TITLE

Optimization of Mass Drug Administration with existing drug regimens for Lymphatic Filariasis and Onchocerciasis

('DOLF' project, Death to onchocerciasis and lymphatic filariasis)

Sponsored by:
Bill and Melinda Gates Foundation: GH 5342

Principal Investigators:
Gary J. Weil, M.D., Peter U. Fischer, Ph.D., and Taniawati Supali, Ph.D.

Draft or Version Number: 5

Day Month Year: 31 December 2013

Statement of Compliance

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

- U.S. Code of Federal Regulations applicable to clinical studies (45 CFR 46)
  http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.htm#46

  ICH GCP E6 Completion of Human Subjects Protection Training
  http://grants.nih.gov/grants/guide/notice-files/NOT-OD-0 1-061 .html

- Bill and Melinda Gates Foundation "grant agreement" terms and conditions

SIGNATURES

The signature below documents the approval of this protocol and the attachments, and provide 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 U.S. federal regulations and ICH guidelines.

Signed: Date (dd/mm/yyyy):

_____ _____

Peter U. Fischer, Ph.D. - Washington University School of Medicine

Title: Principal Investigator, Community MDA Studies, DOLF Project, Objective 1

Taniawati Supali, Ph.D. University of Indonesia

Title: Country Principal Investigator, DOLF Project, Objective 1

Gary J. Weil, M.D. - Washington University School of Medicine

Title: Principal Investigator, DOLF Project

Table of Contents

List of Abbreviations

Alb	Albendazole
APOC	African Programme for Onchocerciasis Control
CFR	Code of Federal Regulations
CRF	Case Report Form
DEC	Diethylcarbamazine
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH
DSMB	Data Safety Monitoring Board
ELF	Elimination of Lymphatic Filariasis
FWA	Federal-Wide Assurance
GCP	Good Clinical Practice
GPELF	Global Programme to Eliminate Lymphatic Filariasis
IATA	International Air Transport Association
ICH	International Conference on Harmonisation
ICIDR	International Collaboration for Infectious Disease Research
ICT	Immunochromatography Test
IntTrans	Interruption of transmission
IRB	Institutional Review Board
IVER	Ivermectin
LF	Lymphatic Filariasis
MDA	Mass Drug Administration
MF	Microfilaria(e)
OCP	Onchocerciasis Control Programme
OHRP	Office for Human Research Protection
ONCHO	Onchocerciasis
OSHA	Occupational Safety and Health Administration
PELF	Program to Eliminate Lymphatic Filariasis
PI	Principal Investigator
SAE	Severe adverse events
SOP	Standard Operating Procedure
STH	Soil transmitted helminths
WHO	World Health Organization

Protocol Summary

Title: Optimization of Mass Drug Administration with existing drug regimens for Lymphatic Filariasis and Onchocerciasis

Population:	**Approximately 6,400 people will participate per year. The study population will include females and males over 5 years of age who live in filariasis endemic areas. Subject selection will not be based on health status.**
Number of Sites:	**Two sites in Indonesia (in separate *B. timori* and *W. bancrofti* endemic areas)**
Study Duration:	**4 years**
Subject Duration:	**Participants will be studied only once in cross-sectional surveys. Some subjects may be included in more than one annual population survey, but this is not a longitudinal study.**
Objectives:	**To evaluate different mass drug administration (MDA) regimens for lymphatic filariasis and its impact on soil transmitted helminthes. MDA is administered by others (e.g., Ministry of Health) and may enhance efforts to control and eliminate these important neglected tropical diseases.**

1. We will compare the relative impact and cost effectiveness of annual vs. twice yearly mass drug administration (MDA) for elimination of lymphatic filariasis (LF).

2. We will also study the impact of annual vs. semiannual MDA on soil transmitted helminth (STH) infection in these populations.

Remarks on the project structure: The Project "Optimization of Mass Drug Administration with existing drug regimens for Lymphatic Filariasis and Onchocerciasis" has 3 major Objectives (Objective 1-Community MDA Trials, Objective 2-Randomized Clinical Trials and Objective 3-Pilot Flubendazole Studies). Objective 1 will be conducted in 4 countries in Africa and 2 countries in the Asia. Although the overall objectives are similar, there are country-specific modifications. <u>This protocol was developed for Indonesia</u>.

December 31, 2013

1 KEY ROLES

Principal Investigator: Peter U. Fischer, Ph.D. Community MDA Studies (DOLF Objective 1)

DOLF project Principal Investigator: Gary J. Weil, M.D.

Project Statistician: Professor Philip Miller

Indonesian collaborating scientist: Taniawati Supali, Ph.D. (Country Principal Investigator, DOLF Objective 1)

Institutions:

Infectious Diseases Division, Washington University School of Medicine, Campus Box 8051, 660 S. Euclid Avenue, St. Louis, Missouri, 63110, USA. Contact: Peter U. Fischer, Ph.D. Tel. +1 314 454 7876. Fax. 314 454 5293. Email: pufische@dom.wustl.edu

Department of Parasitology, Faculty of Medicine, University of Indonesia, Salemba 6, Jakarta 10430, Indonesia. Contact: Taniawati Supali, Ph.D. Tel. + 62 812 9355870, Email: taniawati@yahoo.com

2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Lymphatic filariasis (LF) is a deforming and disabling infectious disease that causes elephantiasis and genital deformity (especially hydroceles). The infection affects some 120 million people in 81 countries in tropical and subtropical regions with well over 1 billion people at risk of acquiring the disease [1]. LF is caused by *Wuchereria bancrofti* and *Brugia* spp. (*B. malayi* and *B.timori*), nematode parasites that are transmitted by mosquitoes. The World Health Organization (WHO) developed a plan for LF elimination that is based on using novel approaches to rapidly map endemic areas and 4 to 6 annual rounds of mass drug administration (MDA) with antifilarial medications [2, 3]. A recent report from WHO reported that more than 1.9 billion doses of MDA were distributed between 2000 and 2007 [4]. Thus, the Global Programme to Eliminate Lymphatic Filariasis (GPELF) is the largest infectious disease intervention program attempted to date based on MDA. MDA has worked better in some areas than others. A recent publication reviewed challenges faced by GPELF [5]. The limited macrofilaricidal activity of current MDA regimens (especially Ivermectin/Albendazole) that necessitate repeated annual rounds of MDA, and the difficulty of achieving high compliance rates for MDA over a period of years. ***It is clear that new dosing schedules for MDA have the potential to greatly improve the number of countries that will successfully eliminate LF by the WHO target date of 2020.***

Drugs used for LF MDA are also active against soil transmitted helminths (STH, e.g., *Ascaris*, Hookworm, and *Trichuris*). The estimated numbers of infected individuals (in millions) for *Ascaris*, hookworm and *Trichuris* range from 807-1227, 576-740, and 604-795 respectively [6]. De-worming campaigns using anthelminthics usually target special groups of the population, such as schoolchildren, and have limited impact on the transmission. Treatment of the total population and semiannual treatments may reduce re-infection considerably and will most likely lead to reduced infection densities and infection prevalences. ***Suppression of STH is an important ancillary benefit of MDA programs for filarial infections*** **[2].**

2.2 Rationale

This study is based on the assumption that currently used MDA regimens and schedules are not optimal for achieving elimination of LF. These regimens (either annual Alb 400 mg plus DEC 6 mg/kg or Alb 400 mg plus Iver 200 µg/kg for LF) were developed more than 10 years ago [3, 7].

WHO (WHA 50.29) has targeted LF for global elimination by the year 2020. GPELF recommends 4 to 6 years of annual MDA [2]. However, the likelihood of this strategy to succeed depends on a number of factors [5]. While some national MDA programs have succeeded, others

are struggling to sustain MDA with high coverage rates for 5 to 7 years according to WHO guidelines, and others have not completed mapping or initiated MDA. Annual DEC/Alb may be sufficient to eliminate LF in endemic areas with low to moderate endemicity when high MDA coverage (at least 75%) can be sustained for 5 or more years. However, in a few countries in South East Asia the endemicity is high in some areas and annual DEC/Alb may not be optimal. In addition, the Iver/Alb regimen used for LF in Africa is less active against adult worms than DEC/Alb, and annual Iver/Alb may not be sufficient to eliminate LF in Africa. We assume that more effective and efficient MDA regimens might tip the balance toward LF elimination in areas that are failing or at risk of failing to reach elimination targets with current regimens. Therefore, the goal of Objective 1 is to compare intensive (twice per year, 2X) MDA with conventional annual MDA (1X) to determine whether improved efficacy of the 2X regimens justifies increased resources required for this more intensive treatment. These trials are based on two major *assumptions*:

A. We assume that 2X MDA regimens will reduce the time needed to reach LF elimination targets. 2X MDA should shorten the time it takes to interrupt transmission (preventing infections that would otherwise have to be treated in later years); depending on the drug combination used and parasite species targeted, 2X treatment should also lead to faster clearance of adult worms. Current MDA treatment strategies are based on interrupting transmission for the reproductive life span of the adult worms (estimated to 5-7 years for species that cause LF). However, these durations may be overly conservative, because they do not factor in the partial macrofilaricidal activity of repeated rounds of treatment. Accelerated MDA programs may improve compliance and reduce costs for social mobilization and training expenses for health personnel. These factors may reduce the time required to reach elimination targets. In addition, more intensive LF elimination programs may be more attractive for public health officials to undertake than programs that require 6 years or longer.

B. Suppression of STH is an important ancillary benefit of MDA programs for LF [2]. We assume that 2X MDA will be more effective for this purpose than annual MDA. The impact of MDA on STH varies for different parasite species and drug regimens [8, 9]. For example, *Ascaris* and hookworm are more susceptible to Albendazole than *Trichuris*. Persistence of *Ascaris* eggs in soil limits the ability of MDA to reduce *Ascaris* transmission.

2.3 Potential Risks and Benefits / Potential Risks

2.3.1 Potential Risks

This research includes risks associated with collection of blood by finger prick

Likely	**Collection of blood causes minor pain**
Less Likely	**Collection of blood can cause a bruise, bleeding or infection at the collection site.** To minimize risks, sterile disposable lancets will be used for blood collection. Blood will be collected by experienced technicians after disinfecting the skin with alcohol. Subjects will be instructed to apply pressure to finger prick sites to minimize bleeding and hematoma formation. The amount of blood being collected (less than 1 ml) is not enough to cause anemia.

There are no risks associated with collection of stool samples.

Long range risks: None

- *Rationale for the necessity of such risks:* **Blood is required for diagnosis of filarial infections. Procedures in this project meet the definition of "minimal risk".**
- *Alternative data gathering procedures that have been considered or will be considered:* **None. Blood is needed for diagnosing filarial infections which are the subject of this project.**
- *Why the value of the information to be gained outweighs the risks involved.* **The potential benefits to individuals, society and science are considerable. The risks are minimal.**

2.3.2 Potential Benefits

- To subjects:
  The study will screen participants for filarial infections and refer infected subjects for free treatment at their local government health center.
- To society:
  Participation will provide information on the impact of MDA on filarial and STH infections that affect the public health in the community.

3 OBJECTIVES

To conduct population-based field studies to determine the relative cost and efficacy of annual vs. semiannual MDA for elimination of lymphatic filariasis.

1. **We will test the hypothesis that twice annual MDA is superior to annual MDA for eliminating lymphatic filariasis.**

2. **We will test the hypothesis that twice annual MDA is superior to annual MDA for controlling soil transmitted helminth infections.**

4 STUDY DESIGN

(Please read this section together with Section 5, "Study Population")

The project is comprised of repeated annual cross-sectional surveys in sentinel communities before and after initiation of MDA for LF. MDA (standard regimens recommended by WHO) will be provided by government health officials. Data collection will include demographic data, history of lymphedema, scrotal swelling (hydrocele), acute filarial fever or adenolymphangitis, prior treatment for LF. Blood samples (400 µL per person) will be collected from systematically sampled households. Blood will be tested for filarial antigenemia with the Binax Now Filariasis Card Test and Alere Filariasis Strip Test (which detects *Wuchereria bancrofti* antigen in blood, manufactured by Alere, Portland ME, USA). Blood from persons with positive filarial antigen tests will also be tested for microfilaremia (see Section 6 below for methods). The Brugia Rapid (BmR1) test will be used to detect antibodies to *Brugia* parasites antibodies in serum or blood samples. Stool examinations will also be performed for diagnosis of STH infections. **Note not all test will be performed on blood and stool samples.**

Safety oversight: Study personnel will monitor subjects for adverse events related to blood collection. No medical monitor or DSMB is needed for this minimal risk, non-intervention study.

Time to collect specimens: Most participants in the community will only be studied once (demographic data, blood, and stool samples). Thus, the **duration** of subject participation will be one day.

Parameters and criteria for assessing the impact of MDA:

Blood tests for *Wuchereria bancrofti* infection, Binax Now Filariasis Card Test and Alere Filariasis Strip Test (with MF testing of antigen positive subjects): We are using these tests as a measure of LF endemicity. The rapid tests detect antigen released from living adult filarial worms potentially capable of resuming MF production in the future. The antigen tests are much more sensitive for *W. bancrofti* infection than tests for microfilaremia. However, antigen tests may

remain positive years after treatment has cleared microfilaremia. The antigen tests will be performed in individual residents over 5 years of age. This will be done by sampling approximately 100 randomly selected households per sentinel site (village) to produce N of 400. There will be 4 sentinel sites for each treatment zone (annual vs twice annual MDA). The target number of samples per village village is close to the minimum sample size of 335 needed to show that the rate of antigenemia (card test) positivity is less than 2% (with 95% confidence and 80% power). In post-MDA populations, this corresponds to a MF rate of less than 0.5%, which is an LF elimination target. We expect to collect 4 to 5 blood samples per household. Subjects with positive card tests will be tested for microfilaremia with 60 µl night blood thick smears. Our decision to detect MF by thick smears of finger prick blood rather than membrane filtration of venous blood is because thick smears are recommended for LF elimination program guidelines. In addition, our prior studies in Egypt have shown that L3 production by *Culex* mosquitoes that feed on MF carriers with negative thick smears is negligible. The justification for collecting blood at night for MF testing is that microfilariae of *W. bancrofti* and *B. timori* in Indonesia are nocturnally periodic with highest counts in peripheral blood between 9 p.m. and 2 a.m.

Blood tests for *Brugia timori* infection *B. timori* infections will be detected by two methods, namely microscopic examination and the BrugiaRapid Test (BmR1 antibody) Cassette (Cellabs, Malaysia). Microscopic examination of thick blood smears prepared with 60 µl of blood collected between the hours of 9 p.m. and 2 a.m. The Brugia Rapid test is a rapid diagnostic test kit for detecting antibodies to filarial parasites using serum or whole blood.

Blood samples will collected from randomly selected households per sentinel site (village) to produce N of 400. There will be 4 sentinel sites for each treatment zone (annual vs twice annual MDA). This sample size number exceeds the minimum target sample size of 335 needed to show that the MF rate is less than 2% (with 95% confidence and 80% power, assuming an actual rate of 0.5%, which is an LF elimination target). We expect to collect 4 to 5 samples per household.

Stool examination for infection with soil transmitted helminths. We will examine stool samples of the study population mentioned above for the presence and the density of STH eggs. We will use the FLOTAC method to examine a single stool sample from each subject [10]. This quantitative method has an excellent sensitivity and will provide relatively accurate data on both prevalence and density of infection. We will use community egg loads as the primary endpoint for assessing the impact of MDA on STH infections, because this combines both prevalence and density of infection.

Hypotheses, outcomes, and methods for statistical testing are provided in Section 7, Analysis plan.

5 STUDY POPULATIONS

Description of human subject involvement in research:

We will perform repeated annual cross-sectional surveys in study communities.
Eligibility: This project will study people who live in communities that are endemic (or that were endemic at the beginning of the study) for LF. The endemicity rate (MF rate) at baseline should be at least 10% and study areas should have limited or no prior experience with MDA for LF.

Gender, minority and child inclusion: Males and females will be included in population-based village studies without regard to race, religion, or ethnic group. There is no reason to exclude pregnant women and older children from population-based studies. Exclusion of children less than 6 years of age from community studies is justified because prevalence rates for filariasis tend to be very low in young children and because of difficulties associated with collecting clinical specimens from this population.

6 STUDY PROCEDURES / LABORATORY EVALUATIONS

Study procedures include collection of blood and testing for microfilaremia, filarial antigenemia and anti-filarial antibodies. We will perform stool examination to detect STH infections.

All assays will be performed in Indonesia (filarial antigen and antibody tests, MF smears, stool examinations). Some samples will be tested in parallel at Washington University for QC.

6.1 Laboratory Evaluations

The **rationale** for performing these tests is described in some detail in Section 4 (study design) above. Briefly, the study calls for collection of blood that will be tested for microfilaremia (60 µl thick blood smear) and filarial antigenemia (75-100 µl blood, Binax Now Filariasis Card Test and/or Alere Filariasis Strip Test) and anti-filarial antibodies ((25-35 µl), BrugiaRapid (BmR1) cassette test-**Sikka district only**) and for collection stool (at least 1g of stool) that will be tested for the presence of STH infections.

Test methods:

Filarial antigen testing: The Binax Now Filariasis Card Test and/or Alere Filariasis Strip Test (Alere, Portland, Maine) will be performed according to manufacturer's instructions. Briefly, the subject's finger is cleaned with an alcohol wipe, dried with sterile cotton, and stuck with a sterile lancet. Finger blood is collected with a 75-100 µl capillary tube and placed on a sample pad on the

one or both filariasis tests. The result is read at 10 minutes. If desired, test results can be fixed by adding a drop of ethanol or methanol to the nitrocellulose strip of either tests..

Anti-Filarial antibody testing: In addition to rapid antigen tests, Brugia Rapid (BmR1) antibody test (Cellabs, Malaysia) will be performed according to manufacturer's instructions but only in the Sikka district where *W. Bancrofti* and *Brugia infections* are co-endemic. Blood will be collected by finger prick into EDTA anticoagulated microcontainer tubes. Sera may be separated by centrifuge. Sera (25 µl) will be tested on the BrugiaRapid Test (BmR1). Results are provided within 15 minutes for sera and 20 minutes for whole blood.

Microfilaria (MF) detection: For both filarial infections three-line thick smears are prepared with a measured total 60 µl of finger prick blood collected between 9 pm and 2 am. Slides are fixed, stained with Giemsa, and examined by microscopy with a 10x objective (higher magnification for suspicious objects). The species of MF will be determined by morphological criteria.

Testing stool for ova of soil transmitted helminths. Stool will be collected in 25 ml containers and preserved in formalin (final concentration 5%). One gram of stool will be examined using the FLOTAC method [10]. This method provides a sensitive means of detecting and quantifying helminth eggs. Some stools will also be tested with traditional parasitological methods currently used in Indonesia for comparison with FLOTAC results. In Indonesia *Ascaris lumbicoides*, hookworms and *Trichuris trichiura* are the mosty prevalent STH. Eggs/larvae of other STH will be recorded, but not statistically analyzed.

6.2 Instructions for Specimen Preparation, Handling, and Storage

Finger prick blood will be collected in Eppendorf or microtainer tubes with EDTA anticoagulant and diagnostic tests will be performed within 48h following collection. rapid LF antigen tests (Binax Now Filariasis Card Test or Alere Filariasis Strip Test) will be read in day light 10 minutes after finishing the procedure as required for the current test. BmR1 will be tested any time after blood collection. All persons will be tested for blood microfilaremia by thick smear examination (60 µl). Blood samples will be stored at RT or 4°C (if available) and away from direct sunlight.

Stool samples will be preserved with formalin (final concentration 5%) and stored at room temperature for later examination for helminth eggs. Samples will be labeled with barcode numbers that can be linked to unique identifiers (ID numbers).

Safety: Project personnel will treat all human blood and stool specimens as if they were infectious, and we will comply with OSHA safety regulations (29CFR part 1910,1030). **Field personnel will wear gloves, and laboratory personnel will wear gloves and lab coats for protection while working with blood or stool samples.**

6.3 Specimen Shipment

All samples will be tested in Indonesia. Some preserved stool samples and questionable blood smears will be tested in parallel in the reference laboratory at Washington University in St. Louis for quality control. This will be performed together with UI scientists. Shipment will comply with University of Indonesia rules and with IATA regulations.

7 STATISTICAL CONSIDERATIONS

7.1 Study Outcome Measures

The following variables will be treated as binary variables: Microfilaremia, antigenemia, anti-filarial antibody, stool parasites (*Ascaris*, Hookworm, and *Trichuris*). Microfilaria counts will also be recorded (MF/60 µl smear), and stool egg counts per species will be recorded as eggs/gram of feces.

7.2 Sample Size Considerations

Except as otherwise noted, all sample sizes in this protocol were calculated using a binomial power calculator (using alpha = .05, two-tailed tests, and power = .80). These sample sizes were chosen to provide confidence that measured prevalence rates will be below specified limits based on assumptions regarding true prevalence rates. The sample size analysis considered various contingencies to assess robustness of the sample size estimates (see Appendix).

Note: Sample size considerations for community surveys for demonstrating that prevalence rates are below pre-defined targets for antigenemia, microfilaremia, antifilarial antibody and STH infections are provided in Section 4, above, and this is not repeated here.

7.3 Participant Enrollment and Follow-Up

This project aims to enroll approximately 6,400 subjects per year for two study areas (total for *W. bancrofti* and *B. timori* sub-projects). Each of the two study areas (one for *Brugia and Wuchereria (Sikka)* and one for *Wuchereria (Java)*) will have cluster surveys of approximately 400 people per village for 4 villages from annual MDA areas and 400 people per village for 4 villages from twice per year MDA areas.

The community/household surveys are cross-sectional with no follow-up planned.

7.4 Statistical Analysis Plan

The following outcome variables will be collected and treated as binary variables: Microfilaremia, antigenemia with the Binax Now Filariasis Card Test and/or the Alere Filariasis Strip Test, anti-filarial antibody (*Brugia* endemic area only) and stool parasites (separate analysis for *Ascaris*, *Trichuris, and* Hookworm). Microfilaria counts (recorded as

MF/60 µl smear) and stool egg counts per species (recorded as eggs/gram of feces) will be taken as count variables.

Data will also be collected for demographic characteristics and covariates including age, gender, history of lymphedema, scrotal swelling (hydrocele), and prior treatment for LF.

For demographic characteristics variables, descriptive statistics will be examined for the individuals who participate in the trials by sentinel site (village) and over years of observation. Frequency will be tabulated for categorical variables. Descriptive statistics (mean, median, range and standard deviation) will be obtained for continuous variables.

Impact of MDA on study outcome measures

1. Testing primary hypothesis

The primary hypothesis is that twice annual MDA is superior to annual MDA for eliminating LF. The binary outcome variables used to test this hypothesis include microfilaremia and antigenemia and anti-filarial antibody (*Brugia* endemic area only). The model which is being used will fit a linear trend over time within each village. The mean slope across villages within each treatment group will then allow estimates of time to target levels for control (microfilaremia < .5% and antigenemia < 2%) for a selection of baseline prevalences. To illustrate the analysis strategy, we use antigenemia as an example.

The antigenemia prevalence is expressed as filarial antigenemia rate (proportion of examined individuals with filarial parasite antigenemia detected with the Binax Now Filariasis Card Test). To estimate the effect of MDA on antigenemia prevalence, we will tabulate mean rates of antigenemia across the four sentinel sites from each treatment zone (annual vs. twice annual MDA) over years of observation including the baseline. A generalized linear mixed model (GLMM) with a logistic link function will be used to account for the correlation of observations clustered within the same household from the same sentinel site. The model to estimate the difference in the odds of antigenemia includes treatment regimen and year of observation, and interaction between treatment regimen and year of observation. Some covariates that will be adjusted for in the analysis include sentinel sites, history of lymphedema, scrotal swelling (hydrocele) and prior treatment for LF as well as demographic variables of interest. Modeling will be performed with use of PROC GLIMMIX in SAS 9.2. CONTRAST statements will be used to test the significance of the log odds of antigenemia between the two treatment regimens at each year of observation. The difference of slope of rate decrease between the two regimens over the years of observations will also be tested with CONTRAST statements in PROC GLIMMIX. The resulting estimates will be converted to odds ratios with 95% confidence interval calculated from the robust estimator of variance. The other binary outcome variables (microfilaremia and anti-filarial antibody) will be analyzed with the same technique.

With microfilaria (per 60 µl of blood) measured as counts, we will display mean counts of microfilaria across the four sentinel sites from each treatment zone (annual vs. twice annual MDA) over years of observation including the baseline. To accommodate zero counts in modeling, we will use Geometric mean intensity (GMI) of circulating microfilaria. GMI is calculated as antilog $[(\Sigma\log(x+1))/n] - 1$, where x is the number of microfilaria per 60 mm^3 of blood and n is number of subjects. A generalized linear mixed model (GLMM) (implemented in PROC GLIMMIX) will be used to account for correlation of observations resulted from clustering. CONTRAST statements will be used to test the significance of differences in GMI of antigenemia between the two treatment regimens at each year of observation. The resulting estimates of GMI will be presented with 95% confidence interval. All statistical tests will be two-sided at a significance level of 0.05, but one-sided p-value will be reported according to the hypothesis. Similar analytic models will be done for community Mf loads and community egg load for each of three intestinal worm eggs.

The cost analysis comparing the two treatment regimens will be conducted by Ann Goldman of the George Washington University School of Public Health and Health Services. The model will also allow cost effectiveness analysis when combined with the efficacy analysis.

2. Testing secondary hypothesis

The secondary hypothesis is that twice annual MDA is superior to annual MDA in controlling STH. The primary endpoint for assessing the impact of MDA on STH infections is community egg load (average stool egg count per species). The analysis strategy for these end points is the same for microfilaria count in testing the primary hypothesis.

Adverse event

Since this is a minimal risk and nonintervention study, adverse events are not expected to be an issue. However, subjects will be monitored for adverse events related to blood collection. Proportion of subjects with an adverse event will be tabulated with 95% confidence interval for each sentinel site at each year of observation.

Treatment coverage rate

Treatment coverage rate of the eligible population will be reported from the repeated cross-sectional surveys for the sentinel sites (villages) during different rounds of treatment. Differences in treatment coverage rates will be evaluated between the two treatment regimens (annual vs. twice annual MDA) over time with the use of Chi-square test.

8 SUBJECT CONFIDENTIALITY

Subject confidentiality will be strictly held in trust by the participating investigators and staff. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participating subjects. This project does not include genetic testing. The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any third party without prior written approval of the DOLF Principal Investigator.

Study monitors or sponsor representatives may inspect all documents and records required to be maintained by the investigator at any time during or after the completion of the study.

Data privacy control:

1- Every individual will have one unique bar code identifier.

2- Every sample collected in the field will be labeled with barcode stickers linked to ID number.

3- The Laboratory will test and report results using sample numbers, not names or ID.

4- Person names and identifying information will be directly entered into Smart phone, and the information will be synchronized daily from these devices to a laptop with a flash drive backup. Names will be removed automatically from the standard project database and will be kept in a separate database that will be password protected and accessed only by the Indonesian Project Data Manager (PDM) to be identified in response to a written request from the DOLF PI (Dr. Weil), the Objective 1 PI (Dr. Fischer) or country PI (Dr. Supali). This is not expected to be a common event and will only be necessary if there are questions about discrepancies between serial/sample numbers and ID numbers.

5- The Data Management Team will be responsible for ensuring that no one in the project can link the the unique bar code identifier with the enrolled person except the PDM and others listed in #4 above. Person ID's will not be released for use in other projects.

6- The data server will keep a log file/data audit trail that will indicate the time/date and identify for every access to the data files. No one outside the Data Management Group will be able to modify any data on the server, and unauthorized persons will not be able to access any of the data files.

7- Each computer, Smartphone with project data will have a separate password. Also, each database will have an access system with user names and passwords that will control the level of access and permissions for each user. The PDM is responsible for controlling these permissions, and she/he is the only one that can change this.

8.1 Future Use of Stored Specimens

Residual specimens (serum, blood smears) will be maintained after the study is completed. Samples will be stripped of unique identifiers and stored in endemic country laboratories or in the PI's laboratory at Washington University. . Sequencing will be performed with DNA isolated from a small number of stool or blood samples to detect and characterize worm parasite DNA (blood or stool) and bacterial DNA (stool). The samples will be deidentified prior to any sequencing procedures. In addition, all human DNA sequences will be filtered out prior to bioinformatic analysis and no genetic testing of humans will be performed.

9 INFORMED CONSENT PROCESS

We will follow procedures outlined in the **DHHS Regulation on Informed Consent 45 CFR Part 46 - Subpart A, 46.116** (http://www. hhs.gov/ohrp/humansubjects/guidance/45cfr46. htm#46. 116).

Informed consent (IC) is a process that is initiated prior to the individual's agreeing to participate in the study and continuing throughout the individual's study participation. Study physicians will read the consent script (with potential risks and benefits) in a locally understood language to subjects and their families and leave a copy of this with each family. The consent document will be reviewed and approved by project IRB's prior to initiation of the study. Study physicians will discuss risks and possible benefits of participation in this study with subjects and their families. Study physicians will explain the purpose of the study to subjects and answer any questions that may arise. The subjects may withdraw consent at any time during the course of the study. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

9.1 Informed Consent/Assent Process (in Case of a Minor or Others Unable to Consent for Themselves.

Inclusion of minor children in community surveys will require consent from at least one parent plus assent of the child.

Human studies for this project are pending at FWA registered IRBs at Washington University (Dr. Fischer) and at IRB of the Faculty of Medicine of the University of Indonesia (Dr. Supali); no human studies will be conducted prior to approval by both IRBs.

10 LITERATURE REFERENCES

1. Michael, E., D.A. Bundy, and B.T. Grenfell, *Re-assessing the global prevalence and distribution of lymphatic filariasis.* Parasitology, 1996. **112 (Pt 4)**: p. 409-28.

2. Ottesen, E.A., *Lymphatic filariasis: Treatment, control and elimination.* Adv Parasitol, 2006. **61**: p. 395-441.

3. Ottesen, E.A., et al., *Strategies and tools for the control/elimination of lymphatic filariasis.* Bull World Health Organ, 1997. **75**(6): p. 491-503.

4. Ottesen, E.A., et al., *The global programme to eliminate lymphatic filariasis: health impact after 8 years.* PLoS Negl Trop Dis, 2008. **2**(10): p. e317.

5. Kyelem, D., et al., *Determinants of success in national programs to eliminate lymphatic filariasis: a perspective identifying essential elements and research needs.* Am J Trop Med Hyg, 2008. **79**(4): p. 480-4.

6. Bethony, J., et al., *Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm.* Lancet, 2006. **367**(9521): p. 1521-32.

7. Remme, J.H., *The African Programme for Onchocerciasis Control: Preparing to launch.* Parasitology Today, 1995. **11**(11): p. 1995.

8. Oqueka, T., et al., *Impact of two rounds of mass drug administration using diethylcarbamazine combined with albendazole on the prevalence of Brugia timori and of intestinal helminths on Alor Island, Indonesia.* Filaria J, 2005. **4**: p. 5.

9. Albonico, M., et al., *Intervention for the control of soil-transmitted helminthiasis in the community.* Adv Parasitol, 2006. **61**: p. 311-48.

10. Cringoli, G., et al., *FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans.* Nat Protoc, 2010. **5**(3): p. 503-15.

SUPPLEMENTS / APPENDICES

Standard Operating Procedure (SOP) Summary of Laboratory Work

Project laboratories will receive, process, and archive biological samples. They will perform assays and report results to the Data Management Team and Project scientists. The Laboratory will perform the following procedures and assays.

Blood samples: The laboratory will use finger blood samples to perform several tests (filarial antigen test and thick smears for MF detection).

Microfilaria testing: Thick blood smears (60 µl in 3 parallel lines of 20 µl each) will be stained with Giemsa and examined with a microscope for MF.

Filarial antigen tests: Binax Now Filariasis Card Test and/or Alere Filariasis Strip Test using finger prick blood (75-100 µL) will be performed in the laboratory according to the kit protocol and read at 10 minutes.

Antifilarial Antibody Testing: The Brugia Rapid Test detects antibodies to filarial parasite with finger prick blood (25 µL for serum/ 35 µL for whole blood). This test will be performed in field sites or in a laboratory according to the kit protocol and read at 15 minutes for serum and 20 minutes for whole blood.

Stool samples for STH examination: Stool samples will be collected in 25 ml containers and preserved in formalin (final concentration 5%). One gram of stool will be examined using the FLOTAC method [10]. This method provides a sensitive means of detecting and quantifying helminth eggs.

Specimen archive: In addition to new samples generated during this study, the Laboratory will be responsible for maintenance of archived samples (used Binax Now Filariasis Card Test, preserved stool samples, etc.) collected during the DOLF project.

QC activities: DOLF investigators will visit study sites to review procedures and results. They will also keep in close contact with Indonesian counterparts by email, telephone, and during visits by collaborating scientists from Indonesia to the USA. All samples will be tested in the endemic country laboratories. Some preserved stool samples and questionable blood smears will be tested in parallel in the reference laboratory at Washington University.

Standard Operating Procedures Summary (SOP) for Data Collection and Field Work

Field work will be conducted by trained physicians and paramedical personnel who will be trained for data and blood collection.

All field team members will be trained by staff physicians with assistance from other project personnel:

1. All field epidemiology personnel (physicians, technicians and field workers) will be trained in GCP topics for research involving human subjects.

2. Field and laboratory personnel will also be trained regarding proper procedures for collecting blood, for preparing blood smears, for performing filarial antigen tests, and for labeling specimens (barcode stickers).

3. Field data will be entered in the field using electronic questionnaires on PDAs or smart telephones. Finger-prick blood (400 µl) will be collected by physicians or trained technicians for laboratory studies. All samples will be labeled in the field.

4. Technicians will be responsible for transferring blood samples to the Laboratory immediately after field trips (same night of collection).

5. Stool samples will be collected by the field epidemiological personnel during the survey or on the next day.

Informed Consent Script to Be Read in Population-Based Field Surveys

Project title: **Optimization of chemotherapy for control and elimination of onchocerciasis and lymphatic filariasis**

Project Director: **Dr. Taniawati Supali (country PI) and Dr. Peter Fischer (PI, DOLF Project Objective 1)**

Sponsored by: **Bill and Melinda Gates Foundation (USA)**

1. Invitation: You (and your family members) are being invited to participate in a project to test the impact of a public health program on parasite infections that are common in this area.

2. Purpose: The purpose of this project is to assess effects of mass drug administration on filariasis and other worm infections in Indonesia. This project is being conducted by the University of Indonesia in Jakarta in cooperation with scientists at Washington University in St. Louis USA.

3. Procedures: Your finger will be cleaned with alcohol, and a few drops of blood will be collected by finger prick. We will keep samples collected now to do other research studies of infectious diseases in the future. We will also collect information (such as name, age, and address or house location). The screening procedure will take a few minutes of your time for collecting information and the blood sample. We will also collect stool samples to test for intestinal worm infections.

4. Risks: There are no significant risks. Collection of blood samples (a few drops by finger prick) causes minor pain and occasionally a small bruise. You will be asked to keep pressure with cotton on your finger for 2 minutes to stop bleeding. The small amount of blood being collected will not harm your health.

5. Number of participants and duration of the project: Approximately 6,400 people will participate in the project per year; the surveys will be repeated annually for 4 years.

6. Benefits: Participation in this health project will benefit you and your family by providing a free screening test for early detection of parasitic worm infections. If you or any of your family members are found to have worm infections, you will be referred to a nearby primary health center for treatment. The project will also help the Ministry of Health in Indonesia by providing information on the effects of their mass drug administration program on filariasis and other worm infections in your community.

7. Confidentiality: Results of any abnormal blood or stool tests will be provided to you and also to local health authorities. All project records may be reviewed by the sponsors of the program. Otherwise, any information linked to your name will be strictly confidential.

8. Questions: You are free to ask any question regarding this health study now. If questions arise later, please contact Dr.Taniawati Supali, who can be reached by telephone at 62-8129355870.

9. Participation is voluntary: Participation by you and your family members in this public health assessment project is entirely voluntary. Children should not be forced to participate against their will. Refusal to participate will not penalize you in any way, and you are free to withdraw at any time.

If you willingly agree to participate, you will provided a copy of this information.

Note: Program personnel must mark each computer record to verify that they have read this script to all participants and answered any questions. A copy of this information form should be left in each house included in the program.