95% CI 0.3-0.79) for the development of the composite outcome of death or bronchopulmonary dysplasia (BPD) amongst non-invasive strategies.
-
- We agree that the early trials comparing non-selective surfactant therapy had no advantage compared to CPAP alone. This may have been due to the negative effects of intubation and positive pressure ventilation with surfactant administration (i.e INSURE). There has not been a trial comparing non-selective LISA administration with early caffeine use. This study would allow us to determine whether giving very early surfactant with the benefit of spontaneous breathing and without the harms of intubation and ventilation has an effect on the need for intubation. Futhermore, we have stratified our study by 24-26 week and 27-29 weeks to determine if there will be a differential effect.

3. If such a trial design is adopted, a contraction of the gestation range should be considered. One imagines that at the two participating centres, the majority of infants born at 24 and 25 weeks gestation would be intubated in the DR as part of resuscitation (despite the best efforts of care providers to transition them on CPAP). Why not limit the gestation range to 26-29 weeks, a group for which we expect non-invasive support (including mask PPV) to suffice during the delivery room transition in most cases?
- Since the study start we have already successfully included 1/3 of enrollment down to 24 and 25 weeks at birth. So we do not feel that there is a need to limit this group.

4. It is difficult to understand why this is being proposed as a trial of caffeine as well as LISA. As the authors state (page 10, line 41), caffeine is already given immediately after birth in both centres. I strongly recommend that the study design (and the study name) be simplified by removal of caffeine as one of the interventions under study. All that then is required is to state that the receipt of caffeine is one of the inclusion criteria. Whilst other studies have not mandated the use of caffeine prior to LISA, it is widely practiced and strongly recommended in recent guidelines
(e.g. Kribs Clin Perinatol 2016, Vento Neonatology 2019).

- We have had many discussions with investigators that have performed LISA both in the United States and Europe and it has not been clear which centers have consistently given caffeine BEFORE LISA. While we agree that early caffeine is now common, most studies (including the OPTIMIST trial) are not requiring caffeine before the LISA procedure.

A study by Dekker et al (Pediatr Res 2017 Aug;82(2):290-296) demonstrated that delivery room caffeine compared to early NICU caffeine showed better minute ventilation, tidal volumes in newborns when given in the delivery room. A trial by our group (Katheria et al American Journal of Perinatology 2018) demonstrated early hemodynamic improvements with early caffeine (given before 2 hours of life.) Yet no trial has made caffeine a co-intervention for LISA.
- We have expanded on this to make it clear that this is a trial of caffeine AND early LISA to prevent intubation.

5. The introduction to the study is not adequate.

- Introduction has been revised to implicate adequate information regarding the CaLI trial

5a. A three-quarter page introduction with a handful of citations is not sufficient to describe the landscape in which the trial is being conducted, and state the case for conducting it.

- Please see response above

5b. The introduction starts with a statement about delivery room resuscitation, but the CaLi study is about early respiratory management.

- Please see response above

5c. The Isayama meta-analysis (JAMA 2016) does indeed find an advantage of LISA over other early respiratory therapies for death/BPD, but of course includes a mixture of DR trials (e.g. COIN/SUPPORT/VON-DRM etc) done in unselected populations, and non-DR trials (almost all of the LISA studies) which are in selected populations of preterm infants. On this basis, it is a leap of faith to propose that the Isayama paper provides support for a DR study of LISA.

The citation of the Isayama meta-analysis is to indicate the odds ratio of the LISA method in reducing the development of BPD or death.

5d. The landscape in which this trial will conducted is very poorly described. Even the details of current management in the infants at the two participating centres would be useful. What proportion of infants 24-29 weeks gestation at each centre are currently intubated immediately after birth. What proportion are thus likely to be eligible?

Sharp Mary Birch has shown that approximately 49% of our infants 24-29+6 weeks' gestation were intubated and mechanically ventilated after 5 minutes of life but within the first 72 hours of life

5e. Further to 5d, there is also a selective quoting on what is an emerging literature on surveys of current practice in relation to the use of thin catheter surfactant techniques (e.g. Klotz 2017, Heiring 2017, Jeffreys 2019, Beltempo 2018, Kurepa 2019 – done in the US). It needs to be clear that LISA/MIST is an emerging therapy around which there is a fair deal of experience (as the surveys attest), and but perhaps not certainty with regard to its benefit, especially not compared with continuation of CPAP. The latest version of the European Consensus Guidelines should be referenced (rather than the 2017 version).

- The 2019 European Consensus Guidelines has been referenced.

5f. It would be useful at this juncture also to document what the local experience of the proposed thin catheter method has been (numbers, safety, success).

- At Sharp Mary Birch Hospital for Women & Newborns, a total of 18 patients have been enrolled since January 2020. Neonatologist, Advanced Life Support Nurses (Registered nurses trained in intubation, umbilical line placement), and Neonatal Nurse Practitioners have been trained in performing the LISA method.
- The Research team has been diligently screening for qualified admissions and obtaining consents prior to deliveries.
- To date, we have not have any serious adverse events or deviations.

5g. The last paragraph (page 6, lines 7-18) contains text that largely belongs in the Protocol itself.

- Revisions made

6. The structure of the protocol requires a great deal of work.

- Revisions made

6a. I urge the authors to draw on the structure of protocols of other studies, including those previously conducted by senior members of the study team.

- Revisions made appropriately

Examples of issues to be addressed:

6b. Under the major heading Specific aims we find primary and secondary outcomes, study timeframe and costs

- Revised

6c. Information about screening and consent is found in several locations, including on page 13, lines 12-27 under the heading Human subjects protection.

-Revised

6d. The section headed Study design should be a major heading with subheadings for all the usual elements that go with a clinical trial.

6e. The section on Data collection should move before Statistical Analysis.
- Revised

6f. Other sections contain text on a mixture of topics. Each sub-section should be reviewed to ensure that the text in that section belongs there.
- Revised

7. The protocol lacks detail and requires clarification in numerous places
- Revised

7a. Some more detail of important aspects of the LISA technique is required.

- Revised to contain more details of LISA technique

i) Will the proceduralists be trained in the technique, and how?

- Proceduralists (Neonatologist, Neonatal nurse practitioners, and advanced life support nurses) have been trained in the LISA method/technique during the launch of the study in November of 2019. A simulation lab during this meeting was scheduled. Videos of current facilities with successful techniques and methods were shown. Each proceduralists were observed and feedback given by the Principal Investigators.

ii) Why use intubation tape to mark the catheter insertion depth (instead of a wax pencil?). Have the authors trialled this method of marking the catheter? Tape generally does not stick well to these catheters, which are made of fluorinated polymer.

- We have found that marking the catheter with intubation tape sticks well and serves the purpose of marking the depth of catheter insertion for the duration of the procedure.

iii) Will premedication or sedation be given? What other measures of comfort will be used?

- In this trial, we opted not to use any premedication or sedation to minimize respiratory deterioration. Our resuscitation room is equipped with vital sign monitoring and infants will be continuously monitored during the LISA procedure. Infants will be bundled and swaddled to maximize comfort during the procedure.

iv) Is videolaryngoscopy an option?

- Yes, video laryngoscopy is an option at Sharp Mary Birch. Other sites are currently not equipped with a video laryngoscope, therefore, they are using conventional laryngoscopes but may be purchasing them at a later time. We will record the approach used and analyze for effect if there is a difference in failure rate with the LISA procedure.

v) How will CPAP be maintained during the procedure?

- If LISA procedure is to be performed in the DR, the catheter will be held secured to the side of the mouth after insertion of the thin catheter and mask CPAP will continue while instilling surfactant via the thin catheter. If LISA performed in the NICU, infant will be on CPAP via nasal prongs and will continue during the insertion of the thin catheter and instillation of surfactant.

7b. Why give caffeine at birth in the CPAP group, and prior to the procedure in the LISA group (page 10, line 27-29)? This is in contradiction to what is stated later (line 40-42).

- Revisions made appropriately

7c. It seems odd to include the criteria for intubation in the delivery room (page 11, lines 3-9), because these would or should apply to infants not yet being considered for the study.

- We have clarified the methods to explain that these criteria are to determine eligibility for the study NOT for intubation after randomization. Intubation criteria in the delivery room was established to inform the neonatal provider that even if the infant is consented for this study, they are not obligated to randomize. If the infant meets intubation criteria, the infant will be intubated and NOT randomized for the study.
-

7d. The criteria for intubation in the NICU (page 11, lines 14-22) require clarification. i) Will it really be necessary to have FiO2 >0.40 for two hours before qualifying for intubation (FiO2 might have got to 0.60 or 0.80 in that time).

- Yes it is our practice to wait at least 2 hours to determine whether the FiO2 can improve with other measures before intubation. However, there are other criteria such as apnea, desaturations or bradycardia can be used to intubate sooner. Similarly abnormal blood gas parameters can allow for an earlier intubation.
-

ii) There must be an upper limit for SpO2 as well as a lower limit.

- Thank you for your comment. Upper limit for SpO2 added.

iii) Why do the blood gases need to be 2 hours after randomisation?

- We acknowledge that early hypercarbia in an infant that is transitioning may not be the same as an infant 2 days old with hypercarbia. Our practice is not to intubate infants solely for an elevated CO2 on the first blood gas but to rather “wait” and see how the infant transitions. Therefore, we agreed to use blood gases at least 2 hours after randomization to allow for and adequate transition. Again, however, for elevated FiO2, apnea or bradycardia the infant can be intubated earlier.

7e. Under Human Subject Protection it is stated (page 12, line 28-33) that ‘both groups will receive the same surfactant…”. But this is not necessarily true – some infants in the CPAP group will not qualify for intubation and thus will not receive surfactant.

- This statement is meant to indicate that the same “type” of surfactant will be given if the infant qualifies (i.e intubated in the CPAP arm is the same surfactant as the LISA group)
-

7f. The statistical analysis plan is rudimentary. It should focus first and foremost on the handling of the primary outcome, and then other outcomes, documenting how alpha error is to be avoided. What are the ‘various hemodynamic parameters’ and why are they specifically mentioned?

- We have deleted ‘various hemodynamic parameters.’ The study is a pilot study and only had sufficient power for the primary outcome. The 22 percent was chosen based on a combination of what would be clinically significant and the number of subjects we would be able to recruit in a grant period. We increased the N to 180 to account for new funding and multiples (see reviewer below) and have revised the protocol to reflect this point.

7g. Why has a 22% absolute risk reduction been chosen?
- See above.

7h. The information on handling of adverse event reporting is detailed, but suggests that there may be a rather burdensome amount of reporting to the DSMC /Ethics committee. The collection of data on secondary outcomes will take care of many of the standard AEs that occur in preterm infants.

- This level of detail was requested by our DSMB members.

7i. Is it really correct to expect that the DSMB will 'protect all study patients'? (page 14, line 37).

- This is correct. We have collaborated with a DSMB to foresee the safety and conduct of this trial.

8. The authors are somewhat dismissive of the possibility of blinding the intervention (page 9, lines 5-6), especially given that the trial is being done in only two centres, both of which one presumes are reasonably well-resourced. Blinding of such an intervention has been achieved previously in an INSURE study (Reininger J Perinatol 2005) and has been written into the design of the OPTIMIST-A trial (Dargaville BMC Pediatrics 2014).

- The intent of the study to evaluate if prophylactic surfactant reduces the need of invasive mechanical ventilation within 72 hours. Included in the risks/benefits of the minimally invasive surfactant procedure is the use of a laryngoscope. A sham procedure that includes the use of a laryngoscope might answer the question regarding surfactant itself, but not the procedure.
OTHER COMMENTS
Abstract
The abstract at present recapitulates most of what can be found in the introduction (or vice versa), and gives no detail on many aspects of the trial design (PICOT, plus numbers of subjects etc).
We have revised the abstract to be in a PICOT format.

Figure 1. The flowchart should be a visual representation of all aspects of the trial processes, including screening/consent/eligibility (inclusions and exclusions)/ randomisation/intervention/post-intervention management. It is lacking several of these elements at present.

We have revised the flowchart.

FORMATTING AMENDMENTS (if any)
Required amendments will be listed here; please include these changes in your revised version:
- Figure Citation Missing

We have noticed that you have uploaded Figure 1. However, we cannot see any citation for this file within the main text. If this file needs to be published, please cite it in the main text.

Intervention:
The infants are planned to be placed on CPAP in the delivery room with a pressure of 5-6 cm of water which seems rather low for infants in the early transition period. The European consensus recommends at least 6 cm water pressure when using CPAP in this setting.

- We will add a minimum CPAP/PEEP of 6

The infants are to be randomized in infants on CPAP who shall receive surfactant by LISA and those who get surfactant after intubation and ventilation. The protocol states that the LISA group will receive surfactant as soon as they are randomized (between 5 minutes and 2 hours of age) regardless
of the severity of lung disease . A within the trial also relatively mature preterm (28-29 weekers) are randomized, which often do need no surfactant at all and can be safely managed on CPAP, this

approach exposes some infant to an unnecessary risk (of surfactant administration) and needs further explanation.

- This trial is meant to evaluate prophylactic surfactant use, therefore, there are infants that will receive surfactant when they do not need any surfactant.

The conventional group, however, is scheduled to be intubated only after having reached a defined grade of disease severity, which includes high oxygen demands or hypercapnia/acidosis for at least 2 hours. Surfactant this will be given to all these infants under no circumstances prior to 2 hours after birth. This is not in line with current evidence. The European guidelines of surfactant administration recommend giving surfactant if infants needed more than 30% oxygen on noninvasive support. By this trial design one does not compare two different methods of surfactant administration but two completely different philosophies (actually early less invasive vs. late invasive surfactant administration).
This is not correct. LISA is not intubation, we are testing a less invasive approach of surfactant delivery to determine whether there is a reduction intubation. BOTH groups will follow the same criterion for intubation. We respectfully acknowledge the European guidelines but the US does not mandate that surfactant has to be given at 30 percent or even 40 percent. The neonatologist can intubate if the infants has events, bradycardia, hypercarbia OR if the FiO2 is at 40 percent. The criteria were set that to be the HIGHEST tolerable, that is they will be REQUIRED to intubate (even if they do not agree) if the infant is at 40 percent oxygen.
No criteria are stated when to give surfactant to the conventional group. I presume it will be given to all intubated babies, but this does not appear from the paper.

- Surfactant will be given to the conventional group once infant is determined to meet intubation criteria.

Uniform criteria should be given when to extubate intubated babies to obviate bias between both groups.
-Intubated infants will be extubated per unit protocol of intubated premature infants.

Caffeine is planned to be given differently in both groups. The LISA group will receive it prior to the LISA procedure, the conventional group as soon as possible after birth. What is the reason for this difference (and bias)?

- Of current enrollment, Caffeine has been administered to both groups immediately after randomization.
-
Criteria for intubation are not specific enough. What does “prolonged hypoxia” and “prolonged PPV” mean in this setting. A clear cut definition is important as it will determine how many infants at all will still be not intubated after 5 minutes and will be available for randomization.
- Our criteria should not be compared to rescue surfactant studies that give surfactant when an infant has a high or low FiO2 requirement. The infants that are felt to be able to transition on CPAP are included. So an infant that is on 40 percent oxygen may actually wean down in the delivery room, but an infant who is bradycardic by 5 minutes may need intubation. The goal for the inclusion criteria is to avoid randomizing infants that would need intubation shortly after birth.

Outcomes:
The primary outcome should be combined with the mortality rate within the first 72 hrs of life because this is a competing risk.

Since every preterm infant in our experience has been intubated before death we would be able to code death and or endotracheal intubation as the same outcome.

As important secondary outcomes should be included:
- FIP or NEC > stage I (the papper von Kribs et al in JAMA Pediatr has seen more babies to be operated on with perforated bowels in the LISA group)

- ROP with need for treatment (because non-invasive support may increase the risk for fluctuating oxygen saturations)

- Death until discharge

Thank you for these suggestions. We have added them.

No definition is given for “requirement of supplemental oxygen at 36 weeks”. Is this tested by room air challenge? What is the threshold Oxygen saturation for supplementing O2? How to proceed with infants on CPAP or HHHFNC without additional oxygen need?

- The definition of requirement of supplemental oxygen at 36 weeks is as defined by the National Institute of Child Health and Human Development Protocol for the Physiologic Definition of Bronchopulmonary Dysplasia (BPD)

No definiton is given for “neurodevelopemntal impairment” and its grading
- Secondary outcomes: Neurodevelopment outcomes at 24 months. We will gather results from the BSID 4th edition

Randomization procedure:
It is always challenging to perform ethical sound research in the delivery room setting. The proposed trial plans to get parental consent prior to birth, which certainly will be the best way. However, the plan also allows for getting consent within the first two hours after birth. This postpones potentially beneficial treatment for the infants (surfactant) or increases the drop-out rate (and makes the study not representative for widespread use) as the infants already had to be intubated prior to consent.

- Only infants that are not intubated and are stable on CPAP will be approached for consent if unable to approach prior to delivery. To date only one of 18 infants included have been consented after birth. The infant that is consented after birth was still randomized and given LISA within 30 minutes. So there does not seem to be an effect of consent after birth. Again no infants are actually getting LISA at 5 minutes. They just cannot get randomized before 5 minutes

Statistics:
As multiples are randomized to the same intervention: was sample size calculation correct for this procedure?

An adjustment of 1.12 derived from the NICHD Neonatal Research Network Generic Database, allowed for multiples to be randomized to the same treatment introducing a clustering effect. (Finer et al, NEJM 2012)

Our increased sample size of 180 infants will allow us to account for any such effects.

Publication:
As the primary study outcome is ventilation within the first 3 days of life it becomes not quite clear why the authors plan to analyze their data only after 24 months. This would considerably delay the dissemination of the primary study result. Certainly, safety concerns are an important reason why

many neonatologists hesitate to perform LISA and the assessment of neurodevelopment at 24 months of age should be undertaken, but wouldn't it be wiser to split the analysis into two publications?

- It is in fact our intent to split the publication, and this will be made more clear in the protocol.

It is stated that LISA is not widely accepted. At least it should be conceded that it is not universally accepted but has found wide acceptance in some areas (e.g. mainland Europe (Klotz D et al, Eur J Ped 2017)).

- Sentence has been clarified to indicate that the LISA procedure is widely accepted in Europe, however, it is still not accepted widely in our country.

The authors want to document esophageal surfactant administration by using a gastric tube. In my experience there is often a spillover of surfactant from the trachea into the pharynx (and consecutively into the stomach) even if your tracheal catheter is placed correctly. So surfactant in the gastric aspirate does not necessarily proof that your catheter was in the wrong position.

- The idea of collecting possible esophageal surfactant administration is to quantify how much of the surfactant dose the infant received and not necessarily proving catheter placement.

The introduction of the abstract is a copy and paste version of the actual introduction and thus should be shortened (including the referral to reference 4).

- The abstract has been significantly modified

No initial dose for caffeine is given in the paper.

- Revised to indicate loading dose of Caffeine at 20 mg/kg

The first author of reference 2 is "Göpel" not "Gopel"

- Revised as suggested

Reference 3 is a retrospective study. The same group has published a large randomized trial in extremely immature infants, which should be cited instead (Kribs et al, JAMA Pediatr 2015).

We have revised this as suggested.

Reference 4 is incompletely cited

- Revised

Reference 7 cites an older version of the European consensus on surfactant administration. The most recent consensus has been published 2019.

- The most recent publication has been referenced

VERSION 2 – REVIEW

REVIEWER	Claudia Roll Department of Neonatology, Pediatric Intensive Care, Sleep Medicine Vest Children's Hospital Datteln Datteln, Germany
REVIEW RETURNED	29-Aug-2020

GENERAL COMMENTS	The authors made strong efforts to improve the manuscript. As the trial already started recruiting patients, there is no point for going into details of the methodology again. Nevertheless, the authors should clarify how caffeine is going to be administered, most so because they believe caffeine to be pivotal for the LISA to be successful. Is caffeine given as single shot or via continuous intravenous infusion (if so, how long)?

REVIEWER	Peter Dargaville Royal Hobart Hospital and University of Tasmania, Hobart, Tasmania, Australia I am the Chief Investigator of the OPTIMIST-A trial, a multicentre RCT investigating minimally-invasive surfactant therapy in preterm infants.
REVIEW RETURNED	07-Sep-2020

GENERAL COMMENTS	Ines and coauthors present a revision of their manuscript describing the protocol of the CALI trial. Some of the criticisms of the reviewers have been addressed, others have been rebutted. Numerous significant concerns regarding the protocol remain. The review of the revision is made more difficult by the lack of a marked up version of the manuscript. MAJOR COMMENTS: 1a) The authors have stated their case in support of this trial examining prophylactic surfactant therapy, along with caffeine. Whilst I made a strong case against this in my previous review, given the trial has now commenced it appears to be locked in as prophylaxis trial. 1b) Given 1a, what now is extremely difficult to accept is that the recruitment period for this surfactant prophylaxis trial extends to 2 hours, a concern clearly shared by both the other reviewers. A surfactant prophylaxis trial is done in a group of preterm infants within a certain gestation range that remain undifferentiated as to the severity of their RDS. It scarcely needs to be said that by two hours most preterm infants have declared themselves in terms of early signs of RDS. This was the meaning of the comment in my previous review that the trial sits between a study of DR care and early respiratory management in the NICU. 1c) Extension of recruitment in this prophylaxis trial to two hours leads to a preposterous situation in which a 29 week gestation infant with no prenatal consent would be eligible for enrolment in the NICU at 90 minutes when on gentle CPAP, room air, SpO2 98%, with no signs of respiratory distress and a clear chest X-ray. Such infants exist, and the protocol as written requires that consent be pursued post-natally to recruit them. To not do so would impose a selection bias, and render the trial as a prophylaxis trial in the DR and a trial of selective surfactant in the NICU. And yet to recruit infants like this in the NICU, exhibiting no features of RDS, would be to randomise them to possibly receive a drug they have no need for, given by a procedure which may destabilise them. 1d) This aspect of the study design is likely to be heavily criticised once the trial is completed. I urge the authors to address it now at an early stage of recruitment.

	1e) The simple remedy still available is to shut down the option of obtaining post-natal consent, and limit the maximum recruitment time to 45 min (as also proposed by reviewer 1). Given that 17 of 18 consents have thus far been obtained prenatally, this would not seem to represent a major alteration to current trial operations, nor a significant loss of recruitment opportunity. 1f) The fact that 17 of 18 consents have been obtained pre-natally also brings the question of how many babies in the gestation range 24-29 weeks have been born in the trial period, and why weren't there more post-natal consents sought and obtained? Does this mean a selective approach to post-natal consent is already being pursued? 2. The revision of the manuscript has led to improvement in some areas, but on the other hand some rather important sections have been altered or omitted to the detriment of the protocol (e.g. Aim, hypotheses). As stated above, without a marked up version (or more explicit comments by the authors about what has been altered) it is very difficult to discern where changes have been made. 3. ABSTRACT: The abstract still fails to encapsulate the main features of the trial. The first sentence describes a meta-analysis that doesn't disclose that preterm infants are the focus. Nowhere is it mentioned that this is a multicentre randomised controlled parallel group trial. The LISA method, surfactant dose, control group management, primary outcome, main secondary outcomes and sample size are not mentioned. As stated in my previous review, the authors should report the main features of the study in the abstract, to the point where it can stand alone as a summary of the trial. 4. INTRODUCTION The revised introduction still barely describes the landscape of the trial, and continues to exhibit a lack of attention to detail. 4a). Page 4, para 1: The statements in the first paragraph regarding evidence-based practices for lung protection must have some references. 4b). Page 4, para 1: The statement that early surfactant therapy is a management strategy to be employed in the delivery room 'with lung protection in mind' has no basis. 4c). Page 4, line 42-47: The AMV trial is referenced as showing a reduction in BPD, but did not show that. 4d). Page 4, lines 49-51: The Isayama meta-analysis is referenced as showing a decrease in invasive mechanical ventilation but this was not an outcome in this meta-analysis. 4e). Page 4, line 54: With only 11% of NICUs using LISA, the Bhayat survey data do not suggest that LISA is "widely adopted in Europe". As mentioned in my previous review, there are numerous reports of surveys which do show wider uptake in Europe. I continue to think that the Kurepa survey of US practice should also be referenced. 4f). There is still no mention of the pre-CALI experience of LISA in the Units involved in the trial. 4g). Page 5, lines 5-10: The last paragraph of the Introduction suggests that the comparator group is 'caffeine, early CPAP and positive pressure ventilation alone', but surely this should be 'caffeine and early CPAP alone'.

	5. METHODS In response to my previous comment that the protocol structure requires work, the authors state that revisions have been made. Nonetheless there remain some significant imperfections. 5a). The SPIRIT checklist for description of the features of a clinical trial in logical sequence has been uploaded by the authors, but not followed. I urge them to follow the sequence suggested by SPIRIT – this will not be hard, and the time spent will be rewarded. This would mean that some objectives, aims and hypotheses would appear up front (currently they have been lost), and, by example, the mention of long term neurodevelopmental outcome would be found in the right place, rather than in sentence 2. 5b). The outcome list needs some further work. Only 7 in-hospital outcomes are now mentioned, including spontaneous intestinal perforation, (but not NEC, as suggested by another reveiwer). Will there be any safety outcomes in relation to the LISA intervention? 5c). Suggest outcome 1 should be worded 'Requirement for endotracheal intubation in the first 72 h' (might be an INSURE procedure without MV). Outcome 2 is actually three outcomes (duration MV, duration CPAP, duration MV+CPAP). Outcome 3 (BPD outcome) needs to be more than 'requirement of supplemental oxygen at 36 weeks' – what about the baby on CPAP in room air? There is now no NEC outcome (even though it is mentioned at page 12, line 24). 5d). The statement regarding randomisation of multiples is mentioned twice (page 6, lines 3-5 and page 7, lines 25-27) 5e). Exclusion criterion 3 should be 'requiring intubation in the delivery room' (not 'requiring intubation prior to randomisation' as they won't be randomised if this happens). 5f). Page 8, CPAP Group: It is stated (lines 30-35) 'premature infants may require CPAP immediately after delivery if they elicit signs of labored breathing…''. But in order to be in the trial the infant must be already on CPAP. This statement is redundant (and confusing). 5g). Page 8, caffeine: The description of the role of caffeine therapy remains confusing. If caffeine is really part of the study, it needs to be stated that it will be given soon after randomisation in all infants, and this needs to be adhered to. 5h). Page 12/13, Statistical Analysis Plan: This now only shows a sample size calculation. 6. FIGURES: Figure 1 (Participant Timeline) shows no timelines, but is just a CONSORT diagram template with no data, and is not helpful. Figure 2 (Study Overview Diagram) is actually more like a study flowchart and is useful. What are LDR and PSCU? The Figures do not need to be mentioned in an Appendix.

VERSION 2 – AUTHOR RESPONSE

Nevertheless, the authors should clarify how caffeine is going to be administered, most so because they believe caffeine to be pivotal for the LISA to be successful. Is caffeine given as single shot or via continuous intravenous infusion (if so, how long)?

Yes we agree that caffeine is pivotal for the success for this trial. The Caffeine drug preparation for this study is Caffeine Citrate with a loading dose of 20 mg/kg given via continuous intravenous infusion over 15 to 30 minutes.

) The authors have stated their case in support of this trial examining prophylactic surfactant therapy, along with caffeine. Whilst I made a strong case against this in my previous review, given the trial has now commenced it appears to be locked in as prophylaxis trial.

Please do not refer to this study as a prophylactic surfactant study. The timing is for early surfactant and early caffeine. We agree that there is a difference in our viewpoint regarding Caffeine. We firmly believe the caffeine was a co-intervention in the early trials from Germany (Kribs et al, Gopel et al) and should be stated from the onset in our trial.

1b) Given 1a, what now is extremely difficult to accept is that the recruitment period for this surfactant prophylaxis trial extends to 2 hours, a concern clearly shared by both the other reviewers. A surfactant prophylaxis trial is done in a group of preterm infants within a certain gestation range that remain undifferentiated as to the severity of their RDS. It scarcely needs to be said that by two hours most preterm infants have declared themselves in terms of early signs of RDS. This was the meaning of the comment in my previous review that the trial sits between a study of DR care and early respiratory management in the NICU.

Again, we have never stated that our trial is a prophylaxis study this has been repeated by the reviewers as such. The two hour time point is consistent with early caffeine and surfactant administration which is the standard of care in the three US centers. The trial by Kattwinkle et al had a median time for early surfacntant at 1.5 hours which will be consistent with our trial (the maximum latest is 2 hours).

1c) Extension of recruitment in this prophylaxis trial to two hours leads to a preposterous situation in which a 29 week gestation infant with no prenatal consent would be eligible for enrolment in the NICU at 90 minutes when on gentle CPAP, room air, SpO2 98%, with no signs of respiratory distress and a clear chest X-ray.

The reviewer is not understanding how the study is designed. We must randomize, place and IV, give caffeine over 15 minutes, and then perform the LISA procedure and then slowly instill caffeine. This would have to happen after a consent is obtained.

No babies would be approached for consent at 90 minutes since it would be too late for consent. Nonetheless, see our response to 1e.

Such infants exist, and the protocol as written requires that consent be pursued post-natally to recruit them. To not do so would impose a selection bias, and render the trial as a prophylaxis trial in the DR and a trial of selective surfactant in the NICU. And yet to recruit infants like this in the NICU, exhibiting no features of RDS, would be to randomise them to possibly receive a drug they have no need for, given by a procedure which may destabilise them.

See comment for 1e.

1d) This aspect of the study design is likely to be heavily criticised once the trial is completed. I urge the authors to address it now at an early stage of recruitment.

Again see 1 e.

1e) The simple remedy still available is to shut down the option of obtaining post-natal consent, and limit the maximum recruitment time to 45 min (as also proposed by reviewer 1). Given that 17 of 18 consents have thus far been obtained prenatally, this would not seem to represent a major alteration to current trial operations, nor a significant loss of recruitment opportunity.

While we disagree that 2 hours is not outside of current practice (given all the things that occur after admission, antibiotics, umbilical lines, Caffeine), we will require that randomization occurs by one hour of life and eliminate post-natal consent.

1f) The fact that 17 of 18 consents have been obtained pre-natally also brings the question of how many babies in the gestation range 24-29 weeks have been born in the trial period, and why weren't there more post-natal consents sought and obtained? Does this mean a selective approach to post-natal consent is already being pursued?

At present, we have only done 1 out of 35 subjects with postnatal consent (none since the last revision), we have removed this option.

2. The revision of the manuscript has led to improvement in some areas, but on the other hand some rather important sections have been altered or omitted to the detriment of the protocol (e.g. Aim, hypotheses). As stated above, without a marked up version (or more explicit comments by the authors about what has been altered) it is very difficult to discern where changes have been made.

This was due to having a new submission (since it took much time to address the comments and we missed the deadline for a resubmission). We have submitted a marked up version for this revision.

3. ABSTRACT: The abstract still fails to encapsulate the main features of the trial. The first sentence describes a meta-analysis that doesn't disclose that preterm infants are the focus. Nowhere is it mentioned that this is a multicentre randomised controlled parallel group trial. The LISA method, surfactant dose, control group management, primary outcome, main secondary outcomes and sample size are not mentioned. As stated in my previous review, the authors should report the main features of the study in the abstract, to the point where it can stand alone as a summary of the trial.

Due to the limitations in the abstract length we are unable to add more here but can add them to the manuscript.

4. INTRODUCTION

The revised introduction still barely describes the landscape of the trial, and continues to exhibit a lack of attention to detail.

4a). Page 4, para 1: The statements in the first paragraph regarding evidence-based practices for lung protection must have some references.

Evidence-based practices and protocol for lung protection is referenced in the European Consensus Guidelines on the Management of Respiratory Distress Syndrome-2019 Update

4b). Page 4, para 1: The statement that early surfactant therapy is a management strategy to be employed in the delivery room 'with lung protection in mind' has no basis.

We have removed this phrase

4c). Page 4, line 42-47: The AMV trial is referenced as showing a reduction in BPD, but did not show that.

Reference has been corrected

4d). Page 4, lines 49-51: The Isayama meta-analysis is referenced as showing a decrease in invasive mechanical ventilation but this was not an outcome in this meta-analysis.

Reference has been corrected

4e). Page 4, line 54: With only 11% of NICUs using LISA, the Bhayat survey data do not suggest that LISA is "widely adopted in Europe". As mentioned in my previous review, there are numerous reports of surveys which do show wider uptake in Europe. I continue to think that the Kurepa survey of US practice should also be referenced.

***Referenced*:** Kurepa D, Perveen S, Lipener Y, Kakkilaya V. The use of less invasive surfactant administration (LISA) in the United States with review of the literature. J Perinatol. 2019 Mar;39(3):426-432. doi: 10.1038/s41372-018-0302-9. Epub 2019 Jan 11. PMID: 30635595.

4f). There is still no mention of the pre-CALI experience of LISA in the Units involved in the trial.

The previous review did not ask us to mention pre-cali experience, rather the training that would be needed for the trial. We had 2 separate site meetings to provide simulation training along with lectures and discussion prepared by Drs. Katheria and Finer. All of the investigators, research staff, and respiratory therapists attended the sessions. We have added this into the protocol.

4g). Page 5, lines 5-10: The last paragraph of the Introduction suggests that the comparator group is 'caffeine, early CPAP and positive pressure ventilation alone', but surely this should be 'caffeine and early CPAP alone'.

Revised to reflect Caffeine and early CPAP alone.

5. METHODS

In response to my previous comment that the protocol structure requires work, the authors state that revisions have been made. Nonetheless there remain some significant imperfections.

5a). The SPIRIT checklist for description of the features of a clinical trial in logical sequence has been uploaded by the authors, but not followed. I urge them to follow the sequence suggested by SPIRIT – this will not be hard, and the time spent will be rewarded. This would mean that some objectives, aims and hypotheses would appear up front (currently they have been lost), and, by example, the mention of long term neurodevelopmental outcome would be found in the right place, rather than in sentence 2.

The website has two checklists, one that is downloadable and one that is only on the website. We see the reviewers point about the second checklist. This was confusing since it seemed the downloaded checklist would be appropriate but have reformatted the entire paper for the other checklist.

5b). The outcome list needs some further work. Only 7 in-hospital outcomes are now mentioned, including spontaneous intestinal perforation, (but not NEC, as suggested by another reveiwer). Will there be any safety outcomes in relation to the LISA intervention?

Outcomes must be limited due to multiple hypothesis testing. For example the OPTIMIST trial only lists 7 secondary outcomes.

We have several outcomes that include safety measures, severe IVH, NEC and death. We have detailed physiologic observations including bradycardia and hypoxia during both lisa and endotracheal intubation procedures which will allow us to assess for differences in the procedures.

5c). Suggest outcome 1 should be worded ‘Requirement for endotracheal intubation in the first 72 h’ (might be an INSURE procedure without MV). Outcome 2 is actually three outcomes (duration MV, duration CPAP, duration MV+CPAP).

We have revised the wording.

Outcome 3 (BPD outcome) needs to be more than ‘requirement of supplemental oxygen at 36 weeks’ – what about the baby on CPAP in room air? There is now no NEC outcome (even though it is mentioned at page 12, line 24).

We have revised the BPD outcome to include CPAP and high flow nasal cannula ≥2 LPM per our practice.

5d). The statement regarding randomisation of multiples is mentioned twice (page 6, lines 3-5 and page 7, lines 25-27)

Second mention of randomization of multiples will be removed

5e). Exclusion criterion 3 should be ‘requiring intubation in the delivery room’ (not ‘requiring intubation prior to randomisation’ as they won’t be randomised if this happens).

We have revised criterion 3.

5f). Page 8, CPAP Group: It is stated (lines 30-35) 'premature infants may require CPAP immediately after delivery if they elicit signs of labored breathing…''. But in order to be in the trial the infant must be already on CPAP. This statement is redundant (and confusing).

Statement has been clarified

5g). Page 8, caffeine: The description of the role of caffeine therapy remains confusing. If caffeine is really part of the study, it needs to be stated that it will be given soon after randomisation in all infants, and this needs to be adhered to.

We have clarified the text that the caffeine therapy MUST be given as soon as possible in both arms after randomization.

5h). Page 12/13, Statistical Analysis Plan: This now only shows a sample size calculation.

We have always developed a detailed SAP once all variables are final and as a separate document. In our multicenter trials this has always been the case since it is an evolving document unlike the trial which should stay the same. For example now that NEC is being considered as a secondary (see comments below) we will have to increase our hypothesis testing for this outcome. So yes for now it is a sample size calculation with the comments that a full SAP will follow.

6. FIGURES: Figure 1 (Participant Timeline) shows no timelines, but is just a CONSORT diagram template with no data, and is not helpful. Figure 2 (Study Overview Diagram) is actually more like a study flowchart and is useful. What are LDR and PSCU? The Figures do not need to be mentioned in an Appendix.

Thank you for the comment. We have created a timeline for figure one with a legend for figure 2 in the text to accompany the figure.

VERSION 3 – REVIEW

REVIEWER	Peter Dargaville Royal Hobart Hospital and University of Tasmania, Hobart, Australia I am the Chief Investigator of the OPTIMIST-A trial, an RCT investigating minimally-invasive surfactant therapy.
REVIEW RETURNED	09-Nov-2020

GENERAL COMMENTS	The resubmission of this trial protocol has dealt with many of the outstanding reviewer comments, but some important deficiencies remain. The authors' submitted Response to Reviewers document also contains some rather glaringly erroneous claims. 1. Is this a prophylaxis study? 1a. The authors' new responses: "Please do not refer to this study as a prophylactic surfactant study. The timing is for early surfactant and early caffeine" and: "Again, we have never stated that our trial is a prophylaxis study this has been repeated by the reviewers as such"

	are completely at odds with what they stated in their previous Response to Reviewers, received along with the R1 manuscript: “Yes, this trial is meant to evaluate prophylactic surfactant” and: “The intent of the study is to evaluate if prophylactic surfactant reduces the need of invasive mechanical ventilation within 72 hours.” The study is most certainly a prophylaxis study, in the true sense of the word, in that the infants are enrolled before exhibiting features of RDS. This design was criticised but finally accepted by this reviewer in our previous transactions. 1b. The authors are confusing the timing of recruitment (i.e. latest time that an infant can be enrolled and randomised) with the timing of intervention. These are two very different things. In the original version of the manuscript it was stated that recruitment was allowable until two hours of life (“Consented premature infants with gestational age from 24 weeks to 29 weeks plus 6 days will be enrolled within 2 hours of life”). In the current Response to Reviewers document the authors appear to state that enrolment (i.e. recruitment) will be allowed up to one hour of age (but only with prenatal consent), with the expectation that the intervention should be completed by 2 hours. This seems reasonable. It just needs to be made clear in the published protocol. 1c. Currently the abstract states (at line 35) that infants will be enrolled “within 2 hours of life”. This is incorrect – based on what has been stated in the latest Response to Reviewers this is now 1 hour. 1d. In the manuscript proper there is now no mention of the time limit of recruitment, certainly no mention of a one hour limit. This must be rectified. 1e. In the CaLi study overview diagram (supp Fig 4) post-natal consent still appears as an option. 1f. In supp Fig 4 it also states that randomisation will take place at 5 minutes of life. This clearly is at odds with the 1 hour timeline mentioned by the authors in their response. 2. Abstract and protocol format. The authors’ claim to have “reformatted the entire paper for the other [SPIRIT] checklist” is incorrect. The order of the protocol does not follow the SPIRIT checklist. This is not a disaster. But I still urge the authors to make two adjustments: 2a. As is stated in a previous review, the protocol at the very least should have some Objectives and Hypotheses set out (SPIRIT checklist #7). 2b. As stated in two previous reviews, the abstract must include some additional information about the trial. At the very least, the primary outcome and the sample size. Ideally also mention the pre-defined intubation criteria applied post-randomisation, and the secondary outcomes. The abstract introduction is currently very long and could easily be shortened so that this much more important information can be included. 3. Documentation of previous experience of the CaLi methods.

	The authors' claim that "The previous review did not ask us to mention pre-cali experience" is incorrect – see Major Comment 5f of my first review. What now appears in the revised protocol is documentation of simulation training, which is helpful. It still leaves the question of what experience the investigators have had with the surfactant delivery method, and what opportunities they took to fine-tune the trial methodology, but these are now moot points as the trial has started. 4. Other issues 4a. The authors state that they have referenced the Kurepa survey of US centres, but it still does not appear in the latest submitted protocol. 4b. As mentioned twice previously, the reference cited in support of the notion that LISA is "widely adopted in Europe" (Bhayat) shows exactly the opposite (11% uptake, England only). 4c. Typographical error in Exclusions in supp file 4. ("of" should be "or)

VERSION 3 – AUTHOR RESPONSE

1. Is this a prophylaxis study?
   1a. The authors' new responses:
   "Please do not refer to this study as a prophylactic surfactant study. The timing is for early surfactant and early caffeine" and: "Again, we have never stated that our trial is a prophylaxis study this has been repeated by the reviewers as such"

   are completely at odds with what they stated in their previous Response to Reviewers, received along with the R1 manuscript:

   "Yes, this trial is meant to evaluate prophylactic surfactant" and: "The intent of the study is to evaluate if prophylactic surfactant reduces the need of invasive mechanical ventilation within 72 hours."

   The study is most certainly a prophylaxis study, in the true sense of the word, in that the infants are enrolled before exhibiting features of RDS. This design was criticised but finally accepted by this reviewer in our previous transactions.

I apologize for the confusion but thank you again for accepting our stance.

1b. The authors are confusing the timing of recruitment (i.e. latest time that an infant can be enrolled and randomised) with the timing of intervention. These are two very different things. In the original version of the manuscript it was stated that recruitment was allowable until two hours of life ("Consented premature infants with gestational age from 24 weeks to 29 weeks plus 6 days will be enrolled within 2 hours of life").

In the current Response to Reviewers document the authors appear to state that enrolment (i.e. recruitment) will be allowed up to one hour of age (but only with prenatal consent), with the expectation that the intervention should be completed by 2 hours. This seems reasonable. It just needs to be made clear in the published protocol.

We have made sure the entire document clearly states that randomization will take place before 1 hour of life and the intervention will be completed by 2 hours of life.

1c. Currently the abstract states (at line 35) that infants will be enrolled “within 2 hours of life”. This is incorrect – based on what has been stated in the latest Response to Reviewers this is now 1 hour.

Yes we have clarified this above.
1d. In the manuscript proper there is now no mention of the time limit of recruitment, certainly no mention of a one hour limit. This must be rectified.

To be clear we will ensure that randomization (not recruitment) will have occurred by 1 hour of life.

1e. In the CaLi study overview diagram (supp Fig 4) post-natal consent still appears as an option.

We apologize for this oversight, it has been removed.

1f. In supp Fig 4 it also states that randomisation will take place at 5 minutes of life. This clearly is at odds with the 1 hour timeline mentioned by the authors in their response.
We have review this in the figure as well and changed it to randomize by 1 hour of life.

2. Abstract and protocol format.
The authors’ claim to have “reformatted the entire paper for the other [SPIRIT] checklist” is incorrect. The order of the protocol does not follow the SPIRIT checklist. This is not a disaster. But I still urge the authors to make two adjustments:

2a. As is stated in a previous review, the protocol at the very least should have some Objectives and Hypotheses set out (SPIRIT checklist #7).

WE have stated objectives and hypothesis in the protocol per checklist #7

2b. As stated in two previous reviews, the abstract must include some additional information about the trial. At the very least, the primary outcome and the sample size. Ideally also mention the pre-defined intubation criteria applied post-randomisation, and the secondary outcomes. The abstract introduction is currently very long and could easily be shortened so that this much more important information can be included.

We have shortened the abstract to allow for more text to include intubation criteria and secondary and sample size.

2. Documentation of previous experience of the CaLi methods.
   The authors’ claim that “The previous review did not ask us to mention pre-cali experience” is incorrect – see Major Comment 5f of my first review. What now appears in the revised protocol is documentation of simulation training, which is helpful. It still leaves the question of what experience the investigators have had with the surfactant delivery method, and what opportunities they took to fine-tune the trial methodology, but these are now moot points as the trial has started.

We agree that the trial has already stated and the training was done a priori. You are correct that the experience of LISA while not as extensive as yours had to be coupled with extensive boot camp training.

4. Other issues
4a. The authors state that they have referenced the Kurepa survey of US centres, but it still does not appear in the latest submitted protocol.

4b. As mentioned twice previously, the reference cited in support of the notion that LISA is “widely adopted in Europe” (Bhayat) shows exactly the opposite (11% uptake, England only).
4c. Typographical error in Exclusions in supp file 4. (“of” should be “or)

We have corrected Kurepa reference and changed the text to refer that LISA is NOT widely used in the United States and England. We have correct the typographical errors in supplemental file 4.