Cross-reactive antigenemia and treatment-related adverse events in loiasis

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1. Ethical clearance request

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To the President of the Cameroon National Ethics Committee for Research in Human Health (CNERSH)

Subject: Request for Ethical Clearance

Dear Mr. President,
I am writing on behalf of the investigators (including myself) of the collaborative research project entitled **'' Cross-reactive antigenemia and treatment-related adverse events in loiasis''** to request for ethical clearance.

Lymphatic Filariasis (LF) is a debilitating parasitic infection endemic in tropical countries (including Cameroon) and targeted for elimination by 2020. The strategy to eliminate LF relies on yearly Mass Drug Administration (MDA) of albendazole and ivermectin (IVM) or diethylcarbamazine (DEC). Despite the success of this strategy (the transmission interruption have been achieved in many countries), none of the 11 loiasis-endemic African nations have interrupted LF transmission, because of (i) cross reactivity of LF-RDT with *Loa loa*, and (ii) severe adverse events (SAEs) potentially fatal that have been reported among persons with very high *L. loa* microfilaria counts treated with IVM or DEC against LF or onchocerciasis. The objective of this study is to determine what are the cross-reactive *L. loa* antigens, and investigate how and when are they released, as well as whether their release contribute to treatment-related SAEs observed in persons with heavy loiasis infections. This study will provide insights in the progress towards elimination of LF.

I remain at your disposal to bring any additional information if needed.
Yours sincerely,

Prof Joseph Kamgno.

2. Contents

3. List of abbreviations

AEs: Adverse Events
CFA: Circulating Filarial Antigen
DALYs: Disability-Adjusted Life Years
DEC: Diethylcarbamazine
FTS: Filariasis Test Strip
GPELF: Global Program to Eliminate Lymphatic Filariasis
ICT: Immuno-Chromatographic Test
IVM: Ivermectin
LF: Lymphatic filariasis
MDA: Mass Drug Administration
Mf: Microfilaria
MMDP: Morbidity Management and Disability Prevention
NTD: Neglected Tropical Disease
RDT: Rapid Diagnostic Test
SAEs: Severe Adverse Events
TaNT: Test and Not Treat
WHO: World Health Organization

4. Summary of the proposal

Lymphatic filariasis (LF) is a disfiguring and disabling, mosquito-borne, parasitic neglected tropical disease. The majority of LF worldwide, and all LF in Africa, is caused by the filarial worm, *Wuchereria bancrofti.* The Global Program to Eliminate Lymphatic Filariasis (GPELF) is the world's largest disease elimination program relying on population-based mass drug administration (MDA). Since its inception in 2000, more than 7.1 billion doses of LF elimination medications have been administered to more than 890 million people. However, none of the 11 loiasis-endemic African nations have interrupted LF transmission, partly due to two major obstacles in loiasis-endemic populations. (1) Severe adverse events (AEs), including coma and death, which sometimes occur when LF MDA medications are given to persons with heavy loiasis infections (>20,000 microfilariae per mL of blood); (2) Up to 6% of persons in loiasis-endemic populations test positive by LF rapid diagnostic tests (RDTs) in the absence of *W. bancrofti* infection. Because a filarial antigenemia prevalence of 1% is the GPELF threshold for implementing MDA for LF, cross-reactivity with loiasis makes it impossible for national programs to determine with certainty where LF MDA is required and when it can be stopped. Therefore, improved RDTs that can reliably distinguish *W. bancrofti* infection from cross-reactive loiasis are urgently needed to prevent unnecessary and potentially harmful mass drug administration in areas where LF is not endemic.

This study aims to determine what the cross-reactive *L. loa* antigens are, how and when they are released, and whether their release contributes to treatment-related SAEs observed in persons with heavy loiasis infections.

5. Résumé du protocole

La filariose lymphatique (FL) est une maladie tropicale négligée parasitaire à transmission vectorielle (moustique) débilitante et invalidante. La plupart des cas d'infection associés à FL dans le monde, et tous ceux retrouvés en Afrique, est due à la filaire *Wuchereria bancrofti.* Le Programme Global pour l'Elimination de la Filariose Lymphatique (GPELF) s'appuie sur la distribution de masse des médicaments aux populations à risque. Depuis sa création en 2000, plus de 7,1 milliards de doses de médicaments (albendazole + ivermectine/diethylcarbamazine) contre la FL ont été

distribués à près de 890 millions de personnes à travers le monde. Cependant, aucun des 11 pays Africains où la loase est endémique n'a réussi à interrompre la transmission de la filariose lymphatique pour deux principales raisons : (1) les effets secondaires graves (ESG) tels que le coma et la mort, qui surviennent souvent lorsque l'ivermectine ou la diethylcarbamazine sont administrés, dans la cadre de la lutte contre la FL, aux personnes ayant une forte charge de loase (> 20 000 microfilaires par ml de sang); (2) près de 6% de personnes dans les zones d'endémie à la loase réagissent (testées positives) aux tests de diagnostic rapide (TDR) de la FL, même en l'absence du parasite responsable de la FL (*W. bancrofti*). Compte tenu du fait qu'un taux d'antigènes filariens de 1% est recommandé par le GPELF pour l'implémentation de la distribution de masse des médicaments contre cette maladie, la réactivité croisée des antigènes de la loase rend impossible l'identification avec certitude des zones où la distribution de masse est nécessaire et là où elle doit être stoppée. Ainsi, une amélioration de l'efficacité des TDRs à distinguer l'infection à *W. bancrofti* de la loase est urgemment nécessaire pour éviter une administration de masse de médicaments inutile et potentiellement dangereuse dans les zones où la FL n'est pas endémique.

Cette étude a pour objectifs de déterminer les antigènes de la loase ayant une réaction croisée avec les TDR dédiés à la FL, quand et comment ces antigènes sont produits et si leur libération contribue aux effets secondaires graves associés au traitement des personnes ayant une forte charge de loase.

6. Introduction

Lymphatic filariasis (LF) is a vector-borne neglected tropical disease (NTD) targeted for elimination as a public health problem. The infection transmitted by different species of mosquitoes (*Culex, Anopheles* and *Aedes*) is caused by thread-like filarial worms (nematodes) (CDC, 2019). In majority of the cases (90%), the infection is caused by *Wuchereria Bancrofti* and remainder by *Brugia* Species (*Brugia malayi* & *Brugia timori*). It is estimated that 120 million people in tropical and subtropical areas of the world are infected with lymphatic filariasis; among which, almost 25 million have genital disease (most commonly hydrocele in men) and almost 15 million (both men and women) have lymphoedema of the leg. The majority (57%) of population requiring preventative chemotherapy lives in the South-East Asia Region (9 countries) and remaining (37%) live

in the WHO Africa Region (35 countries). WHO estimates indicate that around 36 million people are living with these chronic disease manifestations globally. LF accounts for at least 2.8 million Disability-Adjusted Life Years (DALYs); this does not include significant co-morbidity of mental illness commonly experienced by patients and their caregivers (WHO, 2018). Fortunately, LF elimination is possible by interrupting the transmission cycle. Providing treatment on a large-scale to entire communities where the infection is present can stop the spread of infection. A key intervention strategy is MDA in all endemic areas with a microfilaria (Mf) or antigen prevalence of at least 1% (GPELF, 2017).

The Global Programme to Eliminate LF (GPELF), the world's largest disease elimination program relying on population-based mass drug administration, has worked towards elimination for nearly two decades. Since its inception in 2000, more than 7.1 billion doses of LF elimination medications have been administered to more than 890 million people (WHO, 2018). However, the disease is still persisting in 11 countries in Central and West Africa where Loiasis is also endemic. Loiasis, better known as the African eye worm, is a parasitic disease caused by the filarial nematode *Loa loa* and transmitted to humans by tabanids belonging to the genus *Chrysops*. It afflicts approximately 10 million people and clinical symptoms include localized skin swelling and irritation due to migration of the adult worms in subcutaneous tissue, as well as occasional passage of a worm across the eye under the bulbar conjunctiva (Boussinesq, 2006). Although loiasis has traditionally been considered benign and not classified on the WHO' official list of NTD (Metzger WG 2014), it poses two major obstacles for LF elimination. First, Severe adverse events (AEs) including coma and death sometimes occur when LF MDA medications are given to persons with heavy loiasis infections (>20,000 microfilariae per mL of blood) (Gardon *et al.,* 1997). Secondly, up to 6% of persons in loiasis-endemic populations test positive by LF-RDTs in the absence of *W. bancrofti* infection (Pion *et al.,* 2016 ; Wanji *et al.,* 2015). Therefore, there is a need for a rapid test that can reliably distinguish *W. bancrofti* infection from cross-reactive loiasis.

Recently many *L. loa* antigens that cause RDT cross-reactivity in two samples from persons with loiasis were identified. These included both secreted and somatic antigens, suggesting indiscriminate release of *L. loa* antigen from dying adult worms and/or microfilariae (Hertz *et al.,* 2018). A similar pattern of antigen release has been observed

following treatment of microfilaremic LF patients with medications known to kill microfilariae (Anderson and Hertz, unpublished data), this suggests that filarial antigens might be released from dying worms. In addition, it was found that cross-reactivity with LF-RDTs is not constant in many loiasis patients despite persistence of high microfilarial loads. All these data above suggest intermittent release and/or clearance of cross-reactive antigen in persons with loiasis. Understanding how, why, and when cross-reactive Loiasis antigen are released and cleared will be essential to defining strategies to distinguish true LF-RDT positives from cross-reactive loiasis.

7. Research questions

This study addresses the following questions:

- What are the cross-reactive *L. loa* antigens, and how and when are they released?
- Does their release and their quantity correlate with the occurrence and the severity of treatment-related AEs and cytokine profiles?

8. Research hypothesis

- Death of *L. loa* microfilariae releases antigens responsible for RDT cross-reactivity, and an imbalance in release and clearance of these antigens accounts for their intermittent presence in serum.
- The antigen profile of those with spontaneous antigenemia will not differ significantly from ivermectin-induced antigenemia.
- The nature and duration of treatment-related adverse events will correlate with the presence and levels of cross-reactive antigen and cytokine profiles.

9. Objectives

9.1. General objective

- To define the cross-reactive antigen profile of persons with spontaneous loiasis antigenemia and in those treated with Ivermectin, and to determine whether specific *L. loa* antigens are related to treatment-related adverse events.

9.2. Specific objectives

- To characterize and quantify spontaneous cross-reactive *L. loa* antigens;

- To characterize and quantify treatment-induced cross-reactive *L. loa* antigens;
- To compare spontaneous and treatment-induced cross-reactive *L. loa* antigens profiles and define the kinetics of antigen clearance;
- To determine whether specific *L. loa* antigens are associated with the occurrence and severity of treatment related AEs and cytokine profiles.

10. Litterature review

LF is a vector-borne neglected tropical disease targeted for elimination as a public health problem. The infection transmitted by different species of mosquitoes (*Culex*, *Anopheles* and Aedes) is caused by a thread like filarial nematodes roundworms (CDC, 2019). Three species of lymphatic dwelling filarial worms (*Wuchereria bancrofti*, *Brugia malayi* and *B. timori*) cause lymphatic filariasis in humans. The majority of the cases worldwide (90%), and all cases of LF in Africa are caused by *Wuchereria Bancrofti* (Michael *et al.,* 1996). Adult worms reside in the lymphatic vessels interrupting the normal function of the lymphatic system. The worms have life span of about 6–8 years and produce millions of Mf (immature larvae) that circulate in the blood. Mosquitoes are infected with Mf by consuming blood when biting an infected person. Mf then mature into the infective L3 larval stage within the mosquito. When mosquitoes harbouring L3 larvae bite people, the larvae are deposited on the skin from where they can enter the body. The L3 larvae then migrate to the lymphatic vessels where they develop into adult worms, thus continuing a cycle of transmission (CDC, 2019). LF infection can cause a variety of clinical manifestations, including genital disease (hydrocele and/or chylocele) and lymphoedema of the limbs, which can be complicated by recurrent bacterial superinfections called acute attacks, which are extremely painful and are accompanied by fever. The majority of infected people are asymptomatic, but virtually all of them have subclinical lymphatic damage. It takes years to manifest chronic and disfiguring conditions. These conditions lead to mental, social and financial losses contributing to social stigma and poverty (WHO, 2018).

It is estimated that 120 million people in tropical and subtropical areas of the world are infected with lymphatic filariasis; among them, almost 25 million men have genital disease (most commonly hydrocele) and almost 15 million men and women, have lymphoedema of the leg. The majority (57%) of population requiring preventative

chemotherapy live in the South-East Asia Region (9 countries) and remaining (37%) live in the African Region (35 countries). An estimation of WHO indicated that around 36 million people are living with these chronic disease manifestations globally. LF accounts for at least 2.8 million DALYs; this does not include significant co-morbidity of mental illness commonly experienced by patients and their caregivers (WHO, 2018).

Elimination of LF as a public health problem means reducing infection prevalence in an area to below target thresholds and providing the recommended basic package of care in all areas with lymphoedema or hydrocele patients (WHO, 2018). GPELF has worked towards elimination for nearly two decades; first by interrupting transmission by MDA using different regimen combinations of albendazole, IVM and diethylcarbamazine (DEC), and second by alleviating suffering through morbidity management and disability prevention (MMDP) (WHO, 2017). Since its inception in 2000, more than 7.1 billion doses of LF elimination medications have been administered to more than 890 million people (WHO, 2018). Overall significant progress has been made towards elimination, but greater efforts are required in the WHO Africa Region, where many countries remain behind the elimination targets (WHO, 2017). In 2016, the WHO Africa region was estimated to have 371.2 million people requiring MDA across 32 endemic countries, with a reported coverage of 56.9% (WHO, 2017). While this marks an increase in coverage from previous years there are still 17 African countries yet to start or scale-up MDA to full nationwide geographical coverage (Molyneux *et al.,* 2014). The reasons for limited coverage are complex and related to several factors including but not limited to political will, conflict, financial commitment, technical support, difficult to access populations, stakeholder interest, poverty, poor infrastructure, limited human capacity, competing priority diseases, and co-endemicity with *L. loa* (Hope *et al.,* 2018).

Loiasis, also known as African eye-worm disease, is a vector-borne parasitic infection caused by *L. loa*, a filarial worm endemic to Central and West Africa (Pion et *al.,* 2017). It is restricted to the equatorial rain forest regions of Central and West Africa. Epidemiological data collected from 11 African countries indicates that at least 10 million people are infected (Zoure *et al.,* 2011). *L. loa* is transmitted by daytime biting flies of the genus *Chrysops* during a blood meal. The fly injects into the human host infective larvae (L3 development stage) that develop over time into adult worms that reside and migrate

in the subcutaneous tissues. These then mate to produce Mf that circulate in peripheral blood (Pion et *al.,* 2017). The major clinical manifestations of loiasis are Calabar swellings (evanescent episodic angioedema) and occasional subconjunctival migration of the adult worm (eye-worm). Less specific manifestations include urticaria, pruritus, myalgias, and arthralgia (Herrick *et al.,* 2015). Moreover, *L. loa* infection can cause renal, cardiac, pulmonary and neurological diseases, and a recent study found *L. loa* infection to be associated with a decreased life expectancy (Chesnais *et al.,* 2017). The primary health risk from loiasis is the risk for severe or fatal AEs that can occur when individuals with *L. loa* Mf loads >30,000/mL are treated in MDA programs for LF and onchocerciasis (river blindness) (Gardon *et al.,* 1997).

Loiasis has recently emerged as a disease of public health importance, not because of its own clinical manifestations but because of its negative impact on the control of onchocerciasis and LF in areas of co-endemicity (Zouré *et al.,* 2011). In fact, loiasis poses two problems for LF elimination:

- First, severe AEs including coma and death sometimes occur when LF MDA medications are given to persons with heavy loiasis infections (>20,000 microfilariae per mL of blood) (Gardon *et al.,* 1997). In fact, systemic AEs are common following treatment of filarial infections, including LF, onchocerciasis and loiasis, and are thought to be due to inflammation caused by dying filarial worms (Budge et al., 2018). These can be severe or fatal in persons with loiasis and high microfilarial loads. It has been shown that circulating Mf can reach 500,000 Mf/mL in loiasis and the risk of AEs is positively associated with this Mf density, with Mf counts >50,000 Mf/mL conferring high risk for serious/fatal AEs (Gardon *et al.,* 1997). The "Test and Not Treat" (TaNT) studies have recently demonstrate that there was no risk for serious AEs following ivermectin treatment of persons with loiasis Mf counts <20,000 Mf/mL (Kamgno *et al.,* 2018; Kamgno *et al.,* 2017). Among persons with microfilaremic LF or low-level loiasis, treatment-related AEs remain common but are mild and self-limited, usually resolving within 2-3 days following treatment. Typical reactions include fever, headache, myalgias, and malaise, which are similar to those seen following treatment of LF (Budge *et al.,* 2018). Treatment-related AEs in LF are thought to be related to the immune response to filarial or *Wolbachia* antigens released by

dying Mf (Kumaraswami *et al.*, 1988; Taylor *et al.,* 2001). However, since *L. loa* does not harbour endosymbiont *Wolbachia* (McGarry *et al.,* 2003), the pathogenesis of AEs following loiasis treatment may differ. In LF and loiasis, eosinophil counts and serum concentrations of type 2 cytokines increase following treatment with antifilarial drugs (Herrick *et al.,* 2017; Andersen *et al.,* 2018). The relationship between specific cytokine activation and AEs in people with loiasis has not been elucidated. In LF, there is a post treatment increase in circulating filarial antigen and filarial DNA in patients who went on to develop moderate AEs compared to those who had mild or no AEs (Andersen *et al.,* 2018).

- Second, *L. loa* infection complicates the mapping of LF distribution since standard RDTs for LF [e.g. BinaxNOW Filariasis immunochromatographic test (ICT) and/or the new Alere Filariasis Test Strip (FTS Alere, Scarborough, ME, USA)] (Weil *et al.,* 2013), cross-react with *L. loa,* resulting in false positives for *W. bancrofti*, with strong associations between ICT positivity and *L. loa* Mf counts (around 30% and 50% of ICTs reacting to *L. loa* when densities were > 15,00 Mf/ml and > 30,000 Mf/ml respectively) (Pion *et al.,* 2016). Other studies also shown that up to 6% of persons in loiasis-endemic populations test positive by LF-RDTs in the absence of *W. bancrofti* infection (Wanji *et al.,* 2015; Pion *et al.,* 2016). This makes it impossible for national programs to determine with certainty where LF MDA is required and when it can be stopped, given that a filarial antigenemia prevalence of 1% is the GPELF threshold for implementing MDA for LF.

Recently, many *L. loa* antigens that cause LF-RDT cross-reactivity in two samples from persons with loiasis were identified. Surprisingly, these included both secreted and somatic antigens, suggesting indiscriminate release of *L. loa* antigen from dying adult worms and/or Mf (Hertz *et al.,* 2018). A similar pattern of antigen release has been observed following treatment of patients with microfilaremic LF with medications known to kill Mf (Anderson et al, unpublished data). In addition, it was found that cross-reactivity with LF RDTs is not constant in many loiasis patients despite persistence of high Mf loads. These data suggest intermittent release and/or clearance of cross-reactive antigen in persons with loiasis.

11. Methods

11.1. Study design

This study is a prospective cohort study that will include two cohorts of persons with loiasis.

- ***Cohort 1 (spontaneous antigenemia).*** This cohort will prospectively enroll 50 adults with cross-reactive antigenemia and *L. loa* Mf counts >20,000 Mf/mL. Participants will be followed for one year and tested every three months for persistence of antigenemia. This cohort will not receive any filariasis treatment**.** Cohort 1 will allow us to define the cross-reactive antigen profile of persons with spontaneous loiasis antigenemia, and determine whether this varies with time.
- ***Cohort 2 (induced antigenemia)***. This cohort will include 50 adults with low to moderate *L. loa* microfilaremia (greater than 5,000, but less than 18,000 Mf/mL) who do not have cross-reactive antigenemia at the time of enrollment. All will receive ivermectin (150 µg/kg, once) and will be monitored daily for one week post-treatment to document AEs and collect venous blood for measurement of cytokines and cross-reactive *L. loa* antigens. Cohort 2 will allow us to identify cross-reactive *L. loa* antigens released by ivermectin treatment and define the kinetics of antigen clearance, and to determine whether the release of specific *L. loa* antigens or the amount of released antigen correlate with the occurrence and severity of treatment-related AEs and cytokine profiles.

11.2. Study sites

Study site for cohort 1 (spontaneous antigenemia)

The study site for cohort 1 will be the Okola health district (HD). The Okola HD is a degraded forest area located at 20 km from Yaoundé that is highly endemic for loiasis and hypomesoendemic for onchocerciasis (Zoure *et al.,* 2011). Participants in this cohort will be recruited from among those excluded from the ongoing TaNT study due to Mf counts >20,000/mL.

Study site for cohort 2 (induced antigenemia)

This cohort will be recruit and monitored in the Awae HD (Mefou and Afamba Division, Centre Region), which is located about 50 km south of Yaoundé, the political capital city of Cameroon. Because loiasis is highly endemic and onchocerciasis is hypoendemic in the area, no mass ivermectin distribution has ever been implemented in this area.

11.3. Period of the study

This study will be carried out over three years as shown in the chronogram. Data and sample collection in the field will begin in 2019 after ethical clearance have been granted.

11.4. Study population

Individuals eligible for this study will be of both sexes, aged from 18 years old and over, as follow:

- Participants of both sexes aged from 18 years and over in the ongoing TaNT study in Okola that were excluded from treatment due to Mf counts >20,000 Mf/mL will be included in the cohort 1 (spontaneous antigenemia).
- Participants of both sexes aged from 18 years and over in the Awae HD where no MDA of ivermectin is ongoing will be included in cohort 2 (induced antigenemia)

11.5. Selection criteria

11.5.1. Inclusion criteria

Inclusion criteria will be defined according to the cohort:

- Participants who will be included in cohort 1 will meet the following criteria:
  - Age ≥ 18 years
  - Ability to give informed consent
  - Loiasis Mf count > 20,000 Mf/mL
  - Resident of study area
  - No evidence of severe or systemic comorbidities
  - Consent to storage of blood samples for future study

- Participants who will be included in cohort 2 will meet the following criteria:
  - Age ≥ 18 years
  - Ability to give informed consent

- Loiasis Mf count between 5,000 Mf/mL and <18,000 mf/mL (to account for potential imprecision in the Mf counts, we will used a cut-off of 18,000 to ensure that no one with >20,000 Mf/mL is included)
- Resident of study area
- No evidence of severe or systemic comorbidities
- Absence of non-*L. loa* Mf (i.e. *W. bancrofti* or *M. perstans*) on thick blood smear
- Consent to storage of blood samples for future study

11.5.2. Exclusion criteria

Exclusion criteria will differ from one cohort to the other one. Participants who plans to move from study area during subsequent 12 months will be excluded from cohort 1, while participants will be excluded from cohort 2 if they meet the following criteria:

- Pregnancy or breastfeeding
- Alcohol or drug abuse
- History of severe adverse reaction to ivermectin
- Onchocerciasis (history of positive Ov16 test or positive skin snip)
- *M. perstans* infection (positive day blood smear)

11.6. Sample size

The sample size has been calculated according to the objectives and the corresponding cohort:

- The objective Cohort 1 is to identify one or more antigens that are sufficiently prevalent among persons with cross-reactive loiasis, that they can be used as biomarker(s) to distinguish cross-reactive loiasis from LF. We define a potential biomarker as an antigen that is detectable in at least **90%** of the population of interest. Assuming that one or more antigens is present in at least **90%** of the population of interest (those with cross-reactive loiasis), a sample size of **50** patients will give us **95%** confidence in excluding antigens that are present in less than **80%** of samples (1-sided Wilson score lower than the **95%** confidence interval).
- The objectives of cohort 2 are to define the cross-reactive antigen profile released by ivermectin treatment and the kinetics of antigen clearance, and to determine

whether the release of specific *L. loa* antigens or the amount of released antigen correlate with the occurrence and severity of treatment-related AEs and cytokine profiles. The sample size of **50** was chosen for cohort 2 to provide direct comparison to the objective of cohort 1 and will provide a similar level of confidence about the prevalence of antigens for this population.

11.7. Data and sample collection

Data and sample collection will be organized according to the objectives of the different cohorts.

11.7.1. Cohort 1 (recruitment and monitoring)

This cohort will include participants of the ongoing TaNT in Okola, who were excluded from IVM treatment due to Mf count ≥20,000 Mf/mL. Those participants will be visited in their home or other designated location. After providing consent for screening, they will be test for cross-reactivity using FTS. In addition, a LoaScope Mf count will be done for those who have not done this test during the last three months. Those with positive FTS test results who consent to participate to the study will be enrolled in cohort 1. If, after screening all available participants excluded from TaNT due to high Mf counts, fewer than 50 FTS positive subjects are found, we will enroll some of the FTS negative subjects with highest MF loads.

The day of enrolment, each participant will provide 10 mL venous blood for subsequent mass spectrometry (detection of cross-reactive *L. loa* antigens) and quantitative ELISA (determination of the level of cross-reactive *L. loa* antigens). Additionally, daytime calibrated thick blood smear will be performed for *L. loa* and *Mansonella* species detection and quantification. Furthermore, nocturnal calibrated thick blood smears and dried blood spot (DBS) will be done for detection and quantification of *W. bancrofti* by microscopy and PCR.

For this cohort, the follow up will be done 3, 6, 9 and 12 months after enrolment. During each visit, a FTS test will be done for each participant and those who will be positive will provide 10 mL venous blood for subsequent mass spectrometry (detection of cross-

reactive L. loa antigens) and quantitative ELISA (determination of the level of cross-reactive L. loa antigens).

11.7.2. Cohort 2 (recruitment and monitoring)

Screening for Cohort 2 will involve community-based testing of potential participants. Initial screening tests will examine a small amount (~200 µL) of capillary blood (obtained by fingerprick) for the presence of *L. loa* Mf (measured using the LoaScope), and absence of filarial antigen (using the FTS test). Individuals meeting inclusion criteria and providing informed consent will undergo further testing to rule out co-infection with other filarial species (*O. volvulus, M. perstans*) that may contribute to treatment-related AEs. Cohort 2 participants will then be admitted to the local health center for observation. All participants will receive a single dose of ivermectin (150 µg/kg) the day after admission. This dose is the same as that given for onchocerciasis MDA annually. Physical examination will be performed daily starting on day –1 (pre-treatment, the day of admission). Vital signs will be recorded on admission and at least daily through day 7. Venous blood will be drawn before treatment on day 0, and on days 1, 2, 3, and 7 post-treatment. Procedure for data and samples collection for this cohort are resumed in the table below.

Visit	**Cohort 2 activities and assessments**
Screening 1 (0-4 weeks pre-treatment)	• Consent for screening • LoaScope Mf count • FTS • Daytime thick blood smear (*Loa, Mansonella* Mf count) • Skin snips (rule out Onchocerciasis) • Urine pregnancy test (if female)
Enrollment (Day 0)	• FTS (*exclude if positive*) • LoaScope Mf count • Thick blood smear • Admit to health center • Pre-treatment physical examination • Pre-treatment AE questionnaire (*defer if febrile or other systemic illness*) • Venous blood (pre-treatment) for cytokines, antigen ELISA • Ivermectin treatment • Vital signs and AE monitoring q8hrs

Day 1	• FTS • LoaScope Mf count • Venous blood (antigen ELISA, cytokines, +/- mass spec) • Physical exam • Vital signs and AE monitoring q8hrs
Day 2	• FTS (if not positive day 1) • LoaScope Mf count • Venous blood (antigen ELISA, cytokines, +/- mass spec) • Physical exam • Vital signs and AE monitoring q8hrs
Day 3	• LoaScope Mf count • Thick blood smear • Venous blood (antigen ELISA, cytokines, +/- mass spec) • Physical exam • Vital signs and AE monitoring q8hrs
Day 4 – 6	• LoaScope Mf count (if not zero on day 3) • Venous blood (antigen ELISA, cytokines) • Physical exam • Vital signs and AE monitoring daily
Day 7	• FTS • LoaScope Mf count • Thick blood smear • Venous blood (antigen ELISA, cytokines) • Physical exam • Vital signs and AE monitoring daily
Follow-up	• Weekly FTS until negative (if not negative on day 7)

11.7.3. Laboratory evaluation

- **FTS test**

The Alere/Abbott Diagnostics FTS test cassettes will be used, according to manufacturer specifications. Briefly, 75 μL of finger prick blood will be added to a sample application pad that contains dried anti-filarial polyclonal antibody that has been labeled with colloidal gold. The labeled antibody binds to circulating filarial antigen (CFA) if it is present. Blood cells are retained in the sample pad, and the labeled antigen–antibody complexes flow down a nitrocellulose strip with the plasma. The immune complexes are immobilized when they bind to a monoclonal antibody to CFA that has been striped across

the nitrocellulose membrane, and this results in a positive test with a visible "T-line." The procedural control "C-line" develops when excess labeled polyclonal antibody crosses a line that contains a secondary antibody to the immunoglobulin that was in the sample pad. Thus, samples that contain CFA produce visible T- and C-lines in the FTS, while negative samples only produce the C-line (Chesnais *et al.,* 2016).

- **Calibrated thick blood smears**

Nocturnal as well as daytime calibrated thick blood smear will be performed according to established protocol on 70 µL of fingerprick blood. These will be stained and read by experienced microscopists at CRFilMT.

- **LoaScope Mf counts**

LoaScope Mf count will be done on fingerstick blood as described in (Kamgno *et al.,* 2017).

- **Mass spectrometry and ELISA**

Venous blood samples will be separated by centrifugation. Plasma will be frozen and shipped to Washington University in St. Louis for antigen testing (Weil lab ELISA protocol), and for specific antigens by mass spectrometry as previously described (Hertz *et al.,* 2018).

- **Skin Snips**

For cohort 2 participants will have skin snips (one punch over each iliac crest) to rule out onchocerciasis. The skin will be disinfected before a snip is taken with a sterile scleral punch. Each snip will be weighed on an analytical balance and incubated for at least 8 hours in isotonic saline in a well of a flat-bottomed micro-titre plate at ambient temperature. Microfilariae will be counted by microscopy. Mf number and skin snip weight will be recorded; skin microfilarial density will be calculated and recorded as mf/mg.

11.8. Statistical analysis

Data analysis will be specific to the cohorts:

- Cohort 1

The prevalence of antigens on Day 0 and at follow-up will be described using frequency (percentages) and their 95% confidence intervals will be reported. Potential biomarkers and the antigen profile of Cohort 1 will be defined as described according to demographic data (age, sex, village), *L. loa* Mf counts, and presence of *M. perstans*. The presence of detectable cross-reactive antigen at each follow-up study will be analysed as a binary outcome and differences in the proportion of samples positive for cross-reactive antigen compared using Fischer's exact test.

- Cohort 2

Potential biomarkers and the antigen profile for Cohort 2 will be defined as described for Cohort 1. For the purpose of comparing spontaneous antigenemia (Cohort 1) to ivermectin induced antigenemia (Cohort 2), the cross-reactive antigen profile of Cohort 1 will be defined as the collection of antigens found in >90% of baseline samples. Comparison between the antigen profiles of Cohorts 1 and 2 will be assessed as the proportion of samples in Cohort 2 containing detectable amounts of the potential biomarkers identified in Cohort 1. The association of quantitative antigen levels and AE occurrence and severity will be assessed comparing total circulating filarial antigen levels (measured by Weil Lab ELISA) among participants with no AEs to those with mild AEs or moderate AEs and examining differences using t-tests or Mann-Whitney U tests. Data management for Cohort 2 will be the same as for Cohort 1.

12. Ethical considerations

Before the beginning of this study, an ethical clearance will be requested to National Ethics Committee for Research in Human Health of Cameroon and to the Institutional Review Board of the Washington University in St. Louis.

Participation in this study will be voluntary and the refusal to participate will have no consequences for the persons who refuse to take part in the study. The objectives and schedule of the study will be explained to the potential participants and those who agree to participate will sign an informed consent form and keep a copy. The sampling will be performed with non-invasive or sterile and disposable equipment. All participants in Cohort 2 will benefit from general medical consultations with free study-related medication. The laboratory filarial test results will be communicated individually to each

participant in strict compliance with the confidentiality rules. The results of this study will be made available to the Ministry of Public Health and the health services of the targeted districts.

12.1. Risks/Discomfort of the study

Risk of blood draws

Drawing blood may be painful; as such, we shall assure the participants that the prick and venous puncture will last only a few seconds. Blood collection will be performed by trained professionals. Blood drawing will be carried out with sterile and single use materials. The risk is then very limited for the participants.

Risk of treatment (cohort 2 only)

This medication is safe and is provided as MDA to more than 100 million persons in Africa each year by programs to eliminate LF and onchocerciasis. The most common side effects are diarrhea, dizziness and nausea. Persons with filarial infections including onchocerciasis, LF, or loiasis sometimes experience rash, hives, itching, difficulty breathing, tightness in the chest, swelling of the mouth, face, lips, or tongue; eye pain, swelling, or redness. Seizures, fainting, mild decrease in leukocyte counts, elevated liver function tests, and cardiovascular effects that included tachycardia and orthostatic hypotension have been reported, but these adverse events are rare and not necessarily related to IVM treatment. Risks of treatment in this study will be minimized by ruling out onchocerciasis by skin snip prior to treatment, and by including only subjects with *L. loa* Mf counts <20,000 per mL in cohort 2, which has been demonstrated to be safe in this population (Kamgno *et al.,* 2018; Kamgno *et al.,* 2017).

Risk of skin snips (cohort 2 only)

This procedure is associated with a small risk of bleeding and infection. The skin will be disinfected before a snip is taken with a sterile scleral punch. The snip (about 2 mg) will cause a superficial wound of the skin at the iliac crests, which will be covered with a plaster for one day. These procedures are routine and designed to minimize risks of infection or bleeding. The scar will be 2 mm or less in size.

12.2. Benefits of the Study

The main benefit for participants will be to know their status about the diseases tested. Another benefit will be the satisfaction of contributing to the advancement of science.

12.3. Confidentiality

Participant identities, test results, and medical information will be maintained in a confidential manner by the study team and will not be released to anyone outside the staff conducting the project. Confidentiality of individual data will be protected insofar as legally as possible. The report of this study will be transmitted to the Regional Ethic Committee for Research in Human Health for the *Centre* region as well as to the Ministry of Public Health. Participants will be informed about their personal test results will be disclosed only to the participant in a private setting.

13. Information notes

This study will have two separate information notes, according to the objectives and the cohort.

13.1. Information note, Cohort 1

I am Professor Joseph KAMGNO, Medical Doctor, and Director of the Centre for Research on Filariasis and other Tropical Diseases (CRFilMT). Our team works with the Ministry of Public Health and other international organizations to research strategies to control filariasis.

We invite you to participate in this research project entitled **'' Cross-reactive antigenemia and treatment-related adverse events in loiasis''** whose objective is to determine why some people with loiasis test positive for lymphatic filariasis even though they do not have lymphatic filariasis, and whether this varies with time. **Participation in the study is entirely voluntary and you may choose not to participate or to withdraw at any moment.**

Now we will describe the research study and explain to you how it will take place.

1- Study title: Cross-reactive antigenemia and treatment-related adverse events in loiasis.

2- Principal investigator: Joseph KAMGNO

3- Authorization number of the Rational Ethic Committee for Research in Human Health:

4- Background Information

Lymphatic Filariasis (LF) (elephantiasis) and loiasis (African eye worms) are important public health problem in our country. LF manifests itself mainly by lymphedema (swollen limbs), genital diseases (swollen scrotum) and recurrent acute attacks which are extremely painful and accompanied by fever. On the other hand, symptoms of loiasis include specific manifestations as swellings of the hands called Calabar swelling, or migration of the worm that causes loiasis across the white surface of the eye. To eliminate LF, Mass Drugs Administration (MDA) of Ivermectin, and Albendazole are organized each year by the Ministry of Public Health. However, MDA for LF is very difficult in areas where loiasis is common because some people with loiasis test positive for LF even though they do not have the disease. This makes it very difficult to tell whether MDA for LF is actually needed.

5- Purpose of the study

The objective of this study is to determine why some people with loiasis test positive for lymphatic filariasis even when they do not have the disease, and to test whether this varies with time.

6- Research procedures

6.1- Study Population

This study will include people who are at least 18 years old who have loiasis and a positive LF test. People with very high levels of loiasis are more likely to have a positive LF test, so we will be testing people living in the Okala Health District who previously tested (during the TaNT study) and were found to have loiasis levels of at least 20,000 Mf/mL of blood.

6.2- Study Design

If you participate in this study, the study team will visit you in your home or village every three months for one year. At each visit, a study nurse or technician will prick your finger to obtain a few drops of blood that he/she will used to test how heavy your loiasis infection is using a handheld microscope called a LoaScope. This is the same procedure as you have had done previously in the Test and Not Treat Study. At the first study visit, we will also use a few drops of fingerprick blood to test for loiasis and related infections. Additionally, the study team will draw 10 mL of venous blood to test for loiasis antigens to help identify why many people with loiasis test falsely positive for LF. On the first study day the study team will also visit you at night for a second fingerprick to test for *W. bancrofti*, the parasite that causes LF.

At each subsequent study visit (3 months, 6 months, 9 months, and 12 months after the first visit), the study team will visit you in your home of village and will again collect daytime blood by fingerprick and by vein. All these visits will be during the day; the only time you will be asked to give blood at night is at the first study visit.

7- Risks/Discomfort of the study procedures. Drawing blood may be painful; as such, we shall assure the participants that the prick and venous puncture will last only a few seconds. Blood drawing will be carried out by trained professionals using sterile and single use materials.

8- Benefits of the Study

The main benefit for you will be to know your status about these diseases. Another benefit will be the satisfaction of contributing to the advancement of science. At any study visit if you have a health concern, a consultation will be done by a medical Doctor or a nurse, and you will receive generic drug (or prescription if the drug is not available).

9- Compensation/Indemnification/Motivation

Participants in this cohort 1 will receive 5000 CFA francs (~$8.65) at each study visit to compensate for their travel and lost work time.

10- Confidentiality and Reporting of Findings

Your name, test results, and medical information will be maintained in a confidential manner by the study team and will not be released to anyone outside the staff conducting the project. Confidentiality of individual data will be protected insofar as legally as possible. The report of this study will be transmitted to the Regional Ethic Committee for Research in Human Health for centre region as well as to the Ministry of Public Health. However, your personal results will be given to you and you only.

11- Voluntary feature of the participation

Participation in the study is entirely voluntary. You may withdraw from the study at any time. This will not affect at all your health care within the community.

12- New information

Storage of collected samples: your samples will be stored and transferred to the Washington University for further analysis. We will NOT test them for HIV nor perform any human genetic

Commented [BP1]: We work at Washington University (in St. Louis, Missouri). « University of Washington » refers to a different university (in Seattle, Washington State).

exam on your samples. Even in our research team, we will not share your name with any person using those samples. Samples collection supplies will be disposed according to security norms.

13- People to contact for further information

If you have any questions or concerns, or you feel you might have been hurt by the study, please contact Professor Joseph KAMGNO (Tel.: 222 202 442, PO Box 5797, Yaoundé). He is the Principal Investigator of this study.

14. Future Contact

We will keep your contact information. In the future, you may be asked to participate in follow up studies that would help to eliminate LF or Loiasis. At any time, you may choose not to participate in future studies.

13.2. Information note, Cohort 2

I am Professor Joseph KAMGNO, Medical Doctor, and Director of the Centre for Research on Filariasis and other Tropical Diseases (CRFilMT). Our team works with the Ministry of Public Health and other international organizations to research strategies to control filariasis.

We invite you to participate in a research project entitled '' Cross-reactive antigenemia and treatment-related adverse events in loiasis''. The objective of this study is to learn more about what happens in the body when people infected with the parasite, *Loa loa,* are treated with the medication ivermectin. **Participation in the study is entirely voluntary and you may choose not to participate or to withdraw at any moment.**

Now we will describe the research study and explain to you how it will take place.

1- Study title: Cross-reactive antigenemia and treatment-related adverse events in loiasis.

2- Principal investigator: Joseph KAMGNO

3- Authorization number of the Rational Ethic Committee for Research in Human Health:

4- Background Information

Lymphatic Filariasis (LF) (elephantiasis) and loiasis (African eye worms) are important public health problem in our country. LF causes leg or genital swelling, and recurrent acute attacks which are extremely painful skin infections accompanied by fever. On the other hand, symptoms of

loiasis include swelling, of the hands called Calabar swelling, or migration of the parasitic worm across the white surface of the eye. To eliminate LF, Mass Drugs Administration (MDA) of ivermectin and albendazole are organized each year by the Ministry of Public Health. However, MDA for LF is very difficult in areas where loiasis is common because some people with loiasis test positive for LF even though they do not have the disease. This makes it very difficult to tell whether MDA for LF is actually needed. In addition, one of the medications used for LF MDA (ivermectin), can be dangerous for people with loiasis who have very heavy infections.

When ivermectin is used to treat LF or loiasis, it kills the microscopic parasites that live in the blood. When these parasites die, it causes inflammation in the body that may cause headaches, muscle aches, fever, fatigue, weakness, itching, and other symptoms that make people feel ill. When parasite levels are low, these symptoms typically last about one day and resolve on their own. However, when parasite levels are high (more than 20,000 parasites per millilitre of blood), the symptoms caused by dying parasites can be more severe. For people with extremely high parasite levels (more than 50,000 parasites per millilitre of blood), the inflammation caused by dying parasites can be life-threatening. For this reason, ivermectin should not be given to anyone with parasite levels greater than 20,000 Mf/mL of blood.

5- Purpose of the study

The objective of this study is to determine what parasite proteins are released into the blood when people with loiasis are treated with ivermectin, and whether specific parasite proteins are responsible for the inflammatory responses caused by dying parasites.

6- Research procedures

6.1- Study Population

This study will include adults at least 18 years old, who have loiasis at levels that are safe to treat with ivermectin (between 5,000 – 18,000 parasites per millilitre of blood), and who have not been previously treated with ivermectin.

6.2- Study Design

People who wish to enroll in the study will first be tested to determine if they are eligible. This will be done by testing a few drops of blood obtained by fingerprick and examining it under a microscope to count the number of loiasis (*Loa loa)* parasites. A few drops of fingerprick blood will also be tested for the presence of parasite antigens. In addition, two small skin snips (one over each hip) will be take to test for parasites in the skin.

Eligible participants who wish to participate in the study will asked to come to the local health center for treatment, where they will stay for seven days. Each participant will be treated with a single dose of ivermectin (150 µg/kg) and will be monitored closely so the study team can observe and treat any symptoms that might develop. A physical examination and monitoring of vital signs at least once a day. A small amount of venous blood (10 mL) will be collected from each participant on the day prior to treatment, the day of treatment, and on days 1, 2, 3, and 7 post-treatment. This blood will be tested for parasite antigens and for evidence of inflammation caused by dying parasites.

7- Risks/Discomfort of the study procedures

Risk of treatment

Some people with loiasis feel ill for one or two days after receiving ivermectin. Symptoms may include fever, headache, muscle pain, joint pain, cough, chest pain, rash, itching, fatigue, high or low blood pressure, nausea, abdominal pain, or diarrhea. These symptoms are caused the body's response to the dying parasites. In people with less than 20,000 parasites per millilitre of blood, the symptoms are usually mild and resolve on their own. If you participate in the study and experience such symptoms a study physician or nurse will examine you and provide appropriate treatment.

Risk of blood collection

Participants in the study will have a small amount of blood drawn from a vein or fingerprick several times throughout the study. Drawing blood may be painful; but the prick or venous puncture will last only a few seconds. Blood collection will be performed by trained professionals using sterile, single use materials to minimize the risk of bleeding or infection.

Risk of skin snips

There is a small risk of

8- Benefits of the Study

The main benefit for you will be to know your status about these diseases. Another benefit will be the satisfaction of contributing to the advancement of science. Participants with some intestinal worms, lice, or scabies will benefit from receiving ivermectin, which is often used to treat these conditions.

9- Compensation/Indemnification/Motivation

Participants who will undergo both phases of screening will benefit from medical consultation and delivery of generic drugs. Those who enroll will receive XAF 15,000 (~$25) on completion of the study.

10- Confidentiality and Reporting of Findings

Your name, test results, and medical information will be maintained in a confidential manner by the study team and will not be released to anyone outside the staff conducting the project. Confidentiality of individual data will be protected insofar as legally as possible. The report of this study will be transmitted to the Regional Ethic Committee for Research in Human Health for centre region as well as to the Ministry of Public Health. However, your personal results will be given to you and you only.

11- Voluntary feature of the participation

Participation in the study is entirely voluntary. You may withdraw from the study at any time. This will not affect at all your health care within the community.

12- New information

Storage of collected samples: your samples will be stored and transferred to Washington University (USA) for further analysis. We will not test them for HIV nor perform any human genetic tests on your samples. Even in our research team, we will not share your name with any person using those samples. Samples collection supplies will be disposed according to security norms.

13- People to contact for further information

If you have any questions or concerns, or if you feel you might have been hurt by the study, please contact Professor Joseph KAMGNO (Tel.: 222 202 442, PO Box 5797, Yaoundé). He is the Principal Investigator of this study.

14. Future Contact

We will keep your contact information. In the future, you may be asked to participate in follow up studies that would help to eliminate LF or Loiasis. At any time, you may choose not to participate in future studies.

Notices d'information

Cette aura deux notices d'information, ton des objectifs et des cohortes

13.3. Notice d'information (Cohorte 1)

Je suis le Professeur Joseph KAMGNO, Médecin, Directeur du Centre de Recherche sur les Filarioses et autres Maladies Tropicales (CRFilMT). Notre équipe travaille avec le Ministère de la Santé Publique et d'autres Instituts de recherche internationaux sur les meilleurs moyens de lutte contre les filaires.

Nous vous invitons à participer à ce projet de recherche intitulé : **''Réaction croisée des antigènes et effets secondaires liés au traitement de la loase''** dont l'objectif est de définir le profil des antigènes à réactivité croisée chez les personnes avec une antigénémie spontanée et de déterminer si ce profil vari avec le temps**. La participation à cette étude est volontaire et vous pouvez choisir de ne pas y participer ou de vous retirer à n'importe quel moment.**

Maintenant nous allons décrire l'étude et vous présenter comment elle va se dérouler.

1-Titre de l'étude : Réactivité croisée des antigènes et effets secondaires liés au traitement de la loase.

2- Investigateur principal : Joseph KAMGNO.

3-Numéros d'autorisation du Comité National d'Éthique pour la Recherche en Santé Humaine (CNERSH) :

4- Introduction

La filariose lymphatique (FL) et la loase sont d'importants problèmes de santé publique dans notre pays. La FL se manifeste principalement par les lymphœdèmes, les hydrocèles et l'éléphantiasis. La loase quant à elle a pour manifestations spécifiques les œdèmes de Calabar, et la migration sous-conjonctivale des vers adultes. La FL cause la perte d'environ 2,8 million d'espérance de vie corrigée de l'incapacité (EVCI) chaque année due uniquement à l'incapacité des personnes affectées de vaquer à leur occupation. Pour éliminer cette maladie, des campagnes de distribution de masse de médicaments (Ivermectine, Albendazole, Diethylcarbamazine) sont organisées chaque année. Mais cette activité se heurte à un véritable obstacle qu'est la loase. En effet, pour débuter la distribution de masse, la prévalence de la FL (évaluée par les tests de

diagnostic rapide (TDR)) doit être ≥1%. Cependant, dans les zones ou la loase est aussi endémique, près de 6%de la population sont testés positif par les TDR de la FL en l'absence de W. bancrofti, le parasite responsable de la FL. En plus, l'Ivermectin cause des effets secondaires graves chez des personnes avec une charge de loase >20 000 Mf/ml de sang. Il est donc nécessaire d'identifier les antigènes de L. loa responsables des FL-TDR faux positifs et de voir si la production de ces antigènes est responsable des effets secondaires observés lors du traitement de la loase.

5- But de la recherche

L'objectif principal de cette partie de définir le profil des antigènes qui induisent la réction croisée chez les personnes avec une antigénémie spontanée et de déterminer si ce profil vari avec le temps.

6- Procédures de la recherche

6.1- Populations concernées par l'étude

Cette partie de l'étude concerne les personnes âgées de 18 ans et plus, ayant été exclues de l'étude TaNT d'Okola suite à leur charge de loase ≥20 000 Mf/ml de sang.

6.2- Protocole de l'étude

Les examens consisteront à la déterminer la charge de loase et la présence des antigènes spontanés par Loascope et FTS respectivement. Ces deux examens seront faits à partir d'un prélèvement de sang capillaire au bout du doigt. A partir du même prélèvement, les gouttes épaisses calibrées diurne, nocturnes, ainsi que des spots de sang sur papier filtre seront faits. Additionnellement, 10 ml de sang veineux seront prélevés pour l'identification et la quantification des antigènes par spectrométrie de masse et ELISA.

7- Risques/ Inconforts éventuels associés à l'étude

Le prélèvement de sang (capillaire et veineux) est un peu douloureux ; nous rassurerons donc les participants que la piqure ne durera que quelques secondes. Nous aimerions préciser que ce prélèvement sera réalisé par des professionnels expérimentés. Les prélèvements seront réalisés avec du matériel stérile et à usage unique, ce qui réduit le risque pour les participants.

8- Bénéfices potentiels de l'étude

Le principal bénéfice sera de connaître votre statut vis-à-vis de ces maladies. Outre le fait de connaître leur statut vis-à-vis de ces maladies parasitaires, une retombée très importante de cette étude sera la possibilité de participer à l'évolution de la science.

9- Compensation/Indemnisation/Motivation

Une compensation financière de 5000 Francs CFA (~$8.65) est prévue pour chaque visite. Pendant les visites, vous bénéficierez également de consultations médicales.

10- Confidentialité

Le rapport de cette étude sera transmis au Comité Régional d'Ethique pour la Recherche en Santé Humaine (CNERSH) du centre, ainsi qu'au Ministère de la santé publique. Cependant, vos résultats personnels ne seront communiqués qu'à vous et à vous seul.

11- Caractère volontaire de la participation

Votre participation à cette étude est volontaire. Vous pourrez vous retirer de l'étude à tout moment sans que ceci n'affecte vos soins de santé dans votre communauté.

12- Nouveaux renseignements

Conservation des échantillons de prélèvement : vos échantillons seront conservés et transférés à nos collaborateurs de l'Université Washington a St. Louis pour certaines analyses. Par ailleurs, nous n'allons tester aucun de ces échantillons pour le VIH. Nous n'allons pas non plus effectuer un test de génétique humaine sur vos échantillons. Même au sein de notre équipe, nous ne communiquerons pas votre nom à quiconque susceptible d'utiliser ces échantillons. Les échantillons seront conservés aussi longtemps que nécessaire pour aider les programmes d'élimination de la FL. Le matériel de prélèvement sera quant à lui détruit suivant les normes de sécurité.

13. Personnes à contacter en cas de besoin

Si vous avez d'autres questions ou estimez que vos droits n'ont pas été respectés dans le cadre de cette étude, vous pouvez contacter le Professeur Joseph KAMGNO (Tél. : 222 202 442, BP 5797, Yaoundé). Il est le principal investigateur de cette étude.

14. Futur contact Nous conserverons vos informations de contact

Dans l'avenir, vous pourrez être invité à participer à des études de suivi qui faciliteraient les efforts pour éliminer du paludisme et de la schistosomiase. À tout moment vous pouvez choisir de ne pas participer à ces études futures.

13.4. Notice d'information (Cohorte 2)

Je suis le Professeur Joseph KAMGNO, Médecin, Directeur du Centre de Recherche sur les Filarioses et autres Maladies Tropicales (CRFilMT). Notre équipe travaille avec le Ministère de la

Santé Publique et d'autres Instituts de recherche internationaux sur les meilleurs moyens de lutte contre les filaires.

Nous vous invitons à participer à la deuxième partie du projet de recherche intitulé : **"Réactivité croisée des antigènes et effets secondaires liés au traitement de la loase"** dont les objectifs sont (i) de déterminer les antigènes à réactivité croisée de L. loa induits suite au traitement par l'Ivermectine et de (ii) déterminer si la production de ces antigènes spécifiques et/ou leur quantité est en corrélation avec l'apparition et la sévérité des effets secondaires observés suite au traitement par l'Ivermectine. **La participation à cette étude est volontaire et vous pouvez choisir de ne pas y participer ou de vous retirer à n'importe quel moment.**

Maintenant nous allons décrire l'étude et vous présenter comment elle va se dérouler.

1-Titre de l'étude : Réactivité croisée des antigènes et effets secondaires liés au traitement de la loase.

2- Investigateur principal : Joseph KAMGNO.

3-Numéros d'autorisation du Comité National d'Éthique pour la Recherche en Santé Humaine (CNERSH) :

4- Introduction

La filariose lymphatique (FL) et la loase sont d'importants problèmes de santé publique dans notre pays. La FL se manifeste principalement par les lymphœdèmes, les hydrocèles et l'éléphantiasis. La loase quant à elle a pour manifestations spécifiques les œdèmes de Calabar, et la migration sous-conjonctivale des vers adultes. La FL cause la perte d'environ 2,8 million d'espérance de vie corrigée de l'incapacité (EVCI) chaque année due uniquement à l'incapacité des personnes affectées de vaquer à leur occupation. Pour éliminer cette maladie, des campagnes de distribution de masse de médicaments (Ivermectine, Albendazole, Diethylcarbamazine) sont organisées chaque année. Mais cette activité se heurte à un véritable obstacle qu'est la loase. En effet, pour débuter la distribution de masse, la prévalence de la FL (évaluée par les tests de diagnostic rapide (TDR)) doit être ≥1%. Cependant, dans les zones ou la loase est aussi endémique, près de 6%de la population sont testés positif par les TDR de la FL en l'absence de W. bancrofti, le parasite responsable de la FL. En plus, l'Ivermectin cause des effets secondaires graves (incluant le coma et la mort) chez des personnes avec une charge de loase >20 000 Mf/ml de sang. Il est donc nécessaire d'identifier les antigènes de L. loa responsables des FL-TDR faux

positifs et de voir si la production de ces antigènes est responsable des effets secondaires observés lors du traitement de la loase.

5- But de la recherche

Les objectifs de cette partie de l'étude sont (i) de déterminer les antigènes à réactivité croisée de *L. loa* induits suite au traitement par l'Ivermectine et de (ii) déterminer si la production de ces antigènes spécifiques et/ou leur quantité est en corrélation avec l'apparition et la sévérité des effets secondaires observés suite au traitement par l'Ivermectine.

6- Procédures de la recherche

6.1- Populations concernées par l'étude

Cette partie de l'étude va inclure les personnes âgées de 18 ans et plus vivant dans le district de santé d'Awae n'ayant pas suivi un traitement par l'Ivermectine.

6.2- Protocole de l'étude

Les examens consisteront à analyser quelques goutes de sang capillaire (~200 µl) obtenu au bout du doigt. Les microfilaires de L. loa seront mesurées par Loascope et les antigènes qui induisent la réaction croisée par FTS. 10 ml de sang veineux seront prélevés pour l'identification et la quantification des antigènes par spectrométrie de masse et ELISA et les participants seront internés dans un centre de santé. Par la suite ils seront traités par une dose (150mg) d'Ivermectine et seront suivis pendant 07 jours, durant lesquels ils seront surveillés pour les effets secondaires et fourniront 10 ml de sang veineux (pour l'identification et la quantification des antigènes par spectrométrie de masse et ELISA) chaque jour. Pendant l'hospitalisation pour les maux éventuels, vous recevrez des médicaments génériques.

7- Risques/ Inconforts éventuels associés à l'étude

Le prélèvement de sang (capillaire et veineux) est un peu douloureux ; nous rassurerons donc les participants que la piqure ne durera que quelques secondes. Nous aimerions préciser que ce prélèvement sera réalisé par des professionnels expérimentés. Les prélèvements seront réalisés avec du matériel stérile et à usage unique, ce qui réduit le risque pour les participants.

8- Bénéfices potentiels de l'étude

Le principal bénéfice sera de connaître votre statut vis-à-vis de ces maladies. Outre le fait de connaître leur statut vis-à-vis de ces maladies parasitaires, une retombée très importante de cette étude sera la possibilité de participer à l'évolution de la science. Vous bénéficierez également de consultation et recevrez des médicaments génériques.

9- Compensation/Indemnisation/Motivation

Les participants bénéficieront de consultations médicales lors du screening et une somme 15,000 Francs CFA (~$25) sera remis aux participant qui seront hospitalisé à la fin de l'étude pour compenser tout le temps perdu à l'hôpital.

10- Confidentialité

Le rapport de cette étude sera transmis au Comité Régional d'Ethique pour la Recherche en Santé Humaine (CNERSH) du centre, ainsi qu'au Ministère de la santé publique. Cependant, vos résultats personnels ne seront communiqués qu'à vous et à vous seul.

11- Caractère volontaire de la participation

Votre participation à cette étude est volontaire. Vous pourrez vous retirer de l'étude à tout moment sans que ceci n'affecte vos soins de santé dans votre communauté.

12- Nouveaux renseignements

Conservation des échantillons de prélèvement : vos échantillons seront conservés et transférés à nos collaborateurs de l'Université Washington a St. Louis pour certaines analyses. Par ailleurs, nous n'allons tester aucun de ces échantillons pour le VIH. Nous n'allons pas non plus effectuer un test de génétique humaine sur vos échantillons. Même au sein de notre équipe, nous ne communiquerons pas votre nom à quiconque susceptible d'utiliser ces échantillons. Les échantillons seront conservés aussi longtemps que nécessaire pour aider les programmes d'élimination de la FL. Le matériel de prélèvement sera quant à lui détruit suivant les normes de sécurité.

13. Personnes à contacter en cas de besoin

Si vous avez d'autres questions ou estimez que vos droits n'ont pas été respectés dans le cadre de cette étude, vous pouvez contacter le Professeur Joseph KAMGNO (Tél. : 222 202 442, BP 5797, Yaoundé). Il est le principal investigateur de cette étude.

14. Futur contact Nous conserverons vos informations de contact

Dans l'avenir, vous pourrez être invité à participer à des études de suivi qui faciliteraient les efforts pour éliminer du paludisme et de la schistosomiase. À tout moment vous pouvez choisir de ne pas participer à ces études futures.

14. Informed consents

14.1. Assent form (Cohort 1: Participants aged from 18 to 20 years old)

I, the undersigned, M / Ms / Miss ....., attest to having been invited to participate in the research work entitled **''Cross-reactive antigenemia and treatment related adverse events in loiasis''** whose principal investigator is Professor Joseph KAMGNO (Director of the Center for Research on Filariasis and other Tropical Diseases).

- I understood the information note that was provided to me; moreover, this information leaflet has been read and explained by the investigators;
- I understood the purpose and objectives of this study;
- I received all the answers to the questions I asked;
- The risks and benefits of this study were presented to me and explained;
- I understand that I am free to accept or refuse to participate in this study;
- My consent does not discharge the investigators of the research of their responsibilities; I therefore retain all my rights guaranteed by law.

I freely agree to participate in this study under the conditions specified in the information leaflet by (1) answering questions from the investigators, (2) communicating my medical information, (3) submitting myself to the fingerprick as well as venous blood samples for the examinations of filariasis, and (4) to be present at each follow up visit.

I agree that my samples taken for this study may be used in subsequent studies.

☐ yes ☐ no

(*please check one box only)*

Done at …… on ……. /……. / 2019

_____ _____

Signature of the Principal Investigator Fingerprint of the participant

_____

Name and Signature witness

14.2. Assent form (Cohort 2: Participants aged from 18 to 20 years old)

I, the undersigned, M / Ms / Miss ....., attest to having been invited to participate in the research work entitled **''Cross-reactive antigenemia and treatment related adverse events in loiasis''** whose principal investigator is Professor Joseph KAMGNO (Director of the Center for Research on Filariasis and other Tropical Diseases).

- I understood the information note that was provided to me; moreover, this information leaflet has been read and explained by the investigators;
- I understood the purpose and objectives of this study;
- I received all the answers to the questions I asked;
- The risks and benefits of this study were presented to me and explained;
- I understand that I am free to accept or refuse to participate in this study;
- My consent does not discharge the investigators of the research of their responsibilities; I therefore retain all my rights guaranteed by law.

I freely agree to participate in this study under the conditions specified in the information leaflet by (1) answering questions from the investigators, (2) communicating my medical information, (3) submitting myself to the fingerprick as well as venous blood and skin snip samples for the examinations of filariasis, and (4) to be admitted to a health center for treatment with single dose ivermectin and actively monitored for adverse events during seven days.

I agree that my samples taken for this study may be used in subsequent studies.

☐ yes ☐ no

(*please check one box only)*

Done at …… on ……. /……. / 2019

_____ _____

Signature of the Principal Investigator Fingerprint of the participant

_____

Name and Signature witness

14.3. Informed consent form (Cohort 1: Participants aged ≥21 years old)

I, the undersigned, M / Ms / Miss ....., attest to having been invited to participate in the research work entitled **"Cross-reactive antigenemia and treatment related adverse events in loiasis"** whose principal investigator is Professor Joseph KAMGNO (Director of the Center for Research on Filariasis and other Tropical Diseases).

- I understood the information note that was provided to me; moreover, this information leaflet has been read and explained by the investigators;
- I understood the purpose and objectives of this study;
- I received all the answers to the questions I asked;
- The risks and benefits of this study were presented to me and explained;
- I understand that I am free to accept or refuse to participate in this study;
- My consent does not discharge the investigators of the research of their responsibilities; I therefore retain all my rights guaranteed by law.

I freely agree to participate in this study under the conditions specified in the information leaflet by (1) answering questions from the investigators, (2) communicating my medical information, (3) submitting myself to the fingerprick as well as venous blood samples for the examinations of filariasis, and (4) to be present at each follow up visit

I agree that my samples taken for this study may be used in subsequent studies.

☐ yes ☐ no

(*please check one box only)*

Done at …… on ……. /……. / 2019

_____ _____

Signature of the Principal Investigator Signature of the participant

14.4. Inform consent form (Cohort 2: Participants aged ≥21 years old)

I, the undersigned, M / Ms / Miss ....., attest to having been invited to participate in the research work entitled **"Cross-reactive antigenemia and treatment related adverse events in loiasis"** whose principal investigator is Professor Joseph KAMGNO (Director of the Center for Research on Filariasis and other Tropical Diseases).

- I understood the information note that was provided to me; moreover, this information leaflet has been read and explained by the investigators;
- I understood the purpose and objectives of this study;
- I received all the answers to the questions I asked;
- The risks and benefits of this study were presented to me and explained;
- I understand that I am free to accept or refuse to participate in this study;
- My consent does not discharge the investigators of the research of their responsibilities; I therefore retain all my rights guaranteed by law.

I freely agree to participate in this study under the conditions specified in the information leaflet by (1) answering questions from the investigators, (2) communicating my medical information, (3) submitting myself to the fingerprick as well as venous blood and skin snip samples for the examinations of filariasis, and (4) to be admitted to a health center for treatment with single dose ivermectin and actively monitored for adverse events during seven days.

I agree that my samples taken for this study may be used in subsequent studies.

☐ yes ☐ no

(*please check one box only)*

Done at …… on ……. /……. / 2019

_____ _____

Signature of the Principal Investigator Signature of the participant

15. Formulaires de consentement éclairé

15.1. Formulaire d'assentiment (Cohorte 1 : Participants âgés entre 15 et 20 ans)

Je soussigné M/Mme/Mlle …… atteste avoir été invité à participer au travail de recherche intitulé **«Réactivité croisée des antigènes et effets secondaires associés au traitement de la loase»** dont l'investigateur principal est le Professeur Joseph KAMGNO (Directeur du Centre de Recherche sur les Filarioses et autres Maladies Tropicales.

- J'ai bien compris la notice d'information qui m'a été remise concernant l'étude ; par ailleurs, cette notice d'information a été lu et expliqué par les investigateurs ;
- J'ai bien compris le but et les objectifs de cette étude ;
- J'ai reçu toutes les réponses aux questions que j'ai posées ;
- Les risques et bénéfices de cette étude m'ont été présentés et expliqués ;
- J'ai bien compris que je suis libre d'accepter ou de refuser de participer à cette étude ;
- Mon consentement ne décharge pas les investigateurs de la recherche de leurs responsabilités ; je conserve de ce fait tous mes droits garantis par la loi.

J'accepte librement de participer à cette étude dans les conditions précisées dans la notice d'information en (1) répondant aux questions des investigateurs, (2) communiquant mes informations médicales, (3) me soumettant aux prélèvements de sang veineux et capillaire pour la réalisation des examens de filarioses, identification des antigènes par spectrométrie de masse aussi bien que leur quantification par ELISA et enfin (4) d'être présent pour chaque visite de suivi. Je donne mon accord pour que mes échantillons prélevés pour cette étude soient utilisés dans les études ultérieures.

Fait à …… le ……. /……. / 2019

_____ _____

Signature Investigateur Principal Empreinte digitale du participant

_____

Nom et Signature du témoin

15.2. Formulaire d'assentiment (Cohorte 2 : Participants âgés entre 18 et 20 ans)

Je soussigné M/Mme/Mlle …… atteste avoir été invité à participer au travail de recherche intitulé **«Réactivité croisée des antigènes et effets secondaires associés au traitement de la loase»** dont l'investigateur principal est le Professeur Joseph KAMGNO (Directeur du Centre de Recherche sur les Filarioses et autres Maladies Tropicales.

- J'ai bien compris la notice d'information qui m'a été remise concernant l'étude ; par ailleurs, cette notice d'information a été lu et expliqué par les investigateurs ;
- J'ai bien compris le but et les objectifs de cette étude ;
- J'ai reçu toutes les réponses aux questions que j'ai posées ;
- Les risques et bénéfices de cette étude m'ont été présentés et expliqués ;
- J'ai bien compris que je suis libre d'accepter ou de refuser de participer à cette étude ;
- Mon consentement ne décharge pas les investigateurs de la recherche de leurs responsabilités ; je conserve de ce fait tous mes droits garantis par la loi.

J'accepte librement de participer à cette étude dans les conditions précisées dans la notice d'information en (1) répondant aux questions des investigateurs, (2) communiquant mes informations médicales, (3) me soumettant aux prélèvements de sang veineux et capillaire pour la réalisation des examens de filarioses, identification des antigènes par spectrométrie de masse aussi bien que leur quantification par ELISA et enfin (4) être admis dans un centre de santé pour y recevoir un traitement par ivermectine à dose unique et surveiller activement pour les effets indésirables sur une période de sept jours.
Je donne mon accord pour que mes échantillons prélevés pour cette étude soient utilisés dans les études ultérieures.

Fait à …… le ……. /……. / 2019

_____ _____

Signature Investigateur Principal Empreinte digitale du participant

_____

Nom et Signature du témoin

15.3. Formulaire de consentement élaire (Cohorte 1 : Participants âgés de ≥21 ans)

Je soussigné M/Mme/Mlle …… atteste avoir été invité à participer au travail de recherche intitulé **«Réactivité croisée des antigènes et effets secondaires associés au traitement de la loase»** dont l'investigateur principal est le Professeur Joseph KAMGNO (Directeur du Centre de Recherche sur les Filarioses et autres Maladies Tropicales.

- J'ai bien compris la notice d'information qui m'a été remise concernant l'étude ; par ailleurs, cette notice d'information a été lu et expliqué par les investigateurs ;
- J'ai bien compris le but et les objectifs de cette étude ;
- J'ai reçu toutes les réponses aux questions que j'ai posées ;
- Les risques et bénéfices de cette étude m'ont été présentés et expliqués ;
- J'ai bien compris que je suis libre d'accepter ou de refuser de participer à cette étude ;
- Mon consentement ne décharge pas les investigateurs de la recherche de leurs responsabilités ; je conserve de ce fait tous mes droits garantis par la loi.

J'accepte librement de participer à cette étude dans les conditions précisées dans la notice d'information en (1) répondant aux questions des investigateurs, (2) communiquant mes informations médicales, (3) me soumettant aux prélèvements de sang veineux et capillaire pour la réalisation des examens de filarioses, identification des antigènes par spectrométrie de masse aussi bien que leur quantification par ELISA et enfin (4) d'être présent pour chaque visite de suivi. Je donne mon accord pour que mes échantillons prélevés pour cette étude soient utilisés dans les études ultérieures.

Fait à …… le ……. /……. / 2019

_____ _____

Signature Investigateur Principal Signature du participant

15.4. Formulaire de consentement élaire (Cohorte 2 : Participants âgés de ≥21 ans)

Je soussigné M/Mme/Mlle …… atteste avoir été invité à participer au travail de recherche intitulé **«Réactivité croisée des antigènes et effets secondaires associés au traitement de la loase»** dont l'investigateur principal est le Professeur Joseph KAMGNO (Directeur du Centre de Recherche sur les Filarioses et autres Maladies Tropicales.

- J'ai bien compris la notice d'information qui m'a été remise concernant l'étude ; par ailleurs, cette notice d'information a été lu et expliqué par les investigateurs ;
- J'ai bien compris le but et les objectifs de cette étude ;
- J'ai reçu toutes les réponses aux questions que j'ai posées ;
- Les risques et bénéfices de cette étude m'ont été présentés et expliqués ;
- J'ai bien compris que je suis libre d'accepter ou de refuser de participer à cette étude ;
- Mon consentement ne décharge pas les investigateurs de la recherche de leurs responsabilités ; je conserve de ce fait tous mes droits garantis par la loi.

J'accepte librement de participer à cette étude dans les conditions précisées dans la notice d'information en (1) répondant aux questions des investigateurs, (2) communiquant mes informations médicales, (3) me soumettant aux prélèvements de sang veineux et capillaire pour la réalisation des examens de filarioses, identification des antigènes par spectrométrie de masse aussi bien que leur quantification par ELISA et enfin (4) être admis dans un centre de santé pour y recevoir un traitement par ivermectine à dose unique et surveiller activement pour les effets indésirables sur une période de sept jours.
Je donne mon accord pour que mes échantillons prélevés pour cette étude soient utilisés dans les études ultérieures.

Fait à …… le …… /…… / 2019

_____ _____

Signature Investigateur Principal Signature du participant

16. Fiche de collecte de données/Survey form

16.1. Fiche de collecte de données cohorte 1

Code- barre

INFORMATIONS GENERALES SUR LE PATIENT

Région : _____ District de sante : _____ Aire de santé : _____

Date : _____ N° TaNT 2019: |_____| Code équipe : |____|

N° d'ordre dans village : |___|___|___| N° ID final : |___|___|___|___|

Code du patient : |___||___||___| Identifiant final : |____||____||____||____|

Nom : _____ Prénom : _____ Sexe (M/F) : |___|

Age : |___| tel : |_____|

……

MORBIDITE

Œdème de Calabar Oui |___| Non |___| **Urticaire*** Oui |___| Non |___|

Migration vers adulte dans l'œil * Oui |___| Non |___| **Prurit*** Oui |___| Non |___|

** le personnel de santé doit vérifier ces lésions*

……

PRELEVEMENTS

Screening

FTS Positif |____| Négatif |____| (Préciser le grade** si positif : 1 |__| 2 |__| 3 |__|)

Loascope : Oui |___| Non |___| (|_____|/ml)

*** Par rapport au trait contrôle, le trait test est (1) moins intense ; (2) de même intensité ; (3) plus intense*

Jour 0

Prélèvement veineux (10 ml) : Oui |___| Non |___| (|_____|/ml)

GEC diurne: Oui |___| Non |___| ***L. loa* :**(|_____|/ml) ***Mansonella*:**(|_____|/ml)

GEC nocturne Oui |___| Non |___| (|_____|/ml) **BDS nocturne** Oui |___| Non |___|

03 Mois

FTS Positif |____| Négatif |____| (Préciser le grade** si positif : 1 |__| 2 |__| 3 |__|)

Prélèvement veineux (10 ml) : Oui |___| Non |___| (|_____|/ml) ***

*** Par rapport au trait contrôle, le trait test est (1) moins intense ; (2) de même intensité ; (3) plus intense*
**** Fait si FTS positif*

06 Mois

FTS Positif |____| Négatif |____| (Préciser le grade** si positif : 1 |__| 2 |__| 3 |__|)
Prélèvement veineux (10 ml) : Oui |___| Non |___| (|_____|/ml) ***
** Par rapport au trait contrôle, le trait test est (1) moins intense ; (2) de même intensité ; (3) plus intense*
**** Fait si FTS positif*

09 Mois

FTS Positif |____| Négatif |____| (Préciser le grade** si positif : 1 |__| 2 |__| 3 |__|)
Prélèvement veineux (10 ml) : Oui |___| Non |___| (|_____|/ml) ***
** Par rapport au trait contrôle, le trait test est (1) moins intense ; (2) de même intensité ; (3) plus intense*
**** Fait si FTS positif*

12 Mois

FTS Positif |____| Négatif |____| (Préciser le grade** si positif : 1 |__| 2 |__| 3 |__|)
Prélèvement veineux (10 ml) : Oui |___| Non |___| (|_____|/ml) ***
** Par rapport au trait contrôle, le trait test est (1) moins intense ; (2) de même intensité ; (3) plus intense*
**** Fait si FTS positif*

……

REMARQUES

_____

_____

Noms et signature du chef d'équipe (après vérification des informations)

16.2. Fiche de collecte de données cohorte 2

Code- barre

INFORMATIONS GENERALES SUR LE PATIENT

Région : _____ District de sante : _____ Aire de santé : _____

Date : _____ Village : _____ Code village : |_____|

N° d'ordre dans village : |_____|_____|_____| N° ID final : |_____|_____|_____|_____|

Nom : _____ Prénom : _____ Sexe (M/F) : |___| Age : |___|

Tel : |_____|

……

MORBIDITE

Œdème de Calabar Oui |_____| Non |_____| **Urticaire*** Oui |_____| Non |_____|

Migration vers adulte dans l'œil * Oui |_____| Non |_____| **Prurit*** Oui |_____| Non |_____|

** le personnel de santé doit vérifier ces lésions*

……

PRELEVEMENTS

Screening

FTS Positif |____| Négatif |____| (Préciser le grade** si positif : 1 |__| 2 |__| 3 |__|)

Loascope : Oui |___| Non |___| (|_____|/ml)

GEC diurne: Oui |___| Non |___| ***L. loa* :**(|_____|/ml) ***Mansonella*:**(|_____|/ml)

Skin Snip : Oui|___| Non|___| (Snip Gauche: |___|mg, |___|mf / Snip Droit: |___|mg , |___|mf)

Test rapide de grossesse : Oui |___| Non |___| (Positif |___| Négatif |___|)

*** Par rapport au trait contrôle, le trait test est (1) moins intense ; (2) de même intensité ; (3) plus intense*

Jour 0

FTS*** Positif|____| Négatif |____| (Préciser le grade** si positif : 1 |__| 2 |__| 3 |__|)

Loascope : Oui |___| Non |___| (|_____|/ml) **GEC** Oui |___| Non |___| (|_____|/ml)

Admission au centre de santé : Oui |___| Non |___|

Examens cliniques prétraitement : Date _____ Time _____

Questionnaire Effets secondaires prétraitement**** :** Oui |___| Non |___|

Prélèvement veineux (10 ml) prétraitement : Oui |___| Non |___|

Traitement par une dose (150 mg) d'Ivermectine : Oui |___| Non |___| Date _____ ; Time _____

Surveillance des signes vitaux après chaque 8 heures : Oui |___| Non |___|

*** Par rapport au trait contrôle, le trait test est (1) moins intense ; (2) de même intensité ; (3) plus intense*

**** Exclus si positif*

**** Voir questionnaire effets secondaires

Jour 1

FTS Positif|____| Négatif |____| (Préciser le grade** si positif : 1 |__| 2 |__| 3 |__|)

Prélèvement veineux (10 ml) post-traitement: Oui |____| Non |____|

Examens cliniques post-traitement: Oui |____| Non |____|

Questionnaire Effets secondaires post-traitement **:** Oui |____| Non |____|

Surveillance des signes vitaux après chaque 8 heures: Oui |____| Non |____|

*** Par rapport au trait contrôle, le trait test est (1) moins intense ; (2) de même intensité ; (3) plus intense*

**** Voir questionnaire effets secondaires

Jour 2

FTS***** Positif|____| Négatif |____| (Préciser le grade** si positif : 1 |__| 2 |__| 3 |__|)

GEC Prélèvement veineux (10 ml) post-traitement: Oui |____| Non |____|

Examens cliniques post-traitement: Oui |____| Non |____|

Questionnaire Effets secondaires post-traitement **:** Oui |____| Non |____|

Surveillance des signes vitaux après chaque 8 heures: Oui |____| Non |____|

*** Par rapport au trait contrôle, le trait test est (1) moins intense ; (2) de même intensité ; (3) plus intense*

***** *Si pas positif au jour 1*

Jour 3

Loascope : Oui |____| Non |____| (|_____|/ml)

Prélèvement veineux (10 ml) post-traitement: Oui |____| Non |____|

Examens cliniques post-traitement: Oui |____| Non |____|

Questionnaire Effets secondaires post-traitement **:** Oui |____| Non |____|

Surveillance des signes vitaux après chaque 8 heures: Oui |____| Non |____|

Jour 3-6

Prélèvement veineux (10 ml) post-traitement: Oui |____| Non |____|

Examens cliniques post-traitements Oui |____| Non |____|

Questionnaire Effets secondaires post-traitement **:** Oui |____| Non |____|

Surveillance des signes vitaux après chaque 8 heures: Oui |____| Non |____|

****** *Si pas zéro au jour 3*

Jour 7

FTS Positif|____| Négatif |____| (Préciser le grade** si positif : 1 |__| 2 |__| 3 |__|)

Loascope : Oui |___| Non |___| (|_____|/ml) **GEC** Oui |___| Non |___| (|_____|/ml)

Examens cliniques post-traitements Oui |___| Non |___|

Prélèvement veineux (10 ml) prétraitement: Oui |___| Non |___|

*** Par rapport au trait contrôle, le trait test est (1) moins intense ; (2) de même intensité ; (3) plus intense*

……

REMARQUES

_____

_____

Noms et signature du chef d'équipe (après vérification des informations)

17. Chronogram

Years	2019						2020												2021								
Activities	**July**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**June**	**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**June**	**July**	**Aug**	**Sep**
Ethical clearance / administrative authorization																											
Cohort 1																											
Screening and enrolment																											
Follow-up visits																											
Laboratory analysis (LCMS, CFA ELISA)																											
Cohort 2																											
Screening and enrolment																											
Ivermectin treatment and monitoring																											
Laboratory analysis (LCMS, CFA & cytokines ELISA)																											
Valorization																											
Preparation and submit publication																											
Meeting attendance																											
Prepare and submit R01																											
Final Report (Thesis defense)																											

18. Detailed budget and Source of funding

18.1. Detailed budget

18.2. Source of funding

This study will be funded by the Doris Duke Charitable Foundation (650 5th Ave, New York, NY 10019, USA).

19. References

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Herrick JA, Legrand F, Gounoue R, et al. «Posttreatment Reactions After Single-Dose Diethylcarbamazine or Ivermectin in Subjects With Loa loa Infection.» *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, 2017: 64(8):1017-1025.

Herrick JA, Metenou S, Makiya MA, Taylar-Williams CA, Law MA, et al. «Eosinophil-associated processes underlie differences in clinical presentation of loiasis between temporary residents and those indigenous to Loa-endemic areas.» *Clin Infect Dis*, 2015: 60: 55–63.

Hertz MI, Nana-Djeunga H, Kamgno J, et al. «Identification and characterization of Loa loa antigens responsible for cross-reactivity with rapid diagnostic tests for lymphatic filariasis.» *PLoS neglected tropical diseases*, 2018: 12(11):e0006963.

Kamgno J, Nana-Djeunga HC, Pion SD, et al. «Operationalization of the test and not treat strategy to accelerate the elimination of onchocerciasis and lymphatic filariasis in Central Africa.» *Int Health*, 2018: 10(suppl_1):i49-i53.

Kamgno J, Pion SD, Chesnais CB, et al. «A Test-and-Not-Treat Strategy for Onchocerciasis in Loa loa-Endemic Areas.» *N Engl J Med*, 2017: 377(21):2044-2052.

Louise A. Kelly-Hope, Janet Hemingway, Mark J. Taylor and David H. Molyneux. «Increasing evidence of low lymphatic filariasis prevalence in high risk Loa loa areas in Central and West Africa: a literature review .» *Parasites & Vectors* , 2018: 11:349 .

Metzger WG, Mordmuller B. «Loa loa—does it deserve to be neglected? .» *The Lancet Infectious Diseases*, 2014: 14: 353–357.

Michael E, Bundy DA, Grenfell BT. «Re-assessing the global prevalence and distribution of lymphatic filariasis.» *Parasitology*, 1996: 112: 409–428.

Molyneux DH, Hopkins A, Bradley MH, Kelly-Hope LA. «Multidimensional complexities of filariasis control in an era of large-scale mass drug administration programmes: a can of worms.» *Parasit Vectors*, 2014: 7:363.

Paul E. Simonsen, Peter U. Fischer, Achim Hoerauf, Gary J. Weil. «The Filariases.» *Manson's Tropical Diseases, 23rd Edition*, 2014: 54:765.e1.

Pion S, Chesnais C. «Loiasis.» *Switzerland Springer International Publishing*, 2017: 427–444.

Pion SD, Montavon C, Chesnais CB, Kamgno J, Wanji S, Klion AD, et al. «Positivity of antigen tests used for diagnosis of lymphatic filariasis in individuals without Wuchereria bancrofti infection but with high Loa loa microfilaremia.» *Am J Trop Med Hyg*, 2016: 95:1417–23.

Wanji S, Amvongo-Adjia N, Koudou B, et al. «Cross-Reactivity of Filariais ICT Cards in Areas of Contrasting Endemicity of Loa loa and Mansonella perstans in Cameroon: Implications for Shrinking of the Lymphatic Filariasis Map in the Central African Region.» *PLoS neglected tropical diseases*, 2015: 9(11):e0004184.

Weil GJ, Curtis KC, Fakoli L, Fischer K, Gankpala L, Lammie PJ, et al. «Laboratory and field evaluation of a new rapid test for detecting Wuchereria bancrofti antigen in human blood.» *Am J Trop Med Hyg*, 2013: 89:11–5.

WHO. «Summary of global update on preventive chemotherapy implementation in 2016: crossing the billion.» *Wkly Epidemiol Rec*, 2017: 92: 589–93.

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Zouré HGM, Wanji S, Noma M, Amazigo UV, Diggle PJ, Tekle AH, et al. «The Geographic Distribution of Loa loa in Africa: Results of Large-Scale Implementation of the Rapid Assessment Procedure for Loiasis (RAPLOA).» *PLoS Negl Trop Dis* , 2011: 5(6): e1210.

20. Others documents

20.1. Curriculum vitae of investigators

Joseph KAMGNO (MD, MPH, PhD)

Date of birth: January 19, 1965

Nationality: Cameroonian

Gender: Male

Position and address

Posts held: Executive Director (CRFilMT), Deputy Dean in charge of Research and Cooperation (Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I), Technical advisor (NOTF, MoH)

Phone: (Office): 00237 22 20 24 42 **(Mobile):** 00237 77 78 97 36

Fax: 00237 22 20 24 43; **Email:** kamgno@crfilmt.org

Education and trainings

- **Degrees:** MD, MPH, PhD
- **Main proficiencies:** General Medicine; Clinical trials; Biostatistics and Epidemiology; Geographic Information System; Ethics in research; Database construction.

Main Research activities

- **More than 10 clinical trials** for the treatment of filariasis (onchocerciasis, loiasis, lymphatic Filariasis);
- **Mapping** of filariasis (onchocerciasis, loiasis, lymphatic Filariasis) and other tropical diseases;
- **Clinical impact of loiasis;**
- **Impact** of mass drug administration on the prevalence, intensity and transmission of filariasis in Central Africa;
- **Elaboration of the surveillance system and training** for the management of post-ivermectin adverse events in Cameroon, Democratic Republic of Congo, Angola, South Sudan.

Publications (about 100 of which the 05 most significant)

Kamgno J, Pion S, Chesnais C, Bakalar M, D'Ambrosio M, Mackenzie CD, Nana-Djeunga HC, Gounoue-Kamkumo R, Njitchouang GR, Nwane P, Tchatchueng-Mbougua JB, Wanji S, Stolk WA, Fletcher DA, Klion AD, Nutman TB, Boussinesq M (2017). "Test and not treat" for onchocerciasis control in a Loa loa endemic area. *New England Journal of Medicine*. DOI: 10.1056/NEJMoa1705026.

Nana-Djeunga H, Tchouakui M, Njitchouang GR, Tchatchueng-Mbougua J, Nwane P, Domche A, Bopda J, Mbickmen-Tchana S, Akame J, Tarini A, Epee E, Biholong B, Zhang Y, Tougoue JJ, Kabore A, Njiokou F & **Kamgno J** (2017). First Evidence of Lymphatic Filariasis Transmission Interruption in Cameroon: 3 Progress towards Elimination. *PLoS Neglected Tropical Diseases* 11(6): e0005633.

Gardon J., Gardon-Wendel N., Demanga-Ngangue, **Kamgno J.**, Chippaux J.P. & Boussinesq M. (1997). Serious reactions after mass treatment of onchocerciasis with ivermectin in an area endemic for Loa loa infection. Lancet, 350, 18-22.

Gardon J., Boussinesq M., **Kamgno J.**, Gardon-Wendel N., Demanga-Ngangue & Duke B.O.L. (2002). Effects of standard and high doses of ivermectin on adult worms of Onchocerca volvulus: a randomised controlled trial. Lancet, 360, 203-210.

Pion S.D.S., **Kamgno J.**, Demanga-Ngangue & Boussinesq M. (2002). Excess mortality associated with blindness in the onchocerciasis focus of the Mbam Valley, Cameroon. Annals of Tropical Medicine and Parasitology, 96, 181-189.

Society's Membership and expertise

- Member of the American Society of Tropical Medicine and Hygiene (ASTMH)
- Member of the Cameroon Society of Epidemiology (CaSE)
- Member of the Mectizan/Albendazole Expert committee
- Member of the Expert Committee for the Evaluating Moxidectin development

Philip BUDGE (MD, PhD)

CITIZENSHIP: USA

ADDRESS AND TELEPHONE NUMBERS:

Washington University School of Medicine

660 South Euclid Avenue

Campus Box 8051,

St. Louis, MO 60110-1093

(314) 747-5532

pbudge@wustl.edu

PRESENT POSITION:

Assistant Professor of Medicine, Infectious Diseases Division, Washington University School of Medicine, St. Louis, MO

EDUCATION:

1991 – 1998	BS (Molecular Biology), Brigham Young University, Provo, UT
1998 – 2007	MD, PhD (Microbiology and Immunology), Vanderbilt University School of Medicine, Nashville, TN
2004 (Jan – Aug)	Postdoctoral Fellowship, Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, TN
2007 – 2009	Internal Medicine Internship and Residency, Vanderbilt University Medical Center, Nashville, TN
2009 – 2011	Epidemic Intelligence Service, Centers for Disease Control and Prevention (Division of Parasitic Diseases and Malaria), Atlanta, GA
2011 – 2014	Infectious Diseases Fellowship, Vanderbilt University Medical Center, Nashville, TN

ACADEMIC POSITIONS / EMPLOYMENT:

2014 – present	Assistant Professor of Medicine, Division of Infectious Diseases, Washington University School of Medicine, St. Louis, MO

UNIVERSITY AND HOSPITAL APPOINTMENTS AND COMMITTEES:

2011 – 2014	Hospitalist (part time), Scoville Internal Medicine Service, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN
2013	Internal Review Committee, Vascular Surgery Program, Office of Graduate Medial Education, Vanderbilt University School of Medicine, Nashville, TN
2013 – 2014	Hospitalist (part time), Williamson Medical Center, Franklin, TN
2014 – present	Physician, Barnes-Jewish Hospital, St. Louis, MO
2014 – 2016	Governing Board, Infectious Disease Clinic, Washington University in St. Louis School of Medicine
2015	Rapid Improvement Event Team, Infectious Disease Clinic, Washington University in St. Louis School of Medicine

MEDICAL LICENSURE AND BOARD CERTIFICATION:

2009 – 2014	Tennessee medical license 44675
2012 – 2022	American Board of Internal Medicine, certificate in Internal Medicine
2013 – 2023	American Board of Internal Medicine, certificate in Infectious Diseases
2014 – present	Missouri medical license 2014018769

HONORS AND AWARDS:

2000 – 2007	Medical Scientist Training Program, Vanderbilt University School of Medicine
2001 – 2003	Intramural Research Training Award, National Institutes of Health
2007	Max Kade Fellowship, American Austrian Foundation
2007	Outstanding Senior Medical Student in Infectious Diseases, Vanderbilt University School of Medicine
2007	Overall Fellowship, Vanderbilt University School of Medicine
2012	Golden Pen Award, Florida Environmental Health Association
2012 – 2013	Kirschstein National Research Service Award Department of Preventive Medicine, Vanderbilt University Medical Center
2013 – 2014	Kirschstein National Research Service Award Division of Infectious Diseases, Department of Medicine, Vanderbilt University Medical Center
2014	Outstanding Fellow in Infectious Diseases, Vanderbilt University Medical Center

EDITORIAL RESPONSIBILITIES:

Reviewer:	American Journal of Tropical Medicine and Hygiene (2014-2015, 2017-2018)
	BioMed Research International (2013)
	Biomedical Engineering/Biomedizinische Technik (2018)
	The BMJ (2018)
	Journal of Clinical Microbiology (2016)
	Lancet Infectious Diseases (2016)
	Lymphatic Research and Biology (2018)
	Parasitology Research (2019)
	PLoS Neglected Tropical Diseases (2013 – 2019)
	PLoS One (2013 – 2014)
	Transactions of the Royal Society of Tropical Medicine & Hygiene (2015, 2018)
	Tropical Medicine and Infectious Diseases (2017, 2019)

NATIONAL SCIENTIFIC PANELS:

2018	NIAID Small Business Innovation Research (SBIR) Study Section: Topic 59, Diagnostics to Enable Malaria and Neglected Tropical Diseases (NTDs) Elimination

PROFESSIONAL SOCIETIES AND ORGANIZATIONS:

2007 – present	American Society of Tropical Medicine and Hygiene
2011 – present	Infectious Disease Society of America

INVITED PROFESSORSHIPS AND LECTURESHIPS:

Jan 2014	"Global elimination of lymphatic filariasis: progress and challenges", Division of Infectious Diseases, Saint Louis University, St. Louis, MO
Feb 2014	"Global elimination of lymphatic filariasis: progress and challenges", Division of Infectious Diseases, Washington University, St. Louis, MO
Oct 2015 – 2018	"Parasitic Diseases in 35 Minutes", V.T. Andriole Board Review Course, IDSA Annual Meeting (ID Week)
Apr 2017	"Progress towards elimination of LF". ID Grand Rounds, Vanderbilt University, Nashville, TN
Apr 2017	Harrison Society Career Development Panel. Vanderbilt University, Nashville, TN
Jun 2019	"Doxycycline for Filarial Lymphedema". Lymphatic Forum 2019. Austin, TX

RESEARCH SUPPORT:
Active

Governmental NIH/NIAID K08 AI121422-01A1 (07/01/2016 – 06/30/2021)

Improved antigen detection tests for filarial infections

Role: principle investigator

Total direct cost: $855,606

USAID LEDoxy Study (03/14/2016 – 06/11/2019)

A multi-center, double-blind randomized, 24-month study, to compare the efficacy of doxycycline 200 mg [once daily] versus placebo in improving filarial lymphedema (independent of active filarial infection)

Role: co-investigator

Total direct cost: $137,846

Non-Governmental Bill and Melinda Gates Foundation OPP GH 5342 (11/1/2009 – 10/31/2019)

Optimization of chemotherapy for control and elimination of onchocerciasis and lymphatic filariasis

Role: co-investigator

Total direct cost: $29,853,900

Bill and Melinda Gates Foundation (2019 – 2024, pending)

DOLF Next-Gen: Next-generation studies to optimize chemotherapy for elimination of lymphatic filariasis and onchocerciasis.

Role: co-investigator

Total direct cost: $24,731,775

Doris Duke Charitable Foundation

Clinical Scientist Development Award (CSDA) (07/1/2019 – 06/30/2022)

Cross-reactive antigenemia and treatment-related adverse events in loiasis

Role: principle investigator

Total direct cost: $450,000

Past Vanderbilt Physician Scientist Development Award (2014)

Low-Resource Detection of High-Level Loiasis

Role: principle investigator

Total cost: $75,000 (declined)

NIH Loan Repayment Program, NIAID (7/1/2014 – 6/30/2018)

Improved antigen detection tests for filarial diseases

Role: Awardee

Total cost: $57,587

CLINICAL TITLE AND RESPONSIBILITIES:

2014 – present	Attending Physician, Internal Medicine
2014 – present	Consulting Physician, Infectious Diseases

TEACHING TITLES AND RESPONSIBILITIES:

Centers for Disease Control and Prevention

Sep 2010	Lecturer, Haitian Ministry of Health Epidemiology Training Division of Parasitic Diseases and Malaria

Vanderbilt University School of Medicine

2012 – 2013	Small Group Instructor, Epidemiology 1: Research Design

Washington University School of Medicine

Aug 2014	Panelist, Global Health Career Panel, Global Health Scholars Pathway in Internal Medicine
Sep 2014	Panelist, Engineering World Health panel on innovating low cost technology to solve global health problems
Fall 2014 – 2018	Lecturer, Global Health Clinical Cases, Global Health Scholars Pathway in Internal Medicine
Nov 2014	Faculty Host, Forum for International Health and Tropical Medicine (FIHTM) medical student dinner discussion
Dec 2015	Lecturer, Internal Medicine House Staff Noon Conference, parasitic diseases
Fall 2016, 2019	Lecturer, Infectious Disease Core Curriculum (for ID Fellows). Helminths I & II
Spring 2017 – 2019	Lecturer, Anthropology 161: “Gender, Youth, and Global Health”

Spring 2017 – 2019	Small Group Instructor, Medical Microbiology (2nd year medical school curriculum)
Apr 2017	Lecturer, FIHTM Global Health Symposium
Fall 2017	Preceptor, Reading Elective (Tarik Salih): Preventive Medicine in the Primary Care Setting
Dec 2017	Panelist, "Faculty Professional Development", 2017 Midwest Universities for Global Health Meeting
Dec 2017	Lecture: "Global Outbreak Response and Disease Eradication", Forum for International Health and Tropical Medicine (FIHTM) student group
Spring 2019	Lecturer ("Helminths"), Medical Microbiology (2nd year medical school curriculum)

TRAINING / MENTEE RECORD:

Current Trainees / Mentees

2016 – present	Marla Hertz	Postdoctoral Fellow
2018 – present	Irene Hamlin	Undergraduate Student
2019 – present	Aja Drain	Undergraduate Student

Past Trainees / Mentees

2010 – 2011	Amanda Akosa	Volunteer, Centers for Disease Control and Prevention
2016 – 2017	Carly Herbert	Undergraduate Student
2018	Celia Zhou	WUSM Summer Research Program in Global Health
2018 – 2019	Georgia Redd	Undergraduate Student
2018 – 2019	Grant Owen	Undergraduate Student
2018 – 2019	Phil Glaessner	Undergraduate Student

BIBLIOGRAPHY:
Peer Reviewed Manuscripts

1. **Budge PJ**, Lebowitz J, Graham BS. Antiviral activity of RhoA-derived peptides against respiratory syncytial virus is dependent on formation of peptide dimers. Antimicrob Agents Chemother. 2003: 47: 3470-3477. PMID: 14576104.
2. **Budge PJ**, Li Y, Beeler JA, Graham BS, RhoA-derived peptide dimers share mechanistic properties with other polyanionic inhibitors of respiratory syncytial virus (RSV), including disruption of viral attachment and dependence on RSV G. J Virol 2004: 78: 5015-5022. PMID: 15113882.

3. **Budge PJ**, Graham BS, Inhibition of respiratory syncytial virus by RhoA-derived peptides: implications for the development of improved antiviral agents targeting heparin-binding viruses. J Antimicrob Chemother 2004: 54: 299-302. PMID: 15254023.
4. Herzog B, Cardenas J, Hall RK, Villena JA, **Budge PJ**, Giguere V, Granner DK, Kralli A, Estrogen-related receptor alpha is a repressor of phosphoenolpyruvate carboxykinase gene transcription. J Biol Chem 2006: 281: 99-106. PMID: 16267049.
5. Kallen AJ, Brunkard J, Moore Z, **Budge P**, Arnold KE, Fosheim G, Fineli L, Beekmann SE, Polgreen PM, Gorwitz R, Hageman J, Staphylococcus aureus community-acquired pneumonia during the 2006 to 2007 influenza season. Ann Emerg Med 2009: 53: 358-365. PMID: 18534715.
6. Centers for Disease Control and Prevention, Balamuthia mandrillaris transmitted through organ transplantation—Mississippi, 2009. MMWR Morb Mortal Wkly Rep 2010: 59: 1165-1170. PMID 20847719. (**Primary contributor—**MMWR articles did not attribute personal authorship at that time). Reprinted in (1) JAMA 2010: 304: 2008-2012, and (2) Am J Transplant 2011: 11:173-176.
7. Mathieu E, Dorkenoo A, Otogbe FKJ, **Budge PJ**, Sodahlon YK, A laboratory-based surveillance system for Wuchereria bancrofti in Togo: a practical model for resource-poor settings. Am J Trop Med Hyg 2011: 84: 988-993. PMID: 21633038.
8. Pelletreau S, Nyaku M , Dembele M , Sarr B , **Budge P**, Ross R, Mathieu E. The field-testing of a novel integrated mapping protocol for neglected tropical diseases. PLoS Negl Trop Dis 2011: 5: e1380. PMID: 22102921.
9. Lopez C**, Budge P**, Chen J, Bilyeu S, Mirza A, Custodio H, Irazuzta J, Visvesvara G, Sullivan KJ, Primary amebic meningoencephalitis: a case report and literature review. Pediatr Emerg Care 2012: 28: 272-276. PMID: 22391923.
10. Ailes E**, Budge P**, Shankar M, Collier S, Brinton W, Cronquist A, Chen M, Thornton A, Beach MJ, Brunkard JM, Economic and health impacts associated with a Salmonella typhimurium drinking water outbreak—Alamosa, CO, 2008. PLoS One 2013: 8: e57439. PMID: 23526942.
11. **Budge PJ**, Lazensky R, Van Zile KW, Elliott KE, Dooyema CA, Visvesvara GS, Beach MJ, Yoder JS, Primary amebic meningoencephalitis in Florida: a case report and epidemiological review of Florida cases. J Environ Health 2013: 75: 26-31. PMID: 23621053.
12. **Budge PJ,** Little KM, Mues KE, Kennedy ED, Prakash A, Rout J, Fox LM, Impact of community-based lymphedema management on perceived disability among patients with lymphatic filariasis in Orissa State, India. PLoS Negl Trop Dis 2013: 7: e2100. PMID: 23516648.
13. **Budge PJ**, Dorkenoo AM, Sodahlon YK, Fasuyi OB, Mathieu E, Ongoing surveillance for lymphatic filariasis in Togo: assessment of alternatives and nationwide reassessment of transmission status. Am J Trop Med Hyg 2014: 90: 89-95. PMID: 24189363.
14. **Budge PJ**, Griffin MR, Edwards KM, Williams JV, Verastegui H, Hartinger SM, Maeusezahl D, Johnson M, Klemenc JM, Zhu Y, Gil AI, Lanata CF, Grijalva CG; RESPIRA PERU Group, A household-based study of acute viral respiratory illness in Andean children. Pediatr Infect Dis J 2014: 33: 443-447. PMID: 24378948.
15. **Budge PJ,** Griffin MR, Edwards KM, Williams JV, Verastegui H, Hartinger SM, Maeusezahl D, Johnson M, Klemenc JM, Zhu Y, Gil AI, Lanata CF, Grijalva CG; RESPIRA PERU Group, Impact of home environment interventions on the risk of influenza-associated ARI in Andean children: observations from a prospective household-based cohort study. PLoS One 2014: 9: e91247. PMID: 24622044.

16. Mues KE, Deming M, Kleinbaum DG, **Budge PJ**, Klein M, Leon JS, Prakash A, Rout J, Fox LM, Impact of a community-based lymphedema management program on episodes of adenolymphangitis (ADLA) and lymphedema progression—Odisha State, India. PLoS Negl Trop Dis 2014: 8:e3140. PMID: 25211334.

17. Wu A, **Budge PJ**, Williams JV, Griffin MR, Edwards KM, Johnson M, Zhu Y, Hartinger SM, Verastegui H, Gil AI, Lanata CF, Grijalva CG, Incidence and risk factors for respiratory syncytial virus and human metapneumovirus infections among children in the remote highlands of Peru. PLoS ONE 2015: 10: e0130233. PMID: 26107630.

18. **Budge PJ**, Sognikin E, Akosa A, Mathieu EM, Deming M, Accuracy of coverage survey recall following an integrated mass drug administration for lymphatic filariasis, schistosomiasis, and soil transmitted helminthiasis. PLoS Negl Trop Dis. 2016: 10:e4358. PMID: 26766287.

19. Farnon ED, Kokko K, **Budge PJ**, Mbaeyi C, Lutterloh E, Qvanrstrom Y, da Silva AJ, Shieh W, Roy S, Paddock CD, Sriram R, Zaki SR, Visvesvara G, Kuehnert MJ, Transmission of *Balamuthia mandrillaris* by organ transplantation. Clin Infect Dis. 2016. 63: 878-88. PMID 27358357.

20. Yahathugoda C, Weiler MJ, Rao R, De Silva L, Dixon JB, Weerasooriya MV, Weil GJ, **Budge PJ**. Use of a novel portable 3D scanner to measure limb volume and circumference in patients with filarial lymphedema. Am J Trop Med Hyg. 2017: 97(6):1836-1842. PMID: 29141750.

21. **Budge PJ,** Herbert C, Andersen BJ, Weil GJ. Adverse events following single dose treatment of lymphatic filariasis: observations from a review of the literature. PLoS Negl Trop Dis. 2018: 12:e0006454. PMID 29768412.

22. Hertz MI, Nana-Djeuna H, Kamgno J, Njouendou AJ, Chunda VC, Wanji S, Rush A, Fischer PU, Weil GJ, **Budge PJ**. Identification and characterization of *Loa loa* antigens responsible for cross-reactivity with rapid diagnostic tests for lymphatic filariasis. PLoS Negl Trop Dis. 2018: 12:e0006963. PMID 30444866

23. Weil GJ, Bogus J, Christian M, Dubray C, Djuardi Y, Fischer PU, et al. The safety of double- and triple-drug community mass drug administration for lymphatic filariasis: A multicenter, open-label, cluster-randomized study. PLoS Med. 2019;16(6):e1002839. doi:10.1371/journal.pmed.1002839. PubMed PMID: 31233507

Invited Publications

1. Mejia-Chew C, **Budge PJ**. Helminthic Infections. Washington Manual of Infectious Disease Subspecialty Consult.

2. **Budge PJ**, Odom John AR. The longest mile: moving malaria from clinical care to elimination of transmission. Clin Chem. 2019. doi:10.1373/clinchem.2019.303719. PubMed PMID: 31171527

Hugues NANA DJEUNGA (PhD)

Date of birth: July 24, 1982

Nationality: Cameroonian

Gender: Male

Position and address

2012-present: Researcher

Centre for Research on Filariasis and other Tropical Diseases (CRFilMT)

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Tel. Office: 00237 222 20 24 42| **Mobile 1:** 00237 699 07 64 99| **Mobile 2:** 00237 651 88 56 39 | **Fax:** 00237 222 20 24 43 | **Web site:** www.crfilmt.org | E-mail: nanaclotaire@yahoo.fr | nanadjeunga@crfilmt.org

Education and trainings

Degrees: PhD in Molecular Parasitology and Population Genomics, Department of Animal Biology and Physiology, University of Yaoundé I (UYI), Yaoundé, Cameroon

Main proficiencies: Parasitology, Molecular Biology and Bioinformatics; Population Genomics and Genetic Epidemiology; Biostatistics and Epidemiology; Geographic Information System; Ethics in research; Hazardous Waste Management and Biosafety.

Main Research activities

- **Filariasis:** epidemiology and mapping of onchocerciasis, loiasis, lymphatic Filariasis in Cameroon; impact of loiasis; impact of treatments on endemicity and transmission of onchocerciasis and lymphatic filariasis; drug resistance studies; severe adverse events management
- **Sleeping sickness:** analysis of domestic and wild animal reservoir of trypanosomes, assessment of risk factors in Cameroonian foci, design and evaluation of transmission risk indices, interactions between trypanosomes, tsetse flies and endosymbiotic bacteria
- **Malaria:** epidemiology of malaria and genetic diversity of *Plasmodium falciparum* msp-1 block 2, a possible candidate vaccine against malaria
- **Schistosomiasis and Soil Transmitted Helminthiasis:** epidemiology and risk factors of schistosomiasis and soil transmitted helminths in Cameroon

Publications (40 of which the 05 most significant)

Nana-Djeunga HC, Fossuo-Thotchum F, Pion SD, Chesnais CB, Kubofcik J, Mackenzie CD, Klion AD, Boussinesq M, Nutman TB, Kamgno J **(2019)**. *Loa loa* microfilariae in skin snips: consequences for onchocerciasis monitoring and evaluation in *L. loa* endemic areas. Clinical Infectious Diseases. In press.

Chesnais CB, **Nana-Djeunga HC,** Njamnshi AK, Lenou-Nanga CG, Boullé C, Zoung-Kanyi Bissek AC, Kamgno J, Colebunders R, Boussinesq M, (2018). The temporal relationship between onchocerciasis and epilepsy: a population-based cohort study. *Lancet Infectious Diseases*. http://dx.doi.org/10.1016/S1473-3099(18)30425-0.

Kamgno J, Pion S, Chesnais C, Bakalar M, D'Ambrosio M, Mackenzie CD, **Nana-Djeunga HC,** Gounoue-Kamkumo R, Njitchouang GR, Nwane P, Tchatchueng-Mbougua JB, Wanji S, Stolk WA, Fletcher DA, Klion AD, Nutman TB, Boussinesq M (2017). "Test and not treat" for onchocerciasis control in a Loa loa endemic area. *New England Journal of Medicine*. DOI: 10.1056/NEJMoa1705026.

Nana-Djeunga H, Tchouakui M, Njitchouang GR, Tchatchueng-Mbougua J, Nwane P, Domche A, Bopda J, Mbickmen-Tchana S, Akame J, Tarini A, Epee E, Biholong B, Zhang Y, Tougoue JJ, Kabore A, Njiokou F & Kamgno J (2017). First Evidence of Lymphatic Filariasis Transmission Interruption in Cameroon: 3 Progress towards Elimination. *PLoS Neglected Tropical Diseases* 11(6): e0005633.

Nana-Djeunga H, Bourguinat C, Pion SDS, Kamgno J, Gardon J, Njiokou F, Boussinesq M & Prichard RK (2012). Single Nucleotide Polymorphism in β-tubulin selected in *Onchocerca volvulus* following repeated ivermectin treatment: possible indication of resistance selection. *Molecular and Biochemical Parasitology* 185: 10-18

Society's Membership and expertise

- Member of the Cameroon Academy of Young Scientists (CAYS)
- Member of the American Society of Tropical Medicine and Hygiene (ASTMH)
- Member of the African Research Network for Neglected Tropical Diseases (ARNTDs)
- Member of the Cameroon Society of Epidemiology (CaSE)
- Member of the Editorial Board of American Journal of Clinical Immunology and Infectious Diseases, Global Journal of infectious diseases and Clinical Research
- Reviewer for Infectious Diseases and Poverty, BMJ Open, Journal of Public Health and Epidemiology, SciMedCentral, Tropical Medicine and Health, Journal of Medical Chemistry, HINDAWI, Journal of Veterinary Medicine and Animal Health, Jacobs Journal of Clinical Case Reports
- Temporary Consultant for Drugs for Neglected Diseases Initiative (DNDi), World Health Organization (WHO) and the former African Programme for Onchocerciasis Control (APOC)

Marla HERTZ (PhD)

Edit

Linda DJUNE YEMELI (MSc, PhD Candidate)

Date of birth: December 10, 1990

Nationality: Cameroonian

Gender: Female

Position and address

2018-present: Research Assistant

Centre for Research on Filariasis and other Tropical Diseases (CRFilMT)

Street 1302, Quartier Fouda | **PO Box** 5797 Yaounde, Cameroon

Tel. Office: 00237 222 20 24 42| **Mobile 1:** 00237 655 11 96 48 | **Mobile 2:** 00237 670 14 35 88 | **Fax:** 00237 222 20 24 43 | **Web site:** www.crfilmt.org | E-mail: djunelinda@gmail.com

Education and trainings

Degrees: PhD Candidate, Department of Biochemistry, University of Yaoundé I, Yaounde, Cameroon

Main proficiencies: Immunology and immunological technics, molecular biology and biomolecular technics, and other biochemistry technics. Basic technic in parasitology, good understanding of ethics in biomedical research, good understanding of informed consent process.

Other Relevant trainings

- Training on OV-16 ELISA technic by CDC (CRFilMT)
- Certification of First Yaoundé Advance Course in Immunology (University of Yaoundé 1)
- Training on HRP2 ELISA technic, for malaria diagnosis (Laboratory of research on malaria, Centre Pasteur de Yaoundé)

- Training on diagnosis of measles by ELISA
- Training on ELISA and PBMCs isolation (Laboratory of research on malaria, Centre Pasteur de Yaoundé)
- Training on microscopic detection of malaria parasites (Laboratory of Public Health Biotechnology, Biotechnology center)
- Training on stool analysis by Kato-Katz technic (IMPM of Nkomo)
- Training on biomolecular technics (Laboratory of Public Health Biotechnology, Biotechnology center)

Main Research activities

- **Malaria and Schistosomiasis:** molecular and rapid diagnostic of malaria; influence of *Schistosoma mansoni* on *Plasmodium falciparum hrp2* gene deletion, and therefore on malaria HRP2 rapid diagnostic test; Immune interaction between malaria and schistosomiasis parasites (influence of schistosomiasis on malaria pathogenesis)
- **Other Tropical Diseases:** Onchocerciasis, Lymphatic Filariasis and Soil Transmitted Helminthiasis.

Society's Membership and expertise

- Member of the EDCTP-CANTAM project, since December 2018
- Close to 3 years research experience as member of Molecular Diagnosis Group
- Member of the Laboratory of Public Health Biotechnology, Biotechnology center, University of Yaoundé 1

Gary J Weil (MD, PhD)

Edit

20.2. Site Authorizations

MINISTERE DE LA SANTE PUBLIQUE
****
DELEGATION REGIONALE DU CENTRE
****
DISTRICT DE SANTE D'OKOLA
****

REPUBLIQUE DU CAMEROUN
****
Paix-Travail-Patrie
****

Yaoundé, le 27 juin 2019

A Monsieur le Président du Comité National d'Ethique pour le Recherche en Santé Humaine (CNERSH)

Objet : Accord de principe

Monsieur le Président,

J'ai l'honneur de venir auprès de votre haute bienveillance, soutenir la demande de clairance éthique pour l'étude intitulée « **Cross-reactive antigenemia and treatment-related adverse events in loiasis ».** L'investigateur principal de cette étude est le Prof Joseph Kamgno et le laboratoire porteur du projet est le Centre Recherche sur les Filarioses et autres Maladies Tropicales (CRFilMT) dont il est le Directeur.

J'ai pris connaissance du protocole de cette étude qui permettra de (i) définir le profil des antigènes de la loase, responsables des résultats faux-positifs lors du diagnostic de la filariose lymphatique par les tests de diagnostic rapides, et de (ii) déterminer si la production de ces antigènes spécifiques et/ou leur quantité est en corrélation avec l'apparition et la sévérité des effets secondaires observés suite au traitement des personnes avec une forte charge de loase par l'Ivermectine. En effet le district de santé d'Okola est fortement endémique pour la loase, ce qui a rend difficile la cartographie de la filariose lymphatique par TDRs, ainsi que le traitement de masse par l'Ivermectine. L'identification des antigènes de la loase ayant réactivité croisée et leur relation avec les effets secondaires liés au traitement par l'Ivermectine contribuera à la mise au point de TDRs plus fiable et aidera à mieux comprendre la cause des effets secondaires liés au traitement par l'Ivermectine.

Dans l'attente d'une suite favorable, veuillez agréer Monsieur le Président l'expression de ma haute considération.

Chef de Service de Santé du District d'Okola

20.3. Material/data sharing agreement

Preamble

This document defines the arrangements for material transferring and data sharing between the **Centre for Research on Filariasis and other Tropical Diseases (CRFilMT)** and **Washington University in Saint Louis (WUSTL)** under the project **"Cross-reactive antigenemia and treatment-related adverse events in loiasis"** that binds them.

I. PARTIES TO THE AGREEMENT

Institution providing the material and/or data: Centre for Research on Filariasis and other Tropical Diseases (CRFilMT), Yaounde, Cameroon ("Provider")

Contact person: Joseph KAMGNO (MD, MPH, PhD)

Title: Director of the CRFilMT and Vice-Dean in charge of Research and Cooperation at the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I.

Address: Street 1308, Quartier Fouda | PO Box 5797 Yaoundé, Cameroon

Phone number: 00237 677 789 736 | 00237 222 202 442

Email: kamgno@crfilmt.org

Fax number: 00237 222 202 443

Institution receiving the material and/or data: Washington University in St. Louis ("Recipient")

Contact person: Philip Budge, MD, PhD

Title: Assistant Professor of Medicine and Infectious Diseases

Address: 4444 Forest Park Ave room 4186c, St. Louis, MO, 63108. USA

Phone number: +1-314-747-5532 or +1-314-747-5198

Email: pbudge@dom.wustl.edu

Fax number: +1-314-454-5293

II. PURPOSE AND TERM OF AGREEMENT

A. Purpose of material and/or data sharing

To investigate what are the cross-reactive *L. loa* antigens, how and when are they released, and if their releases contribute to treatment-related adverse events observed in individuals with heavy loiasis infection. This study is of great importance for mapping, transmission assessment survey, surveillance and validation of the elimination of lymphatic filariasis in areas where loiasis is co-endemic. Indeed, it was demonstrated that people harboring high *L. loa* microfilarial densities can exhibit false-positive rapid diagnostic tests result while targeting LF.

B. Legality and credibility of parties

1. The Centre for Research on filariasis and other Tropical Diseases (CRFilMT) is a non-profit organization working in the medical field whose mission is to contribute to the improvement of the living conditions of the populations through an appropriate and applied scientific and medical research. The CRFilMT had signed a collaboration agreement with the Ministry of health of Cameroon as well as with many other national and international institutions and was positioned in 2009 as reference in Central Africa for surveillance of different filariasis. In addition, the CRFilMT also received in 2009 an official testimony of satisfaction from the Ministry of health of Cameroon.
2. Washington University in St. Louis, a corporation established by special act of the Missouri General Assembly approved February 22, 1853 and acts amendatory thereto, having its principal office at One Brookings Drive, St. Louis, MO 63130, USA (WUSTL) is among the top-ranked medical research institutions in the United States.

C. Period of performance

This Agreement shall be effective when signed by both parties and shall continue until terminated pursuant to the termination clause contained herein.

III. MATERIAL AND DATA MANAGEMENT PLAN

Sample collection will be done after approval by WUSTL Institutional Review Board (IRB) and Cameroon Regional Ethic Committee for Research in Human Health for *Centre* region, only on those individuals who will agree to participate in the study by signing a consent form. Thick blood smears will be prepared and stored at the CRFilMT in a locked cabinet designated for sample storage. Venous blood samples will be used for the preparation of dried blood spots and isolation of plasma samples. These plasma samples will be transferred in anonymously recoded 4.8 ml cryovial tubes containing and stored at -20°C at the CRFilMT. These anonymous samples ("Samples") will then be shipped to WUSTL where they will be processed, blinded from the personal information of the subjects ("Results"). Hard copies with personal information of subjects will be stored at CRFilMT in a locked cabinet designated for data storage. Samples collection materials or used Samples will be destroyed according to the validated security norms.

IV. ACCESS TO MATERIAL AND/OR DATA

A. Method of access and transfer

Thick blood smears slides and hard copies of data collection forms, stored in locked cabinet, will only be accessible at the CRFilMT after specific authorizations. DBS and plasma Samples shipped at WUSTL will be processed to determine and quantify cross-reactive loiasis antigens and cytokines. Secured electronic databases generated by CRFilMT and WUSTL will be shared between both parties.

B. Persons having access to data

Only principal investigators (and/or authorized designee) will have access to data and/or stored material. Prior to the transfer of any data, team members and researchers who will have access to the data should request specific authorization to the principal investigators (both at CRFilMT and WUSTL).

C. Frequency of data exchange

Data will be exchanged as needed to meet reporting requirements as well as on an ongoing basis between CRFilMT and WUSTL teams for the entire length of the project.

V. SECURITY OF DATA AND CONFIDENTIALITY OF SUBJECT RECORDS

Neither CRFilMT team, nor WUSTL team will attempt to identify individuals' records by any method. Datasets containing protected personal information shall be encrypted. All reasonable precautions shall be taken to secure the data from individuals who do not specifically have authorized access. Data shall be kept on a password-protected file server located in a secure environment, both at CRFilMT and at WUSTL. Project data will be kept in a separate directory on the server which is also password-protected and will be accessible only by principal investigators or designee with specifically authorized access.

The report of this study will be transmitted to the Cameroon Regional Ethic Committee for Research in Human Health for *Centre* region as well as to the Ministry of Public Health. However, subject personal results will be given to them in strict compliance with privacy rules.

VI. WARRANTY AND LIMITATION OF LIABILITY

A. Warranty

Any Samples or Results delivered pursuant to this Agreement is understood to be experimental in nature and may have hazardous properties. THE PARTIES MAKE NO REPRESENTATIONS AND EXTEND NO WARRANTIES OF ANY KIND, EITHER EXPRESSED OR IMPLIED. THERE ARE NO EXPRESS OR IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, OR THAT THE USE OF THE SAMPLES OR THE RESULTS WILL NOT INFRINGE ANY PATENT, COPYRIGHT, TRADEMARK, OR OTHER PROPRIETARY RIGHTS

B. Limitation of Liability

Except to the extent prohibited by law, the Recipient assumes all liability for damages which may arise from its use, storage or disposal of the Samples. The Provider will not be liable to the Recipient for any loss, claim or demand made by the Recipient, or made against the Recipient by any other party, due to or

arising from the use of the Samples by the Recipient, except to the extent permitted by law when caused by the gross negligence or willful misconduct of the Provider.

Except to the extent prohibited by law, the Provider assumes all liability for damages which may arise from its use of the Results. The Recipient will not be liable to the Provider for any loss, claim or demand made by the Provider or made against the Provider by any other party, due to or arising from the use of the Material by the Provider, except to the extent permitted by law when caused by the gross negligence or willful misconduct of the Recipient.

This agreement must be formally approved and signed by both parties before any material transfer or data sharing takes place. Both parties will ensure that the material transfer and the data sharing agreements are known and understood by all staff involved in the process.

Centre for Research on Filariasis and other Tropical Diseases (CRFilMT)

_____	_____
Name	Position
_____	_____
Signature	Date

Washington University in Saint Louis (WUSTL)

_____	_____
Name	Position
_____	_____
Signature	Date

20.4. Receipt for Evaluation fees