Version 1.3 - August 2021

Neonates and Azithromycin, an Innovation in the Treatment of Children in Burkina Faso NAITRE

Manual of Operations and Procedures

Centre de Recherche en Santé de Nouna

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Contents

Abbreviations

CRSN: Centre de Recherche en Santé de Nouna
DCC: Data Coordinating Center
DSMC: Data and Safety Monitoring Committee
GPS: global positioning system
HDSS: Health and Demographic Surveillance Site
IHPS: Infantile Hypertrophic Pyloric Stenosis
IRB: Institutional Review Board
MUAC: mid-upper arm circumference
NP swabs: nasopharyngeal swabs
PCR: polymerase chain reaction
STGG: skim milk tryptone glucose glycerin media
UCSF: University of California San Francisco
WHO: World Health Organization

1. Chapter 1: Overview

1.1 Executive summary

Although under-5 mortality rates are declining globally, neonatal mortality remains persistently high in many regions of sub-Saharan Africa.[1] Mass azithromycin distribution to children aged 1-59 months has been shown to reduce childhood mortality in Niger, Tanzania, and Malawi.[2] This study did not evaluate the effect of azithromycin administered during the neonatal period. Observational evidence from high income countries has suggested that macrolides, including erythromycin and azithromycin, may be associated with increased risk of development of infantile hypertrophic pyloric stenosis (IHPS).[3,4] However, these studies are limited by confounding by indication, as infants only receive antibiotics when they are ill.

We proposed an individually randomized trial of azithromycin versus placebo to establish the efficacy and safety of administration of a dose of azithromycin during the neonatal period. Our long-term goal is to generate evidence that can be used by neonatal and child survival programs related to the use of azithromycin in the youngest children who have the highest risk of mortality.

1.2 Objectives

1: Establish the safety and efficacy of a single dose of azithromycin among neonates. Neonates aged 8 to 27 days will be randomized to a single dose of azithromycin or placebo. *We hypothesize that neonates randomized to a single dose of azithromycin will have significantly lower all-cause mortality by 6 months of age, compared to those randomized to placebo.*

2: Evaluate the impact of Neonatal azithromycin administration on long term microbiome and resistome in children enrolled in the NAITRE study two to five years after treatment administration. *We hypothesize that neonates randomized to a single dose of azithromycin will have a significant difference in resistome and microbiome compared to children randomized to receive a single dose of placebo.*

1.3 Study Sites

This study will be conducted in several regions of Burkina Faso, including peri-urban areas of Ouagadougou and Nouna town, and rural areas that are within 4 hours drive of a pediatric facility with capacity for performing pyloromyotomy.

2 Chapter 2: Context

Child mortality in West Africa is among the highest in the world. Although child health and mortality are improving worldwide, children in the Sahel and sub-Sahel regions of West Africa have the greatest risks of mortality.[1,5] Burkina Faso's current under-5 mortality rate is estimated 110 per 1,000 live births[5]. Similar to other countries in the region, the major causes of child mortality in Burkina Faso are malaria, respiratory tract infection, and diarrhea. Malnutrition acts as a major underlying contributor to mortality.[6,7] Neonatal mortality remains persistently high, with approximately 1/5th of neonatal mortality due to pneumonia, meningitis, and sepsis.[8] Interventions that address these underlying causes may be particularly efficacious for reducing mortality.

Younger children are at a higher risk of mortality. Approximately 2/3rd of under-5 deaths occur during the first year of life.[5] In general, the child mortality rate decreases as age increases. While some improvement has been observed, neonatal mortality is declining at a slower rate than post-neonatal childhood mortality.[5] Many child health interventions are designed specifically for children over 6 months of age, such as vitamin A supplementation, seasonal malaria chemoprevention, and lipid-based nutritional supplementation. Identification of strategies that are safe and effective for the youngest children will be required to address persistently high rates of neonatal and infant mortality.

The MORDOR I study demonstrated a significant reduction in all-cause child mortality following biannual mass azithromycin distribution. Across three diverse geographic locations in sub-Saharan Africa (Malawi, Niger, and Tanzania), biannual mass azithromycin distribution over a two-year period led to a 14% decrease in all-cause child mortality. In Niger, 1 in 5-6 deaths were averted. These results are qualitatively similar to those of a previous study of mass azithromycin distribution for trachoma control in Ethiopia, which found reduced odds of all-cause mortality in children in communities receiving mass azithromycin compared to control communities.[9]

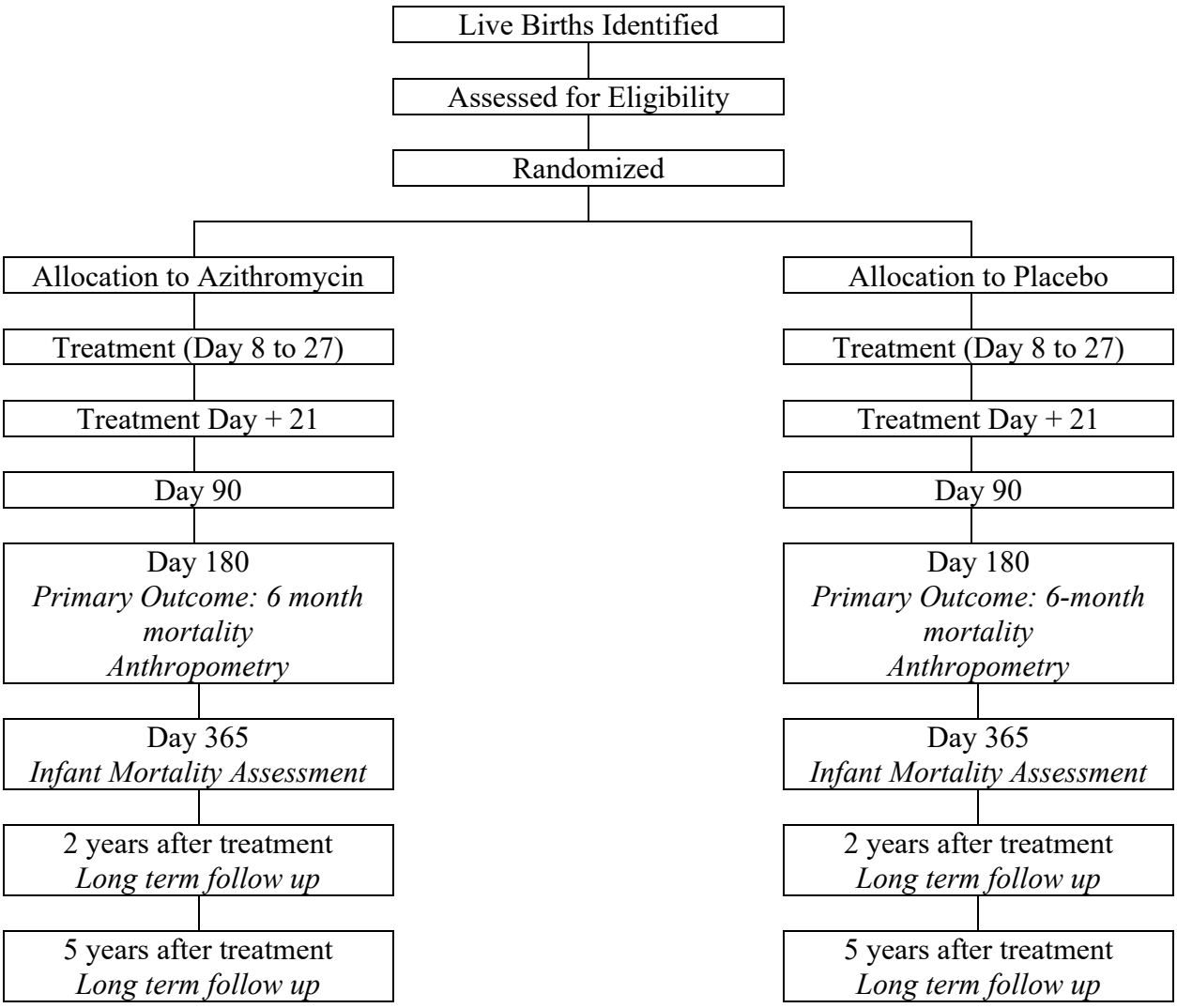
In MORDOR I, the strongest effect of azithromycin was in the youngest cohort of children. Across all three countries, the strongest effect of azithromycin was consistently in children 1-5 months of age, with an approximately 25% reduction in all-cause mortality. However, MORDOR I was not optimized to target the youngest age groups. Although children as young as 1 month were eligible, biannual distributions might not reach some children until 7 months of age. On average, children were first treated at 4 months. Given that there may be a substantial benefit to treating children at younger ages, azithromycin strategies that are designed to target younger age groups may be even more beneficial for reducing child mortality.

Here, we propose a randomized controlled trial designed to evaluate the efficacy of a dose of azithromycin administered during the neonatal period for prevention of mortality within in the first year of life. We propose to randomize births in several geographic regions of Burkina Faso to a single dose of azithromycin or placebo between day 8 and 27 of life. This study is designed to provide evidence of the efficacy of azithromycin treatment for the youngest children.

3 Chapter 3: Study Design

We will enroll and follow children from birth until 6 months of age. Figure 1 shows the trial profile.

Figure 1. Trial profile



3.1 Recruitment

Mothers and infants will be recruited either during antenatal care visits, via facility births, or via a key informant who notifies study staff of a birth.

3.1.1 Antenatal clinic recruitment

Women in the third trimester of their pregnancy who attend antenatal care services in health facilities in the geographic catchment areas of the study will be approached for possible inclusion in the study. Study staff members will approach women in late pregnancy and explain and discuss the study with the woman. If the woman is interested in participating, her contact information and approximate due date will be recorded by the study staff member. For women who are recruited during pregnancy, we will obtain verbal consent from the woman to follow the pregnancy until delivery. Once the baby is born we will obtain individual written consent to enroll the infant in the study. Formal eligibility assessment and enrollment will occur after birth.

3.1.2 Facility birth recruitment

Newborns and mothers will be recruited in facilities that have delivery services. Postpartum women will be approached by study staff, who will explain the study to the woman and assess the newborn's eligibility for the study. Newborns will be enrolled on the day of recruitment if they meet eligibility criteria (Chapter 4).

3.1.3 Key informant-based recruitment

In some rural areas and in areas with Health and Demographic Surveillance Sites (HDSS) we will employ key informants who will alert study staff of new births in their catchment area. Key informants will receive a small remuneration for reporting births.

3.2 Enrollment

Enrollment will occur the same day the child will be treated.
For each child assessed, a study staff member will review the eligibility criteria and fill out an eligibility assessment form (Appendix 2). If a child meets all of the eligibility criteria, a written informed consent process will be undertaken with the child's caregiver (Chapter 9. After eligibility assessment and consent, the child will be formally enrolled in the trial, a study identification number will be assigned to the child, and the child will be randomized a treatment arm.

3.3 Randomization

3.3.1 Unit of randomization

The unit of randomization is the neonate. Children will be randomized to azithromycin or placebo.

3.3.2 Randomizing treatment

Children will be randomized in a 1:1 fashion to a single dose of azithromycin or placebo. Randomization will occur via the tablet based on the child's identification number. Children will be assigned a random letter that corresponds to the treatment bottle. To prevent accidental unmasking,

a total of 8 letters will be used in the study, with 4 referring to azithromycin and 4 to placebo. Each facility will be stocked with medication bottles labeled with each of the 8 letters.

4 Chapter 4: Eligibility

4.1 Eligible Communities

To be eligible for the trial, a community must meet the following criteria:

1. Within 4 hours of a facility that can provide services for pyloromyotomy (Ouagadougou or Bobo Dioulasso)
2. Accessible during the rainy season
3. Ultrasound machine available OR a facility in which an ultrasound machine could be placed is within 2 hours

4.2 Eligible Participants

Eligible participants are neonates who are screened within the first week of life. Specific inclusion and exclusion criteria are described below.

Inclusion Criteria (all must be met):

- Weight ≥2500 g
- Able to feed orally
- Family intends to stay in study area for at least 6 months
- Appropriate consent from at least one caregiver
- No known allergy to azalides
- Not living within one of the communities included in the community study (CHAT/CHATON)
- No hepatic failure manifested by neonatal jaundice

Exclusion Criteria (any excludes):

- Weight <2500 g
- Unable to feed orally
- Family planning to move
- Mother/caregiver not willing to participate
- Allergic to azalides
- Living in one of the communities included in the community study (CHAT/CHATON)
- Hepatic failure manifested by neonatal jaundice

Only children who meet all inclusion criteria and do not meet any exclusion criteria will be enrolled in the trial.

Inclusion criteria for the long term follow up

- **Enrolled in the NAITRE study**
- **Written consent of one guardian is obtained**
- **Randomly selected to participate in the long term follow up**

5 Chapter 5: Procedures

A general overview of study procedures is shown in Table 1. Eligibility assessment, enrollment, and randomization are covered in detail in Chapters 3 and 4. Appendix 2 contains all study forms.

Table 1. Overview of study procedures

Day (since birth)	Activity	Study Form
0 - 7	• Birth • Sensitization	
8 to 27	• Assessment of eligibility • Enrollment • Randomization • Anthropometry (weight, Length, MUAC) • Treatment	Form 1: Eligibility assessment Form 2: Baseline Form 3: Treatment
Treatment Day + 21	• Field worker safety assessment including vital status	Form 4: Vital status Form 5: Neonate AE and IHPS risk
90	• Field worker safety assessment including vital status	Form 4: Vital status Form 5: Neonate AE and IHPS risk
180 (*primary outcome*)	• 6 months vital status assessment • Anthropometry	Form 6: 6 months vital status Form Form 5: Neonate AE and IHPS risk
365	• Vital status assessment	Form 4: vital status
2 years after treatment	• Long-term follow-up in a randomly selected subgroup of children including, vital status assessment, survey on antibiotic use and biological sample collection	Form 8: long term follow up form Form 9: long term survey
5 years after treatment	• Long-term follow-up in a randomly selected subgroup of children including, vital status assessment, survey on antibiotic use and biological sample collection •	Form 8: long term follow up form Form 9: long term survey

5.1 Treatment

Details on study medication are provided in Chapter 8. No child will be treated before Day 8 or after Day 27. After treatment, the study staff member will record the treatment or if the child did not receive the treatment, and for those who were not treated, the reason the child did not receive the treatment.

5.2 Field worker safety assessment

Field workers will conduct an initial home visit 21 days after treatment, followed by a home visit at 3 and 6 months after birth. At 21 days following treatment, field workers will conduct a survey of adverse events, including if the child had any of the following symptoms:

- Fever
- Diarrhea
- Vomiting
- Abdominal pain
- Skin rash
- Constipation

The field worker will assess whether the caregiver had sought care for the child since the last time they spoke to the study team, and if so, what the reason was for the health care visit and if the child was hospitalized.

All caregivers who report that the child has been vomiting since the last study visit will be asked additional follow-up questions to screen for IHPS, including questions related to progression of vomiting, if the vomiting is projectile, and if the child is not gaining or losing weight. Those who respond affirmatively to those questions will be referred to the study pediatrician for additional follow-up and possible referral to a tertiary facility. (Appendix 3)

All caregivers will be given a pamphlet explaining the signs of IHPS. We will also include contact information and instructions on what to do if the infant develops symptoms. (Appendix 4).

5.3 Vital status assessment

A vital status assessment will occur at each follow-up study assessment. Field workers will record on the tablet if the child is alive, died, moved, or unknown.

5.4 Anthropometry assessment

We will record every enrolled child's weight and length at baseline and at 6 months of age. The anthropometry measurements will be taken at baseline when the neonate is enrolled and during the in person follow-up visit on day 180. Weight will be measured using a digital scale. Length will be taken using a Shorrboard to the nearest cm. We will also record the middle upper arm circumference of every enrolled child. (See appendix: anthropometric measurements protocol).

5.5 Passive Surveillance

In the Centre de Santé et de Promotion Sociale (CSPS, community health facility), we will conduct morbidity passive surveillance. Each CSPS will be equipped with a tablet for electronic capture of health facility visits. Each visit will be recorded, including the reason for the visit (e.g., fever, diarrhea, malnutrition, etc), the village

of residenc, the person's age and sex, diagnosis (e.g., malaria, pneumonia, etc), treatment (e.g., antibiotic, antimalarial, etc), and timing of the visit (e.g., first versus follow-up visit). Note that this data is already routinely collected on paper forms. Identifying information like names will not be collected.

5.6 Long term follow up

Long term follow up will be performed on a randomly selected subgroup of children 2 years and 5 years after treatment in the same subset of children.

The long term follow up will include a vital status check, a survey on antibiotic use, rectal sample, nasopharyngeal samples and blood will be collected to assess for antimicrobial resistance, microbiome diversity and blood transcriptome.

Antibiotic survey

The antibiotic survey will be performed by medical personnel previously trained during the long term follow up. Data will be recorded electronically using an mobile device with custom made application.

Question of the survey will be asked to the caregiver of the child. The survey will ask whether the child have sought care to the health center or hospital since the beginning of the study and if they did it will ask if antibiotic were prescribed and what type. Pictures of most commonly used antibiotics will be used to help the caregiver answer the questions.

Rectal swabbing

Rectal swabbing will be performed during the long term follow up visits at 2 years and 5 years after treatment in a random subset of participants. The collection will be performed by medical personnel previously trained. Rectal swab will be collected in the following way:

1. Put on a clean pair of gloves.
2. Partially open the fecal swab package and remove the top section of the collection vial (this can be discarded).
3. Position the child:
    - Lie the child on his/her back, hold legs in the air (it is useful to have assistance).
    - Or have the child lay on his/her stomach across the mother/guardian's lap
4. Remove the swab from the package. Take care that the cotton tip is not touched. If it is touched, throw the swab away and begin with a new one.
5. Insert the tip of the swab into the child's anus only as far as needed to contact fecal material (1-3cm) and rotate 180 degrees. The tip should be a brownish color when removed.
6. Place swab into the preservative in the collection tube. Make sure the swab is fully submerged in the liquid preservative and then break the swab off using the pre-scored breaking point.
7. Screw the cap back on the tube and make sure that it's tightened. Wrap the area where the cap meets the tube with Parafilm to ensure that the sample will not leak, and then place the tube into the appropriate sample box.
    - If the swab cannot be broken off while the tip is fully submerged in the liquid, try twirling the swab in the liquid first (to release the contents of the sample into the preservative) before breaking it off. Avoid rubbing the sample on the tip of the swab

off on the side of the tube where there is no liquid.

8. Place a random number label on the collection tube.
9. Place the tube the rectal swab container.
10. Swab storage for Genetic analysis: Store samples at room temperature. According to the manufacturer, the preservative in the tube will preserve DNA for 5 months at room temperature. Samples can be frozen for long time storage

Nasopharyngeal swabbing

Nasopharyngeal swabbing will be performed during the long term follow up visits at 2 years and 5 years after treatment in a random subset of participants. The collection will be performed by medical personnel previously trained and will be collected in the following way:

The examiner will:

1. Place a pediatric flocked swab with a nylon tip through the right nostril and down the nasopharynx of each participant. Note that if the swab is not perpendicular to the frontal plane of the face, it is likely not in the inferior turbinate.

2. Reach the nasopharynx, rotate the swab 180° and remove the swab from the nose.

3. Place the swab in a tube containing 1.0 mL DNA/RNA shield media by Zymo media, cut the handle off using sterile scissors, and close the cap of the tube with the swab immersed.

4. The nasopharyngeal swab samples in DNA/RNA shield media will be stored in ambient temperature in the field. Then transferred to a refrigerator or freezer.

5. The scissors used to cut calcium alginate swabs will be sterilized with alcohol pads or cleaned with bleach wipes between participants. When collecting specimens in DNA/RNA shield, scissors will be cleaned between participants - first with bleach wipes, and then with alcohol pads.

Do not attempt to collect the NP swab if you are not successful after three attempts.

Nasopharyngeal swabs will be stored in DNA/RNA shield media by Zymo research, and will be assessed for the most common genetic resistant determinants (ermB and mefA) using a PCR-based assay. Serotype will be assessed using a nested PCR reaction for the most common serotypes, followed by the Quellung reaction for any untyped isolates.

Blood collection

Blood collection will be performed during the long term follow up visits at 2 years and 5 years after treatment in a random subset of participants. The collection will be performed by medical personnel previously trained and will be collected in the following way:

Determination of the maximum allowable blood draw volume

See the chart below in order to determine the maximum allowable blood draw volume from children and infants, based on body weight.

Body wt in kg	Max drawn in one blood draw
	2.5% of total blood volume
1 kg	2.5 ml
2 kg	4.5 ml
3 kg	6 ml
4 kg	8 ml
5 kg	10 ml
6 kg	12 ml
7 kg	14 ml
8 kg	16 ml

9 kg	18 ml
10 kg	20 ml
11 thru 15 kg	22 - 30 ml
16 thru 20 kg	32 - 40 ml
21 thru 25 kg	42 - 50 ml
26 thru 30 kg	52 - 60 ml
31 thru 35 kg	62 - 70 ml
36 thru 40 kg	72 - 80 ml
41 thru 45 kg	82 - 90 ml
46 thru 50 kg	92 - 100 ml
Greater than 50 kg	100 ml

Citation for above chart: Seattle Children's Hospital Powered by Mayo Medical Laboratories. "Maximum Allowable Blood Draw from Infants." https://seattlechildrenslab.testcatalog.org/show/1000721-1

Identification du patient:

Identify the child by scanning her/his study identity card.

Verify the identity of the child with the mother/guardian of the child: check the name, study identification number, sex, age and the name of the mother and father of the child.

Supplies

2 chairs

1 examination table

1 pillow

1 Cotton ball

1 Alcohol wipes

1 Wing needle 23G with tubing and holder 1 needle container

1 trash bag

1 small bandage 1 pair of gloves

1 tube to collect blood

1 tourniquet

1 tablet

1 unique identification number

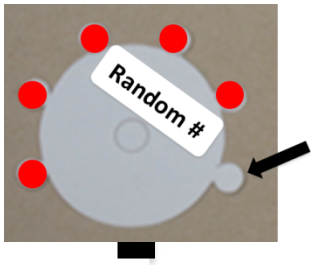
1 blue pad to put under the arm of the child

Procedure

1. Collect supplies and equipment
2. Wash your hands
3. Look at the arms of the child to try to find a good venipuncture site
4. Wash your hands
5. Position the child
6. Put the tourniquet on the child about 2 fingers widths above the venipuncture site chosen
7. Put on your gloves
8. Attach the end of the tubing to the end of the holder
9. Remove the plastic sleeve from the needle
10. Using the alcohol swab, disinfect the venipuncture site and allow it to dry
11. Draw the skin using your tumb and stick the needle in the vein
12. Push the tube onto the holder until the needle is in it
13. Blood should begin to flow into the tube
14. Fill the tube until it is full, remove the tube
15. Release the tourniquet
16. Place a dry coton ball over the venipuncture site and withdraw the needle
17. Ask the parent/guardian to continue applying mild pressure
18. Collect dried blood spots using the whole blood
19. Dispose of the needle in the sharps container
20. Dispose of all contaminated supplies in the trash
21. Label the tube with a label containing a unique number
22. Put a bandage on the child's arm and ask the mom to remove it 1h later
23. Gently and completely invert the tube 10 times in order ro mix blood with the tube preservation agents
24. Remove gloves, put them in the trash
25. Wash your hands

How to fill out the filter paper:

1. Label the filter paper with a random number sticker.
2. Grip the filter paper on the side without small circles. Place a droplet of blood directly from the thumb or finger onto five of the six circles, leaving the right one blank. Be sure to fill each circle completely.



Leave last circle blank

Area to hold the filter paper.
Do not touch the small circles.

3. The recorder will scan the QR code.
4. Carefully slide the filter paper onto a pencil to air dry for at least an hour. There should be about 1 cm in between each sample. Secure the pencil into a Styrofoam surface in a box or container to protect from dust.
5. When the filter paper is dry, place each sample into a small zip plastic bag (individually). Place the small bags into a larger Ziploc bag with five desiccant packets.
6. Ensure the large Ziploc bag is sealed tightly, as moisture will damage the samples. Transport these filter paper samples to a freezer.

If venipuncture is not possible at the study site, we will collect blood using fingerprick method.

6 Chapter 6: Adverse Event Monitoring and Safety Assessment

There is some concern that azithromycin administered during the neonatal period will increase the risk of infantile hypertrophic pyloric stenosis (IHPS). Follow-up assessments have been designed to carefully monitor for IHPS following administration of study treatment.

6.1 Background on Infantile Hypertrophic Pyloric Stenosis in Neonates

IHPS is a condition in which the pylorus of the stomach becomes thickened, resulting in gastric outlet obstruction.[10] In developed countries, the incidence of IHPS is approximately 2 per 1,000 infants in the general population. While surgery is generally curative, the condition is lethal in the absence of surgery. The cause of IHPS is unknown and likely complex, and both genetic[11,12] and environmental[13,14] factors are thought to contribute to its development. Male neonates are disproportionately affected by IHPS, with a 4-5:1 male to female ratio.[10,12,15] Other risk factors for IHPS include prone sleeping position, bottle/formula feeding,[14,16,17] preterm birth,[13,14] cesarean section delivery,[13,14] and birth order.[10,13-15] IHPS incidence appears to be decreasing over time, which some have attributed to public health interventions promoting supine sleeping position and exclusive breastfeeding.[18,19]

In addition to genetic and environmental factors, erythromycin is associated with increased risk of infantile hypertrophic pyloric stenosis (IHPS).[20] Given that azithromycin is a related compound, there is some concern that azithromycin may lead to increased risk of IHPS in neonates. Limited

evidence exists of the risk of IHPS among neonates treated with azithromycin.[21] Two randomized controlled trials have assessed the use of intravenous azithromycin for prevention of bronchopulmonary dysplasia (BPD) in low birthweight infants (<1,250g) compared to placebo within 72 hours of birth.[22,23] Of 263 neonates enrolled in the two studies (N=130 receiving IV azithromycin), no cases of IHPS were reported. A third non-placebo controlled randomized trial of azithromycin prophylaxis for BPD in premature neonates additionally reported no cases of IHPS among 53 neonates receiving azithromycin.[24] An observational study of 58 infants receiving azithromycin following exposure to a healthcare worker with pertussis did not identify any cases of IHPS.[25] The largest study of azithromycin exposure in neonates is a retrospective cohort of more than one million infants in the TRICARE Management Activity military health system, which reported an overall IHPS rate of 2.3 per 1,000 (95% CI 2.2 to 2.4) among children in the first 90 days of life.[4] Of 4,875 infants prescribed azithromycin, there were 8 cases IHPS, 3 of which occurred when azithromycin was prescribed during the first 14 days of life and 5 of which during the first 15-42 days of life. Overall, there was no significant difference in IHPS rate in infants treated with azithromycin versus IHPS rates among infants who had not received an antibiotic during the first 90 days of life were not presented by time since birth. Table 2 lists the rates of IHPS following azithromycin, erythromycin, or cephalexin prescription.

Table 3. Unadjusted rate of IHPS in infants with oral azithromycin, per 1,000

	Azithromycin	**Erythromycin**	**Cephalexin**
0-14 days[1]			2.2 (0.05-
15-42 days[1]	20.3 (4.2-59.2)	30.9 (14.1-58.7)	12.2)
43-90 days[1]	6.9 (2.2-16.1)	9.3 (3.0-21.8)	3.1 (1.0-7.2)
Overall	0 (0-0.9)	2.8 (0.6-8.1)	0 (0-1.6)
	1.6 (0.7-3.2)	8.9 (5.2-14.3)	1.4 (0.5-3.0)

[1]Day of azithromycin prescription, from birth

There is relatively little evidence of the epidemiology of IHPS in sub-Saharan Africa. A study in Nigeria documented 57 cases of IHPS from 1978-2008 at a university teaching hospital, with only a single case from 2003-2008.[19] A study of 102 cases of IHPS over a 5-year period in Tanzania documented a 4.9% mortality rate despite surgical intervention.[26] The risk of mortality was higher in infants under 2 weeks of age and those with delayed presentation to care. A retrospective study at a tertiary hospital in Ethiopia found that 12.9 per 1,000 admissions were due to IHPS, with a 3.3% mortality rate.[15] In Ouagadougou, an unpublished case series of 32 infants treated for IHPS showed a 6.3% mortality rate, which was attributed to late presentation due to the infant traveling from far outside the city.

6.2 Screening for IHPS

Field workers will conduct home visits with caregivers 21 days following treatment, as described in Chapter 5. Any child suspected of having IHPS will be immediately referred to the study pediatrician for evaluation. Evaluation will include ultrasonography and physical exam. Physical exam will include assessment of the pyloric olive (a thickened and elongated pylorus). Any child with a positive physical exam for a pyloric olive will be immediately transferred to the pediatric surgical unit in Ouagadougou or Bobo.

Images will be taken including the longitudinal pylorus with canal length measurement, transverse pylorus with muscle thickness measurement, and the relationship of the pylorus to the gallbladder. IHPS will be strongly suspected in infants with a permanently closed pylorus and exaggerated, retrograde gastric peristalsis. Diagnostic measurements include pyloric muscle thickness (diameter of a single muscular wall on a transverse image) >4 mm, length (longitudinal measurement) >15 mm, and pyloric volume >1.5 cc.[27] The child should be placed with their right side down, and the pylorus watched to determine if it opens. Small infants not below the pathologic limits with a permanently closed pylorus will also be considered for further workup.[28] Any child with a pyloric muscle thickness >4 mm will be immediately transferred to the pediatric surgical unit in Ouagadougou. In addition any child with normal measurements, but no food is passing, will be transferred.

All children will be assessed for electrolyte disturbance and dehydration, and rehydration and correction of electrolyte disturbance will occur prior to surgery.

Any children receiving surgery for IHPS will be followed-up with 1 week after the procedure. The follow-up will be a phone call or at-home visit. The child's vital status information will be collected. Another follow-up visit will take place 4 weeks after the procedure.

7 Chapter 7: Training

CRSN and UCSF will work together prior to the start of the study to standardize all study procedures. We will review the format, general logistics, and procedures for the recruitment, enrollment, randomization and anthropometric measurements. We will review all study protocols, including informed consent procedures and documentation and adverse event monitoring. The importance of screening for IHPS and safety protocols will be stressed. Refresher trainings will occur as needed and supervisors and study investigators will routinely review data and procedures to ensure fidelity to protocol.

8 Chapter 8: Study Medication

Neonates enrolled in the study will be offered weight-based, directly observed, oral suspension azithromycin or placebo. We will monitor adverse events following treatment as described in Chapter 6.

8.1 Study Medication Description (from Pfizer, Inc.)

Azithromycin

Zithromax® for oral suspension is supplied in bottles containing azithromycin dehydrate powder equivalent to 1200mg per bottle and the following inactive ingredients: sucrose; tribasic anhydrous sodium phosphate; hydroxypropyl cellulose; xanthan gum; FD&C Red #40; and flavoring including spray dried artificial cherry, crème de vanilla, and banana. After constitution, a 5mL suspension contains 200mg of azithromycin.

8.2 Dosage Information

Azithromycin and placebo will be administered as a single dose, in oral suspension form for children. Dosing will follow the WHO recommendations for treatment of active trachoma:

- Single dose of 20mg/kg in children (up to the maximum adult dose of 1g)

Individuals who are allergic to macrolides/azalides will not be treated.

8.3 Medication Procurement/Donation

Azithromycin (Zithromax®) and the placebo have been donated by the Pfizer Corporation. There will be no costs to acquiring the study medication. Pfizer, Inc. will ship azithromycin and placebo directly to the study sites. Representatives of each study site will manage the customs process and transport the medication from the port to storage sites.

8.4 Medication Quality Control

Study medication will be shipped by Pfizer directly to CRSN and stored at CRSN research offices prior to distribution to each study site. The study coordinator and other staff will regularly check and record the study medication expiration dates. The expiration dates on the medication containers will be strictly monitored and all expired study medicine will be discarded appropriately. The study coordinator will work with each health facility to ensure that they have appropriate stock of all study medications.

8.5 Study Treatment

Study treatment procedures are detailed in Chapter 5.

8.6 Serious Adverse Events

Any serious adverse events (SAE) will be reported to Pfizer. An **IIR SAE Form** (*Investigator-Initiated Research Serious Adverse Events Form*) will be completed for each event. (See Appendix for form and complete instructions.)

According to Pfizer, an SAE is any adverse event that:

- Results in death
- Is life-threatening (i.e., causes an immediate risk of death)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Results in a congenital anomaly or birth defect

Or that is considered to be:

- An important medical event

All participants will be advised to alert a village health worker if they experience, within one week of treatment, a serious adverse event (by the preceding definition). An SAE report must be submitted for all deaths in the study – regardless of the time of treatment. The local health worker will report to the study coordinator; who must, within 24 hours, submit a Pfizer **IIR SAE Form** to mordor.burkina.sae@gmail.com. AS and TL will review, and forward to Pfizer and/or the Medical Monitor, as appropriate. SAEs must be submitted to Pfizer within 24 hours of receipt from the on-site coordinator. AS and TL will also forward SAE to DSMC if meets criteria of being possibly related to study drug. All deaths reported that are not serious adverse events resulting from the treatment will be reported to Pfizer on a quarterly basis.

One or more qualified investigators will be posted in the health centers at the recruitment site. Mothers of included children will be encouraged to visit these health centers in the event of an adverse event. Any adverse events occurring during the trial will be covered by the study free of charge. Participants with a serious event will be cared for in the nearest hospital. The research team will be in constant contact with pediatricians from regional and university hospitals and pediatric surgeons to manage potential serious adverse events. Surgical units will be involved in the diligent management of pyloric stenosis cases. All recruitment sites will be within a two-hour drive of a

Regional Hospital Centre where there is a paediatrician and a radiologist to enable rapid diagnosis of pyloric stenosis and within 4 hours of Bobo-Dioulasso or Ouagadougou to enable rapid management.

The reporting of any serious adverse event will follow national procedures in Burkina Faso.
- In the event of a non-serious adverse event, the CSPS will process the cases and the national reporting form will be completed.
- In the event of serious events, the CSPS will contact the study doctors on the same day. The patient will then be evacuated to an appropriate level of care for management.
- The declaration will be made to the National Agency for Pharmaceutical Regulation in accordance with the regulations and within the deadlines (7 days in the event of death or life-threatening prognosis, 15 days in other serious and unexpected cases, 15 days in new facts) at pharmacovigilance.burkina@sante.gov.bf
- The ethics committees will also be informed of the occurrence of this event within the same time frame.
- The sponsor will be informed within 24 hours in the event of a serious event.
Pfizer will be reported within 24 business hours from the date of knowledge of the serious adverse event

8.7 Adverse Events Data

We will keep records and report all adverse events of azithromycin to the DSMC. We will report both efficacy and side effects of azithromycin For any "sudden deaths" believed to be associated with azithromycin treatment, key informants will immediately notify the verbal autopsy interviewer via SMS message or another appropriate form of rapid communication.

9 Chapter 9: Protection of Human Subjects

Before the study begins, CRSN and UCSF will obtain formal ethical approval from their respective ethics committees as well as national ethical approval in Burkina Faso. In addition, local staff will approach community leaders to describe the study and answer any questions. Study staff will proceed only if local leadership consents to participate. At the individual level, we will obtain written consent from a parent or guardian for all study activities with patient contact, including following pregnancies for potential enrollment, treatment, and follow-up visits.

If, at any time, a parent or guardian elects to withdraw a family member from the study, they will be free to do so. Individuals who withdraw will be offered the same medical treatment outside the study.

9.1 Institutional Review Board Approval

UCSF Committee on Human Research
UCSF's Committee on Human Research will annually review study protocol for ethical approval.

CRSN Comité Institutionnel d'Ethique
The study protocol will be reviewed and granted ethical approval by the Comité Institutionnel d'Ethique at the CRSN headquarters before any patient-related research activities begin.

National Health Ethics Committee of Burkina Faso.
The study protocol will be reviewed and granted ethical approval by the National Health Ethics Committee of Burkina Faso before any patient-related research activities begin and annually.

9.2 Informed Consent

First, the chairman of each village will be asked for permission to include the village in the study. Additionally, the study will be discussed with all adults in the village by team members who speak the local language(s).

Informed consent scripts will be translated into local languages before the study can begin. Consent scripts will then be back-translated by a different party to ensure comprehension. Consent scripts will be submitted and approved by national IRB committees in Burkina Faso prior to study implementation. Then they will be read aloud to each study participant (and his/her parent/guardian) by a local team member who is a native speaker of the local language to ensure that they understand the risks and benefits of participating in all study activities. Young adults and children under 18 years of age, who cannot give consent by law, will be included in the study only following the receipt of verbal informed consent from a parent or guardian. If, at any time, a parent or guardian elects to withdraw themselves or a family member from the study it will be made clear that they can, without consequences.

10 Chapter 10: Data and Safety Monitoring Committee Charter

This Charter is for the Data Safety and Monitoring Committee (DSMC) for *Neonatal Azithromycin to Prevent Infant Mortality. (OPP 1187628)*

The Charter will define the primary responsibilities of the DSMC, its relationship with other trial components, its membership, and the purpose and timing of its meetings. The Charter will also provide the procedures for ensuring confidentiality and communication, statistical monitoring guidelines to be implemented by the DSMC, and an outline of the content of the Open and Closed Reports that will be provided to the DSMC.

10.1 Primary Responsibilities of the DSMC

The DSMC will be responsible for safeguarding the interests of trial participants, assessing the safety and efficacy of the interventions during the trial, and monitoring the overall conduct of the trial. The DSMC will provide recommendations about stopping or continuing the trial. To contribute to the integrity of the trial, the DSMC may also formulate recommendations relating to the selection/recruitment/retention of participants, to protocol-specified regimens, and the procedures for data management and quality control.

The DSMC will be advisory to the trial leadership group, hereafter referred to as the Steering Committee (SC). The SC will be responsible for promptly reviewing the DSMC recommendations and determining, whether to continue or terminate the trial, and to determine whether amendments to the protocol are required. If needed, the DSMC may seek the advice of a content expert outside of the committee.

10.2 DSMC Membership

The DSMC is an independent multidisciplinary group consisting of epidemiologists, biostatisticians, bioethicists, and clinicians that collectively has experience in pediatrics, the management of infectious diseases, and in the conduct and monitoring of randomized clinical trials including subsaharan Africa.

10.3 Conflicts of Interest

The DSMC membership has been restricted to individuals free of apparent conflicts of interest. The source of these conflicts may be financial, scientific, or regulatory. Thus, neither study investigators nor individuals employed by the sponsor, nor individuals who might have regulatory responsibilities for the trial products, are members of the DSMC.

The DSMC members will disclose to fellow members any consulting agreements or financial interests they have with the sponsor of the trial, with the contract research organizations (CRO) , or with other sponsors having products that are being evaluated or that are competitive with those in the trial. The DSMC will be responsible for deciding whether these consulting agreements or financial interests materially impact their objectivity.

The DSMC members will be responsible for advising fellow members of any changes in any of the membership requirements that occur during the course of the trial. It may be appropriate for DSMC members who develop significant conflicts of interest resign from the DSMC.

DSMC membership is to be for the full duration of the trial. If any members leave the DSMC, the SC, in consultation with the DSMC, will promptly appoint a replacement.

10.4 Timing and Purpose of the DSMC Meetings

Organizational Meeting

The initial meeting of the DSMC will be an Organizational Meeting. This is during the final stages of protocol development and the purpose is to provide advisory review of scientific and ethical issues relating to study design to discuss the standard operating procedures and to discuss the format and content of the Open and Closed Reports that will be used to present trial results.

The Organizational Meeting will be attended by all DSMC members, lead trial investigators, and the trial biostatistician. The DSMC will be given the drafts of the trial protocol, the Statistical Analysis Plan, the DSMC Charter, and the current version of the case report forms. At subsequent meetings, committee members will receive Open and Closed Data Reports.

Formal Interim Analysis Meetings

One or more 'Formal Interim Analysis' meetings will be held to review data relating to treatment safety and efficacy, and quality of trial conduct. There will be at least two interim decisions to be made by the DSMC, at approximately 12 months and 24 months into the study.

10.5 Procedures to Ensure Confidentiality and Proper Communication

To enhance the integrity and credibility of the trial, procedures will be implemented to ensure the DSMC has access to all emerging information from the trial regarding comparative results of efficacy and safety, aggregated by treatment arm.

Closed Sessions
Sessions involving only DSMC members and, where appropriate, those unmasked trial investigators (on the Data Coordinating Committee) who generate the Closed Reports (called Closed Sessions) will be held to allow discussion of confidential data from the trial, including information about the relative efficacy and safety of interventions.

At a final Closed Session, the DSMC will develop a consensus on its list of recommendations, including that relating to whether the trial should continue.

Open Session
In order for the DSMC to have access to information provided, by study investigators, or members of regulatory authorities, a joint session between these individuals and DSMC members will be held between the Closed Sessions.

Open and Closed Reports
For each DSMC meeting, Open and Closed Reports will be provided. Open Reports, will include data on recruitment and baseline characteristics, pooled data on eligibility violations, and completeness of follow-up and compliance. The study statistician (TCP) will prepare these Open Reports.

Closed reports, available only to those attending the Closed Sessions of the meeting, will include analyses of primary and secondary efficacy endpoints, including subgroup and adjusted analyses, AEs and symptom severity, , and Open Report analyses that are displayed by intervention group. These Closed Reports will be prepared by the study biostatistician.

The Open and Closed Reports should provide information that is accurate, with follow-up that is complete to within two months of the date of the DSMC meeting. The Reports should be provided to DSMC members approximately three days prior to the date of the meeting.

Minutes of the DSMC Meeting
The research team will prepare minutes for the open portion of the meeting, including the DSMC's recommendations.

Recommendations to the Steering Committee (SC)
At each meeting of the DSMC during the trial, the committee will make a recommendation to the Steering Committee to continue or terminate. This recommendation will be based primarily on safety and efficacy considerations and will be guided by statistical monitoring guidelines defined in this Charter.

Recommendations to amend the protocol or conduct of the study made by the DSMC will be considered and accepted or rejected by the SC. The SC will be responsible for deciding whether to continue or to stop the trial based on the DSMC recommendations.

The DSMC will be notified of all changes to the protocol or to study conduct. The DSMC concurrence will be sought on all substantive recommendations or changes to the protocol or study conduct prior to implementation.

The SC may communicate information in the Open Report to the sponsor and may inform them of the DSMC recommended alterations to study conduct or early trial termination in instances in which the SC has reached a final decision agreeing with the recommendation. The SC will maintain confidentiality of all information it receives other than that contained in the Open Reports until after the trial is completed or until a decision for early termination has been made.

10.6 Statistical Monitoring Guidelines

The SC will propose statistical rules for a futility stopping rule (requested by the sponsor) and an efficacy stopping rule at the first DSMC meeting. A decision will be made whether the efficacy stopping rule is appropriate for the study.

11 Chapter 11: Data Collection, Management, and Security

11.1 Scope of Data

Mortality and morbidity data will be collected in this trial. Data include treatment, vital status, adverse events, demographic and birth-related characteristics, and specimen collections.

Demographic and Birth Data
At baseline, trained field workers will collect information on the child's gestational age and birthweight and basic demographic information about the mother (age, education, etc).

Treatment Data
Trained field workers who administer treatment will record each treatment dose in the mobile application at the time of treatment of the child. Data will include the child's weight (used to calculate the dose), the dose, if the child received the treatment, and if not, why the child did not receive treatment.

Vital Status Data
Trained field workers will collect information on the vital status of each infant enrolled in the study at multiple pre-specified time points during the study. Vital status will include alive, died, moved, or unknown.

Adverse Events
Trained health workers will collect data on adverse events that arise during the study, including gastrointestinal symptoms, hospitalizations, and other healthcare visits.

11.2 Data Storage, Management, and Security

Data will be recorded electronically using handheld mobile devices with custom-made software applications and uploaded daily onto a secure, password protected, central server. Rapid transfer of electronically captured data will allow nearly real-time monitoring of activity at the study site. Each study site will have a local data coordinating center within the study area. All handheld devices and data entry coordinating centers will be password protected, and all changes in data will be noted, including the date of the change, and the person who made the change. To ensure the quality of the data, we will conduct training sessions before each biannual census where needed. The central database application will use hard disk encryption and physical protection of the server (which is to be maintained in a locked room accessible only to authorized personnel). The database will be based on mySQL (which supports standard SQL queries). Data will be backed up off site (providing integrity in case of the physical loss of the server). Data will never be deleted from mobile capture devices until at least one offsite backup has been completed. Data security during electronic transfer will be achieved through use of the Advanced Encryption Standard (AES).

11.3 Data Monitoring and Cleaning

Data monitoring and cleaning will be overseen by the data coordinating center (DCC) at the coordinating site. Data collection will be monitored on a weekly basis by the site study coordinator

using a dashboard function. The dashboard will consist of the following reports by study site: Date Household Census Completed, Number of Households Census Completed by Village, Percent Household Census Completed by village, Treatment Status by Worker, Age Distribution by Worker, Sex Distribution by Worker, GPS Missing by Worker, GPS Missing by Village, Number of Records Synced by Date, Assigned Treatment by Given Treatment, Treatment Status by Age, Treatment Status by Village, Age Distribution by Village, and Sex Distribution by Village.

The DCC will ensure that the site study coordinators log on to the dashboard at least weekly to confirm the status of the dashboard. In addition, upon each village census completion, the DCC will create and maintain a Stata program to identify data quality concerns. Any such concerns, which must be addressed at the site-specific level, will be queried by the DCC. At every phase, as each village is completed and the data is considered cleaned, the data will be locked and a list of deaths will be generated and provided to each site for verbal autopsy.

Appendix

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