Protocol

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

Protocol for: King CL, Suamani J, Sanuku N, et al. A trial of a triple-drug treatment for lymphatic filariasis. N Engl J Med 2018;379:1801-10. DOI: 10.1056/NEJMoa1706854

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Center for Global Health and Diseases

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This supplement contains the following items for the manuscript entitled "A Randomized Controlled Trial of a Novel Triple Drug Treatment for Lymphatic Filariasis"; Christopher King, MD, Ph.D.

1. Original protocol (version dated March 29, 2012), final protocol (version dated October 4, 2016), summary of changes (version dated November 7, 2017).

2. Original statistical analysis plan (version dated March 29, 2012), final statistical analysis plan (version dated October 4, 2016), summary of changes (version dated November 7, 2017)

Mailing Address:
Center for Global Health and Diseases
CWRU School of Medicine
Biomedical Research Building
10900 Euclid Avenue LC: 4983
Cleveland, Ohio 44106

Visitors and Deliveries:
Center for Global Health and Diseases
Case Western Reserve University
Biomedical Research Building 4th floor
2109 Adelbert Road West Administrative Offices
Cleveland, Ohio 44106

Phone: 216-368-4818
Fax: 216-368-4825

Title of the Protocol

Evaluate Triple Drug Therapy with Diethylcarbamize (DEC), Albendazole (ALB) and Ivermectin (IVM) That Could Accelerate LF Elimination Outside of Africa

Principal Investigators

Christopher L. King, M.D., Ph.D. (USA)[1]
James W. Kazura, M.D. (USA)[1]
Peter M. Siba, Ph.D. (Papua New Guinea)[2]

Co-Investigators

Inoni Betuela, MBBS, M.Med. [2]
Lui Apolos, MBBS, M.Med.[2]
Manasseh Baea[2]
Edward K. Thomsen[1]

Lay Summary

This study will determine if a combination of 3 drugs used to treat the infection that cause lymphatic filariasis (LF) due to *Wuchereria bancrofti* infection are more effective in killing or sterilizing the adult worms compared to just 2 of the 3 drugs that usually given to treat this infection. The three drugs used together are called albendazole (ALB), ivermectin (IVM) and diethylcarbamazine (DEC). The usual treatment in Papua New Guinea (PNG) for lymphatic filariasis are DEC and ALB. A combination of these 3 drugs has not been previously used to treat LF.

Abstract

This study will determine if individuals infected with *Wuchereria bancrofti* and treated with combination of ALB, IVM and DEC compared to the standard treatment of DEC plus ALB in LF endemic areas outside of African can accelerate elimination of LF because of enhanced killing or sterilization of adult worms. Such a drug regimen could reduce the frequency of MDA necessary to control LF in endemic area

Purpose, Including Specific Aims and/or Hypotheses

Primary Objective is to determine whether a combination of three drugs ALB, IVM and DEC will enhance killing or sterilization of adult *Wuchereria bancrofti* infections, the major causative agent of lymphatic filariasis (LF), compared to the standard treatment of DEC plus ALB.

Background and Significance

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. 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 medication. The most recent summary from WHO reported that more than 1.9 billion doses of MDA were distributed between 2000 and 2007. Thus, the Global Program 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. There are a number of challenges faced by GPELF. These include (among others) inability to conduct MDA programs in areas of Africa where *Loa loa* is coendemic because of the unacceptable risk of Serious Adverse Events (SAE's) with Ivermectin in persons with heavy *L. loa* infections, 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. Drugs used for LF and Oncho MDA are also active against soil transmitted helminths (STH, e.g., *Ascaris*, Hookworm, and *Trichuris*). Suppression of STH is an important ancillary benefit of MDA programs for filarial infections.

It is clear that new (or reformulated) drugs and/or 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.

Rationale for the current study

GPELF policies and mathematical models suggest that current regimens will require many years of MDA with high compliance to achieve elimination (i.e. reduction of transmission rates below sustainable levels). None of the current drugs used for MDA are highly effective in themselves and combination therapy hopes to increase their activity. DEC is the best available drug that is active against both the larval stage in the blood or microfilaria (MF) and is partially effective in killing adult worms. Albendazole has little activity against MF, but some activity against adult worms [1]. Ivermectin is primarily active against MF, but has some activity against adult worms [2]. In this study, a single 400 μg/kg dose of Iver cleared Mf completely in 35% of subjects and reduced the geometric mean Mf level by >98% at 1 year; 2 years after the single treatment, 20% of participants remained Mf negative, and geometric mean Mf levels remained reduced by > 90%. Similar but less profound effects were observed for single dose Iver in a trial conducted in Tanzania . Taken together, these data show that Iver at doses previously shown to be safe and effective for treatment of LF (400 μg/kg) may have embryostatic or embryocidal activity against *W. bancrofti [3]*. Based on the superior activity of IVM against Mf, we assume that the triple therapy regimen of Iver/Dec/Alb will be much more

effective than DEC/Alb for clearing Mf from the blood. It is further hypothesized that the triple regimen will be more effective in killing adult filarial worms and leading to their sterilization. Thus, the triple regimen has the potential to markedly reduce the number of MDA treatments needed to achieve transmission interruption and elimination of LF outside of Africa compared to the time frame of 5 to 7 years projected for DEC/Alb.

DEC, IVM and ALB are very safe and highly effective anti-filarial drugs when given singly or in combination (i.e. DEC with ALB, or IVM with ALB) [4]. In addition, billions of doses of these drugs have administered over the past decade as part of global LF elimination effort, with an outstanding safety profile with the exception of co-infections with *L. loa* as noted above [5]. IVM is unlikely to interfere with pharmacokinetics or pharmacodynamics of DEC and ALB since IVM is primarily excreted through the kidney whereas DEC and ALB are metabolized in the liver, as described in further detail below.

ALB causes degenerative alterations in the tegument and intestinal cells of the worm by binding to the colchicine-sensitive site of tubulin, thus inhibiting its polymerization or assembly into microtubules [6]. The loss of cytoplasmic microtubules leads to impaired uptake of glucose by larval and adult stages of the parasite, and depletes glycogen stores. Degenerative changes in endoplasmic reticulum and mitochondria of the germinal layer, and the subsequent release of lysosomal enzymes result in decreased production of adenosine triphosphate, which is the source of energy required for survival of the helminth. Due to diminished energy production, the parasite is immobilized and eventually dies.

ALB has weak ability to kill Mf, and more effective on killing or sterilizing adult worms compared to IVM, but much less so than DEC.

The drug has been shown occasionally (<1% of treated patients) to cause reversible reductions in total white blood cell count. It has also been associated with slight increases in transaminases in ~16% of patients. The enzymes return to normal levels with cessation of treatment. These abnormalities are associated primarily with prolonged treatment for such diseases as neurocysticercosis and hydatid diseases, not single dose treatment which is being proposed here.

IVM is an avermectin compound of macrocyclic lactones derived from the bacterium *Streptomyces avermitilis* [7]. The mechanism by which IVM kills LF microfilariae is not known with certainty, but the drug interferes with glutamate gated ion channels that can affect parasite contractility and release of immunomodulatory molecules by the parasite [8]. IVM also has a direct effect on the central nervous system and muscle function as it enhances strength of inhibitory neurotransmission pathways.

The main concern with the use of IVM in animals and humans is neurotoxicity, which can be manifest as ataxia. Neurotoxicity is usually associated with prolong therapy with the drug and has not been observed in humans given single dose IVM for LF or other

parasitic infections [9]. IVM has been used to treat millions of people with LF and onchocerciasis [9]. Peak IVM serum concentrations are reached approximately 4-5 hours after administration. The half-life of IVR in various populations ranges from 12 to 56 hours [10]. There is no evidence of drug:drug interaction between ALB and IVM [11].

IVM can cause nausea, dizziness and occasionally pruritus, but these are infrequent, transient and usually mild. Major side effects occur with heavy infections of *L. loa*; however, this parasite is not endemic in Papua New Guinea.

DEC is an anthelminthic drug that is structurally distinct from ALB and IVM [12]. DEC inhibits arachidonic acid metabolism by LF, and inducible nitric oxide synthase and the cyclooxygenase pathway may be essential for activity *in vivo* [13]. DEC also has anti-inflammatory properties. The mechanisms of active of DEC remain poorly understood. Its ability to kill Mf and adult worm depends on the host immune responses since the drug has little direct activity on parasites in vitro. The drug has potent activity against LF microfilaria. DEC has about 50-70% efficacy in killing or sterilization of adult worms [14]. The drug is rapidly absorbed from the gastrointestinal tract, has a serum half-life of 12 to 14 hours, and is excreted in the urine with little modification by liver metabolism. There are few side effects to drug other than killing of adult worms.

Study Design

This will determine whether 1 or 2 annual treatments with the triple drug regimen of DEC/Iver/Alb is equally or more effective than repeated annual treatments with DEC/Alb in inducing sustained clearance of Mf in LF infected subjects previously determined to have >100Mf/ml, indicative of a moderate to heavy infection. Subjects will be treated and monitored in several common areas near study subject residences.

There will be 3 treatment arms as follows:

1. The *comparator* (standard treatment) DEC 6 mg/kg + Alb 400 mg administered annually (at 0, 12, and 24 months).

2. DEC 6 mg/kg + Alb 400 mg given once

3. DEC 6 mg/kg + Alb 400 mg + Iver 200 μg/kg administered once only at the beginning of the RCT (0 month).

Primary endpoint will be

Percentage of subjects with total clearance of in *W. bancrofti* Mf at 36 months .

Secondary endpoints

1. *Percentag*e of subjects with total clearance of Mf at 24 months.

2. Percent reduction in Mf at 24 months compared to baseline.

3. Percent reduction in *W. bancrofti* antigen levels by Og4C3 assay compared to baseline measured at 24 and 36 months

4. Percent of subjects *who become* ICT negative at 24 months and 36 months after the beginning of the study.

5. Percent reduction in viable worm nests based on scrotal ultrasound of males at 12, 24, and 36 months.

6. Changes in clinical manifestations associated with LF (e.g. lymphedema, hydrocele, lymphangititis/lymphadenitis, and other scrotal or genital abnormalities at 12, 24, and 36 months.

RESEARCH PLAN

Study Procedures

The study will be conducted as an open labelled trial where subjects and people administering the drugs will be unblinded to the treatment. Subjects who do not receive drugs at 12 and 24 months will be given multivitamin tablets. The laboratory personnel, however, who measure Mf levels, ICT card tests, circulating antigens levels or performing the ultrasound studies will be blinded to the treatment groups.

Randomization procedure A random number table equal to the total number of subjects to be enrolled in the 3 arms with values from 1 to 3. As individuals are enrolled into the study, they will be assigned a number corresponding to one of the 3 arms in sequence from the table.

Treatment: Subjects will be given the treatment medications under direct observation by the study nurse along with a fatty snack (crackers covered with margarine or peanut butter) because fats help absorption of the some of the drugs.

Screening for eligibility and monitoring safety variables: There will 4 steps for the study

1. Subjects will be initially consented and then screened for the presence of LF. At the time of consent it will be made clear to subjects that if they are uninfected or if infected and do not have >100Mf/ml they will not be eligible for the study. Screening will entail a fingerstick night blood sample (after 2100 hours) obtained from adults aged 18-65 years to determine if they are infected with LF and to identify individuals with >100Mf/ml.
2. At a subsequent visit subjects identified with >100Mf/ml will have blood and urine tests performed to evaluate whether they meet the inclusion/exclusion criteria described below.

3. Once individuals have been found to meet the inclusion/exclusion criteria, they will be asked to participate in the study, have an ultrasound examination performed to determine the presence a number of worm nests and be administered the study medications. They will be asked to come to the local health center if they have any fevers, pains consistent with adverse drug reactions. There will be passive surveillance for adverse events up to 1 week post treatment.
4. Individuals will then undergo similar evaluations and receive additional treatment or multivitamins tablets depending on the treatment arms one, two and three years later.

Laboratory Tests and Analysis

Analyses to be performed at PNGIMR Laboratories Maprik and Madang:

Detection of Lymphatic filariasis for diagnosis and monitoring drug efficacy

1) The presence of MF will be determined by examining 60 ul of fingerstick blood for screening purposes. The blood will be placed on a glass slide, dried, fixed and stained with Geimsa and then examined under microscopy for the presence and number of Mf. Immediately prior to treatment and at 12, 24 and 36 months mf levels will be determined by filtering 1 ml of anticoagulated blood through a nucleopore filter, dried, fixed and stained with Geimsa and then examined under microscopy for the presence and number of Mf

2) ICT card test (now manufactured by Alere rather than Binax Inc.)– is a lateral flow card test based on mAB AD12.1 (marketed as the Filariasis Now ICT) to detect antigens released by living adult *W. bancrofti* worms in whole blood from infected subjects.

3) Og4C3 antigen detection ELISA assay marketed as the TropBio Filariasis Antigen, CELISA) based on the mAb Og4C3. The assay detects a heat stable antigen released by living adult *W. bancrofti* worms.. This assay will be performed as described by manufacturer.

4) Preparation of Mf slides and ICT assays will be performed in Dreikiker area, Maprik and/or Madang. The reading of slides for Mf levels will be performed in PNGIMR laboratories in. Maprik and/or Madang.

Additional tests may be performed related to detection of infection and related to drug efficacy such as, but not limited to, humoral and cellular test for immune function.

Ultrasound

All men in the study will be examined by ultrasonography at 0, 12, 24 and 36 months after their first treatment for the presence of viable worm nests as determined by cluster of actively moving adult worms using a linear transducer or "filarial dance sign." The

procedures for the performing the ultrasound have been previously described [15, 16]. Abnormal ultrasound findings will be photographed and recorded on digital recordings and given to health care professionals in PNG for any necessary interventions.

Clinical tests

Hemoglobin, AST/ALT, creatinine and urinanalysis will be performed prior to exclude hematologic and renal abnormalities.

Source of Drugs Used

Albendazole and DEC will be provided by Global Program to Eradicate Lymphatic Filariasis (GPELF) to PNG. Ivermectin (Stromectol by Merck) will be purchased that is identical to the Ivermectin that is used for mass drug administration (MDA).

Study Population

The study team will contact potentially eligible individuals in the community by community health workers that will employed by study. Individuals with Mf levels >100/ml will be offered the opportunity to participate, providing they meet the inclusion and exclusion criteria indicated below. Infected individuals ineligible for the RCT will be offered the standard treatment of DEC plus ALB.

The study population will reside in filarial endemic areas in East Sepik Province, Papua New Guinea.

Inclusion criteria:

- Men and women 18-65 years
- >100mf/ml in finger stick blood samples
- Willing to give informed consent

Exclusion criteria

- Prior treatment for LF within last 5 years
- Pregnant (do pregnancy test)
- Hemoglobin < 7 g/dl
- permanent disability, serious medical illness that prevents or impedes study participation and/or comprehension
- AST/ALT and creatinine > 1.5 upper limit of normal.
- Urine dipstick with glucose ≥ 2+ and/or protein ≥ 2+

Any one or more of the above criteria is sufficient to exclude study participation. Anemia will be treated according to Papua New Guinea national standard treatment guidelines.

Subjects who prematurely discontinue from protocol treatment should follow the same evaluation schedule as those who complete protocol treatment. *No follow-up is needed for subjects who do not receive at least one dose of treatment.*

Criteria for Termination from Study

If a subject refuses to have any blood samples taken following the initial treatment they can be terminated from the study. Also if a subject move away from the area such that cannot be examined for any of subsequent annual blood draws, they can also be terminated from the study.

Assessment of Resources

This study, which is part of a larger study at Washington University School of Medicine/St. Louis directed by Dr. Gary Weil, is being funded by the Bill and Melinda Gates Foundation.

Subjects for this study will be recruited from filarial endemic areas of East Sepik Province in Papua New Guinea. The investigators have conducted research studies in Papua New Guinea for over 20 years in this area and have a strong collaborative relationship with the PNG IMR. As a joint project with the Institute of Medical Research in Papua New Guinea support is available for specimen collection, storage and laboratory testing.

Albendazole and DEC will be provided by Global Program to Eradicate Lymphatic Filariasis (GPELF) to PNG. Ivermectin will be purchased that is equivalent in drug concentration to that used for mass drug administration and is manufactured under good laboratory practices

Storage of Specimens

At the time of each survey, specimen collected for biochemical analysis will be evaluated in Maprik or Madang. The circulating antigen determined by Og4C3 assay will be frozen and sent to the PNG laboratories at Madang for subsequent determination. Serum/plasma and whole blood containing filarial parasite peripheral blood lymphocytes will be stored in freezer in Maprik or Madang In the event that samples are sent outside of Papua New Guinea for testing or storage they will be de-identified. Samples may be stored at one or more of these sites for 35 years. In some cases blood samples will be sent to CWRU or the Laboratory of Parasitic Diseases at the National Institutes of Health for additional analysis for host immune responses, presence of circulation parasite antigens and/or analysis of parasite DNA.

Risks and Discomforts and How Minimized

Risks of Blood Draw

Blood collection via peripheral venipuncture and finger stick is considered to be minimal risk and little or no discomfort is anticipated, similar to a prick. Possible bruising may occur at the venipuncture site. There is a risk of infection but use of sterile technique minimizes the risk for infection. On occasion, a subject may faint, but research staff will be alert to subject reactions after the blood draw and will provide aid as needed.

Risks of Study Drugs

The combinations of Ivermectin plus Albendazole or DEC plus Albendazole are widely used for MDA. There have been clinical trials of DEC plus Ivermectin [18]. Any risk of medications, particularly the combination of IVM, ALB and DEC will be initially assessed in the pharmacodynamics and pharmacokinetics studies performed prior to this study [UHCMC IRB # 01-11-01/IMR IRB #1119].

Risks of each drug separately, with some indication of how likely these are to occur, are summarized below.

> DEC – most common side effects are itching and swelling of face, headache, joint pain, unusual tiredness or weakness. These are transient. Less common are dizziness, nausea or vomiting. Fever, painful and tender glands in groin, neck armpits or skin rash can occur, and are usually associated with high burdens of infection as judged by the level of blood microfilaria.
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> ALB - Headache, nausea, stomach pain and vomiting are most common, and usually associated with heavy geohelminth infections. Severe allergic reactions occur rarely, and include rash, hives, itching, difficulty breathing, tightness in the chest, swelling of the mouth, face, lips, or tongue, dark urine, Mild elevation in liver transaminases can occur, but normalize with cessation of treatment. These side effects are usually associated with prolonged ALB therapy.
>
> IVM – The most common side effects are diarrhea, dizziness and nausea. Rare side effects include rash; hives; itching; difficulty breathing; tightness in the chest; swelling of the mouth, face, lips, or tongue; eye pain, swelling, or redness; fainting; fast heartbeat, Specifically mild decrease in leukocyte counts, elevated liver function tests, and cardiovascular effects that included tachycardia, orthostatic hypotension. Infrequently treatment can exacerbate bronchial asthma. These side effects are also associated with prolonged therapy.

Compensation for Injuries

There is no compensation available in the event of injury. It is not anticipated that any injuries will results from this study because the drugs used have been widely used for treatment of lymphatic filariasis with serious side effects.

Benefits to Subject

There will be direct benefit for participation in this study, in that they will be treated for the LF infection. A broader community benefit is that the triple regimen has the potential to markedly reduce the number of MDA treatments needed to achieve transmission interruption and elimination of LF compared to the time frame of 5 to 7 years projected for DEC and Alb. If the intervention proves successful, the triple therapy is likely to be widely adopted in many LF endemic areas. In order to facilitate such an uptake of triple therapy into national treatment policies, the study will liaise closely with national and provincial health authorities.

Costs to the Subject

There is no cost to the subject to participate in this study.

Alternative(s) To Participation

Study subjects may decline participation in this study with no consequences. Subjects with known LF will be referred to local health centers and treated per national guidelines (single annual doses of DEC + ALB for 5-7 years).

Payment to the Subjects (Reimbursement and Incentives)

Subjects will not be paid for participation in this study. The study will cover costs related to laboratory tests as part of the study and clinical monitoring.

Plan for obtaining informed consent (Informed Consent Process)

Description of the Informed Consent Process:

Following standard practice for Papua New Guinea Institute of Medical Research (PNGIMR) field studies, the informed consent process starts several months before enrollment of participants and involves both community and individual consent. The study will be discussed with representatives of provincial Department of Health representatives and mission health services as well as senior community members to assess both feasibility and community acceptability of different study design features and field procedures. The informed consent process recognizes the community and cultural values in Papua New Guinea and the East Sepik region where the study participants reside. Extensive discussion of risks and possible benefits of participation in this study will be provided to the community, the study participants and their relatives. This is

accomplished through a series of community meetings (*tok saves*) in which the Principal Investigators, co-investigators and/or PNGIMR representatives explain and discuss the purposes of the research study to residents at study sites and PNGIMR field assistants who may refer study participants for more information about this study. The investigators and study personnel who will obtain consent from study participants all have or will receive training in human subjects regulations and good clinical practices (GCP).

Following consent by community leaders to include their village into the study, the study team will invite all interested adult community members in areas known to have levels of LF infection to attend information meetings held in several central locations throughout the study areas. At these meetings, the study team will describe the purpose and significance of the study, the procedures to be followed, the risks and benefits of participation, and state that participation in the study is voluntary and that declining to participate will not reduce the level of, or access to, health care for the eligible individuals.

Consent forms describing in detail the study procedures and risks will be read at *tok saves* in Melanesian pidgin (Tok Pisin). Formal, written informed consent will be obtained for individuals willing to participate in study. Consent forms will be approved by the UH/Case Medical Center IRB and Papua New Guinea Institute of Medical Research IRB. The subject will be asked to read, or have read to them, and review the informed consent documents. Upon reviewing the document, the investigator and/or study staff will explain the research study to the subject and answer any questions that may arise.

Only the principal investigators and study staff authorized and certified to obtain consent will consent subjects for this study. Only individuals who have signed the consent form and meet eligibility criteria will be enrolled in the study.

Additional Consent Issues

Training of Papua New Guinea Study Staff in Human Subjects Research Ethics

The Principal and co-investigators will conduct on-site training sessions for Papua New Guinea study staff who will be collecting study information, specimens, and/obtaining consent from subjects in the study. The study will be explained in detail to the local study staff. The basic principles of informed consent process, documentation of informed consent, protection of subjects' rights, confidentiality, and handling of data will be covered in these training sessions. All training sessions will be documented, and study staff monitored by the on-site project manager on a regular basis to ensure compliance with the principles of informed consent. The Principal Investigator will provide training and readings materials on human subjects regulations with an emphasis on informed consent. The field staff in PNG has difficulty with the use of modern technology (computers, mouse etc.) In addition, field staff do not have separate email accounts allowing on-line certification. Obtaining human subjects training certfication using an on-line tutorial such at the CWRU/CITI or NIH on-line program is not feasible with the

field staff in PNG. The Principal Investigators will provide the specified training (as outlined in the protocol) and submit a signed attestation for this human subjects training.

It is not the practice of the Center for Global Health & Diseases to ask or require that a subject initial each page of the consent form. The investigators will accept either signed (cursive) or printed signatures or a witnessed mark in the case of illiterate study participants on the consent form.

Because this study takes place in Papua New Guinea and the Principal Investigators (of record) is based in the United States (at CWRU), it is not possible for the PI to sign the consent form within 30 days as required by the UHCMC IRB for studies conducted in Cleveland. Also, the Papua New Guinea PI may not always be available at the time of consent to sign the forms. Consent forms will be reviewed at each visit and the PI will sign the consent forms to verify subject eligibility and that informed consent has been obtained. The onsite Coordinator/Manager will review each consent form to be sure that all required signatures are obtained and subjects meet eligibility criteria. Co-investigators on the study may also review the consent forms and sign on behalf of the Principal Investigators.

Entry into the study and participation will be strictly voluntary. It will be made clear that refusal to participate or withdraw can occur at any time throughout the course of the study and will not influence their rights or the care they receive at local health facilities. It is stated that all information is confidential and coded without personal identifiers. It is also indicated that no monetary or other gains are offered in exchange for participation.

Comprehension of informed consent:

After the *tok save* and at the time the consent form is given to the study participant, the participant will have an opportunity to ask additional questions. To assess comprehension of informed consent and the study, the principal investigator and/or authorized consent study staff will ask the following questions:

Do you understand the consent form?
Do you have to participate in this study?
Will we take blood from you during this study?
Can you refuse to participate in the study at any time?
Is there any charge for being in the study?
Will you receive any money for being in the study?
Do you know who to call if you have questions?

The responses will be documented on the consent form, signed by the research staff or principal investigator, and a copy given to the study volunteer. In the event a subject indicates a lack of understanding of the study, or any aspect of it, the Principal Investigator/study staff will invite questions and offer explanations of any particular point. If, in the judgment of the Principal Investigators, the subject's response still does not reflect an understanding of the study, the subject will not be enrolled in the study.

Provisions for Subjects from Vulnerable Populations

Plan to Address Women Who are or Who Become Pregnant While on Study

Pregnant or breast-feeding women will not be eligible to participate in this study because of the unknown effects of the drugs and drug combination proposed in this study. Women of child bearing age who wish to enroll in the study will have a pregnancy test prior to enrolment in the study to ensure they are not pregnant. Pregnancy tests will be repeated before every drug administration.

Plan for Inclusion of Illiterate Subjects

Study participants, if illiterate, will have the information sheet or consent form read to them by a trained field assistant in the local language. Their signature or mark will be witnessed on the consent form.

Plan for Inclusion of Non-English Speaking Individuals

Subjects who do not speak or read English are neither specifically included nor excluded from this study. It is anticipated that most (if not all) the study participants will be residents of Papua New Guinea. The country has over 800 distinct languages and dialects; the common language used in the country is Melanesian pidgin (*tok pisin*). The consent form will be translated from English into *tok pisin* by a native Papua New Guinean resident who is knowledgeable about research and the study. Once the English consent form is approved it will be translated into *tok pisin* and submitted to the UH Case Medical Center IRB and PNG IMR IRB for review and approval.

Subject Privacy and Data Confidentiality

Privacy of the study participants will be maintained by assigning study participants a unique study identification number (UNID). All data, blood samples and laboratory results will be recorded and analyzed by UNID with no personal identifiers. All information collected, including demographic information about enrolled subjects will be kept confidential and available only to the investigators and authorized study personnel such as the data manager. After the public *tok save's*, potential participants in the study will have an opportunity to sign the consent form in a private place if they choose to be part of the study.

All written forms (i.e. consent and data collection forms) will be stored in the CWRU-PNG IMR designated study areas in Maprik and/or Madang, PNG. All forms will be labeled and filed in filing cabinets with the Study Protocol Number, Principal Investigators' names and collection dates. These cabinets will be metal and have functioning locks. Keys will be kept with the CWRU-PNG IMR Project and/or Data Managers. All data will be double entered by trained PNG Data Entry Clerks and cleaned by the Data Manager. The data base will be password protected and access to

password will be authorized by the Principal Investigators and/or Project Manager. Electronic data files will be stored on a CWRU-operated and -dedicated server located in the assigned CWRU-PNG IMR data rooms. The paper forms will be stored for the duration of the study plus three years per IRB protocol for primary data storage. The electronic database will be stored indefinitely by the PIs.

The Principal Investigators, Co-investigators and key personnel may use the results of this study for publications, presentations at scientific meetings or preliminary data for subsequent grant applications. Confidentiality of study participants will be maintained by not using names or personal identifiers.

The study site Project Coordinator will permit access to all documents and records that may require inspection by the respective funding agencies, governmental regulatory agencies, institutional review boards (both UH IRB and IMR IRB) or its authorized representatives.

Data Collected on Subjects

This study will collect the following types of data on each study participant as part of the study. The only information that will be recorded in the subject's permanent medical record is that they were diagnosed with LF and received a single or multiple treatments.

- Demographic data (at enrollment only). This will include age, sex, location of subjects, etc.
- Prior history of any symptoms related to LF; chronic symptoms and signs of lymphedema and/or hydroceles and history of acute symptoms of lymphangitis and lymphadenitis at onset of the study and throughout the 3 years of evaluation as determined by questionnaires and/or physical examination.
- Prior treatment for LF (although no mass drug therapy has been performed in this population).
- Subjective behavioral and clinical data (e.g. feeling of nausea, neurological or other subjective data related to possible side effects) within the first week following treatment
- Hemoglobin levels AST/ALT creatinine urinanalysis at initial evaluation. These will only be collected at the beginning of the study unless the subject experience severe side effects that might affect these laboratory parameters.
- Prevalence and intensity of mf levels and geohelminths in stool.
- The determination of circulating antigen using the Og4C3 mAb will be measured in the Madang laboratories and biochemical analysis in the clinical laboratory at clinical laboratories in hospitals in either Maprik, and/or Madang.

Request for Waiver of HIPAA

A request for waiver of HIPAA for PHI based on the cultural environment and customs in Papua New Guinea accompanies this submission.

Data Analysis Plan

The *primary endpoint* will be the percentage of people with total clearance in *W. bancrofti* Mf compared to baseline measured at 3 years after treatment. Since the goal is to see if adding IVM to DEC plus albendazole is superior to eliminating mf positivity at 3 years after a single dose, the primary hypothesis will be whether DEC+ALB+IVM is superior to DEC+ALB given once. The second hypothesis is that DEC+ALB+IVM given once will be non-inferior to the percent reduction in microfilaremia obtained by the comparator arm of DEC+ALB given annually x 3. We anticipate the DEC+ALB given once (arm 2) to show the lowest percent reduction in mf at 36 months and the comparator arm, DEC+ALB given at 0, 12, and 24 months to show greatest reduction.

A similar analysis between the same groups will be analyzed for the secondary endpoints listed above:

Since the study groups will be randomized, comparison of the primary endpoints will examined using a Student's *t* test for continuous variables and chi-square analysis for percentages.

Sample size determination:

Since the goal is to see if adding IVM to DEC plus albendazole is clearly superior to eliminating mf positivity at 3 years after a single dose, the primary hypothesis will be whether DEC+ALB+IVM administered once is superior to DEC+ALB given once. Based on preliminary data and the literature we anticipate that DEC+ALB+IVM will achieve a 75% clearance compared to 50% to DEC+ALB given once at 36 months among individuals with moderate to high parasitemia levels (i.e. >100mg/ml). For this comparison, 46 is required in each arm with a power of 0.8 and one-side test at alpha=0.05. The second hypothesis is that DEC+ALB+IVM given once will be non-inferior to the percent reduction in microfilaremia obtained by the comparator arm of DEC+ALB given annually x 3. Based on preliminary data the comparator arm will achieve a 95% clearance at 36 months, if the single triple drug is non-inferior to standard treatment that is significantly less than 15%, we will need 54 in each, for a power of 0.8 and alpha=0.05. Therefore given a dropout rate of 30% over 3 years we will need 70 individuals per group. Given the possibility of even a higher drop-out rate we aim to recruit 75 individuals per arm or a total of 225 subjects.

Data and Safety Monitoring Plan

Procedures to be followed in the event of serious adverse reactions after drug intake

If SAE occurs the subject will be referred to the Boram Hospital in Wewak where investigation and treatment will be provided through the East Sepik provincial government facilities. SAEs will be reported to the institutional review boards per guidelines and timelines. In addition, all SAEs must be reported to the DSMB and the sponsor.

There is a formal Data Safety Monitoring Committee or Board (DSMB). See Appendix #2 for a description of the committee and membership.

Plans for the Subjects at the End of the Protocol

Prior to publication of the results, PNGIMR, the study population, district health services, and members of the study team will be informed of general findings arising from analysis of study data and the study outcomes. This will occur through written correspondences and face-to-face discussions with collaborators at PNGIMR. Communications with district health services will occur based on advice from the collaborators at PNGIMR through written correspondences and face-to-face discussions. Presentation of general results from the study to local community leaders, reporters and community-based study participants will occur through village-based tok saves. Individuals in the study will continue annual treatment with Alb and DEC annually for up to 5 years according PNG national guidelines.

Approval by Foreign/Local IRB

In addition to review and approval by the UH Case Medical Center IRB, this study will be submitted to the Papua New Guinea Institute of Medical Research's IRB (IMR IRB) for review and approval of the protocol and the potential use of previously collected blood samples under MRAC approved protocol. As a new study, this protocol will also be submitted by the IMR IRB to the Medical Research Advisory Council (MRAC) for final approval. The study will not begin until all required ethical and regulatory reviews are completed.

FWA 00000123 (expires March 10, 2013)

Dr. Michael Mel, Chairperson or
Dr. William Pomat, Deputy Chairperson
Papua New Guinea Institute of Medical Research
Institutional Review Board
PO Box 60
Goroka, EHP 441 Papua New Guinea
Contact: Mrs. Norries Pomat norries.pomat@pngimr.org.pg

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Appendices

Appendix 1: List of Abbreviations Used
Appendix 2: Data and Safety Monitoring Plan/Board (separate document)
Appendix 3: Data collection sheets (separate document to be submitted later)
Appendix 4: Informed Consent Documents (separate documents)
Appendix 5: PNG Standard treatment Guidelines for Anemia

Appendix 1 – List of Abbreviations

ALB	Albendazole sulfoxide
CGHD	Centre of Global Health & Disease, Case Western Reserve University
CRF	Case Report Form
DEC	Diethylcarbamazine
GPELF	Global Program to Eliminate Lymphatic Filariasis
Hb	Hemoglobin
HC	Health Center
HEO	Health Extension Officer
IVER/IVM	Ivermectin
LF	Lymphatic Filariasis
LM	Light microscopy
MDA	Mass Drug Administration
Mf	microfilaria
N	Number (typically refers to subjects)
PI	Principal Investigator
PNG	Papua New Guinea
PNGIMR	PNG Institute of Medical Research
PNG IMR IRB/ IMR IRB	PNG Institute of Medical Research Institutional Review board (IRB)
Og4C3	Monoclonal Antibody that detects a *W. bancrofti* antigen
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
USS	Ultrasound scan
UHCMC IRB/UH IRB	University Hospital Case Medical Center Institutional Review Board
WHO	World Health Organization

Title of the Protocol

Evaluate Triple Drug Therapy with Diethylcarbamize (DEC), Albendazole (ALB) and Ivermectin (IVM) That Could Accelerate LF Elimination Outside of Africa

Principal Investigators

Christopher L. King, M.D., Ph.D. (USA)[1]
James W. Kazura, M.D. (USA)[1]
Peter M. Siba, Ph.D. (Papua New Guinea)[2]

Co-Investigators

Jessica Kumar, D.O., M.P.H.[1]
Peter A. Zimmerman, Ph.D.[1]

Lay Summary

This study will determine if a combination of 3 drugs used to treat the infection that cause lymphatic filariasis (LF) due to *Wuchereria bancrofti* infection are more effective in killing or sterilizing the adult worms compared to just 2 of the 3 drugs that usually given to treat this infection. The three drugs used together are called albendazole (ALB), ivermectin (IVM) and diethylcarbamazine (DEC). The usual treatment in Papua New Guinea (PNG) for lymphatic filariasis are DEC and ALB. A combination of these 3 drugs has not been previously used to treat LF.

Abstract

This study will determine if individuals infected with *Wuchereria bancrofti* and treated with combination of ALB, IVM and DEC compared to the standard treatment of DEC plus ALB in LF endemic areas outside of African can accelerate elimination of LF because of enhanced killing or sterilization of adult worms. Such a drug regimen could reduce the frequency of MDA necessary to control LF in endemic area.

Purpose, Including Specific Aims and/or Hypotheses

Primary Objective is to determine whether a combination of three drugs ALB, IVM and DEC will enhance killing or sterilization of adult *Wuchereria bancrofti* infections, the major causative agent of lymphatic filariasis (LF), compared to the standard treatment of DEC plus ALB.

Background and Significance

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. 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 medication. The most recent summary from WHO reported that more than 1.9 billion doses of MDA were distributed between 2000 and 2007. Thus, the Global Program 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. There are a number of challenges faced by GPELF. These include (among others) inability to conduct MDA programs in areas of Africa where *Loa loa* is coendemic because of the unacceptable risk of Serious Adverse Events (SAE's) with Ivermectin in persons with heavy *L. loa* infections, 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. Drugs used for LF and Oncho MDA are also active against soil transmitted helminths (STH, e.g., *Ascaris*, Hookworm, and *Trichuris*). Suppression of STH is an important ancillary benefit of MDA programs for filarial infections.

It is clear that new (or reformulated) drugs and/or 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.

Rationale for the current study

GPELF policies and mathematical models suggest that current regimens will require many years of MDA with high compliance to achieve elimination (i.e. reduction of transmission rates below sustainable levels). None of the current drugs used for MDA are highly effective in themselves and combination therapy hopes to increase their activity. DEC is the best available drug that is active against both the larval stage in the blood or microfilaria (MF) and is partially effective in killing adult worms. Albendazole has little activity against MF, but some activity against adult worms [1]. Ivermectin is primarily active against MF, but has some activity against adult worms [2]. In this study, a single 400 μg/kg dose of Iver cleared Mf completely in 35% of subjects and reduced the geometric mean Mf level by >98% at 1 year; 2 years after the single treatment, 20% of participants remained Mf negative, and geometric mean Mf levels remained reduced by > 90%. Similar but less profound effects were observed for single dose Iver in a trial conducted in Tanzania. Taken together, these data show that Iver at doses previously shown to be safe and effective for treatment of LF (400 μg/kg) may have embryostatic or embryocidal activity against *W. bancrofti [3]*. Based on the superior activity of IVM against Mf, we assume that the triple therapy regimen of Iver/Dec/Alb will be much more

effective than DEC/Alb for clearing Mf from the blood. It is further hypothesized that the triple regimen will be more effective in killing adult filarial worms and leading to their sterilization. Thus, the triple regimen has the potential to markedly reduce the number of MDA treatments needed to achieve transmission interruption and elimination of LF outside of Africa compared to the time frame of 5 to 7 years projected for DEC/Alb.

DEC, IVM and ALB are very safe and highly effective anti-filarial drugs when given singly or in combination (i.e. DEC with ALB, or IVM with ALB) [4]. In addition, billions of doses of these drugs have administered over the past decade as part of global LF elimination effort, with an outstanding safety profile with the exception of co-infections with *L. loa* as noted above [5]. IVM is unlikely to interfere with pharmacokinetics or pharmacodynamics of DEC and ALB since IVM is primarily excreted through the kidney whereas DEC and ALB are metabolized in the liver, as described in further detail below.

ALB causes degenerative alterations in the tegument and intestinal cells of the worm by binding to the colchicine-sensitive site of tubulin, thus inhibiting its polymerization or assembly into microtubules [6]. The loss of cytoplasmic microtubules leads to impaired uptake of glucose by larval and adult stages of the parasite, and depletes glycogen stores. Degenerative changes in endoplasmic reticulum and mitochondria of the germinal layer, and the subsequent release of lysosomal enzymes result in decreased production of adenosine triphosphate, which is the source of energy required for survival of the helminth. Due to diminished energy production, the parasite is immobilized and eventually dies.

ALB has weak ability to kill Mf, and more effective on killing or sterilizing adult worms compared to IVM, but much less so than DEC.

The drug has been shown occasionally (<1% of treated patients) to cause reversible reductions in total white blood cell count. It has also been associated with slight increases in transaminases in ~16% of patients. The enzymes return to normal levels with cessation of treatment. These abnormalities are associated primarily with prolonged treatment for such diseases as neurocysticercosis and hydatid diseases, not single dose treatment which is being proposed here.

IVM is an avermectin compound of macrocyclic lactones derived from the bacterium *Streptomyces avermitilis* [7]. The mechanism by which IVM kills LF microfilariae is not known with certainty, but the drug interferes with glutamate gated ion channels that can affect parasite contractility and release of immunomodulatory molecules by the parasite [8]. IVM also has a direct effect on the central nervous system and muscle function as it enhances strength of inhibitory neurotransmission pathways.

The main concern with the use of IVM in animals and humans is neurotoxicity, which can be manifest as ataxia. Neurotoxicity is usually associated with prolong therapy with the drug and has not been observed in humans given single dose IVM for LF or other

parasitic infections [9]. IVM has been used to treat millions of people with LF and onchocerciasis [9]. Peak IVM serum concentrations are reached approximately 4-5 hours after administration. The half-life of IVR in various populations ranges from 12 to 56 hours [10]. There is no evidence of drug:drug interaction between ALB and IVM [11].

IVM can cause nausea, dizziness and occasionally pruritus, but these are infrequent, transient and usually mild. Major side effects occur with heavy infections of *L. loa*; however, this parasite is not endemic in Papua New Guinea.

DEC is an anthelminthic drug that is structurally distinct from ALB and IVM [12]. DEC inhibits arachidonic acid metabolism by LF, and inducible nitric oxide synthase and the cyclooxygenase pathway may be essential for activity *in vivo* [13]. DEC also has anti-inflammatory properties. The mechanisms of active of DEC remain poorly understood. Its ability to kill Mf and adult worm depends on the host immune responses since the drug has little direct activity on parasites in vitro. The drug has potent activity against LF microfilaria. DEC has about 50-70% efficacy in killing or sterilization of adult worms [14]. The drug is rapidly absorbed from the gastrointestinal tract, has a serum half-life of 12 to 14 hours, and is excreted in the urine with little modification by liver metabolism. There are few side effects to drug other than killing of adult worms.

In the pilot study [*Pharmacodynamics and Pharmacokinetics Studies for Triple Drug Therapy to Treat Human Lymphatic Filariasis (LF): Diethylcarbamize (DEC), Albendazole (ALB) and Ivermectin (IVM)* [IRB #01-11-01/IMR IRB #1119] comparing 3 versus 2 drug treatment for LF we found that some individuals following the 2 drug treatment had poor clearance of microfilaremia at 36h and 7 days. It is possible that there are strain-specific differences in responses to anti-filarial responses. Recent studies indicate individual patient infections of Wb contain a complex mixture of strains. Data collected from 5 patients for cytochrome oxidase I (COI) revealed the presence of 10-30 strains per individual patient infection. By characterizing the genetic diversity within an infection we can recover and responses to drug exposure.

To determine whether subjects clear their microfilaremia within the first 6 months after initial drug treatment, and whether delay or impaired clearance is related to presence of microfilaria at one year following treatment, and whether there certain strains of parasite that appear less susceptible to treatment we will collect two additional optional blood samples at 6 and 12 months.

Study Design

This will determine whether 1 or 2 annual treatments with the triple drug regimen of DEC/Iver/Alb is equally or more effective than repeated annual treatments with DEC/Alb in inducing sustained clearance of Mf in LF infected subjects previously determined to have >50 Mf/ml, indicative of a moderate to heavy infection. Subjects will be treated and monitored in several common areas near study subject residences.

There will be 3 treatment arms as follows:

1. The *comparator* (standard treatment) DEC 6 mg/kg + Alb 400 mg administered annually (at 0, 12, and 24 months).

2. DEC 6 mg/kg + Alb 400 mg given once

3. DEC 6 mg/kg + Alb 400 mg + Iver 200 µg/kg administered once only at the beginning of the RCT (0 month).

Primary endpoint will be

Percentage of subjects with total clearance of in *W. bancrofti* Mf at 36 months .

Secondary endpoints

1. *Percentag*e of subjects with total clearance of Mf at 24 months.

2. Percent reduction in Mf at 24 months compared to baseline.

3. Percent reduction in *W. bancrofti* antigen levels by Og4C3 assay compared to baseline measured at 24 and 36 months

4. Percent of subjects *who become* ICT negative at 24 months and 36 months after the beginning of the study.

5. Changes in clinical manifestations associated with LF (e.g. lymphedema, hydrocele, lymphangititis/lymphadenitis, and other scrotal or genital abnormalities at 12, 24, and 36 months.

RESEARCH PLAN

Study Procedures

The study will be conducted as an open labelled trial where subjects and people administering the drugs will be unblinded to the treatment. Subjects who do not receive drugs at 12 and 24 months will be given multivitamin tablets. The laboratory personnel, however, who measure Mf levels, ICT card tests or circulating antigens levels will be blinded to the treatment groups.

Randomization procedure A random number table equal to the total number of subjects to be enrolled in the 3 arms with values from 1 to 3. As individuals are enrolled into the study, they will be assigned a number corresponding to one of the 3 arms in sequence from the table.

Treatment: Subjects will be given the treatment medications under direct observation by the study nurse along with a fatty snack (crackers covered with margarine or peanut butter) because fats help absorption of the some of the drugs.

Screening for eligibility and monitoring safety variables: There will 4 steps for the study

1. Subjects will be initially consented and then screened for the presence of LF. At the time of consent it will be made clear to subjects that if they are uninfected or if infected and do not have >50Mf/ml they will not be eligible for the study. Screening will entail a fingerstick night blood sample (after 2100 hours) obtained from adults aged 18-65 years to determine if they are infected with LF and to identify individuals with >50Mf/ml.
2. At a subsequent visit subjects identified with >50Mf/ml will have blood and urine tests performed to evaluate whether they meet the inclusion/exclusion criteria described below.
3. Once individuals have been found to meet the inclusion/exclusion criteria, they will be asked to participate in the study, and be administered the study medications. They will be asked to come to the local health center if they have any fevers, pains consistent with adverse drug reactions. There will be passive surveillance for adverse events up to 1 week post treatment.
4. Individuals will then undergo similar evaluations and receive additional treatment or multivitamins tablets depending on the treatment arms one, two and three years later.

Optional blood draws: Three optional additional blood samples will be collected from study participants. The purpose of these additional samples is to determine how well treatment eliminates parasites from the peripheral blood. The first would be 1 day following the initial treatment for LF, the second at 3 months and the third at 6 months following initial treatment. Pilot studies suggest individuals differ greatly in the rate parasites clearance from peripheral blood after treatment. These studies would help to determine whether there are certain strains of parasites less susceptible to treatment and whether differences in host immune response effects parasite clearance. The 24 hour post-treatment sample would also help to determine why some individuals develop more severe adverse effects following treatment. We will also look at the release of Wolbachia antigens (a bacterial endosymbiont of LF) from dying parasites that are thought to contribute to adverse reactions immediately following treatment. At each collection time-point subjects would be asked for a 10 mL vacutainer of whole blood be obtained between 2200 and 0200 hrs when MF are present in circulation. From this, 1-5 mL blood will be analyzed for by filtration for MF count. Remaining blood will provide sufficient material for DNA and RNA preparation. In the event of low MF counts observed by filtration efforts will be made to concentrate MF by Percoll density gradient centrifugation.

Laboratory Tests and Analysis

Analyses to be performed at PNGIMR Laboratories Maprik and Madang:

Detection of Lymphatic filariasis for diagnosis and monitoring drug efficacy

1) The presence of MF will be determined by examining 60 ul of fingerstick blood for screening purposes. The blood will be placed on a glass slide, dried, fixed and stained with Geimsa and then examined under microscopy for the presence and number of Mf. Immediately prior to treatment and at 12, 24 and 36 months mf levels will be determined by filtering 1 ml of anticoagulated blood through a nucleopore filter, dried, fixed and stained with Geimsa and then examined under microscopy for the presence and number of Mf

2) ICT card test (now manufactured by Alere rather than Binax Inc.)– is a lateral flow card test based on mAB AD12.1 (marketed as the Filariasis Now ICT) to detect antigens released by living adult *W. bancrofti* worms in whole blood from infected subjects.

3) Og4C3 antigen detection ELISA assay marketed as the TropBio Filariasis Antigen, CELISA) based on the mAb Og4C3. The assay detects a heat stable antigen released by living adult *W. bancrofti* worms.. This assay will be performed as described by manufacturer.

4) Preparation of Mf slides and ICT assays will be performed in Dreikiker area, Maprik and/or Madang. The reading of slides for Mf levels will be performed in PNGIMR laboratories in. Maprik and/or Madang.

Additional tests may be performed related to detection of infection and related to drug efficacy such as, but not limited to, humoral and cellular test for immune function.

Clinical tests

Hemoglobin, AST/ALT, creatinine and urinanalysis will be performed prior to exclude hematologic and renal abnormalities.

Source of Drugs Used

Albendazole and DEC will be provided by Global Program to Eradicate Lymphatic Filariasis (GPELF) to PNG. Ivermectin (Stromectol by Merck) will be purchased that is identical to the Ivermectin that is used for mass drug administration (MDA).

Study Population

The study team will contact potentially eligible individuals in the community by community health workers that will employed by study. Individuals with Mf levels >50mf/ml will be offered the opportunity to participate, providing they meet the inclusion

and exclusion criteria indicated below. Infected individuals ineligible for the RCT will be offered the standard treatment of DEC plus ALB.

The study population will reside in filarial endemic areas in East Sepik Province, Papua New Guinea.

Inclusion criteria:

- Men and women 18-65 years
- >50mf/ml in finger stick blood samples
- Willing to give informed consent

Exclusion criteria

- Prior treatment for LF within last 5 years
- Pregnant at time of enrollment (do pregnancy test)
- Hemoglobin < 7 g/dl
- permanent disability, serious medical illness that prevents or impedes study participation and/or comprehension
- AST/ALT and creatinine > 1.5 upper limit of normal.
- Urine dipstick with glucose ≥ 2+ and/or protein ≥ 2+

Any one or more of the above criteria is sufficient to exclude study participation. Anemia will be treated according to Papua New Guinea national standard treatment guidelines.

Subjects who prematurely discontinue from protocol treatment should follow the same evaluation schedule as those who complete protocol treatment. *No follow-up is needed for subjects who do not receive at least one dose of treatment.*

Criteria for Termination from Study

If a subject refuses to have any blood samples taken following the initial treatment they can be terminated from the study. Also if a subject move away from the area such that cannot be examined for any of subsequent annual blood draws, they can also be terminated from the study.

Assessment of Resources

This study, which is part of a larger study at Washington University School of Medicine/St. Louis directed by Dr. Gary Weil, is being funded by the Bill and Melinda Gates Foundation.

Subjects for this study will be recruited from filarial endemic areas of East Sepik Province in Papua New Guinea. The investigators have conducted research studies in Papua New Guinea for over 20 years in this area and have a strong collaborative relationship with the PNG IMR. As a joint project with the Institute of Medical Research

in Papua New Guinea support is available for specimen collection, storage and laboratory testing.

Albendazole and DEC will be provided by Global Program to Eradicate Lymphatic Filariasis (GPELF) to PNG. Ivermectin will be purchased that is equivalent in drug concentration to that used for mass drug administration and is manufactured under good laboratory practices

Storage of Specimens

At the time of each survey, specimen collected for biochemical analysis will be evaluated in Maprik or Madang. The circulating antigen determined by Og4C3 assay will be frozen and sent to the PNG laboratories at Madang for subsequent determination. Serum/plasma and whole blood containing filarial parasite peripheral blood lymphocytes will be stored in freezer in Maprik or Madang In the event that samples are sent outside of Papua New Guinea for testing or storage they will be de-identified. Samples may be stored at one or more of these sites for 35 years. In some cases blood samples will be sent to CWRU or the Laboratory of Parasitic Diseases at the National Institutes of Health for additional analysis for host immune responses, presence of circulation parasite antigens and/or analysis of parasite DNA.

Risks and Discomforts and How Minimized

Risks of Blood Draw

Blood collection via peripheral venipuncture and finger stick is considered to be minimal risk and little or no discomfort is anticipated, similar to a prick. Possible bruising may occur at the venipuncture site. There is a risk of infection but use of sterile technique minimizes the risk for infection. On occasion, a subject may faint, but research staff will be alert to subject reactions after the blood draw and will provide aid as needed.

Risks of Study Drugs

The combinations of Ivermectin plus Albendazole or DEC plus Albendazole are widely used for MDA. There have been clinical trials of DEC plus Ivermectin [18]. Any risk of medications, particularly the combination of IVM, ALB and DEC will be initially assessed in the pharmacodynamics and pharmacokinetics studies performed prior to this study [UHCMC IRB # 01-11-01/IMR IRB #1119].

Risks of each drug separately, with some indication of how likely these are to occur, are summarized below.

> DEC – most common side effects are itching and swelling of face, headache, joint pain, unusual tiredness or weakness. These are transient. Less common are dizziness, nausea or vomiting. Fever, painful and tender glands in groin, neck

armpits or skin rash can occur, and are usually associated with high burdens of infection as judged by the level of blood microfilaria.

ALB - Headache, nausea, stomach pain and vomiting are most common, and usually associated with heavy geohelminth infections. Severe allergic reactions occur rarely, and include rash, hives, itching, difficulty breathing, tightness in the chest, swelling of the mouth, face, lips, or tongue, dark urine, Mild elevation in liver transaminases can occur, but normalize with cessation of treatment. These side effects are usually associated with prolonged ALB therapy.

IVM – The most common side effects are diarrhea, dizziness and nausea. Rare side effects include rash; hives; itching; difficulty breathing; tightness in the chest; swelling of the mouth, face, lips, or tongue; eye pain, swelling, or redness; fainting; fast heartbeat, Specifically mild decrease in leukocyte counts, elevated liver function tests, and cardiovascular effects that included tachycardia, orthostatic hypotension. Infrequently treatment can exacerbate bronchial asthma. These side effects are also associated with prolonged therapy.

Compensation for Injuries

There is no compensation available in the event of injury. It is not anticipated that any injuries will results from this study because the drugs used have been widely used for treatment of lymphatic filariasis with serious side effects.

Benefits to Subject

There will be direct benefit for participation in this study, in that they will be treated for the LF infection. A broader community benefit is that the triple regimen has the potential to markedly reduce the number of MDA treatments needed to achieve transmission interruption and elimination of LF compared to the time frame of 5 to 7 years projected for DEC and Alb. If the intervention proves successful, the triple therapy is likely to be widely adopted in many LF endemic areas. In order to facilitate such an uptake of triple therapy into national treatment policies, the study will liaise closely with national and provincial health authorities.

Costs to the Subject

There is no cost to the subject to participate in this study.

Alternative(s) To Participation

Study subjects may decline participation in this study with no consequences. Subjects with known LF will be referred to local health centers and treated per national guidelines (single annual doses of DEC + ALB for 5-7 years).

Payment to the Subjects (Reimbursement and Incentives)

Subjects will not be paid for participation in this study. The study will cover costs related to laboratory tests as part of the study, study drugs, and clinical monitoring.

Plan for obtaining informed consent (Informed Consent Process)

Description of the Informed Consent Process:

Following standard practice for Papua New Guinea Institute of Medical Research (PNGIMR) field studies, the informed consent process starts several months before enrollment of participants and involves both community and individual consent. The study will be discussed with representatives of provincial Department of Health representatives and mission health services as well as senior community members to assess both feasibility and community acceptability of different study design features and field procedures. The informed consent process recognizes the community and cultural values in Papua New Guinea and the East Sepik region where the study participants reside. Extensive discussion of risks and possible benefits of participation in this study will be provided to the community, the study participants and their relatives. This is accomplished through a series of community meetings (*tok saves*) in which the Principal Investigators, co-investigators and/or PNGIMR representatives explain and discuss the purposes of the research study to residents at study sites and PNGIMR field assistants who may refer study participants for more information about this study. The investigators and study personnel who will obtain consent from study participants all have or will receive training in human subjects regulations and good clinical practices (GCP).

Following consent by community leaders to include their village into the study, the study team will invite all interested adult community members in areas known to have levels of LF infection to attend information meetings held in several central locations throughout the study areas. At these meetings, the study team will describe the purpose and significance of the study, the procedures to be followed, the risks and benefits of participation, and state that participation in the study is voluntary and that declining to participate will not reduce the level of, or access to, health care for the eligible individuals.

Consent forms describing in detail the study procedures and risks will be read at *tok saves* in Melanesian pidgin (Tok Pisin). Formal, written informed consent will be obtained for individuals willing to participate in study. Consent forms will be approved by the UH/Case Medical Center IRB and Papua New Guinea Institute of Medical Research IRB. The subject will be asked to read, or have read to them, and review the informed consent documents. Upon reviewing the document, the investigator and/or study staff will explain the research study to the subject and answer any questions that may arise. Subjects will be given a copy of the consent form and information sheet for their records . In situations when photocopying services are not available subjects will only be given copies of the information sheet.

Only the principal investigators and study staff authorized and certified to obtain consent will consent subjects for this study. Only individuals who have signed the consent form and meet eligibility criteria will be enrolled in the study.

Additional Consent Issues

Training of Papua New Guinea Study Staff in Human Subjects Research Ethics

The Principal and co-investigators will conduct on-site training sessions for Papua New Guinea study staff who will be collecting study information, specimens, and/obtaining consent from subjects in the study. The study will be explained in detail to the local study staff. The basic principles of informed consent process, documentation of informed consent, protection of subjects' rights, confidentiality, and handling of data will be covered in these training sessions. All training sessions will be documented, and study staff monitored by the on-site project manager on a regular basis to ensure compliance with the principles of informed consent. The Principal Investigator will provide training and readings materials on human subjects regulations with an emphasis on informed consent. The field staff in PNG has difficulty with the use of modern technology (computers, mouse etc.) In addition, field staff do not have separate email accounts allowing on-line certification. Obtaining human subjects training certfication using an on-line tutorial such at the CWRU/CITI or NIH on-line program is not feasible with the field staff in PNG. The Principal Investigators will provide the specified training (as outlined in the protocol) and submit a signed attestation for this human subjects training.

It is not the practice of the Center for Global Health & Diseases to ask or require that a subject initial each page of the consent form. The investigators will accept either signed (cursive) or printed signatures or a witnessed mark in the case of illiterate study participants on the consent form.

Because this study takes place in Papua New Guinea and the Principal Investigators (of record) is based in the United States (at CWRU), it is not possible for the PI to sign the consent form within 30 days as required by the UHCMC IRB for studies conducted in Cleveland. Also, the Papua New Guinea PI may not always be available at the time of consent to sign the forms. Consent forms will be reviewed at each visit and the PI will sign the consent forms to verify subject eligibility and that informed consent has been obtained. The onsite Coordinator/Manager will review each consent form to be sure that all required signatures are obtained and subjects meet eligibility criteria. Co-investigators on the study may also review the consent forms and sign on behalf of the Principal Investigators.

Entry into the study and participation will be strictly voluntary. It will be made clear that refusal to participate or withdraw can occur at any time throughout the course of the study and will not influence their rights or the care they receive at local health facilities. It is stated that all information is confidential and coded without personal identifiers. It is also indicated that no monetary or other gains are offered in exchange for participation.

Comprehension of informed consent:

After the *tok save* and at the time the consent form is given to the study participant, the participant will have an opportunity to ask additional questions. To assess comprehension of informed consent and the study, the principal investigator and/or authorized consent study staff will ask the following questions:

Do you understand the consent form?
Do you have to participate in this study?
Will we take blood from you during this study?
Can you refuse to participate in the study at any time?
Is there any charge for being in the study?
Will you receive any money for being in the study?
Do you know who to call if you have questions?

The responses will be documented on the consent form, signed by the research staff or principal investigator, and a copy given to the study volunteer. In the event a subject indicates a lack of understanding of the study, or any aspect of it, the Principal Investigator/study staff will invite questions and offer explanations of any particular point. If, in the judgment of the Principal Investigators, the subject's response still does not reflect an understanding of the study, the subject will not be enrolled in the study.

Provisions for Subjects from Vulnerable Populations

Plan to Address Women Who are or Who Become Pregnant While on Study

Women who are pregnant or breast-feeding at the time of initial enrollment will not be eligible to participate in this study because of the unknown effects of the drugs and drug combination proposed in this study. Women of child bearing age who wish to enroll in the study will have a pregnancy test prior to enrolment in the study to ensure they are not pregnant. Pregnancy tests will be repeated before every drug administration.

This study involved three arms which participants are randomized into at the initial visit. Based on the participants initial randomization, how they will be managed if they become pregnant during the study will vary depending on the group they were initially randomized to. . Below is a description of how pregnancy in each group will be managed:

1. The *comparator* (standard treatment) DEC 6 mg/kg + Alb 400 mg administered annually (at 0, 12, and 24 months).
    - A female participant is pregnant at the 12 month follow up she will moved to Group 2, which received the same dose of study medication (DEC 6 mg/kg + Alb 400 mg) at the initial visit. The participant will be offered a multivitamin pill and will provide blood and urine sample per protocol. The participant will remain in Group B for the remainder to the study.)

- If a female participant is pregnant at the 24 month follow up, she will not be given any study medications for the remainder of the study. She will be offered a multivitamin tablet and will follow the same blood draw collection schedule as those currently receiving active treatment.

2. DEC 6 mg/kg + Alb 400 mg given once
    - No change in study participation. Female participant will be offered a multivitamin pill and continue with the same evaluation schedule
3. DEC 6 mg/kg + Alb 400 mg + Iver 200 µg/kg administered once only at the beginning of the RCT (0 month).

    No change in study participation. Female participant will be offered a multivitamin pill and continue with the same evaluation schedule

Plan for Inclusion of Illiterate Subjects

Study participants, if illiterate, will have the information sheet or consent form read to them by a trained field assistant in the local language. Their signature or mark will be witnessed on the consent form.

Plan for Inclusion of Non-English Speaking Individuals

Subjects who do not speak or read English are neither specifically included nor excluded from this study. It is anticipated that most (if not all) the study participants will be residents of Papua New Guinea. The country has over 800 distinct languages and dialects; the common language used in the country is Melanesian pidgin (*tok pisin*). The consent form will be translated from English into *tok pisin* by a native Papua New Guinean resident who is knowledgeable about research and the study. Once the English consent form is approved it will be translated into *tok pisin* and submitted to the UH Case Medical Center IRB and PNG IMR IRB for review and approval.

Subject Privacy and Data Confidentiality

Privacy of the study participants will be maintained by assigning study participants a unique study identification number (UNID). All data, blood samples and laboratory results will be recorded and analyzed by UNID with no personal identifiers. All information collected, including demographic information about enrolled subjects will be kept confidential and available only to the investigators and authorized study personnel such as the data manager. After the public *tok save's*, potential participants in the study will have an opportunity to sign the consent form in a private place if they choose to be part of the study.

All written forms (i.e. consent and data collection forms) will be stored in the CWRU-PNG IMR designated study areas in Maprik and/or Madang, PNG. All forms will be labeled and filed in filing cabinets with the Study Protocol Number, Principal Investigators' names and collection dates. These cabinets will be metal and have functioning locks. Keys will be kept with the CWRU-PNG IMR Project and/or Data Managers. All data will be double entered by trained PNG Data Entry Clerks and

cleaned by the Data Manager. The data base will be password protected and access to password will be authorized by the Principal Investigators and/or Project Manager. Electronic data files will be stored on a CWRU-operated and -dedicated server located in the assigned CWRU-PNG IMR data rooms. The paper forms will be stored for the duration of the study plus three years per IRB protocol for primary data storage. The electronic database will be stored indefinitely by the PIs.

The Principal Investigators, Co-investigators and key personnel may use the results of this study for publications, presentations at scientific meetings or preliminary data for subsequent grant applications. Confidentiality of study participants will be maintained by not using names or personal identifiers.

The study site Project Coordinator will permit access to all documents and records that may require inspection by the respective funding agencies, governmental regulatory agencies, institutional review boards (both UH IRB and IMR IRB) or its authorized representatives.

Data Collected on Subjects

This study will collect the following types of data on each study participant as part of the study. The only information that will be recorded in the subject's permanent medical record is that they were diagnosed with LF and received a single or multiple treatments.

- Demographic data (at enrollment only). This will include age, sex, location of subjects, etc.
- Prior history of any symptoms related to LF; chronic symptoms and signs of lymphedema and/or hydroceles and history of acute symptoms of lymphangitis and lymphadenitis at onset of the study and throughout the 3 years of evaluation as determined by questionnaires and/or physical examination.
- Prior treatment for LF (although no mass drug therapy has been performed in this population).
- Subjective behavioral and clinical data (e.g. feeling of nausea, neurological or other subjective data related to possible side effects) within the first week following treatment
- Hemoglobin levels AST/ALT creatinine urinanalysis at initial evaluation. These will only be collected at the beginning of the study unless the subject experience severe side effects that might affect these laboratory parameters.
- Prevalence and intensity of mf levels and geohelminths in stool.
- The determination of circulating antigen using the Og4C3 mAb will be measured in the Madang laboratories and biochemical analysis in the clinical laboratory at clinical laboratories in hospitals in either Maprik, and/or Madang.

Request for Waiver of HIPAA

A request for waiver of HIPAA for PHI based on the cultural environment and customs in Papua New Guinea accompanies this submission.

Data Analysis Plan

The *primary endpoint* will be the percentage of people with total clearance in *W. bancrofti* Mf compared to baseline measured at 3 years after treatment. Since the goal is to see if adding IVM to DEC plus albendazole is superior to eliminating mf positivity at 3 years after a single dose, the primary hypothesis will be whether DEC+ALB+IVM is superior to DEC+ALB given once. The second hypothesis is that DEC+ALB+IVM given once will be non-inferior to the percent reduction in microfilaremia obtained by the comparator arm of DEC+ALB given annually x 3. We anticipate the DEC+ALB given once (arm 2) to show the lowest percent reduction in mf at 36 months and the comparator arm, DEC+ALB given at 0, 12, and 24 months to show greatest reduction.

A similar analysis between the same groups will be analyzed for the secondary endpoints listed above:

Since the study groups will be randomized, comparison of the primary endpoints will examined using a Student's *t* test for continuous variables and chi-square analysis for percentages.

Sample size determination:

Since the goal is to see if adding IVM to DEC plus albendazole is clearly superior to eliminating mf positivity at 3 years after a single dose, the primary hypothesis will be whether DEC+ALB+IVM administered once is superior to DEC+ALB given once. Based on preliminary data and the literature we anticipate that DEC+ALB+IVM will achieve a 75% clearance compared to 50% to DEC+ALB given once at 36 months among individuals with moderate to high parasitemia levels (i.e. >100mg/ml). For this comparison, 46 is required in each arm with a power of 0.8 and one-side test at alpha=0.05. The second hypothesis is that DEC+ALB+IVM given once will be non-inferior to the percent reduction in microfilaremia obtained by the comparator arm of DEC+ALB given annually x 3. Based on preliminary data the comparator arm will achieve a 95% clearance at 36 months, if the single triple drug is non-inferior to standard treatment that is significantly less than 15%, we will need 54 in each, for a power of 0.8 and alpha=0.05. Therefore given a dropout rate of 30% over 3 years we will need 70 individuals per group. Given the possibility of even a higher drop-out rate we aim to recruit 75 individuals per arm or a total of 225 subjects.

Data and Safety Monitoring Plan

Procedures to be followed in the event of serious adverse reactions after drug intake

If SAE occurs the subject will be referred to the Boram Hospital in Wewak where investigation and treatment will be provided through the East Sepik provincial

government facilities. SAEs will be reported to the institutional review boards per guidelines and timelines. In addition, all SAEs must be reported to the DSMB and the sponsor.

There is a formal Data Safety Monitoring Committee or Board (DSMB). See Appendix #2 for a description of the committee and membership.

Plans for the Subjects at the End of the Protocol

Prior to publication of the results, PNGIMR, the study population, district health services, and members of the study team will be informed of general findings arising from analysis of study data and the study outcomes. This will occur through written correspondences and face-to-face discussions with collaborators at PNGIMR. Communications with district health services will occur based on advice from the collaborators at PNGIMR through written correspondences and face-to-face discussions. Presentation of general results from the study to local community leaders, reporters and community-based study participants will occur through village-based tok saves. Individuals in the study will continue annual treatment with Alb and DEC annually for up to 5 years according PNG national guidelines.

Approval by Foreign/Local IRB

In addition to review and approval by the UH Case Medical Center IRB, this study will be submitted to the Papua New Guinea Institute of Medical Research's IRB (IMR IRB) for review and approval of the protocol and the potential use of previously collected blood samples under MRAC approved protocol. As a new study, this protocol will also be submitted by the IMR IRB to the Medical Research Advisory Council (MRAC) for final approval. The study will not begin until all required ethical and regulatory reviews are completed.

FWA 00000123 (expires March 1, 2018)

Dr. Michael Mel, Chairperson or
Dr. William Pomat, Deputy Chairperson
Papua New Guinea Institute of Medical Research
Institutional Review Board
PO Box 60
Goroka, EHP 441 Papua New Guinea
Contact: Mrs. Norries Pomat norries.pomat@pngimr.org.pg

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Appendices

Appendix 1: List of Abbreviations Used
Appendix 2: Data and Safety Monitoring Plan/Board (separate document)
Appendix 3: Data collection sheets
Appendix 4: Informed Consent Documents (separate documents)
Appendix 5: PNG Standard treatment Guidelines for Anemia

Appendix 1 – List of Abbreviations

ALB	Albendazole sulfoxide
CGHD	Centre of Global Health & Disease, Case Western Reserve University
CRF	Case Report Form
DEC	Diethylcarbamazine
GPELF	Global Program to Eliminate Lymphatic Filariasis
Hb	Hemoglobin
HC	Health Center
HEO	Health Extension Officer
IVER/IVM	Ivermectin
LF	Lymphatic Filariasis
LM	Light microscopy
MDA	Mass Drug Administration
Mf	microfilaria
N	Number (typically refers to subjects)
PI	Principal Investigator
PNG	Papua New Guinea
PNGIMR	PNG Institute of Medical Research
PNG IMR IRB/ IMR IRB	PNG Institute of Medical Research Institutional Review board (IRB)
Og4C3	Monoclonal Antibody that detects a *W. bancrofti* antigen
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
UHCMC IRB/UH IRB	University Hospital Case Medical Center Institutional Review Board
WHO	World Health Organization

Summary of Protocol Changes for 17-06854 – A Randomized Controlled Trial of a Novel Triple Drug Treatment for Lymphatic Filariasis; Christopher King, MD, PhD

Initial Protocol Version – March 29, 2012

Amendment #1: Protocol Version updated to October 23, 2012

Rationale for amendment: In the pilot study [*Pharmacodynamics and Pharmacokinetics Studies for Triple Drug Therapy to Treat Human Lymphatic Filariasis (LF): Diethylcarbamize (DEC), Albendazole (ALB) and Ivermectin (IVM)* [IRB #01-11-01/IMR IRB #1119] comparing 3 versus 2 drug treatment for LF we found that some individuals following the 2 drug treatment had poor clearance of microfilaremia at 36h and 7 days. It is possible that there are strain-specific differences in responses to anti-filarial responses. Recent studies indicate individual patient infections of Wb contain a complex mixture of strains. Data collected from 5 patients for cytochrome oxidase I (COI) revealed the presence of 10-30 strains per individual patient infection. By characterizing the genetic diversity within an infection we can recover and responses to drug exposure. To determine whether subjects clear their microfilaremia within the first 6 months after initial drug treatment, and whether delay or impaired clearance is related to presence of microfilaria at one year following treatment, and whether there certain strains of parasite that appear less susceptible to treatment we will collect two additional optional blood samples at 6 and 12 months.

We would like to analyze the complexity of Wb infection in patients participating in this study. This study is already planning to collect blood samples suitable for Wb DNA sequencing experiments at pre-treatment and Day-7 time points. In this amendment we are requesting that two optional additional blood samples be collected from subjects participating in the study. These samples would be collected at 6- and 12-month time-points. At each collection time-point we request that a 5 mL vacutainer of whole blood be obtained between 2200 and 0200 hrs when MF are present in circulation. From this, 1 mL blood will be analyzed for by filtration for MF count. Remaining blood will provide sufficient material for DNA preparation. In the event of low MF counts observed by filtration efforts will be made to concentrate MF by Percoll density gradient centrifugation.

Subjects who may already be enrolled in the study will be re-consented with the revised consent document indicating the two optional blood draws at 6 and 12 months.

This amendment does not increase risk to study participants.

Two co-investigators are being deleted from the study (Edward Thomsen and Luis Apolos). Two investigators are being added to the study (Peter A. Zimmerman, Ph.D. and Jessica Kumar, D.O.).

The iRIS application form is revised to reflect the amendment comments and the deletion/addition of co-investigators.

The consent documents are revised to reflect the addition of two optional blood draws at 6 and 12 months.

Summary of Protocol Changes for 17-06854 – A Randomized Controlled Trial of a Novel Triple Drug Treatment for Lymphatic Filariasis; Christopher King, MD, PhD

Amendment #2

This is an administrative revision to the protocol dated October 23, 2012 to add Peter A. Zimmerman, Ph.D. back in as a co-investigator on the study. There are no other changes to the protocol. The consent documents have not been changed and retain the date of October 23, 2012.

The iRIS Application has been changed to reflect this addition of Peter Zimmerman as a co-investigator.

Amendment #3 (included with Annual Review) Protocol Version updated to July 8, 2013

The protocol and consent documents are being revised to add an additional blood draw (in addition to the two previously approved optional blood draws). (See pages 6-7.)

"Optional blood draws: *Three optional additional blood samples will be collected from study participants. The purpose of these additional samples is to determine how well treatment eliminates parasites from the peripheral blood. The first would be 1 day following the initial treatment for LF, the second at 3 months and the third at 6 months following initial treatment. Pilot studies suggest individuals differ greatly in the rate parasites clearance from peripheral blood after treatment. These studies would help to determine whether there are certain strains of parasites less susceptible to treatment and whether differences in host immune response effects parasite clearance. The 24 hour post-treatment sample would also help to determine why some individuals develop more severe adverse effects following treatment. We will also look at the release of Wolbachia antigens (a bacterial endosymbiont of LF) from dying parasites that are thought to contribute to adverse reactions immediately following treatment. At each collection time-point subjects would be asked for a 10 mL vacutainer of whole blood be obtained between 2200 and 0200 hrs when MF are present in circulation. From this, 1-5 mL blood will be analyzed for by filtration for MF count. Remaining blood will provide sufficient material for DNA and RNA preparation. In the event of low MF counts observed by filtration efforts will be made to concentrate MF by Percoll density gradient centrifugation."*

The consent documents have been updated to reflect the addition of an optional blood draw as well as a new version date to reflect the new version date of the protocol—July 8, 2013.

The protocol is also being revised to indicate that **alternative/comparable study sites in West Sepik Province and Madang Province** may be substituted for study sites in East Sepik Province, PNG. This will allow flexibility in subject recruitment when villages in the study site provinces are not available for recruitment as planned. The main consent document (English and translated) were modified to added "or West Sepik Province or Madang Province" to reflect this revision.

There are no other changes to the protocol or consent documents. These changes do not increase subject risk.

Summary of Protocol Changes for 17-06854 – A Randomized Controlled Trial of a Novel Triple Drug Treatment for Lymphatic Filariasis; Christopher King, MD, PhD

New Title (only version date change):

Evaluate Triple Drug Therapy with Diethylcarbamize (DEC), Albendazole (ALB) and Ivermectin (IVM) That Could Accelerate LF Elimination Outside of Africa **[version dated July 8, 2013]**

Amendment #4

The Application is being amended to add Dr. Jessica Kumar as a person to obtain consent on this study. The CWRU/CITI expiration dates for selected investigators have been updated. No other changes to this study.

Amendment #5

The following are being added as study staff in Papua New Guinea who will be able to obtain consent for this study:

Nelly Sanuku
Delma Beaso
James Suamani
Elite Maki
Barth Lombore
Samson Santofan

The study staff from the Papua New Guinea Institute of Medical Research were trained on November 16, 2013 in Papua New Guinea.

A copy of the signed Human Subjects Training Attestation Form accompanies this submission.

Amendment #6 (included with Annual Review) Protocol Version updated to June 9, 2014

The protocol is being amended to change the inclusion criteria from >100mf/ml in finger stick blood samples to >50mf/ml. Most screening failures occurred because of insufficient microfilaria levels to meet the inclusion criterion.

This change will not increase subject risk.

There are no changes to the consent documents with the exception of the version date in the footer to correspond to the revised date of the protocol.

Dr. Inoni Betuela is being deleted as a co-investigator on the study; Manasseh Baea is being deleted as study staff. Yao-Chieh "Jack" Cheng is being added as study staff with ability to obtain consent.

Summary of Protocol Changes for 17-06854 – A Randomized Controlled Trial of a Novel Triple Drug Treatment for Lymphatic Filariasis; Christopher King, MD, PhD

Amendment #7
The consent documents are being revised to change the study contact coordinators and contact phone numbers. The version date of the consent documents remains the same--June 9, 2014.

Amendment #8 (included with Annual Review) version updated to May 27, 2015
Because the study is conducted in PNG, photocopying may not always be available and it may not be logistically possible to provide subjects with copies of both the consent form and information sheet for their own personnel record. All subjects will be provided with and sign the approved consent form prior to initiation of any study procedure. When copying services are not available, subjects will be provided with a copy of the information sheet only for their own personnel record. The following information was added to the iRIS application under section 10.3 (Plan for Obtaining Informed Consent) and in the protocol on page 12 of 20; "Subjects will sign the approved consent form prior to initiation of any study related procedure. Subjects will be given a copy of the consent form and information sheet for their own personnel record. In situations when photocopying services are not available subjects will only be given copies of the information sheet to keep for their personnel record.

The Information Sheet (English and Tok Pidgin/Melanesian Pidgin) have been revised to include the telephone number for the PNG IMR IRB.

Amendment #9 At the continuing review, changes were made to the protocol, iRIS application & consent form. It was note when the approval was received that the version date was not updated in the protocol title, consent form footer (English & translated), information sheet footer and protocol. This amendment is being submitted to update the version dates of the study documents to May 27, 2015.

Amendment #10 change of version date to November 23, 2015
This protocol amendment was initiated as a result of a protocol deviation submission (iRIS ref# 054131) which involved a female participant who the study team discovered was pregnant at the time of her 12 month visit. The currently approved protocol lists pregnancy as an exclusion criteria. The study is being amendment to allow the inclusion of pregnant women to be followed if they become pregnant during the course of the study. As part of the approved protocol all female participants have a pregnancy test prior to study drug administration. Because this study involves three randomization arms, the arm the female participant is randomized to will determine how she is managed if she becomes pregnant during the study. See summary below:
1. The *comparator* (standard treatment) DEC 6 mg/kg + Alb 400 mg administered annually (at 0, 12, and 24 months).
   - A female participant who is pregnant at the 12 month follow up will be moved to Group 2, which received the same dose of study medication (DEC 6 mg/kg + Alb 400 mg) at the initial visit. The participant will be offered a multivitamin pill and will provide blood and urine sample per protocol. The participant will remain in Group B for the remainder to the study.

   - If a female participant is pregnant at the 24 month follow up, she will not be given any study medications for the remainder of the study. She will be offered a multivitamin

tablet and will follow the same blood draw collection schedule as those currently receiving active treatment.

2. DEC 6 mg/kg + Alb 400 mg given once
   - No change in study participation. Female participant will be offered a multivitamin pill and continue with the same evaluation schedule

3. DEC 6 mg/kg + Alb 400 mg + Iver 200 μg/kg administered once only at the beginning of the RCT (0 month).
   - No change in study participation. Female participant will be offered a multivitamin pill and continue with the same evaluation schedule

Amendment #11 (included with CR) (iRIS reference #057098)
The protocol and iRIS application have been revised to ensure that both documents reflect the inclusion criteria that participants have >50mf/mL.

Amendment #12 Protocol version changed to October 4, 2016
The protocol, iRIS application and consent forms (English & Tok Pisin) are being revised to remove ultrasound procedures from this study.

This amendment is related to a protocol deviation submitted (iRIS ref#063397) in response to a monitoring visit conducted by the UH ORC Office.

The initial approved protocol included a plan to preform ultrasound examinations on all male participants at 0, 12, 24, and 36 months after treatment for the presence of worm nests. Once the study got underway, an individual was hired to go to PNG and train the study staff on how to perform the ultrasound. A week before the trainer was scheduled to be in PNG this individual has a heart attack and was unable to travel. Because there was no local sonographer in PNG who was qualified to train study staff this portion of the study was not conducted. Not conducting the ultrasounds did not adversely affect the participant's rights, their safety, welfare, or the integrity of the study data.

Since there was no qualified sonographer in PNG to train study staff, the decision was made to not conduct this portion of the study.

The study team informed participants during their study visits that the ultrasound portion of the study would not be conducted.

Final Protocol Version – October 4, 2016

Original Statistical Analysis Plan for: 17-06854 – A Randomized Controlled Trial of a Novel Triple Drug Treatment for Lymphatic Filariasis; Christopher King, MD, PhD

Data Analysis Plan

The *primary endpoint* will be the percentage of people with total clearance in *W. bancrofti* Mf compared to baseline measured at 3 years after treatment. Since the goal is to see if adding IVM to DEC plus albendazole is superior to eliminating mf positivity at 3 years after a single dose, the primary hypothesis will be whether DEC+ALB+IVM is superior to DEC+ALB given once. The second hypothesis is that DEC+ALB+IVM given once will be non-inferior to the percent reduction in microfilaremia obtained by the comparator arm of DEC+ALB given annually x 3. We anticipate the DEC+ALB given once (arm 2) to show the lowest percent reduction in mf at 36 months and the comparator arm, DEC+ALB given at 0, 12, and 24 months to show greatest reduction.

A similar analysis between the same groups will be analyzed for the secondary endpoints listed above:

Since the study groups will be randomized, comparison of the primary endpoints will examined using a Student's *t* test for continuous variables and chi-square analysis for percentages.

Sample size determination:

Since the goal is to see if adding IVM to DEC plus albendazole is clearly superior to eliminating mf positivity at 3 years after a single dose, the primary hypothesis will be whether DEC+ALB+IVM administered once is superior to DEC+ALB given once. Based on preliminary data and the literature we anticipate that DEC+ALB+IVM will achieve a 75% clearance compared to 50% to DEC+ALB given once at 36 months among individuals with moderate to high parasitemia levels (i.e. >100mg/ml). For this comparison, 46 is required in each arm with a power of 0.8 and one-side test at alpha=0.05. The second hypothesis is that DEC+ALB+IVM given once will be non-inferior to the percent reduction in microfilaremia obtained by the comparator arm of DEC+ALB given annually x 3. Based on preliminary data the comparator arm will achieve a 95% clearance at 36 months, if the single triple drug is non-inferior to standard treatment that is significantly less than 15%, we will need 54 in each, for a power of 0.8 and alpha=0.05. Therefore given a dropout rate of 30% over 3 years we will need 70 individuals per group. Given the possibility of even a higher drop-out rate we aim to recruit 75 individuals per arm or a total of 225 subjects.

Final Statistical Analysis Plan for: 17-06854 – A Randomized Controlled Trial of a Novel Triple Drug Treatment for Lymphatic Filariasis; Christopher King, MD, PhD

Data Analysis Plan

The *primary endpoint* will be the percentage of people with total clearance in *W. bancrofti* Mf compared to baseline measured at 3 years after treatment. Since the goal is to see if adding IVM to DEC plus albendazole is superior to eliminating mf positivity at 3 years after a single dose, the primary hypothesis will be whether DEC+ALB+IVM is superior to DEC+ALB given once. The second hypothesis is that DEC+ALB+IVM given once will be non-inferior to the percent reduction in microfilaremia obtained by the comparator arm of DEC+ALB given annually x 3. We anticipate the DEC+ALB given once (arm 2) to show the lowest percent reduction in mf at 36 months and the comparator arm, DEC+ALB given at 0, 12, and 24 months to show greatest reduction.

A similar analysis between the same groups will be analyzed for the secondary endpoints listed above:

Since the study groups will be randomized, comparison of the primary endpoints will examined using a Student's *t* test for continuous variables and chi-square analysis for percentages.

Sample size determination:

Since the goal is to see if adding IVM to DEC plus albendazole is clearly superior to eliminating mf positivity at 3 years after a single dose, the primary hypothesis will be whether DEC+ALB+IVM administered once is superior to DEC+ALB given once. Based on preliminary data and the literature we anticipate that DEC+ALB+IVM will achieve a 75% clearance compared to 50% to DEC+ALB given once at 36 months among individuals with moderate to high parasitemia levels (i.e. >100mg/ml). For this comparison, 46 is required in each arm with a power of 0.8 and one-side test at alpha=0.05. The second hypothesis is that DEC+ALB+IVM given once will be non-inferior to the percent reduction in microfilaremia obtained by the comparator arm of DEC+ALB given annually x 3. Based on preliminary data the comparator arm will achieve a 95% clearance at 36 months, if the single triple drug is non-inferior to standard treatment that is significantly less than 15%, we will need 54 in each, for a power of 0.8 and alpha=0.05. Therefore given a dropout rate of 30% over 3 years we will need 70 individuals per group. Given the possibility of even a higher drop-out rate we aim to recruit 75 individuals per arm or a total of 225 subjects.

Summary of all Amendments to the Statistical Analysis Plan Summary for 17-06854 – A Randomized Controlled Trial of a Novel Triple Drug Treatment for Lymphatic Filariasis; Christopher King, MD, PhD

There are no changes between the initial and final version of the Statistical Analysis plan for the protocol referenced above.