Protocol

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

Protocol for: Mitjà O, Houinei W, Moses P, et al. Mass treatment with single-dose azithromycin for yaws. N Engl J Med 2015;372:703-10. DOI: 10.1056/NEJMoa1408586

Supplement documents - Protocols and Statistical analysis plans

Supplement to: Mitjà O, Houinei W, Moses P, et al. Mass treatment with azithromycin for yaws

This supplement contains the following items:

1. Original protocol, including the original statistical analysis plan
2. Final protocol, including the final statistical analysis plan
3. Summary of changes.

Yaws elimination on Lihir Island, New Ireland Province, PNG: A proof of principle of Mass Drug Treatment with azithromycin

Acronym:	YERA – Yaws eradication
Date of protocol:	07-Mar-2013
Protocol version number:	v6.0
Investigators:	Dr Oriol Mitjà; Dr Murray Koka, Mr Raymond Paru, Ms Wedny Houinei, Dr Sibauk Bieb, Prof Sheila Lukehart, Dr Russel Hays.
Sponsors:	Newcrest Mining, InternationalSOS-Niugini
Main centre:	Lihir Medical Centre, International SOS, Lihir Island, New Ireland Province, Papua New Guinea
Ethical approval:	Government of Papua New Guinea, Medical Research Advisory Committee Approval Number 12.36

Protocol Approval and Authorization

Protocol Approval

Statement of Compliance

The study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following:

- ICH GCP E6
- Completion of Human Subjects Protection Training

SIGNATURE PAGE

The signature below documents the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality and according to local legal and regulatory requirements and to the principles outlined in applicable United States federal regulations and ICH guidelines.

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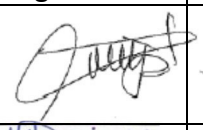
Dr Oriol Mitjà	Co-Principal Investigator, Lihir Medical Center oriolmitja@hotmail.com		22/11/12
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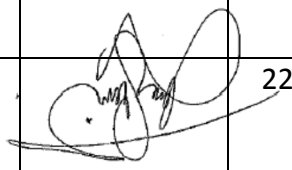
Dr Murray Koka	Co-Principal Investigator, Lihir Medical Center murray.koka@newcrest.com.au		22/11/12
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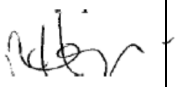
Dr Russell Hays	Lihir Medical Center senior.medicalofficer@newcrest.com.au		22/11/12

Protocol Outline

1. Synopsis

The trial that we are proposing is a proof of principle to determine the efficacy of the new WHO yaws eradication strategy. We aim to assess the impact of mass distribution of single-dose oral azithromycin on yaws in the Lihir Island, PNG, population (n=16,000). We will also monitor the rate of macrolide resistance in yaws before and after the introduction of azithromycin for the treatment of yaws

Project Overview	**Results**	**Results Measurement**
Strategy:		
To perform a non-randomized clinical trial of 2-years mass drug administration (MDA) of oral azithromycin on Lihir Island, coupled with 4-years of surveillance for prevalence of infection.	Adequate MDA coverage and adequate recruitment of patients for prevalence surveys.	Clinical trial performed with adequate standards.
Project Goal:		
To achieve elimination of yaws as a result of the total community treatment (TCT) of oral azithromycin followed by 3 rounds of total targeted treatment (TTT) for 18 months.	Zero clinical cases reported in the community, and no evidence of transmission among children < 5 years.	Whole population medical examination for signs of clinical yaws during MDA, and cross-sectional sero-surveys at months 0, 6, 12, 18, 24, 36 and 48.
Objectives:		
1. To implement **large-scale treatment** (TCT or TTT) with azitromycin biannually during 2 years.	Number of individuals having received the drug; Coverage rates in the total censed population.	Observed coverage survey of directly observed treatment with azithromycin.
2. To conduct **clinical surveys for active yaws** in the whole population.	Number and prevalence of individuals with clinical yaws infection.	Clinical surveys for active yaws (whole population medical examination) during TCT and TTT.
2. To conduct yaws prevalence **surveys for latent disease** in a subset of children.	Number and prevalence of children with latent yaws infections (determined by serology) in community surveys.	Cross-sectional serological surveys in a random sample of children.
3. To evaluate the development of **yaws bacterial resistance** to azithromycin throughout the study.	Macrolides sensitivity profile of *T. p. pertenue* in individuals receiving the intervention.	Proportion of macrolide resistance in yaws at each cross-sectional survey.

2. Introduction

2.1 – Project purpose and Background

The trial that we are proposing is a proof of principle to determine the efficacy of the new WHO yaws eradication strategy. We aim to assess the efficacy of an initial Total Community Treatment (TCT) with single-dose oral azithromycin, followed by 3 rounds of Total Targeted Treatment (TTT) resurveys every 6 months, to completely eliminate yaws infection on Lihir Island, Papua New Guinea (PNG), population 16,000. We will also monitor the rate of macrolide resistance in yaws and the impact of azithromycin MDA on incidence of other macrolide-susceptible infections in the community.

Yaws is an infectious disease caused by *Treponema pallidum* subspecies p*ertenue*, a bacterium which closely resembles the causative agent of syphilis, and is spread by skin to skin contact in humid tropical regions. It causes disfiguring, and sometimes painful lesions of the skin and bones. Like syphilis, the clinical manifestations can be divided into three stages, but mother-to-child transmission does not occur. A major campaign to eradicate yaws in the 1950s and 1960s, by mass treatment of affected communities with long acting, injectable penicillin, reduced the number of cases by 95% worldwide, but yaws has enjoyed a resurgence in recent years in Africa, Asia and the Western Pacific. A single, oral dose of azithromycin was recently shown to be as effective as intramuscular penicillin, and a new initiative for yaws eradication was launched by the World Health Organisation in 2012.

Yaws is spread by direct person-to-person non-sexual contact with the exudate from early infectious lesions. Untreated primary yaws papules are highly infectious (rich in treponemes) and usually last 3-6 months. Early secondary yaws lesions may appear on the skin near the initial lesion or elsewhere in the body, including bone and cartilage. These last around 6 months and usually heal spontaneously. Thereafter the disease enters a latent non-infectious period which may last the lifetime of the patient. This latent state can be interrupted at any time by the reappearance of infectious yaws lesions, up to 5 years after the initial infection. The total duration of infectiousness for an untreated yaws patient, including relapses, is probably around 12-18 months.(1) Unless diagnosed and treated in the early stages, yaws can become a chronic, relapsing and disfiguring disease and can lead to severe deforming bone lesions in the long term. (2)

For routine purposes, the diagnosis of yaws requires the use of traditional and rapid serological tests results together with clinical manifestations, while carefully taking into account the epidemiologic and demographic characteristics of yaws. (3)(4) The serological tests used to

diagnose yaws are the same as those used to diagnose syphilis. The non-treponemal agglutination tests (rapid plasma reagin [RPR] or Venereal Disease Research Laboratory [VDRL] are positive in untreated cases, and can be used as a test of cure, since they usually revert to negative after successful treatment. Both are simple to perform. The RPR can be read with the naked eye, whereas the VDRL requires a microscope. The non-treponemal tests may give rise to false positives in patients with other conditions, including malaria, leprosy and rheumatological diseases. (5) They are often performed on serial dilutions of serum, giving a quantitative read out, or titre, defined as the highest dilution that gives a positive result. They become positive within 2 – 4 weeks of the appearance of the primary lesion. (6)

Long-acting penicillin, given as a single intramuscular dose, has been the mainstay of yaws treatment and eradication efforts for 60 years, (7) having been shown to be effective against yaws in 1948. (8) Benzathine benzylpenicillin, as a single intramuscular dose of 1·2 MU for adults and 0·6 MU for children, is the recommended regimen. Cure rates for patients with early, active lesions are >95%. However there are a few reports of possible penicillin treatment failures in yaws. In PNG, apparent treatment failures were reported in 11 of 39 (28%) cases on Karkar Island, (9) and a few penicillin treatment failures have also been observed in Ecuador.

A single oral dose of azithromycin (30 mg/Kg) has recently been conclusively shown to be as effective as intramuscular benzathine benzylpenicillin. (10) At 6-month follow-up, 96% of patients in the azithromycin group were cured by clinical and serological criteria, as were 93% in the benzathine benzylpenicillin group. These data correspond to a treatment difference of –3·4% (95% CI –9·3 to 2·4), meeting the pre-specified criteria for non-inferiority.

Pharmacokinetic data from **clinical studies show that a 30 mg/kg dose of azithromycin, administered as a single dose**, provides drug exposure equivalent to at least a 5-day regimen. (11) Both regimens maintain azithromycin levels in tissue sites of infection above the MIC of treponemes for several days after administration has ceased. The oral bioavailability of azithromycin is high (approximately 37%), and tissue concentrations exceed serum concentrations by as much as 100-fold following a single oral dose, with high concentrations being found in skin and bones, the principal target tissues for yaws.

The WHO revised policies for the treatment of yaws in 2012, (12) specifically responding to the growing evidence of yaws resurgence and the need to develop new strategies to facilitate eradication. Based on the results of the trial in PNG (10) azithromycin is now recommended as equivalent to the standard regimen of benzathine benzylpenicillin for yaws treatment and eradication.

2.2. Rationale

Substituting a single dose of an oral antibiotic for a painful penicillin injection is a significant advantage:

i) Infection control measures required for injection of Penicillin will no longer be required; attempts to control yaws by mass treatment with injectable drugs have previously led to the transmission of blood-borne viruses, such as hepatitis C.(13)

ii) Injectable penicillin causes anaphylactic shock in a small proportion of patients. Although the risk is small (<1 patient in 50 000), the consequences can be severe, and control programmes using penicillin need to train staff and provide them with the means to treat anaphylaxis (14).

iii) Treatment will be more acceptable to communities who need it.

The early global eradication programmes in the 1950s highlighted the importance of subclinical, or latent cases as a source of reinfection. It was estimated that the ratio of clinically apparent to latent cases could be as high as 1:6, and treatment of active cases only had little impact on the prevalence of yaws one year later (15). Most of the active cases found at resurveys were in persons who were in the latent stage originally and had not received treatment with injectable penicillin.(16) In contrast, high-coverage (95%) treatment of the entire population, as was witnessed in a yaws eradication campaign conducted in the 1950s in Nsukka, Nigeria, resulted in a rapid reduction in prevalence within 6–12 months.(17)

Mass drug administration of a single dose of oral azithromycin (30 mg/kg; maximum 2 g) to given to entire populations in areas known to harbor yaws is more proactive and aggressive strategy in order to deal with all potential contacts and latent cases. We expect that transmission can be interrupted in a reasonably short time, leading to elimination as witnessed in Nigeria. (17)

2.3. Potential Risks and Benefits

2.3.1 Potential Benefits

Yaws is still a substantial cause of morbidity in PNG. The National Department of Health (NDoH) estimated the number of yaws cases to be 15,936 nationwide in 2007. The number has increased to more than 29,000 in 2010 and 2011, of which 7,300 were in New Ireland Province and another 7,200 in the neighbouring province of West New Britain. (Miriam Pahun, personal communication)

Yaws has been endemic in many lowland areas of PNG for many decades. There were general mass treatment —eradication programs in that time, spread over large areas of PNG as well as

targeted —mass treatments. A good example is Karkar Island in Madang, which has had several mass treatment programs with intramuscular penicillin. Yaws was said to be eradicated but when the NDoH stopped the wide spread use of intra-muscular procaine penicillin and introduced oral amoxicillin in its stead for the treatment of a plethora of conditions, yaws started to be seen again.

There is currently need for methods to control yaws, of which mass administration of a single-dose oral treatment is the most definitive. The most obvious benefit that will have subjects participating in the study will be the cure of the disease active and latent yaws. From the point of view of public health, by eliminating yaws in the whole population, we will also prevent future infections.

Also community-based mass treatment programmes using this antibiotic for the control of trachoma have been well accepted by rural communities in many parts of Africa, are highly effective in reducing the prevalence of trachoma and could have collateral benefits. In one study in Ethiopia, mass treatment every year with azithromycin reduced all-cause mortality by 50% in children aged 1–5 years.(18)

2.3.2 Potential risks

Drug resistance as a collateral effect

A note of caution on the use of azithromycin is the biological evidence that selective pressure can select for resistant strains, as has occurred with *T. p. pallidum* in a number of sexual networks in developed countries.(19)(20)(21) Background macrolide use for unrelated infections (mainly respiratory) contributes to the increased prevalence of macrolide-resistant *T. p. pallidum* by providing a selective pressure.(22) Interestingly, macrolide-resistant *T. p. pallidum* has not been found in Uganda, (23) Tanzania, (24) or Madagascar (25) - where macrolides are not widely used. (26) Nonetheless, the recognition of this possibility is a reminder that there is no room for complacency. Surveillance for treatment failures, and biological markers of resistance, will be essential if azithromycin is widely used for the eradication of yaws.

The effect of mass treatment with azithromycin on resistance in *Streptococcus pneumoniae* is also of considerable public-health importance and may impact on the management of acute respiratory infections in children. This phenomenon has been evaluated after mass treatment campaigns to control trachoma and the results have been somewhat contradictory. (27) Surveillance studies have demonstrated short-term changes in susceptibility patterns of the bacteria, although these did not generally persist.

3. Study Objectives

Project goal: To achieve elimination of yaws as a result of the total community treatment (TCT) of oral azithromycin followed by 3 rounds of total targeted treatment (TTT) for 18 months.

3.1 Primary objective:

1. To assess the efficacy of total community treatment (TCT) of oral azithromycin followed by 3 rounds of 6 monthly total targeted treatments (TTT) in Lihir Island.

3.2 Secondary objectives:

2. To estimate coverage of Mass Drug Administration (MDA) in the targeted population. Report at a village level of number of persons treated compared to census.
3. To estimate the prevalence of individuals with clinical yaws infection in the whole population of Lihir after mass treatment.
4. To estimate the prevalence of children with latent yaws infection (determined by serology) in community surveys after mass treatment.
5. To estimate the rate of macrolide resistance in *T. p. pertenue* in patients with active yaws both before and after the MDA.

4. Methods and Intervention

4.1 Outcome measures:

4.1.1 Primary outcome measures:

- Prevalence of clinically active yaws infections in the whole population determined by WHO definitions (and serology confirmation) at 0, 6, 12, 18, 24, 36, and 48 months
- Prevalence of latent yaws infections (determined by serology) in a random sample of children < 15 years, at 0, 6, 12, 18, 24, 36 and 48 months in the Lihir resident population.

4.1.2 Secondary outcome measures:

- Coverage rates during MDA in the total censed population.
- Proportion of macrolide resistance in yaws at each cross-sectional survey.

4.2 Study population:

Inclusion criteria:

- Whole resident population of Lihir Island for MDA and clinical surveys, and
- Subset of 875 children 1 - 15 years in sentinel sites for sero-surveys.

Exclusion criteria:

- Children younger than 2 months and pregnant women;
- Known allergy to macrolide antibiotics;
- Refusal of individual or guardian (for individual inclusion).

[these will be offered benzathine penicillin]

4.3 Case definitions:

Clinically active case:

Persons resident in endemic areas with one or more of the symptoms below and positive serological test (rapid treponemal tests or qualitative and quantitative RPR)

Clinically active yaws:	
Infectious	1. Initial lesion(s) – papilloma 2. Multiple papillomata 3. Plantar and palmar papillomata 4. Ulcers 5. Other early skin lesions (macules, papules, micropapules, nodules, plaques)
Non-infectious	1. Hyperkeratosis 2. Bone and joint lesions
Inactive yaws	Late active yaws: gummata, ulcers, gangosa, sabre tibia

4.4 Design and general approach

Design:

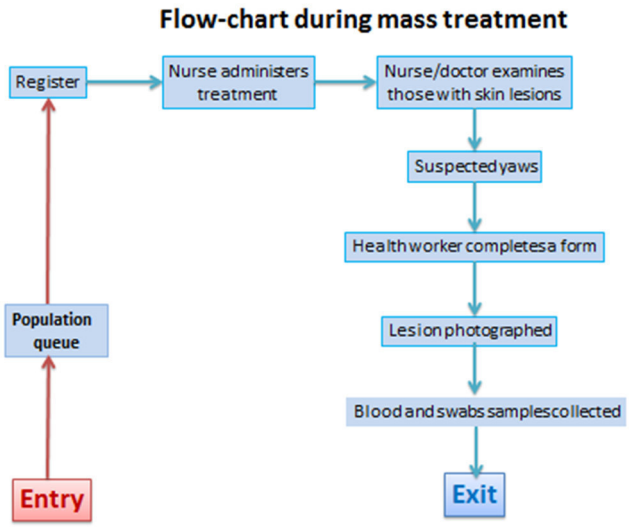
- We intend to perform a mass drug administration exercise of oral azithromycin for yaws, coupled with annual surveillance for prevalence of yaws infection.

, 12, 18, 24, 36, and 48

4.4.1. Large-scale treatment with azithromycin to the whole resident target population (n=16,000):

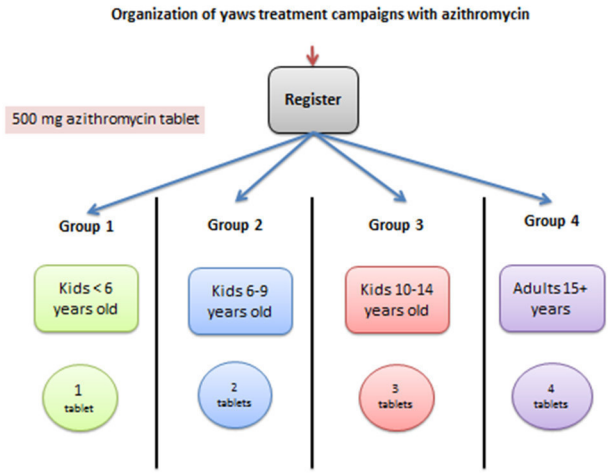
- **Training of health workers and community agents:** Health workers will be trained for one day on clinical diagnosis, administration of azithromycin, recording and surveillance, new eradication policies and strategy, community mobilization and mass treatment campaign, follow-up activities etc.
- **IEC and community mobilization:** After training, the village health workers will be responsible for informing the communities about the planned mass treatment. They will ensure that each household gets a copy of the WHO pictorial booklet. Yaws posters will be put in all strategic places in the community to ensure full knowledge of the disease and impending mass treatment. The objective of the community mobilization is to ensure that almost 100% of the eligible population will be seen during the first round of the mass treatment.
- **Treatment of population:** Communities will receive an initial Total Community Treatment (treat the entire village), followed by 3 rounds of 6 monthly Total Targeted Treatment (treat all active clinical cases, and their contacts). During TTT, all the resident population will undergo medical examination for signs of clinically active yaws. Benzathine benzylpenicillin will be reserved as a backup for those very few who cannot be treated with azithromycin, and who are not allergic to penicillin.

Figure 1. Flow chart of large-scale treatment interventions



- **Dose calculation:** WHO will purchase generic azithromycin (500mg tablet) from Medopharm (India), and supply for the pilot. The drugs will be shipped to PNG around mid-January 2013. The dosages per age are as shown in the figure below. The quantities required for each community will be determined in advance based on the population distribution using excel formula for estimating azithromycin needs.

Figure 2. Dose calculation of azithromycin by age



- **Coverage of MDA versus an updated census:** will be estimated for each round. The program will aim to deliver directly observed treatment to at least 90% of the population in each of the 4 rounds of MDA. Each round of MDA will report, at village level, on coverage. Those on the census not present will be followed up with an appointment letter during the following 2 weeks. The census list will be updated at each round.

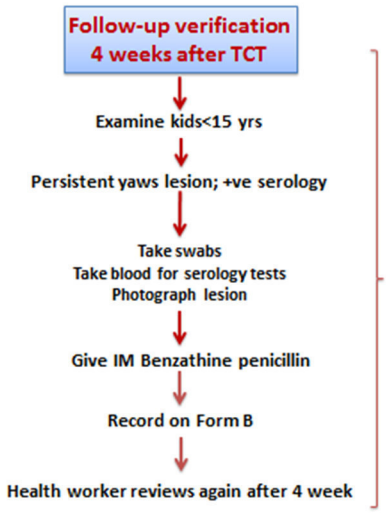
4.4.2 Clinical surveys for active yaws (n=16,000)

During MDA campaigns (TCT and TTT) all the resident population will undergo medical examination by a clinician (doctor or nurse) for signs of clinically active yaws and prevalence of active yaws will be estimated. Those with suspected yaws lesions will be further examined and information on each person will be recorded on a Case Report Form A **(Annex 3).** Swabs (from papilloma and ulcer) and scrapings from squamous macule and papule lesions will be collected to help determine baseline resistance to azithromycin (4.4.4 for macrolide resistance monitoring).

- **At 4 weeks follow-up**

Four weeks after the mass treatment, the health workers will visit each village and examine those who were treated and whose serology tests during the mass treatment confirmed yaws. Any participant with persistent lesion at 4 weeks and serology results are positive will be considered to have failed on azithromycin and a detail examination will be carried out, additional specimens will be taken and benzathine penicillin will be offered in this instance. Information will be recorded on Form B**.**

Figure 3. Flow chart for 4-weeks follow-up visit



- **Post-treatment clinical surveys**

After the initial 2 years we will conduct bi-annual active surveillance for yaws cases with visits to the communities. Between repeat surveys, health facilities will have stocks of azithromycin in order to respond to cases identified in the community.

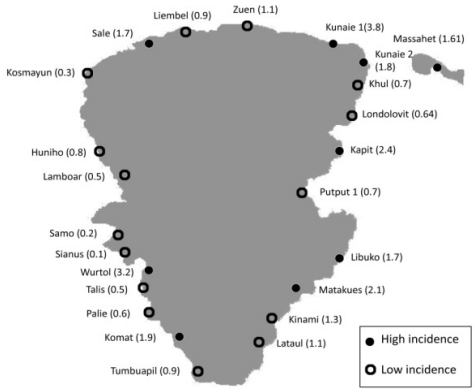
4.4.3. Serological prevalence survey for latent yaws in children (n=1,000; 1-15 in the island):

Since longitudinal intensive serological monitoring of impact of intervention to a total population is labor intensive, we plan sentinel site monitoring in a smaller population group.

To detect latent yaws, we will collect a 5 mL venous sample in a dry vacutainer serum tube and transported at the end of each clinic day to the Lihir Medical Centre Laboratory where it will be centrifuged and serum separated. The laboratory will perform the qualitative and quantitative RPR testing and *Treponema pallidum* hemagglutination assay (TP-HA). The standard external quality control procedures for the RPR and TPHA testing (i.e. 5% of positive and negative samples) will be performed at the Microbiology Unit of Sullivan Nicolaides Pathology (Queensland, Australia).

Household census data will be compiled and all children 1 - 15 in the six sentinel villages will be examined including RPR and TPHA testing in all cross-sectional surveys throughout the study. The screening serologic tests used for the diagnosis of syphilis [i.e. RPR, TPHA] have been evaluated and are used for the diagnosis of yaws. The first 4 surveys will coincide with the MDA activities in the village at 0, 6, 12, and 18 months. The other surveys of the same individuals plus the new under 15 year cohort will occur at 24, 36 and 48 months after the first MDA round.

Figure 4: Map of Lihir Island with locations of high incidence of yaws infections (Mitja, 2011) (32)



4.4.4 Monitoring for causative etiological agent and potential macrolide resistance (n=100)

We will evaluate the presence of *T. p. pertenue* containing the macrolide resistance mutation in a convenience subsample of patients. Resistance to azithromycin is largely conferred by an A2058G or A2059G mutation in the 23S ribosomal RNA (rRNA) gene of *Treponema pallidum*. Swab samples will be obtained from ulcer lesions (n = 100) and transported in medium for DNA preservation. The specimens will be tested at the University of Washington laboratory by nested polymerase chain reaction amplification of one 23S ribosomal DNA region, followed by restriction digestion of the amplicon as previously described (Lukehart, 2004). Based on past experience we expect that *T. pallidum* DNA could be detected in at least 80% of specimens collected, and that of samples containing detectable *T. pallidum* DNA, 23S rRNA gene could be amplified in more than 90%. **(Annex 4)**

4.4.5. Side effects: reporting and quantification

- **Passive surveillance:** Passive surveillance for adverse events will be undertaken throughout the study at the Lihir Medical Centre and all peripheral health posts. Specific training for all health staff will be conducted before the beginning of the interventions, with special emphasis on reporting to the study staff any allergic event or any other adverse event deemed possibly related to the intervention using a standard AE case report form.
- **Active surveillance:** We will also perform household surveys 1 week after the initial distribution of antibiotics to monitor for potential adverse events. We will conduct the surveys in 60 randomly selected households from 28 villages. All drug-related adverse events will be assessed by study investigators and grading will be reported according to standardized criteria.

Table 1. Grading criteria for drug-related adverse events.

	Grade 1	Grade 2	Grade 3	Grade 4
Abdominal pain	Mild	Moderate no treatment needed	Moderate treatment needed	Severe-hospitalization for treatment
Nausea	Able to eat	Oral intake significantly decreased	No significant intake	Requiring IV fluids
Vomiting	1 episode in 24 hours	2-5 episodes in 24 hours over pretreatment	> 6 episodes in 24 hours or need for IV fluids	-
Diarrhea	Increase of < 4 stools / day over pre-treatment	Increase of 4-6 stools/day, or nocturnal stools	Increase of > 7 stools/day or incontinence; or need for parenteral support for dehydration	
Fever	37.7 – 38.5 C or 100.0 – 101.5 F	38.6 – 39.5 C or 101.6 – 102.9 F	39.6 – 40.5 C or 103 – 105 F	> 40.5 C or > 105
Headache	Mild pain not interfering with function	Moderate pain	Severe pain	Disabling
Allergic reaction / hypersensitivity	Transient rash, drug fever < 38°C (<100.4°F)	Urticaria, drug fever > 38°C (>100.4°F), and/or asymptomatic bronchospasm	Symptomatic bronchospasm, requiring parenteral medication(s), with or without urticaria; allergy-related edema/angioedema	Anaphylaxis

5. Schedule of study procedures

Program **timelines and deliverables** will be according to the schedule below:

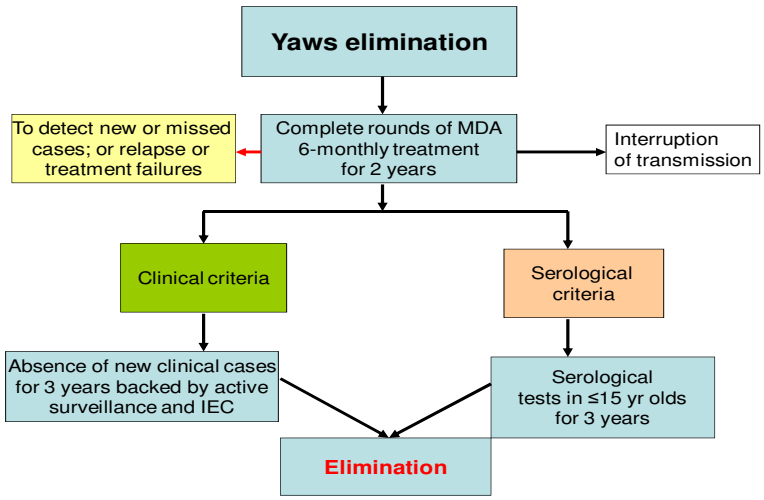
Activity / Months	**0**	**6**	**12**	**18**	**24**	**30**	**36**	**42**	**48**
Mass drug administration; coverage surveys	X	X	X	X					
Clinical surveys for active yaws	X	X	X	X	X		X		X
Serological surveys for latent yaws	X	X	X	X	X		X		X
Macrolide resistance monitoring	X	X	X	X	X		X		X

6. Major Assumptions

- **Elimination at month 24**

For this project, we assume that we will achieve zero prevalence of yaws at month 24, as per previous experience in Nigeria (Zahra,1956). Years 3-4 will be used for surveillance to declare the elimination of yaws (3 consecutive years without reported cases) and to monitor the potential re-introduction of infection. There is the threat of re-introduction of yaws from other endemic regions of PNG due to the large "in-migration" in Lihir. The migrant census is updated regularly by Newcrest Social Impact Monitoring team. The project will work closely with this team to ensure that all in-migrants receive treatment on arrival or soon thereafter (within 3 months).

Figure 5: Proposed yaws elimination protocol



- **Good acceptance of interventions**

We assume also that the MDA campaign will be well accepted by the Lihir communities, since MDA has been well accepted in previous MDA for filariasis elimination and that coverage will be high. Given the large population of the island living in remote areas there could be delays in administering the medication or coverage may be less than 80%. The clinical examinations and blood collection will be undertaken by trained community field workers who are already engaged in an island-wide filariasis control program, but also logistic support from LMC and significant in-kind support from the local communities and health services are measures to strengthen the screening team if necessary.

7. Statistical analysis plan

7.1 Data management

Only de-identified data will be used for analysis. All clinicians and researcher accessing the database will sign confidentiality agreements. The primary data will be collected by study staff at the community, health facility site and laboratory staff at the different field and laboratory sites. The study coordinator (or his/her designee) will cross check all CRF‘s for completeness, validity and legibility. All data from CRFs and laboratory worksheets in PNG will double-enter data in Microsoft Access software (version 14.0); all discrepancies will be checked against the original forms. Data entry will be done in batches. First entry will be done by trained data entry staff at the designated data entry unit directly after receipt of CRF and worksheet copies from the LMC field stations. Second entry will be once a batch of first entry is completed. Discrepancies, missing values, and out-of-range values will be detected in queries run on a batch-by-batch basis by the data base manager. If necessary queries will be send back to the field site for resolution. All corrections made to the database by the data managers (or designees) will be recorded.

7.2 Data ownership

The state must be custodian of the data. In all written publications concerning the research project, the National Department of Health and the Lihir Medical Centre will have co-responsibility for the scientific results. The Lihir Medical Centre, represented by Oriol Mitjà will contact the National Department of Health before publication of any results related to the research project.

7.3 Type of analysis

7.3.1 Primary analyses at 12 months

We will calculate the prevalence rates of active yaws measuring everyone in the population at three time-points (0,6,12 months). We will estimate the prevalence of latent yaws measuring a subset of children in randomly selected villages.

We will estimate the evolution of odds ratio (OR) in active and latent yaws prevalence rates at all time-points (0,6,12) and compare the values to describe the decrease in prevalence across the study time. For this analysis we will use a logistic regression model. We will use Anova test to compare the age of children in the three seroprevalence surveys and the prevalence of sex will be compared with Chi-Square. We will evaluate the decline in proportion of PCR detected infection across the time study using the multinomial logistic regression model. Statistical significance will be defined at a p-value less than 0•05.

7.3.2 Primary analyses at 24 months

We will calculate the prevalence rates of active yaws measuring everyone in the population at all time-points (0,6,12,18,24 months). We will estimate the prevalence of latent yaws measuring a subset of children in randomly selected villages.

We will estimate the evolution of odds ratio (OR) in active and latent yaws prevalence rates at all time-points (0,6,12,18,24) and compare the values to describe the decrease in prevalence across the study time. For this analysis we will use a logistic regression model. We will evaluate the decline in proportion of PCR detected infection across the time study using the multinomial logistic regression model. Statistical significance will be defined at a p-value less than 0•05.

7.4 Sample size and power calculation

7.4.1 Prevalence of latent yaws at 12 months

A standard power calculation indicated that to estimate prevalence of high titer latent yaws with a precision of 1.5%, 875 children were required to achieve 80% power at 5% (two-sided) significance. We assumed the expected prevalence of latent yaws at 12 months to be 5%.

Estimating prevalence at month 12 with precision (ω)

Formula

Sample size

$$n = \frac{Np(1-p)z^2_{1-\alpha/2}}{N\omega^2 + p(1-p)z^2_{1-\alpha/2}}$$

Precision

$$\omega = z^2_{1-\alpha/2}\sqrt{\frac{(N-n)}{Nn}p(1-p)}$$

where z1-α/2 is standard normal deviate corresponding to the specified size of the critical region (α), ω is the precision, p stands for prevalence and N for target population size.

7.4.2 Prevalence of latent yaws at 24 months

At 24 months, we assume that we will achieve prevalence of high titer latent yaws near-zero. With a sample size of approximately 1,000 children in the 6 sentinel villages, an estimate of the prevalence of yaws would have the following 95% confidence intervals:

True prevalence (%)	95CI (%)
0.0	0.0 – 0.3
0.5	0.2 – 1.1
1.0	0.5 – 1.8
1.5	0.9 – 2.4
2.0	1.3 – 3.0

8. Organizational Experience and Collaborative Partnerships

This study will be conducted by CRESIB, and CRESIB contracted local-staff working at Lihir Medical Centre (LMC) in Papua New Guinea in collaboration with the National Department of Health. The interest and priorities of the department will be the driving force in any given situation. The NDoH-NTDs program will be also part of this study. Finally there will be a collaborative partnership with University of Washington who will test for macrolide resistance.

- **CRESIB:** The Barcelona Center for International Health Research (CRESIB) has large experience in elimination/eradication of endemic infectious diseases, with a particular focus on NTDs and malaria. CRESIB investigators conducted the research in oral treatment of yaws (Mitja, 2011) that led to the revision of the WHO policies.
- **LMC:** The Lihir Medical Centre (LMC) has a local research team employed by CRESIB who will implement the azithromycin large-scale treatment programm and yaws prevalence surveys. Clinical examinations (16,000) and blood collection (1,000) will be undertaken by a yaws specifically trained field workers team. The LMC laboratory has capacity to undertake the syphilis serological testing in this study (accredited to perform these tests by the Australasian College of Pathologists quality assurance program (QAP, North Ryde NSW).
- **University of Washington:** Sheilla Lukehaart's laboratory will test swab samples from lesions in persons with symptomatic yaws to determine whether the *Treponema pallidum* strains harbor resistance mutations. Sheilla Lukehaart's lab developed rapid assays for the A2058G and the A2059G mutations in the 23S rRNA gene (both are associated with macrolide resistance). They have years of expertise in these assays and the technical aspects of the evaluation.

8.1. Key roles

- **Doctor Oriol Mitja.** Oriol Mitja will take the lead role in this project including overall leadership and responsibility for the trial, including community consultation, liaison with collaborating organizations, logistics, data management, field work, and communication of results.
- **Doctor Murray Koka.** Koka will take responsibility for supervising the project in the field, including performing and supervising medical examinations of all participants (n=16,000) over the two year MDA period, supervising quality assurance in laboratory specimen collection and handling of specimens for transport, managing clinical cases as required in the field, supervision of clinical research staff, supervising data collection in the field, contributing to data analysis and interpretation and writing of reports and papers

- **Ms Wendy Houinei.** Ms Houinei representing the NDoH-NTDs program will be part of this study; she will be involved in the implementation activities, monitoring and data analyses.

8.2. Capacity building proposal

8.2.1 Publication of articles: All co-authors will benefit of publications of work done for the duration of research. This work will undoubtedly be a state-of-the art in science of eradication for yaws world-wide.

8.2.2 Technical meetings to report on the progress of the investigation. This meeting is proposed to be hold on a quarterly basis and may coincide with the NTD-TWG meeting.

8.2.3 Field team: We will ensure that the personnel in charge of screening the patients had the skills and knowledge that enables them to perform effectively the mass treatment for yaws. Certification of clinical assessors to screen patients for yaws will require a chance corrected agreement (kappa statistic ≥0.6) with an experienced clinician (OM, PM) over the scoring signs of clinically yaws (Active infectious, active non-infectious and inactive yaws in the WHO system) in validation exercises in both the classroom (photographic collection) and the field.

8.2.4 Laboratory technicians: will participate in a training course to upgrade their skills on serological diagnosis of yaws.

8.2.5 Study Investigator Ms Houinei should be involved with the project so that she can either use it for degree or PhD / MPH.

- Involvement in the implementation of activities of the campaign to eliminate yaws. She will learn the logistics of the project, and gain experience in diagnosis, treatment and monitoring yaws. This experience could be eventually applied in other districts of the country.
- Support and tutoring to develop MPH / PhD. She could either be enrolled in UPNG / Divine University or in University of Barcelona (Spain). For the latter she would need to have completed the master degree.

The results of **this project will promote the development of capacities in PNG**, and the strengthening of the research collaborations. Research towards the development of strategies to eradicate yaws represents a very attractive area of research which would increase PNG research potential. Ultimately all of this contributes towards **increasing Papua New Guinean excellence and competitiveness** in research and development.

9. Ethics

9.1 - Ethical conduct of the study

This study will be conducted in compliance with the study protocol. The patient's informed consent will be obtained according to the ethical principles stated in the Declaration of Helsinki 2000 version (amended in Seoul 2008), and the applicable laws and regulations of Papua New Guinea.

9.2- Patient information and consent

The informed consent document will be used to explain the risks and benefits of study participation to the parent or guardian of the patient in simple terms before the patient will be entered into the study. The informed consent document contains a statement that the consent is freely given, that the parent or guardian of the patient is aware of the risks and benefits of entering the study, and that the patient is free to withdraw from the study at any time. Written consent must be given by the legal representative, after the receipt of detailed information on the study. Where appropriate, the children will be asked to provide assent.

Appropriate forms for obtaining written informed consent and the patient information sheet will be provided by the Investigator (see **Annex 1-2**).

Given that some patients for inclusion in this study may not be capable of giving legal consent, written consent must be obtained from their next of kin. In the case where the next of kin are unable to read, an impartial witness should be present during the entire informed consent discussion. After the representative has orally consented to participate in the study, the witness's signature on the form will attest that the information in the informed consent form and patient information sheet was accurately explained and understood. In case of a parent or guardian of the patient who cannot provide informed consent in writing, an 'X' to indicate consent in the presence of two witnesses is acceptable. Permission from the parent or guardian must be obtained in the case of assenting minors.

The principal Investigator(s) at each centre will ensure that the parent or guardian of the patient is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. Patients and their parent or guardian must also be notified that they are free to discontinue from the study at any time. The patient and parent or guardian should be given the opportunity to ask questions and should be allowed time to consider the information provided.

9.3 Patient data protection

The Informed Consent Form will explain that study data will be stored in a computer database, maintaining confidentiality in accordance with ICH GCP and relevant local data protection laws. Patients in this database will be identified by initials or patient number only. The Informed Consent Form will also explain that for data verification purposes, authorised representatives of Sponsor, a regulatory authority, an EC may require direct access to parts of the hospital or practice records relevant to the study, including patient medical history.

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Annex 1. Inform consent (English)

"Yaws elimination on Lihir Island, New Ireland Province, PNG: A pragmatic trial of Mass Drug Treatment with azithromycin"

Lihir Medical Center, International SOS
Study location: Lihir Island, New Ireland Province

Yaws is a highly contagious infection and people can get it through direct contact with infected individuals. The condition starts with an ulcer. If it is left untreated, the ulcers will penetrate all the way to the bone and it can be quite painful. The treatment of yaws has long been a single injection of benzathine penicillin. Today, we have a drug called azithromycin (comes in tablets form) that is swallowed once (single-dose treatment) and has the same effect as injection benzathine penicillin. The drug is safe and has been used to treat other diseases.

Some people may be infected (have the germ which causes yaws in their blood) but show no lesions on the skin. Such people can only be identified through a simple blood test. If they are not treated, they will develop skin lesions in a few weeks or month and can be a source of new infection in the village (stress the need for prophylactic treatment).

Today, we are in your village because yaws occurs here. Our aim is to give everyone living in this village the new treatment which can completely cure everyone and remove yaws from your village within a very short time. Only children less than 2 months and women who are pregnant will be not be treated with azithromycin. Rather, they will be treated with benzathine penicillin.

Some child's blood might be required to be tested for yaws. We use a sort of lottery to decide who will be tested for yaws. Neither you, nor the doctor can decide who will be tested. At the start of the study, the children that are tested for yaws will have the finger pricked for blood slide and to put small amount of blood (250µl) in a container for testing for yaws. Your children blood will be tested once a year during 4 years.

Voluntary Participation
You must know that you can choose to be in the research study or choose not to be in the research study and that you can stop whenever you want.

Confidentiality
Information obtained about you for this study will be kept confidential to the extent allowed by law. The results of the treatment may be published for scientific purposes. However, your identity will not be given out.

Right to Refuse or Withdraw
Whether or not you take part in this study is your choice. There will be no penalty if you decide not to be in the study. You are free to withdraw from this research study at any time. Your choice to leave the study will not affect your relationship with this institution.

If you have questions about the study or if you think you or your child has a study-related injury, you can talk to any study team member, the study doctors;

Dr Oriol Mitja
Lihir Medical Center
Telephone number: 9867188

Annex 1. Inform consent (Tok Pisin)

"Pinism sik Yaws long Lihir Ailan, New Ireland Province, Papua Niugini: Treatim wantaim marasin, Azithromycin"

Husait igo pas long dispel wok: Oriol Mitja
Lihir Medical Center
Ples: Lihir Ailan, New Ireland Province

Yaws i save spread isi tru na ol manmeri na pikinini ken kisim yaws taim ol stap wantaim husait ol manmeri igat yaws. Sik yaws save stat wantaim sua. Sapos yu no kisim marasin, sua wea istap lo skin ken go olgeta long bun na iken pen nogut tru. Yu ken kisim marasin (azithromyin) onepela taim o kisim sut marasin (penicillin) onepela taim tasol lo pinisim sik yaws.

Mipela i lukim olsem yaws save spread isi tru long peles hia lo Lihir na long ol narapela hap tu long Papua Niiugini. Planti ol manmeri pikinini igat dispel sik yaws na ol ino save. Olsem na mipela i laik givim marasin long olgeta man meri na pikinini long Lihir lo stopim dispela long spread. Dispela marasin (azithromycin), ol i bin testim na usim pinis long treatim yaws na ol it ok orait long usim dispel marasin (azithromycin) long treatim yaws.

Sapos yu o pikinini blong yu tok orait long stap insait long dispel study, yu bai kisim marasin long start blong dispela study na long wan wan yia inap long tupela yia.

Sampela ol pikinini bai mipela kisim blut bilong ol long testim sik yaws. Bai mipela pikim nem olsem long laki lotto tiket. Ino inap yu o dokta bai makim husait bai kisim blut test.

Long stat bilong study ol pikinini husait bai mipela testim bulut; bai mipela sutim finga blong ol na testim blut olsem malaria test na kisim sampla blut na putim long ol botol long testim sik yaws bihain olsem piksa i soim.Blut bilong ol pikinini bai mipela testim wan wan yia inap long fourpela yia olgeta.

Tok Orait
Yu mas save olsem yu ken tok orait long take part long dispel study or tok nogat. Na yu ken stop long take part long dispel study anytime.

Toktok Hait
Wanem kain ol toktok/stori yu givim mipela long dispel study bai stap namel long yu na mipelatasol. Ino gat narapela man bai save. Ol results bilong dispela study bai mipela publishim long halivim ol sikman tasol. Mipela ino inap long tokaut long nem na stori bilong yu.

Yu gat rite long tok nogat
Em i laik bilong yu long take part long dispel study. Inogat asua long tok nogat long take part.Wanem choice yu mekim, Lihir Medical Senta em still hausik bilong yu, yu ken kam na kisim marasin anytime yu sik.
Sapos yu gat askim long dispela study yu ken toktok wantaim ol study team members,
study dokta Dokta Oriol Mitja or ringim Lihir Hausik long fone numba 9867188

Annex 2. Individual consent form

Study title: "**Yaws elimination on Lihir Island, New Ireland Province, PNG: A pragmatic trial of Mass Drug Treatment with azithromycin."**

Individual consent form:

We have been given information on this mass treatment exercise by the health workers.

□**I am a participant less than 15 years old**

I (first/surname):_____of _____ _____ give consent for my child _____to be a participant and allow specimens to be collected to confirm yaws in the laboratory.

Parent/Guardian's signature or Right thumb print (date)	Health works signature
_____/_____/_____ Date (dd/mm/yyyy)	_____/_____/_____ Date (dd/mm/yyyy)

□**I am a participant 15 years or older**

I (first/surname):_____of _____ _____ give consent to be a participant and allow my specimens (blood or swabs or scrapings) to be collected to confirm yaws in the laboratory.

Signature or Right thumb print (date)	Health works signature
_____/_____/_____ Date (dd/mm/yyyy)	_____/_____/_____ Date (dd/mm/yyyy)

Annex 2. Individual consent (Tok Pisin)

Nem Bilong dispela study: Pinism sik Yaws long Lihir Ailan, New Ireland Province wantaim marasin, Azithromycin

Taim yu signim dispel pepa yu i tokim mipela olsem yu:
* Yu save long dispela study
* Tok orait long pikinini bilong yu long take part long dispel study
* Yu kisim study infomasin pepa
* Yu bin askim ol askim long kisim kilia tingting long dispela study
* Yu mekim fri decision long take part long study
* Yu tok tok wanatim femili na tok orait long pikinini bilong yu long take part long dispel study

Taim yu signim tok orait ol study team bai mekim wok bilong ol. Wanpela copy bilong tok orait form bai yu kisim.

_____
Nem Bilong Man/Meri/Pikinini

Tok orait Bilong Papa na Mama:
_____ Dait:
Nem Bilong Papa/Mama or Man lukautim pikinini

_____
Mak Bilong Papa/Mama or Man Lukautim pikinini Papa,Mama o Man lukautim pikinini

Dispela tok orait em i witnesim:
_____ Dait:
Nem Bilong Study wokman

_____
Mak Bilong Study Wokman

Annex 3. FORM A

Recording form for participants with skin lesions consistent with clinical yaws at the time of the initial mass treatment

(To be filled in by health worker)

Consent signed: □ Yes □ No *(only fill in questionnaire if consent signed)*

Name of Father:		**Name of Mother:**
Section A: Demographic data		
1	Name of Participant:	
2	Participant ID number:	
3	Date of Birth: (dd)____(mm)___(yyyy)_____	Sex: □ Male □ Female
	Age (yrs):_____	
4	Village:	District:
Section B: History and clinical examination		
5	Duration of illness in weeks:	
6	Clinical forms of yaws (*Refer to WHO pictorial guide*) □ Papilloma □ Ulcers □ Squamous macules □ Bone lesions □ Palmar and plantar	
Section C: Conclusions of clinical Assessment		
7	□ likely yaws □ possible yaws □ unlikely yaws	
8	Tick the below boxes once done. □ Blood collection □ Swabs/scrapings	**ONLY FOR PARTICIPANTS WITH SUSPECTED YAWS LESIONS**
10	Treatment given: □ Azithromycin (number of 500 mg tablets): _____ □ Benzathine penicillin (check): _____0.6 MU or _____1.23 MU	

Annex 4: Sampling, storage and transport of samples for yaws testing

SPECIMENS NEEDED:

- Swabs from primary and moist secondary lesions.

EXCLUSION CRITERIA:

- Antibiotics potentially active against *T. pallidum* within the previous month.

PROCEDURE FOR COLLECTING SPECIMENS:

Primary lesions and moist secondary lesions:

- **Use the swabs* and lysis buffer** specified here**
- Squeeze and swab the lesion vigorously, then place the swab into the tube containing the 1X lysis buffer. Break or cut off the shaft of the swab and leave the tip in the buffer. Replace cap, and vortex or shake vigorously.
- Label with date and pt study number **using special Cryolabel tough tags and permanent marker.**
- Freeze as soon as possible in -70 C freezer (-20 is OK if -70 is not available).

SHIPMENT:

- Samples are shipped frozen (dry ice) to address below.
- Send copy of a log sheet with samples.
- Notify charmie@u.washington.edu when shipment is picked up by FedEx or other carrier.
- UW contact: Charmie Godornes 01 206 897-5360

Ship to:

Sheila A. Lukehart, Ph.D.
University of Washington
Harborview Medical Center
Research and Training, Room 603
300 Ninth Ave.
Seattle WA 98104
USA

*Swabs: Dacron Swabs (Fitzco Inc), order from VWR Product no. 22222-046
**Lysis buffer: 10mM Tris, pH 8.0; 0.1M EDTA, pH 8.0; 0.5% SDS

Yaws elimination on Lihir Island, New Ireland Province, PNG: A proof of principle of Mass Drug Treatment with azithromycin

Acronym:	YERA – Yaws eradication
Date of protocol:	02-Nov-2013
Protocol version number:	V7.0
Investigators:	Dr Oriol Mitjà; Dr Penias Moses, Mr Raymond Paru, Ms Wendy Houinei, Dr Sibauk Bieb, Prof Sheila Lukehart, Dr Russel Hays.
Sponsors:	Newcrest Mining, InternationalSOS-Niugini
Main centre:	Lihir Medical Centre, International SOS, Lihir Island, New Ireland Province, Papua New Guinea
Ethical approval:	Government of Papua New Guinea, Medical Research Advisory Committee Approval Number 12.36

Protocol Approval and Authorization

Protocol Approval

Statement of Compliance

The study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following:

- ICH GCP E6
- Completion of Human Subjects Protection Training

SIGNATURE PAGE

The signature below documents the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality and according to local legal and regulatory requirements and to the principles outlined in applicable United States federal regulations and ICH guidelines.

Principal Investigators

Name	Affiliation
Dr Oriol Mitjà	Co-Principal Investigator, Lihir Medical Center oriolmitja@hotmail.com
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Dr Russell Hays	Lihir Medical Center senior.medicalofficer@newcrest.com.au

Protocol Outline

1. Synopsis

The trial that we are proposing is a proof of principle to determine the efficacy of the new WHO yaws eradication strategy. We aim to assess the impact of mass distribution of single-dose oral azithromycin on yaws in the Lihir Island, PNG, population (n=16,000). We will also monitor the rate of macrolide resistance in yaws before and after the introduction of azithromycin for the treatment of yaws

Project Overview	**Results**	**Results Measurement**
Strategy:		
To perform a non-randomized clinical trial of 2-years mass drug administration (MDA) of oral azithromycin on Lihir Island, coupled with 4-years of surveillance for prevalence of infection.	Adequate MDA coverage and adequate recruitment of patients for prevalence surveys.	Clinical trial performed with adequate standards.
Project Goal:		
To achieve elimination of yaws as a result of the total community treatment (TCT) of oral azithromycin followed by 3 rounds of total targeted treatment (TTT) for 18 months.	Zero clinical cases reported in the community, and no evidence of transmission among children < 5 years.	Whole population medical examination for signs of clinical yaws during MDA, and cross-sectional sero-surveys at months 0, 6, 12, 18, 24, 36 and 48.
Objectives:		
1. To implement **large-scale treatment** (TCT or TTT) with azitromycin biannually during 2 years.	Number of individuals having received the drug; Coverage rates in the total censed population.	Observed coverage survey of directly observed treatment with azithromycin.
2. To conduct **clinical surveys for active yaws** in the whole population.	Number and prevalence of individuals with clinical yaws infection.	Clinical surveys for active yaws (whole population medical examination) during TCT and TTT.
2. To conduct yaws prevalence **surveys for latent disease** in a subset of children.	Number and prevalence of children with latent yaws infections (determined by serology) in community surveys.	Cross-sectional serological surveys in a random sample of children.
3. To evaluate the development of **yaws bacterial resistance** to azithromycin throughout the study.	Macrolides sensitivity profile of *T. p. pertenue* in individuals receiving the intervention.	Proportion of macrolide resistance in yaws at each cross-sectional survey.

2. Introduction

2.1 – Project purpose and Background

The trial that we are proposing is a proof of principle to determine the efficacy of the new WHO yaws eradication strategy. We aim to assess the efficacy of an initial Total Community Treatment (TCT) with single-dose oral azithromycin, followed by 3 rounds of Total Targeted Treatment (TTT) resurveys every 6 months, to completely eliminate yaws infection on Lihir Island, Papua New Guinea (PNG), population 16,000. We will also monitor the rate of macrolide resistance in yaws and the impact of azithromycin MDA on incidence of other macrolide-susceptible infections in the community.

Yaws is an infectious disease caused by *Treponema pallidum* subspecies p*ertenue*, a bacterium which closely resembles the causative agent of syphilis, and is spread by skin to skin contact in humid tropical regions. It causes disfiguring, and sometimes painful lesions of the skin and bones. Like syphilis, the clinical manifestations can be divided into three stages, but mother-to-child transmission does not occur. A major campaign to eradicate yaws in the 1950s and 1960s, by mass treatment of affected communities with long acting, injectable penicillin, reduced the number of cases by 95% worldwide, but yaws has enjoyed a resurgence in recent years in Africa, Asia and the Western Pacific. A single, oral dose of azithromycin was recently shown to be as effective as intramuscular penicillin, and a new initiative for yaws eradication was launched by the World Health Organisation in 2012.

Yaws is spread by direct person-to-person non-sexual contact with the exudate from early infectious lesions. Untreated primary yaws papules are highly infectious (rich in treponemes) and usually last 3-6 months. Early secondary yaws lesions may appear on the skin near the initial lesion or elsewhere in the body, including bone and cartilage. These last around 6 months and usually heal spontaneously. Thereafter the disease enters a latent non-infectious period which may last the lifetime of the patient. This latent state can be interrupted at any time by the reappearance of infectious yaws lesions, up to 5 years after the initial infection. The total duration of infectiousness for an untreated yaws patient, including relapses, is probably around 12-18 months.(1) Unless diagnosed and treated in the early stages, yaws can become a chronic, relapsing and disfiguring disease and can lead to severe deforming bone lesions in the long term. (2)

For routine purposes, the diagnosis of yaws requires the use of traditional and rapid serological tests results together with clinical manifestations, while carefully taking into account the epidemiologic and demographic characteristics of yaws. (3)(4) The serological tests used to

diagnose yaws are the same as those used to diagnose syphilis. The non-treponemal agglutination tests (rapid plasma reagin [RPR] or Venereal Disease Research Laboratory [VDRL] are positive in untreated cases, and can be used as a test of cure, since they usually revert to negative after successful treatment. Both are simple to perform. The RPR can be read with the naked eye, whereas the VDRL requires a microscope. The non-treponemal tests may give rise to false positives in patients with other conditions, including malaria, leprosy and rheumatological diseases. (5) They are often performed on serial dilutions of serum, giving a quantitative read out, or titre, defined as the highest dilution that gives a positive result. They become positive within 2 – 4 weeks of the appearance of the primary lesion. (6)

Long-acting penicillin, given as a single intramuscular dose, has been the mainstay of yaws treatment and eradication efforts for 60 years, (7) having been shown to be effective against yaws in 1948. (8) Benzathine benzylpenicillin, as a single intramuscular dose of 1·2 MU for adults and 0·6 MU for children, is the recommended regimen. Cure rates for patients with early, active lesions are >95%. However there are a few reports of possible penicillin treatment failures in yaws. In PNG, apparent treatment failures were reported in 11 of 39 (28%) cases on Karkar Island, (9) and a few penicillin treatment failures have also been observed in Ecuador.

A single oral dose of azithromycin (30 mg/Kg) has recently been conclusively shown to be as effective as intramuscular benzathine benzylpenicillin. (10) At 6-month follow-up, 96% of patients in the azithromycin group were cured by clinical and serological criteria, as were 93% in the benzathine benzylpenicillin group. These data correspond to a treatment difference of –3·4% (95% CI –9·3 to 2·4), meeting the pre-specified criteria for non-inferiority.

Pharmacokinetic data from **clinical studies show that a 30 mg/kg dose of azithromycin, administered as a single dose**, provides drug exposure equivalent to at least a 5-day regimen. (11) Both regimens maintain azithromycin levels in tissue sites of infection above the MIC of treponemes for several days after administration has ceased. The oral bioavailability of azithromycin is high (approximately 37%), and tissue concentrations exceed serum concentrations by as much as 100-fold following a single oral dose, with high concentrations being found in skin and bones, the principal target tissues for yaws.

The WHO revised policies for the treatment of yaws in 2012, (12) specifically responding to the growing evidence of yaws resurgence and the need to develop new strategies to facilitate eradication. Based on the results of the trial in PNG (10) azithromycin is now recommended as equivalent to the standard regimen of benzathine benzylpenicillin for yaws treatment and eradication.

2.2. Rationale

Substituting a single dose of an oral antibiotic for a painful penicillin injection is a significant advantage:

i) Infection control measures required for injection of Penicillin will no longer be required; attempts to control yaws by mass treatment with injectable drugs have previously led to the transmission of blood-borne viruses, such as hepatitis C.(13)

ii) Injectable penicillin causes anaphylactic shock in a small proportion of patients. Although the risk is small (<1 patient in 50 000), the consequences can be severe, and control programmes using penicillin need to train staff and provide them with the means to treat anaphylaxis (14).

iii) Treatment will be more acceptable to communities who need it.

The early global eradication programmes in the 1950s highlighted the importance of subclinical, or latent cases as a source of reinfection. It was estimated that the ratio of clinically apparent to latent cases could be as high as 1:6, and treatment of active cases only had little impact on the prevalence of yaws one year later (15). Most of the active cases found at resurveys were in persons who were in the latent stage originally and had not received treatment with injectable penicillin.(16) In contrast, high-coverage (95%) treatment of the entire population, as was witnessed in a yaws eradication campaign conducted in the 1950s in Nsukka, Nigeria, resulted in a rapid reduction in prevalence within 6–12 months.(17)

Mass drug administration of a single dose of oral azithromycin (30 mg/kg; maximum 2 g) to given to entire populations in areas known to harbor yaws is more proactive and aggressive strategy in order to deal with all potential contacts and latent cases. We expect that transmission can be interrupted in a reasonably short time, leading to elimination as witnessed in Nigeria. (17)

2.3. Potential Risks and Benefits

2.3.1 Potential Benefits

Yaws is still a substantial cause of morbidity in PNG. The National Department of Health (NDoH) estimated the number of yaws cases to be 15,936 nationwide in 2007. The number has increased to more than 29,000 in 2010 and 2011, of which 7,300 were in New Ireland Province and another 7,200 in the neighbouring province of West New Britain. (Miriam Pahun, personal communication)

Yaws has been endemic in many lowland areas of PNG for many decades. There were general mass treatment —eradication programs in that time, spread over large areas of PNG as well as

targeted —mass treatments. A good example is Karkar Island in Madang, which has had several mass treatment programs with intramuscular penicillin. Yaws was said to be eradicated but when the NDoH stopped the wide spread use of intra-muscular procaine penicillin and introduced oral amoxicillin in its stead for the treatment of a plethora of conditions, yaws started to be seen again.

There is currently need for methods to control yaws, of which mass administration of a single-dose oral treatment is the most definitive. The most obvious benefit that will have subjects participating in the study will be the cure of the disease active and latent yaws. From the point of view of public health, by eliminating yaws in the whole population, we will also prevent future infections.

Also community-based mass treatment programmes using this antibiotic for the control of trachoma have been well accepted by rural communities in many parts of Africa, are highly effective in reducing the prevalence of trachoma and could have collateral benefits. In one study in Ethiopia, mass treatment every year with azithromycin reduced all-cause mortality by 50% in children aged 1–5 years.(18)

2.3.2 Potential risks

Drug resistance as a collateral effect

A note of caution on the use of azithromycin is the biological evidence that selective pressure can select for resistant strains, as has occurred with *T. p. pallidum* in a number of sexual networks in developed countries.(19)(20)(21) Background macrolide use for unrelated infections (mainly respiratory) contributes to the increased prevalence of macrolide-resistant *T. p. pallidum* by providing a selective pressure.(22) Interestingly, macrolide-resistant *T. p. pallidum* has not been found in Uganda, (23) Tanzania, (24) or Madagascar (25) - where macrolides are not widely used. (26) Nonetheless, the recognition of this possibility is a reminder that there is no room for complacency. Surveillance for treatment failures, and biological markers of resistance, will be essential if azithromycin is widely used for the eradication of yaws.

The effect of mass treatment with azithromycin on resistance in *Streptococcus pneumoniae* is also of considerable public-health importance and may impact on the management of acute respiratory infections in children. This phenomenon has been evaluated after mass treatment campaigns to control trachoma and the results have been somewhat contradictory. (27) Surveillance studies have demonstrated short-term changes in susceptibility patterns of the bacteria, although these did not generally persist.

3. Study Objectives

Project goal: To achieve elimination of yaws as a result of the total community treatment (TCT) of oral azithromycin followed by 3 rounds of total targeted treatment (TTT) for 18 months.

3.1 Primary objective:

1. To assess the efficacy of total community treatment (TCT) of oral azithromycin followed by 3 rounds of 6 monthly total targeted treatments (TTT) in Lihir Island.

3.2 Secondary objectives:

2. To estimate coverage of Mass Drug Administration (MDA) in the targeted population. Report at a village level of number of persons treated compared to census.
3. To estimate the prevalence of individuals with serologically confirmed clinical yaws infection in the whole population of Lihir after mass treatment.
4. To estimate the prevalence of children with high-titer (rapid plasma reagin [RPR] ≥ 1:16) latent yaws infection (determined by serology) in community surveys after mass treatment.
5. To estimate the rate of macrolide resistance in *T. p. pertenue* in patients with active yaws both before and after the MDA.

4. Methods and Intervention

4.1 Outcome measures:

4.1.1 Primary outcome measures:

- Prevalence of serologically confirmed clinically active yaws infections in the whole population determined by WHO definitions (and serology confirmation) at 0, 6, 12, 18, 24, 36, and 48 months
- Prevalence of high-titer (rapid plasma reagin [RPR]≥ 1:16) latent yaws infections (determined by serology) in a random sample of children < 15 years, at 0, 6, 12, 18, 24, 36 and 48 months in the Lihir resident population.

4.1.2 Secondary outcome measures:

- Coverage rates during MDA in the total censed population.
- Proportion of ulcers caused by *T. p. pertenue* (using PCR).
- Proportion of macrolide resistance in yaws samples at each cross-sectional survey.

4.2 Study population:

Inclusion criteria:

- Whole resident population of Lihir Island for MDA and clinical surveys, and
- Subset of 875 children 1 - 15 years in randomly selected villagesfor sero-surveys.

Exclusion criteria:

- Children younger than 2 months and pregnant women;
- Known allergy to macrolide antibiotics;
- Refusal of individual or guardian (for individual inclusion).

[these will be offered benzathine penicillin]

4.3 Case definitions:

Clinically active case:

Persons resident in endemic areas with one or more of the symptoms below and positive serological test (rapid treponemal tests or qualitative and quantitative RPR)

Clinically active yaws:	
Infectious	1. Initial lesion(s) – papilloma 2. Multiple papillomata 3. Plantar and palmar papillomata 4. Ulcers 5. Other early skin lesions (macules, papules, micropapules, nodules, plaques)

Non-infectious	1. Hyperkeratosis 2. Bone and joint lesions
Inactive yaws	Late active yaws: gummata, ulcers, gangosa, sabre tibia

Latent yaws infection: Asymptomatic children (1 - 15 years old) found positive by RPR and confirmed by TPHA.

Latent yaws by titer	**Remarks**
Latent yaws	All asymptomatic subjects with reactive TPHA test and a RPR titer ≥ 1:2.
High-titer latent yaws	High RPR titers (≥1:16). It is possible that a high proportion of sero-reactors with low titers (≤1:8) would be previously treated RPR-serofast cases.
Latent yaws by age	
1 – 5 years	Sero-reactivity in young children (1 - 5 years age-group) can reasonably indicate a recent infection as they are new entrants in the potential pool of infection.
6 – 15 years	---
> 15 years	Subjects >15 years are not included in serosurveys to reduce the likelihood of reactive serology due to venereal syphilis.

4.4 Design and general approach

Design:

- We intend to perform a mass drug administration exercise of oral azithromycin for yaws, coupled with annual surveillance for prevalence of yaws infection.

We will undertake three types of surveys in villages included in a yaws-elimination program: (1) clinical surveys to assess active yaws prevalence; (2) serological surveys to assess latent yaws prevalence in children; (3) and PCR-surveillance to determine ulcer etiology and monitor the appearance of macrolide resistance mutations. These surveys will be conducted at baseline, 6, 12, 18, 24, 36, and 48 after initial intervention.

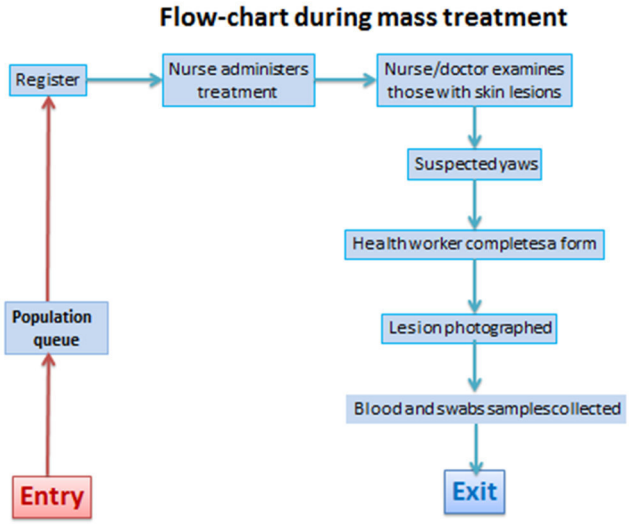
4.4.1. Large-scale treatment with azithromycin to the whole resident target population (n=16,000):

- **Training of health workers and community agents:** Health workers will be trained for one day on clinical diagnosis, administration of azithromycin, recording and surveillance,

new eradication policies and strategy, community mobilization and mass treatment campaign, follow-up activities etc.

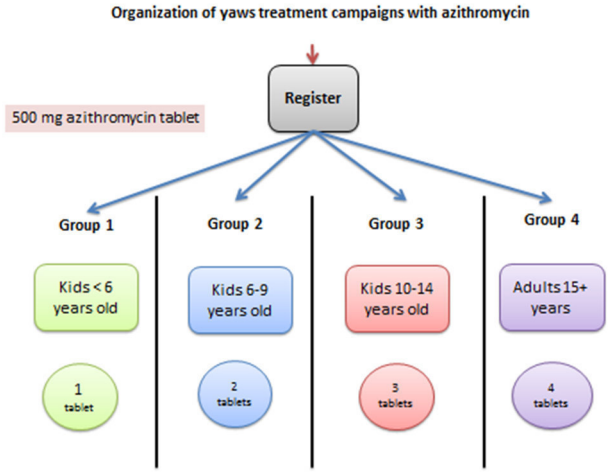
- **IEC and community mobilization:** After training, the village health workers will be responsible for informing the communities about the planned mass treatment. They will ensure that each household gets a copy of the WHO pictorial booklet. Yaws posters will be put in all strategic places in the community to ensure full knowledge of the disease and impending mass treatment. The objective of the community mobilization is to ensure that almost 100% of the eligible population will be seen during the first round of the mass treatment.
- **Treatment of population:** Communities will receive an initial Total Community Treatment (treat the entire village), followed by 3 rounds of 6 monthly Total Targeted Treatment (treat all active clinical cases, and their contacts). During TTT, all the resident population will undergo medical examination for signs of clinically active yaws. Benzathine benzylpenicillin will be reserved as a backup for those very few who cannot be treated with azithromycin, and who are not allergic to penicillin.

Figure 1. Flow chart of large-scale treatment interventions



- **Dose calculation:** WHO will purchase generic azithromycin (500mg tablet) from Medopharm (India), and supply for the pilot. The drugs will be shipped to PNG around mid-January 2013. The dosages per age are as shown in the figure below. The quantities required for each community will be determined in advance based on the population distribution using excel formula for estimating azithromycin needs.

Figure 2. Dose calculation of azithromycin by age



- **Coverage of MDA versus an updated census:** will be estimated for each round. The program will aim to deliver directly observed treatment to at least 90% of the population in each of the 4 rounds of MDA. Each round of MDA will report, at village level, on coverage. Those on the census not present will be followed up with an appointment letter during the following 2 weeks. The census list will be updated at each round.

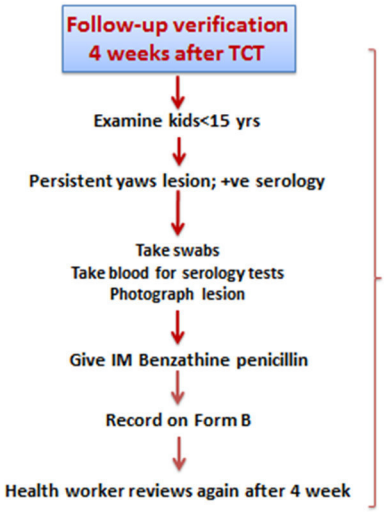
4.4.2 Clinical surveys for active yaws (n=16,000)

During MDA campaigns (TCT and TTT) all the resident population will undergo medical examination by a clinician (doctor or nurse) for signs of clinically active yaws and prevalence of active yaws will be estimated. Those with suspected yaws lesions will be further examined and information on each person will be recorded on a Case Report Form A **(Annex 3).** Serological tests RPR and TPHA will be used to confirm yaws-suspected cases. Swabs (from papilloma and ulcer) and scrapings from squamous macule and papule lesions will be collected to help determine baseline resistance to azithromycin (4.4.4 for macrolide resistance monitoring).

- **At 4 weeks follow-up**

Four weeks after the mass treatment, the health workers will visit each village and examine those who were treated and whose serology tests during the mass treatment confirmed yaws. Any participant with persistent lesion at 4 weeks and serology results are positive will be considered to have failed on azithromycin and a detail examination will be carried out, additional specimens will be taken and benzathine penicillin will be offered in this instance. Information will be recorded on Form B**.**

Figure 3. Flow chart for 4-weeks follow-up visit



- **Post-treatment clinical surveys**

After the initial 2 years we will conduct bi-annual active surveillance for yaws cases with visits to the communities. Between repeat surveys, health facilities will have stocks of azithromycin in order to respond to cases identified in the community.

4.4.3. Serological prevalence survey for latent yaws in children (n=1,000; 1-15 in the island):

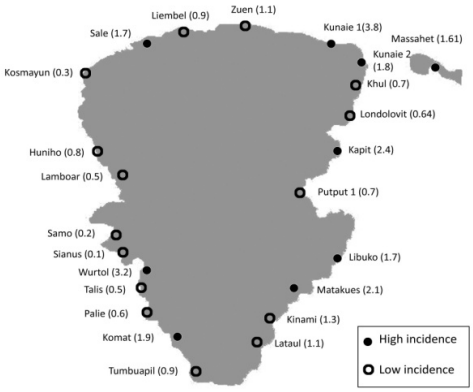
Since longitudinal intensive serological monitoring of impact of intervention to a total population is labor intensive, we plan to conduct serological screening on a subset of asymptomatic children 1 – 15 years in randomly selected villages. We will select six villages at each time point using computer-generated random numbers, and the random sample will be regenerated at each survey.

To detect latent yaws, we will collect a 5 mL venous sample in a dry vacutainer serum tube and transported at the end of each clinic day to the Lihir Medical Centre Laboratory where it will be centrifuged and serum separated. The laboratory will perform the qualitative and quantitative RPR testing and *Treponema pallidum* hemagglutination assay (TP-HA). The standard external quality control procedures for the RPR and TPHA testing (i.e. 5% of positive and negative samples) will be performed at the Microbiology Unit of Sullivan Nicolaides Pathology (Queensland, Australia).

Household census data will be compiled and all children 1 - 15 in the six randomly selected villages will be examined including RPR and TPHA testing in all cross-sectional surveys throughout the study. The screening serologic tests used for the diagnosis of syphilis [i.e. RPR, TPHA] have been evaluated and are used for the diagnosis of yaws. The first 4 surveys will

coincide with the MDA activities in the village at 0, 6, 12, and 18 months. The other surveys of the same individuals plus the new under 15 year cohort will occur at 24, 36 and 48 months after the first MDA round.

Figure 4: Map of Lihir Island with locations of high incidence of yaws infections (Mitja, 2011) (32)



4.4.4 Monitoring for causative etiological agent and potential macrolide resistance (n=100)

During clinical surveys we will swab papilloma and ulcers of each subject, using a highly standardized swabbing technique. Two swabs will be inserted in pre-labeled cryotubes containing 1ml of transport medium (Assay Assure) and will be stored in a freezer (-20 oC) within eight hours after collection, and then flown on dry ice to University of Washington (Seattle). PCR methods will consist of primary screening for the presence or absence of *T. p. pertenue* DNA, using a highly sensitive qualitative PCR assay and testing for other possible aetiological agents (i.e. *H. ducreyi*).

We will evaluate the presence of *T. p. pertenue* containing the macrolide resistance mutation in a convenience subsample of patients. Resistance to azithromycin is largely conferred by an A2058G or A2059G mutation in the 23S ribosomal RNA (rRNA) gene of *Treponema pallidum*. Swab samples will be obtained from ulcer lesions (n = 100) and transported in medium for DNA preservation. The specimens will be tested at the University of Washington laboratory by nested polymerase chain reaction amplification of one 23S ribosomal DNA region, followed by restriction digestion of the amplicon as previously described (Lukehart, 2004). Based on past experience we expect that *T. pallidum* DNA could be detected in at least 80% of specimens collected, and that of samples containing detectable *T. pallidum* DNA, 23S rRNA gene could be amplified in more than 90%. **(Annex 4)**

4.4.5. Side effects: reporting and quantification

- **Passive surveillance:** Passive surveillance for adverse events will be undertaken throughout the study at the Lihir Medical Centre and all peripheral health posts. Specific training for all

health staff will be conducted before the beginning of the interventions, with special emphasis on reporting to the study staff any allergic event or any other adverse event deemed possibly related to the intervention using a standard AE case report form.

- **Active surveillance:** We will also perform household surveys 1 week after the initial distribution of antibiotics to monitor for potential adverse events. We will conduct the surveys in 60 randomly selected households from 28 villages. All drug-related adverse events will be assessed by study investigators and grading will be reported according to standardized criteria.

Table 1. Grading criteria for drug-related adverse events.

	Grade 1	Grade 2	Grade 3	Grade 4
Abdominal pain	Mild	Moderate no treatment needed	Moderate treatment needed	Severe-hospitalization for treatment
Nausea	Able to eat	Oral intake significantly decreased	No significant intake	Requiring IV fluids
Vomiting	1 episode in 24 hours	2-5 episodes in 24 hours over pretreatment	> 6 episodes in 24 hours or need for IV fluids	-
Diarrhea	Increase of < 4 stools / day over pre-treatment	Increase of 4-6 stools/day, or nocturnal stools	Increase of > 7 stools/day or incontinence; or need for parenteral support for dehydration	
Fever	37.7 – 38.5 C or 100.0 – 101.5 F	38.6 – 39.5 C or 101.6 – 102.9 F	39.6 – 40.5 C or 103 – 105 F	> 40.5 C or > 105
Headache	Mild pain not interfering with function	Moderate pain	Severe pain	Disabling
Allergic reaction / hypersensitivity	Transient rash, drug fever < 38°C (<100.4°F)	Urticaria, drug fever > 38°C (>100.4°F), and/or asymptomatic bronchospasm	Symptomatic bronchospasm, requiring parenteral medication(s), with or without urticaria; allergy-related edema/angioedema	Anaphylaxis

5. Schedule of study procedures

Program **timelines and deliverables** will be according to the schedule below:

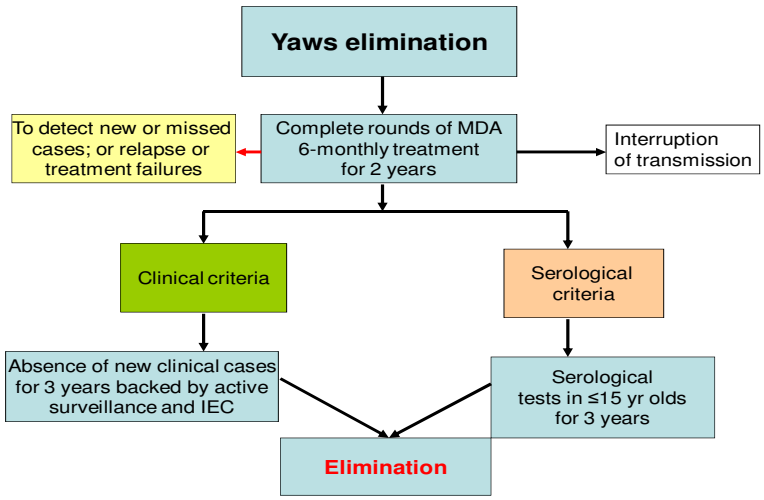
Activity / Months	**0**	**6**	**12**	**18**	**24**	**30**	**36**	**42**	**48**
Mass drug administration; coverage surveys	X	X	X	X					
Clinical surveys for active yaws	X	X	X	X	X		X		X
Serological surveys for latent yaws	X	X	X	X	X		X		X
Macrolide resistance monitoring	X	X	X	X	X		X		X

6. Major Assumptions

- **Elimination at month 24**

For this project, we assume that we will achieve zero prevalence of yaws at month 24, as per previous experience in Nigeria (Zahra,1956). Years 3-4 will be used for surveillance to declare the elimination of yaws (3 consecutive years without reported cases) and to monitor the potential re-introduction of infection. There is the threat of re-introduction of yaws from other endemic regions of PNG due to the large "in-migration" in Lihir. The migrant census is updated regularly by Newcrest Social Impact Monitoring team. The project will work closely with this team to ensure that all in-migrants receive treatment on arrival or soon thereafter (within 3 months).

Figure 5: Proposed yaws elimination protocol



- **Good acceptance of interventions**

We assume also that the MDA campaign will be well accepted by the Lihir communities, since MDA has been well accepted in previous MDA for filariasis elimination and that coverage will be high. Given the large population of the island living in remote areas there could be delays in administering the medication or coverage may be less than 80%. The clinical examinations and blood collection will be undertaken by trained community field workers who are already engaged in an island-wide filariasis control program, but also logistic support from LMC and significant in-kind support from the local communities and health services are measures to strengthen the screening team if necessary.

7. Statistical analysis plan

7.1 Data management

Only de-identified data will be used for analysis. All clinicians and researcher accessing the database will sign confidentiality agreements. The primary data will be collected by study staff at the community, health facility site and laboratory staff at the different field and laboratory sites. The study coordinator (or his/her designee) will cross check all CRF‘s for completeness, validity and legibility. All data from CRFs and laboratory worksheets in PNG will double-enter data in Microsoft Access software (version 14.0); all discrepancies will be checked against the original forms. Data entry will be done in batches. First entry will be done by trained data entry staff at the designated data entry unit directly after receipt of CRF and worksheet copies from the LMC field stations. Second entry will be once a batch of first entry is completed. Discrepancies, missing values, and out-of-range values will be detected in queries run on a batch-by-batch basis by the data base manager. If necessary queries will be send back to the field site for resolution. All corrections made to the database by the data managers (or designees) will be recorded.

7.2 Data ownership

The state must be custodian of the data. In all written publications concerning the research project, the National Department of Health and the Lihir Medical Centre will have co-responsibility for the scientific results. The Lihir Medical Centre, represented by Oriol Mitjà will contact the National Department of Health before publication of any results related to the research project.

7.3 Type of analysis

7.3.1 Primary analyses at 12 months

We will calculate the prevalence rates of active yaws measuring everyone in the population at three time-points (0,6,12 months). We will estimate the prevalence of latent yaws measuring a subset of children in randomly selected villages.

We will estimate the evolution of prevalence ratio (PR) in active and latent yaws prevalence rates at all time-points (0,6,12) and compare the values to describe the decrease in prevalence across the study time. For this analysis we will use a log-binomial regression model. We will evaluate the decline in proportion of PCR detected infection across the time study using the multinomial logistic regression model. Statistical significance will be defined at a p-value less than 0•05.

7.3.2 Primary analyses at 24 months

We will calculate the prevalence rates of active yaws measuring everyone in the population at all time-points (0,6,12,18,24 months). We will estimate the prevalence of latent yaws measuring a subset of children in randomly selected villages.

We will estimate the evolution of prevalence ratio (PR) in active and latent yaws prevalence rates at all time-points (0,6,12,18,24) and compare the values to describe the decrease in prevalence across the study time. For this analysis we will use a log-binomial regression model. We will evaluate the decline in proportion of PCR detected infection across the time study using the multinomial logistic regression model. Statistical significance will be defined at a p-value less than 0•05.

7.4 Sample size and power calculation

7.4.1 Prevalence of latent yaws at 12 months

A standard power calculation indicated that to estimate prevalence of high titer latent yaws with a precision of 1.5%, 875 children were required to achieve 80% power at 5% (two-sided) significance. We assumed the expected prevalence of latent yaws at 12 months to be 5%.

Estimating prevalence at month 12 with precision (ω)

Formula

Sample size

$$n = \frac{Np(1-p)z^2_{1-\alpha/2}}{N\omega^2 + p(1-p)z^2_{1-\alpha/2}}$$

Precision

$$\omega = z^2_{1-\alpha/2}\sqrt{\frac{(N-n)}{Nn}p(1-p)}$$

where z1-α/2 is standard normal deviate corresponding to the specified size of the critical region (α), ω is the precision, p stands for prevalence and N for target population size.

7.4.2 Prevalence of latent yaws at 24 months

At 24 months, we assume that we will achieve prevalence of high titer latent yaws near-zero. With a sample size of approximately 1,000 children in the 6 sentinel villages, an estimate of the prevalence of yaws would have the following 95% confidence intervals:

True prevalence (%)	95CI (%)
0.0	0.0 – 0.3
0.5	0.2 – 1.1
1.0	0.5 – 1.8
1.5	0.9 – 2.4
2.0	1.3 – 3.0

8. Organizational Experience and Collaborative Partnerships

This study will be conducted by CRESIB, and CRESIB contracted local-staff working at Lihir Medical Centre (LMC) in Papua New Guinea in collaboration with the National Department of Health. The interest and priorities of the department will be the driving force in any given situation. The NDoH-NTDs program will be also part of this study. Finally there will be a collaborative partnership with University of Washington who will test for macrolide resistance.

- **CRESIB:** The Barcelona Center for International Health Research (CRESIB) has large experience in elimination/eradication of endemic infectious diseases, with a particular focus on NTDs and malaria. CRESIB investigators conducted the research in oral treatment of yaws (Mitja, 2011) that led to the revision of the WHO policies.
- **LMC:** The Lihir Medical Centre (LMC) has a local research team employed by CRESIB who will implement the azithromycin large-scale treatment programm and yaws prevalence surveys. Clinical examinations (16,000) and blood collection (1,000) will be undertaken by a yaws specifically trained field workers team. The LMC laboratory has capacity to undertake the syphilis serological testing in this study (accredited to perform these tests by the Australasian College of Pathologists quality assurance program (QAP, North Ryde NSW).
- **University of Washington:** Sheilla Lukehaart's laboratory will test swab samples from lesions in persons with symptomatic yaws to determine whether the *Treponema pallidum* strains harbor resistance mutations. Sheilla Lukehaart's lab developed rapid assays for the A2058G and the A2059G mutations in the 23S rRNA gene (both are associated with macrolide resistance). They have years of expertise in these assays and the technical aspects of the evaluation.

8.1. Key roles

- **Doctor Oriol Mitja.** Oriol Mitja will take the lead role in this project including overall leadership and responsibility for the trial, including community consultation, liaison with collaborating organizations, logistics, data management, field work, and communication of results.
- **Doctor Penias Moses.** Moses will take responsibility for supervising the project in the field, including performing and supervising medical examinations of all participants (n=16,000) over the two year MDA period, supervising quality assurance in laboratory specimen collection and handling of specimens for transport, managing clinical cases as required in the field, supervision of clinical research staff, supervising data collection in the field, contributing to data analysis and interpretation and writing of reports and papers

- **Ms Wendy Houinei.** Ms Houinei representing the NDoH-NTDs program will be part of this study; she will be involved in the implementation activities, monitoring and data analyses.

8.2. Capacity building proposal

8.2.1 Publication of articles: All co-authors will benefit of publications of work done for the duration of research. This work will undoubtedly be a state-of-the art in science of eradication for yaws world-wide.

8.2.2 Technical meetings to report on the progress of the investigation. This meeting is proposed to be hold on a quarterly basis and may coincide with the NTD-TWG meeting.

8.2.3 Field team: We will ensure that the personnel in charge of screening the patients had the skills and knowledge that enables them to perform effectively the mass treatment for yaws. Certification of clinical assessors to screen patients for yaws will require a chance corrected agreement (kappa statistic ≥0.6) with an experienced clinician (OM, PM) over the scoring signs of clinically yaws (Active infectious, active non-infectious and inactive yaws in the WHO system) in validation exercises in both the classroom (photographic collection) and the field.

8.2.4 Laboratory technicians: will participate in a training course to upgrade their skills on serological diagnosis of yaws.

8.2.5 Study Investigator Ms Houinei should be involved with the project so that she can either use it for degree or PhD / MPH.

- Involvement in the implementation of activities of the campaign to eliminate yaws. She will learn the logistics of the project, and gain experience in diagnosis, treatment and monitoring yaws. This experience could be eventually applied in other districts of the country.
- Support and tutoring to develop MPH / PhD. She could either be enrolled in UPNG / Divine University or in University of Barcelona (Spain). For the latter she would need to have completed the master degree.

The results of **this project will promote the development of capacities in PNG**, and the strengthening of the research collaborations. Research towards the development of strategies to eradicate yaws represents a very attractive area of research which would increase PNG research potential. Ultimately all of this contributes towards **increasing Papua New Guinean excellence and competitiveness** in research and development.

9. Ethics

9.1 - Ethical conduct of the study

This study will be conducted in compliance with the study protocol. The patient's informed consent will be obtained according to the ethical principles stated in the Declaration of Helsinki 2000 version (amended in Seoul 2008), and the applicable laws and regulations of Papua New Guinea.

9.2- Patient information and consent

The informed consent document will be used to explain the risks and benefits of study participation to the parent or guardian of the patient in simple terms before the patient will be entered into the study. The informed consent document contains a statement that the consent is freely given, that the parent or guardian of the patient is aware of the risks and benefits of entering the study, and that the patient is free to withdraw from the study at any time. Written consent must be given by the legal representative, after the receipt of detailed information on the study. Where appropriate, the children will be asked to provide assent.

Appropriate forms for obtaining written informed consent and the patient information sheet will be provided by the Investigator (see **Annex 1-2**).

Given that some patients for inclusion in this study may not be capable of giving legal consent, written consent must be obtained from their next of kin. In the case where the next of kin are unable to read, an impartial witness should be present during the entire informed consent discussion. After the representative has orally consented to participate in the study, the witness's signature on the form will attest that the information in the informed consent form and patient information sheet was accurately explained and understood. In case of a parent or guardian of the patient who cannot provide informed consent in writing, an 'X' to indicate consent in the presence of two witnesses is acceptable. Permission from the parent or guardian must be obtained in the case of assenting minors.

The principal Investigator(s) at each centre will ensure that the parent or guardian of the patient is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. Patients and their parent or guardian must also be notified that they are free to discontinue from the study at any time. The patient and parent or guardian should be given the opportunity to ask questions and should be allowed time to consider the information provided.

9.3 Patient data protection

The Informed Consent Form will explain that study data will be stored in a computer database, maintaining confidentiality in accordance with ICH GCP and relevant local data protection laws. Patients in this database will be identified by initials or patient number only. The Informed Consent Form will also explain that for data verification purposes, authorised representatives of Sponsor, a regulatory authority, an EC may require direct access to parts of the hospital or practice records relevant to the study, including patient medical history.

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Annex 1. Inform consent (English)

"Yaws elimination on Lihir Island, New Ireland Province, PNG: A pragmatic trial of Mass Drug Treatment with azithromycin"

Lihir Medical Center, International SOS
Study location: Lihir Island, New Ireland Province

Yaws is a highly contagious infection and people can get it through direct contact with infected individuals. The condition starts with an ulcer. If it is left untreated, the ulcers will penetrate all the way to the bone and it can be quite painful. The treatment of yaws has long been a single injection of benzathine penicillin. Today, we have a drug called azithromycin (comes in tablets form) that is swallowed once (single-dose treatment) and has the same effect as injection benzathine penicillin. The drug is safe and has been used to treat other diseases.

Some people may be infected (have the germ which causes yaws in their blood) but show no lesions on the skin. Such people can only be identified through a simple blood test. If they are not treated, they will develop skin lesions in a few weeks or month and can be a source of new infection in the village (stress the need for prophylactic treatment).

Today, we are in your village because yaws occurs here. Our aim is to give everyone living in this village the new treatment which can completely cure everyone and remove yaws from your village within a very short time. Only children less than 2 months and women who are pregnant will be not be treated with azithromycin. Rather, they will be treated with benzathine penicillin.

Some child's blood might be required to be tested for yaws. We use a sort of lottery to decide who will be tested for yaws. Neither you, nor the doctor can decide who will be tested. At the start of the study, the children that are tested for yaws will have the finger pricked for blood slide and to put small amount of blood (250µl) in a container for testing for yaws. Your children blood will be tested once a year during 4 years.

Voluntary Participation
You must know that you can choose to be in the research study or choose not to be in the research study and that you can stop whenever you want.

Confidentiality
Information obtained about you for this study will be kept confidential to the extent allowed by law. The results of the treatment may be published for scientific purposes. However, your identity will not be given out.

Right to Refuse or Withdraw
Whether or not you take part in this study is your choice. There will be no penalty if you decide not to be in the study. You are free to withdraw from this research study at any time. Your choice to leave the study will not affect your relationship with this institution.

If you have questions about the study or if you think you or your child has a study-related injury, you can talk to any study team member, the study doctors;

Dr Oriol Mitja
Lihir Medical Center
Telephone number: 9867188

Annex 1. Inform consent (Tok Pisin)

"Pinism sik Yaws long Lihir Ailan, New Ireland Province, Papua Niugini: Treatim wantaim marasin, Azithromycin"

Husait igo pas long dispel wok: Oriol Mitja
Lihir Medical Center
Ples: Lihir Ailan, New Ireland Province

Yaws i save spread isi tru na ol manmeri na pikinini ken kisim yaws taim ol stap wantaim husait ol manmeri igat yaws. Sik yaws save stat wantaim sua. Sapos yu no kisim marasin, sua wea istap lo skin ken go olgeta long bun na iken pen nogut tru. Yu ken kisim marasin (azithromyin) onepela taim o kisim sut marasin (penicillin) onepela taim tasol lo pinisim sik yaws.

Mipela i lukim olsem yaws save spread isi tru long peles hia lo Lihir na long ol narapela hap tu long Papua Niiugini. Planti ol manmeri pikinini igat dispel sik yaws na ol ino save. Olsem na mipela i laik givim marasin long olgeta man meri na pikinini long Lihir lo stopim dispela long spread. Dispela marasin (azithromycin), ol i bin testim na usim pinis long treatim yaws na ol it ok orait long usim dispel marasin (azithromycin) long treatim yaws.

Sapos yu o pikinini blong yu tok orait long stap insait long dispel study, yu bai kisim marasin long start blong dispela study na long wan wan yia inap long tupela yia.

Sampela ol pikinini bai mipela kisim blut bilong ol long testim sik yaws. Bai mipela pikim nem olsem long laki lotto tiket. Ino inap yu o dokta bai makim husait bai kisim blut test.

Long stat bilong study ol pikinini husait bai mipela testim bulut; bai mipela sutim finga blong ol na testim blut olsem malaria test na kisim sampla blut na putim long ol botol long testim sik yaws bihain olsem piksa i soim.Blut bilong ol pikinini bai mipela testim wan wan yia inap long fourpela yia olgeta.

Tok Orait
Yu mas save olsem yu ken tok orait long take part long dispel study or tok nogat. Na yu ken stop long take part long dispel study anytime.

Toktok Hait
Wanem kain ol toktok/stori yu givim mipela long dispel study bai stap namel long yu na mipelatasol. Ino gat narapela man bai save. Ol results bilong dispela study bai mipela publishim long halivim ol sikman tasol. Mipela ino inap long tokaut long nem na stori bilong yu.

Yu gat rite long tok nogat
Em i laik bilong yu long take part long dispel study. Inogat asua long tok nogat long take part.Wanem choice yu mekim, Lihir Medical Senta em still hausik bilong yu, yu ken kam na kisim marasin anytime yu sik.
Sapos yu gat askim long dispela study yu ken toktok wantaim ol study team members,
study dokta Dokta Oriol Mitja or ringim Lihir Hausik long fone numba 9867188

Annex 2. Individual consent form

Study title: "**Yaws elimination on Lihir Island, New Ireland Province, PNG: A pragmatic trial of Mass Drug Treatment with azithromycin."**

Individual consent form:

We have been given information on this mass treatment exercise by the health workers.

□**I am a participant less than 15 years old**

I (first/surname):_____of _____ _____ give consent for my child _____to be a participant and allow specimens to be collected to confirm yaws in the laboratory.

Parent/Guardian's signature or Right thumb print (date)	Health works signature
_____/_____/_____ Date (dd/mm/yyyy)	_____/_____/_____ Date (dd/mm/yyyy)

□**I am a participant 15 years or older**

I (first/surname):_____of _____ _____ give consent to be a participant and allow my specimens (blood or swabs or scrapings) to be collected to confirm yaws in the laboratory.

Signature or Right thumb print (date)	Health works signature
_____/_____/_____ Date (dd/mm/yyyy)	_____/_____/_____ Date (dd/mm/yyyy)

Annex 2. Individual consent (Tok Pisin)

Nem Bilong dispela study: Pinism sik Yaws long Lihir Ailan, New Ireland Province wantaim marasin, Azithromycin

Taim yu signim dispel pepa yu i tokim mipela olsem yu:
* Yu save long dispela study
* Tok orait long pikinini bilong yu long take part long dispel study
* Yu kisim study infomasin pepa
* Yu bin askim ol askim long kisim kilia tingting long dispela study
* Yu mekim fri decision long take part long study
* Yu tok tok wanatim femili na tok orait long pikinini bilong yu long take part long dispel study

Taim yu signim tok orait ol study team bai mekim wok bilong ol. Wanpela copy bilong tok orait form bai yu kisim.

_____
Nem Bilong Man/Meri/Pikinini

Tok orait Bilong Papa na Mama:
_____ Dait:
Nem Bilong Papa/Mama or Man lukautim pikinini

_____
Mak Bilong Papa/Mama or Man Lukautim pikinini Papa,Mama o Man lukautim pikinini

Dispela tok orait em i witnesim:
_____ Dait:
Nem Bilong Study wokman

_____
Mak Bilong Study Wokman

Annex 3. FORM A

Recording form for participants with skin lesions consistent with clinical yaws at the time of the initial mass treatment

(To be filled in by health worker)

Consent signed: □ Yes □ No *(only fill in questionnaire if consent signed)*

Name of Father:		**Name of Mother:**
Section A: Demographic data		
1	Name of Participant:	
2	Participant ID number:	
3	Date of Birth: (dd)____(mm)___(yyyy)_____	Sex: □ Male □ Female
	Age (yrs):_____	
4	Village:	District:
Section B: History and clinical examination		
5	Duration of illness in weeks:	
6	Clinical forms of yaws (*Refer to WHO pictorial guide*) □ Papilloma □ Ulcers □ Squamous macules □ Bone lesions □ Palmar and plantar	
Section C: Conclusions of clinical Assessment		
7	□ likely yaws □ possible yaws □ unlikely yaws	
8	Tick the below boxes once done. □ Blood collection □ Swabs/scrapings	**ONLY FOR PARTICIPANTS WITH SUSPECTED YAWS LESIONS**
10	Treatment given: □ Azithromycin (number of 500 mg tablets): _____ □ Benzathine penicillin (check): _____0.6 MU or _____1.23 MU	

Annex 4: Sampling, storage and transport of samples for yaws testing

SPECIMENS NEEDED:

- Swabs from primary and moist secondary lesions.

EXCLUSION CRITERIA:

- Antibiotics potentially active against *T. pallidum* within the previous month.

PROCEDURE FOR COLLECTING SPECIMENS:

Primary lesions and moist secondary lesions:

- **Use the swabs* and lysis buffer** specified here**
- Squeeze and swab the lesion vigorously, then place the swab into the tube containing the 1X lysis buffer. Break or cut off the shaft of the swab and leave the tip in the buffer. Replace cap, and vortex or shake vigorously.
- Label with date and pt study number **using special Cryolabel tough tags and permanent marker.**
- Freeze as soon as possible in -70 C freezer (-20 is OK if -70 is not available).

SHIPMENT:

- Samples are shipped frozen (dry ice) to address below.
- Send copy of a log sheet with samples.
- Notify charmie@u.washington.edu when shipment is picked up by FedEx or other carrier.
- UW contact: Charmie Godornes 01 206 897-5360

Ship to:

Sheila A. Lukehart, Ph.D.
University of Washington
Harborview Medical Center
Research and Training, Room 603
300 Ninth Ave.
Seattle WA 98104
USA

*Swabs: Dacron Swabs (Fitzco Inc), order from VWR Product no. 22222-046
**Lysis buffer: 10mM Tris, pH 8.0; 0.1M EDTA, pH 8.0; 0.5% SDS

Summary of changes (initial protocol versus final protocol)

Original protocol (v. 6.0) – Submitted and approved by the ethics committee (MRAC, PNG) on 07-03-2013

Final protocol (v. 7.0) – Completed on 02-11-2013

- The secondary objectives of the study were defined in greater detail.
- We included a secondary outcome: 'the proportion of ulcers caused by *T. p. pertenue* compared to other aetiological agents (i.e. *H. ducreyi*) detected using PCR methods'.
- We modified the sampling frame for latent yaws sero-surveys: 'all children in six randomly selected villages at each time point being the random sample regenerated at each survey'. This differed from 'all children in six randomly selected sentinel-site villages' initially proposed.
- More information on definitions of latent yaws infection was included in a table format.
- Further information was provided on procedures to collect swabs for monitoring macrolide resistance and aetiological agents detected by PCR.
- It was clearly mentioned how grading of adverse events was going to be assessed by study investigators.
- Change in site-coordinator for Lihir from Dr Murray Koka to Dr Penias Moses.
- No changes were implemented with regard to the statistical analysis plan within the protocol.