**NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH (NMIMR)**
**COLLEGE OF HEALTH SCIENCES, UNIVERSITY OF GHANA, LEGON**

**INSTITUTIONAL REVIEW BOARD**

# FORM A – INITIAL PROTOCOL SUBMISSION FORM

**SECTION A –**

**Title of Proposal:** Identification of Novel agents for HIV Cure or Remission

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**Type of Research:** Biomedical ☒

Social/Behavioural ☐

Others (please specify _____

**Duration of project**: 5 years

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**PROPOSAL OUTLINE**

**Abstract/Executive Summary** (Not more than 250 words)

HIV continues to be a major public health problem in Africa. Although combination antiretroviral therapy (cART) has reduced mortality and improved lifespan, it does not provide cure. Patients must take medications daily for the rest of their lives with side effects, unsustainable costs, and development of resistance. The main obstacle to HIV cure is the persistence of the provirus in resting CD4+ T cells, which act as a reservoir to produce virus once treatment is interrupted. Of the approaches being investigated to cure HIV, the 'shock and kill' approach is the most promising. This approach seeks to reactivate and kill the T cells harboring the virus, while patient are on cART, with the idea that patients could then stop treatment after the reservoir cells are eliminated. Despite some modest successes, this approach has not been able to reduce the size of the HIV reservoir, due to inefficient reactivation. Since HIV replication is controlled by surrounding chromatin (epigenetics), we hypothesize that epigenetic modifying compounds will be more effective reactivation agents. Here, we propose to screen an epigenetic library of compounds, select the most effective and evaluate them in resting T cells isolated from HIV patients on cART. To do this, we will follow a cohort of patients, measure their viral loads and select those who have virologic suppression for the reactivation studies. This study will provide useful information on virologic suppression among Ghanaian patients, effectiveness of current therapy and discover novel compounds for the 'shock and kill' approach to HIV cure.

**Introduction/Rationale** (Not more than 5 pages)

HIV continues to be a global public health priority with over one million deaths annually, most of which are in sub-Saharan Africa (http://www.unaids.org/en/resources/fact-sheet). The introduction of combination antiretroviral therapy (cART) has significantly reduced mortality in Africa and increased lifespan. Despite the WHO recommendation to treat all HIV positive patients, less than 50% of patients in sub-Saharan Africa are on treatment and even a lower number are suppressed virologically. It is well known that cART, does not provide a cure for HIV, patients must take medications daily for the rest of their lives (Ananworanich, 2015; Passaes & Saez-Cirion, 2014). Even if we are able to get all patients on cART, there is also the issue of cost, access and side effects. Therefore, some have argued that cART alone will not be enough to end the pandemic(Johnston & Barre-Sinoussi, 2012). There is an urgent need for remedies that will either provide sustained remission or cure. Several groups including the US National Institutes of Health and the International AIDS Society have made HIV cure a priority (Deeks et al., 2016; Tucker, Gilbertson, Lo, & Vitoria, 2016; Walensky, Auerbach, & Office of, 2015) and many consortia and groups are working towards this end. However, not much of these studies are being done in Africa, although the continent bears the brunt of the epidemic. This is a critical gap that needs to be filled. There is the need to have adequate capacity to screen drugs originating in Africa and elsewhere for HIV cure or remission.

In addition, all regions of Africa need to develop the capacity to position themselves for the necessary trials as curative/remission agents become available.

The main obstacle to HIV cure is the persistence of the latent provirus in resting CD4+ T cells. These cells act as a reservoir to produce virus when treatment is interrupted (Ruelas & Greene, 2013; Siliciano & Greene,

2011). Approaches to HIV cure being investigated and tried around the world include boosting the host immune system, genetic approaches to disable co-receptors and the viral genome, and using latency reversing agents (LRAs)(Ananworanich, 2015; Battistini & Sgarbanti, 2014; Choudhary & Margolis, 2011). The use of LRAs to reactivate the latent provirus -- the 'shock and kill' approach -- is the most clinically advanced strategy with several ongoing and completed clinical studies (Archin et al., 2012; Archin & Margolis, 2014; Darcis, Van Driessche, & Van Lint, 2016, 2017; Harper, 2016; Rasmussen et al., 2014; Spivak & Planelles, 2016). The premise behind this idea is that while patients are on cART, they are given an agent that reactivate the latent virus in the resting T cells. Because viral replication is toxic to activated T cells, reactivation will result in death of these T cells. Since patients will be on cART at this stage, any virus produced will not be able to infect bystander cells. Also once viral production begins in the resting cells, they will be recognized by the immune system for clearance. With the clearance of the infected resting T cells, patients could then stop taking cART and undergo occasional monitoring. Of all the approaches being investigated, this seem the most scalable and practical for Africa, since it does not need sophisticated equipment and infrastructure to implement. So far several clinical studies have shown that this approach is based on a sound premise: viral RNA increases in serum when patients on cART are treated with LRAs. However, the challenge has been that the size of the viral reservoir does not significantly change indicating that the agents being used are not potent enough to reactivate most of the infected resting T cells. Therefore, there is the need to find novel compounds that will be more effective for this approach.

The PI and his collaborators have recently shown that a combination of a novel histone deacetylase inhibitors (called Largazoles) and bryostatin analogues are very effective reactivation agents (Albert et al., 2017). Preparations are being made to try this combination in HIV latency animal models for toxicity profiles before being tried in humans. So far histone deacetylate inhibitors (HDACIs) have been the most effective single agents for reactivation that do not lead to global activation of T cells. Since HDACIs affect epigenetic modifications, we hypothesize that screening of compounds that have effect on epigenetics is likely to yield drugs that will be more efficient LRAs(Ay et al., 2013; Choudhary & Margolis, 2011; Saayman, Roberts, Morris, & Weinberg, 2015; Tripathy, Abbas, & Herbein, 2011; Van Lint, Bouchat, & Marcello, 2013). Therefore, in this study, we propose to screen a panel of epigenetic modifying compounds for the ability to reactivate HIV from latency. The choice of this study is strategic in that it will enable us to go through a whole spectrum: from patients, to laboratory and back to patients. It will also enable us to train students in basic laboratory techniques needed for HIV studies.

**Aims or Objectives of study**

***Aim 1: To characterize a cohort of HIV patients and evaluate their knowledge and attitude towards participation in HIV cure research***

Sub-aim 1.1: Establish and follow a cohort of HIV patients and determine virologic suppression, CD4 count and baseline comorbidities. This will enable us to select appropriate patients for Aim 3.

Sub-aim 1.2: To evaluate patients' knowledge and attitudes about HIV treatment options and cure studies. We will determine what patients know and whether they want to participate in cure studies.

Significance and impact: Though recommended by national guidelines, due to lack of resources, viral load testing may not be performed regularly for patients. Patients may have adequate CD4 count with low level viremia that will lead to resistance. Knowing the characteristics of patients who are fully suppressed, and whether low level viremia exist on current therapy is highly significant information for our control programs and may act as a reason to put more resources in and accelerate viral load testing. Patients' knowledge about treatment options and attitude to cure research studies is important to know, as this will inform what risks patients are likely to tolerate.

***Aim 2: To screen a panel of 150 epigenetic modifying compounds for ability to reactivate HIV from latency in a cell line and primary cell model of latency*.**

Significance and impact: This activity will help establish the laboratory procedures and trainings needed at Noguchi, first to screen the compounds in this study but also have the capacity to screen other compounds, such as African herbal extracts in future.

***Aim 3: To evaluate top 10 lead compounds in resting CD4 T cells isolated from patients suppressed on cART for more than 6 months***

Significance and impact: This aim is likely to yield compounds that will be potent reactivation agents. Following this study, if the compound has already been used in humans, then clinical studies in patients could begin. If not, then animal studies for pharmacokinetics and toxicity could begin.

***Aim 4: To assemble a well-characterized HIV patient's biobank to serve as a repository of samples for future research and student training.***

Significance and impact: Lack of well characterized specimens is often a barrier to student training. This will make training of future scientists easier and help strengthen capacity in HIV basic and clinical research at the University of Ghana.

**Methodology** (Include Inclusion and Exclusion Criteria)

*Aim 1: To characterize a cohort of HIV patients and evaluate their knowledge and attitude towards participation in HIV cure research*

Rationale: This aim will enable us to build a cohort of patients on cART treatment and follow them over time for the following reasons: (i) to determine what percentage of patients are virologically suppressed on current therapy. (ii) to enable us recruit appropriate virologically suppressed patients for Aim 3 (ii) to get a broad idea of how patients feel about HIV research in general and cure research in particular.

Patients will be recruited from three HIV treatment centers in Accra: the Korle-Bu Teaching Hospital (KBTH) the largest teaching hospital in Ghana with over 2000 beds and a large center for HIV treatment, the La Polyclinic a district hospital run by the Government and Manna Mission Hospital, a private mission hospital in the inner city of Accra.

*Sample size:* In all we intend to recruit and follow 300 participants. Two hundred samples will be collected from KBTH, seventy samples from the Lekma Hospital and thirty from the University Hospital, Legon. This distribution is based on the numbers of patients that attend HIV clinics in these facilities. All HIV-infected adults (≥ 18 years) attending clinic at the study sites, who are about to initiate antiretroviral therapy will be eligible for the study. At each study sites, convenient sampling will be used to consecutively enroll clients attending clinic within the sampling period, who are due to start ART and who provide written informed consent, into the study until the target number for that site is achieved. Baseline blood samples will be collected from participants before they initiate ART. Two small studies (total 64 patients) performed in Ghanaian adults put the viral suppression rate anywhere from 30 to 70%(Nii-Trebi et al., 2017; Nii-Trebi et al., 2013). Assuming a 30% suppression rate 300 participants will enable us to get 90 eligible patients for the cure studies in Aim 3, where we need suppression for at least 6 months (viral load less than 50 copies per ml). Then assuming consent rate of 60% for we will have 54 patients eligible for the cure studies. All patients will have demographic characteristics collected including age, sex, educational status, income level, occupation and comorbidities. Patient TB status, including prior treatment will be obtained. For those already tested, information will be obtained from medical records. For those not tested, TB testing will be done after symptom screen. Baseline blood count, basic metabolic panel, CD4 and HIV viral load will be obtained. Ten milliliters of blood will be taken per study visit for these tests. Part of this blood will be stored for use in future research analysis. Written consent will be sought from participants before part of their blood is stored for future research.

Inclusion criteria:

- Adults 18 years old or more
- Must be starting or already on cART

Exclusion criteria

- Children less than 18 years old

Children are excluded from this study because of the amount of blood we wish participants to donate in Aim 3.Patients will then be followed every 3 months to assess: blood count, viral load, CD4 count and adherence.

Our criteria for virologic suppression is viral load less than 50 copies per ml. This is different from the standard of care in Africa where viral load of <1000 is considered adequate. A viral load of 1000copies/ml is not considered adequate for reactivation studies. Measuring viral loads every 3 months is also different from standard of care which is 6 months after starting therapy, then 6 months after that, followed by once per year. If we followed the standard of care, we will not be able to achieve the goals of the research. This is because for the purposes of this study, we need to ensure that virologic suppression is maintained.

Embedded in these follow up of patients will be out two sub-aims:

(1) Determination of patient knowledge and attitude towards participation in HIV research and cure research in particular. Very few studies has been done on this subject, mostly in the developed world (Dube et al., 2017; Etokidem, Ogaji, & Okokon, 2014; Evans, 2017; Moodley, Staunton, de Roubaix, & Cotton, 2016; Protiere et al., 2017). Since HIV cure studies, especially when it comes to clinical trials, could involve substantial risks, it is critical that patients are involved in choosing what kinds of research are acceptable. This will be a cross-sectional survey using a questionnaire as the instrument. Questionnaire will be administered to all 300 participants. Questionnaire will be developed, tried at all the sites on 5 patients each and revised based on feedback. Questions to be asked include knowledge about HIV, HIV treatment, whether they have heard about cure research, willingness to participate in trials, willingness to undergo treatment interruption during cure trials, risks without obvious benefits, how much blood they are willing to donate, among others. Initial questionnaire is attached.

(2) We will follow patients to determine what percentage of patients are suppressed virologically over a period of 2 years, factors affecting suppression such as age, socioeconomic status, type of antiretroviral therapy and adherence. We will measure viral load using the COBAS ® AMPLICOR Monitor test (Roche Diagnostic Systems, Branchburg, NJ, USA), available at the at Korle-Bu Teaching Hospital.

*Aim 2: To screen a panel of 150 epigenetic modifying compounds, cocoa and other herbal extracts for ability to reactivate HIV from latency in a cell line and primary cell model of latency*.

Rationale: The shock and kill approach is the most viable approach to HIV cure so far.

Although HIV transcription is very sensitive to epigenetic changes, no epigenetic screen has been done to evaluate HIV reactivation from latency. We hypothesize that screening epigenetic modifying compounds will yield candidate compounds that will be potent HIV latency reversing agents. The 150-compound library will be purchased from the Sellekchem.

(http://www.selleckchem.com/screening/epigenetics-compound-library.html)

The screen will be done using the JLAT 10.6 cell culture system which the PI has extensively used in his lab at Washington University. This cell line has been well described (Lassen, Hebbeler, Bhattacharyya, Lobritz, & Greene, 2012; Spina et al., 2013). The cells are Jurkat cells with HIV inserted at one position in the genome.

The HIV inserted is modified as follows: the viral envelope is disabled and green fluorescent protein (GFP) is engineered in place of the viral protein Nef. HIV is latent in these cells, therefore, expression of GFP is an indication of HIV transcription and production of virus. Typically, less than 2% of cells are positive for GFP at baseline in the JLAT10.6 cells. However, upon adding LRA like TNF alpha up to 90% of cells can show GFP positivity in fluorescent activated cell sorting (FACS) analysis. Because the cells are easily activated, they are a good choice for reactivation screen. We will use TNF alpha and Vorinostat as positive controls.

We chose TNF alpha because it gives a robust reactivation of HIV in this cell type and Vorinostat because it is the gold standard latency reversing agent. Briefly, JLAT 10.6 cells will be plated in 96-well plates. Two concentrations of the compounds (1 uM and 10 uM) will be added in triplicates. After 24 h, FACS will be run for GFP expression using TNF alpha and Vorinostat as positive controls and DMSO as negative control since the compounds come in DMSO. This procedure will be repeated until all the compounds are evaluated. The Noguchi Memorial Institute for Medical Research has the requisite equipment for these experiments.

Selection of lead compounds: Since Vorinostat is the most studied and the gold standard LRA, we will use it as the benchmark. Typically, 1 uM of Vorinostat reactivate about 60% of JLAT cells from latency. Therefore, any compound that reactivate JLAT 10.6 cells up to 40% or more will be considered a positive hit. The positive hits will be rescreened in a primary cell latency model system. For this, we will use the Greene primary cell latency model which the applicant has used in the past (Albert et al., 2017; Lassen et al., 2012). This model is chosen because it is quick, easy to use and easily transferrable to the Ghanaian setting. Briefly, resting CD4+ T cells will be isolated from HIV negative donors (blood will be obtained from the National Blood Bank in Accra, unused blood with no donor identifiers). Cells will be infected with full length replication competent HIV that has luciferase as a marker. Infection will be done with spinoculation at 3000 rpm for 2 hours at 37$^{o}$C and further incubated for 2 hours. Afterwards, cells will be washed, pulled and incubated for 48 hours in the presence of protease inhibitor (Darunavir) to prevent new cycles of infection. The infected cells (which at this point has latent HIV inserted) will be plated in 96-well plates and treated with the compounds being tested in the presence of integrase inhibitor Raltegravir to prevent new integration events. After 48 hours of incubation, HIV reactivation will be measured in the cell lysates as luciferase readouts in the cell lysates. For this part of the screen, CD3/CD28 beads, PMA/Ionomycin and vorinostat will be used as positive controls. Negative control is DMSO. Again, compounds that can reactivate up to the level of Vorinostat or more will considered appropriate for further evaluation in Aim 3. Resting CD4+ T cells isolation will be done with the EasySep CD4 isolation kit from Stem Cell Technologies which typically produces over 96% purity.

Compounds that are deemed as hits will be evaluated for cytotoxicity. Primary resting T cells will be treated with the compounds for 48 hours at the concentrations that was able to reactivate HIV from latency. Viability of cells will be measured using the MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay which has been well described and frequently used in toxicity assays (Maha, Cheong, Leong, & Seow, 2008). Level of toxicity will be measured against that of DMSO (which is non-toxic to resting T cells). With the viability of DMSO treated cells set at 100%, any compound with cell viability 80% or more will be taken as non-toxic and considered for further evaluation.

Aim 3. *To evaluate top 10 lead compounds in resting CD4 T cells isolated from patients suppressed on cART for more than 6 months*

The best 10 candidates that meet the following minimum criteria will be selected for evaluation in patient cells: (i) must reactivate HIV in both the JLAT 10.6 and Greene models (ii) non-toxic to primary cells as defined above (iii) have drug-like structure amenable to medicinal chemistry modifications.

Inclusion criteria:

- Must be part of the original 300 patients being followed (see Aim 1)
- 18 years old or more
- CD4 count 350 or more
- Viral load less than 50 copies per ml for a minimum of 6 months
- Willingness to donate 100 ml (2/5 cup) of blood

Exclusion criteria

- Less than 18 years old
- Not part of the patients being followed
- CD4 count less than 350

From our previous experience, we do not get adequate numbers of resting CD4 T cells for our studies in patients with CD4 count less than 300, hence the 350 threshold. All participants who are virologically suppressed (viral load ≤50copies/ml) are eligible to partake in study Aim 3. However, only those that satisfy the inclusion criteria will be enrolled for Aim 3 and only those enrolled for Aim 3 will be asked to donate 100ml of blood.

The following procedure will be followed: One hundred milliliters of blood taken from patients will be processed in the lab to isolate peripheral blood mononuclear cells (PBMCs), followed by isolation of resting CD4+ T cells using negative selection with a kit form Stem Cell Technologies. Typically 100 mls of blood yields 25 million resting CD4+ T cells in our experience. Cells will be plated in 6 well plates at 5 million cells each and treated with positive controls (CD3/CD28) and negative control (DMSO). The three wells remaining will be treated with three different compounds. After 48 h incubation, supernatant and cell-associated HIV mRNA will be measured with quantitative RTPCR using target primer/probe sets previously described and used by the applicant (Bullen, Laird, Durand, Siliciano, & Siliciano, 2014). Each compound will be tested in 5-10 different patient cells.

Aim 4. *To assemble a well-characterized HIV patient's biobank to serve as a repository of samples for future research and student training.*

All patients recruited for Aim 1 and 3 will be asked for consent for part of their specimens (blood and urine)

to be stored and used for future studies. Anticipated studies include biomarkers of HIV latency, viral resistance testing, biomarkers of HIV suppression, and markers of remission. Another study anticipated is HIV, ageing, and inflammation markers. Yet another area of interest for future study is why some patients' cells are more amenable to HIV reactivation than others. There seem to be genetic differences in reactivation efficiencies from person to person. Since some form of 'shock and kill' therapy is likely to get into clinic, it will be useful if we could determine which patients are likely to benefit, making it a form of precision medicine. Such a biobank of suppressed patient cells could be used to answer some of these questions.

**Statistics and Reproducibility**

All experiments will be performed at least 3 times and appropriate T-statistic or analysis of variance employed. Student T test will be used for pairwise comparisons while ANOVA will be done for multiple comparisons. Experiments will be performed with authenticated cell lines and chemicals and antibodies will be obtained from recognized vendors and catalog and batch numbers recorded and disclosed in all publications. For patient studies in Aim 3, numbers were chosen to show proof of principle that these compounds can reactivate HIV from patient cells. For Aim 1, summary of patient characteristics will employ descriptive statistics such as percentages and quartiles. Mixed models will be used in the longitudinal analyses to evaluate differences in viral load, viral suppression as it relates to medications, age, gender, socioeconomic status among others.

**Feasibility**

The PI is an HIV clinician and scientist with extensive experience in HIV molecular biology and patient care. He has led a recent study from its conception (design, securing approvals, hands-on experimentation, supervising technicians, students and postdocs) at Washington University (WU) to screen histone deacetylase inhibitors that can reactivate HIV from latency (Albert et al., 2017). He has recruited patients at the WU HIV clinic for his translational studies on HIV latency. The NMIMR also has multiple sites for clinical studies, some of which have been chosen for this project. For the laboratory aspects of the study, the NMIMR has all the major equipment like biosafety level 3 facilities, biosafety hoods, ultracentrifuges, FACS machines, qRTPCR machines, all of which are available for the study. Therefore, the applicant has the needed experience and institutional support to successfully accomplish the aims of the study.

**Expected key deliverables and future studies**

This study will have the largest patient cohort in Ghana on antiretroviral therapy, well characterized and engaged in clinical studies. This patient population could be engaged in all kinds of clinical trials and clinical studies. For instance, viral resistance testing for those not suppressed is not part of this study due to budgetary constraints. However, due to our biobank collection, this study could be performed on a large cohort of patients in Ghana. Another key deliverable is the identification of novel compounds for HIV reactivation. The expertise and capacity built for this part of the study will be available for future studies of this kind or other cure-related studies in Ghana. In addition, the compounds found could point to other mechanisms of HIV latency which could be investigated further in future studies.

**Ethical Considerations:** (i.e. consent procedures, confidentiality, privacy, risks and benefit, etc.)
Eligible participants will be identified by the principal investigator or designee, and asked if they are interested in participating. The designee will be a trained nurse who has experience in clinical studies. The PI/designee will review the consent document with the potential participant. The PI/designee will answer any questions the patient may have without coercion or influence. Once all questions are answered, the participant will decide if they want to sign consent. If consent is signed the participant will be enrolled in the study. The PI/designee will make clear to the participant that their care in the clinic will not be affected by their decision to take part in the study or otherwise. In addition, they can change their mind to not participate even after signing a consent. Blood will be taken by venipuncture (10 milliliters during Aim 1) and 100 mls during Aim 3. The blood draw could take approximately 10 minutes. It will be made clear to patients that they can withdraw their consent at each point in the process. Participants will be made aware that this is a research study and does not replace regular check up with their doctor. The benefit to them may be that their viral loads and CD4 will be checked more frequently than if they were not part of the study. The risks include bruising at the venipuncture site, remote possibility of bleeding from the site and that sometimes some people may feel faint after blood is taken from them. These risks will be mitigated by observing participants for at least 5 minutes after blood is taken.

The process will be done in the language that the patient is most comfortable with. Interpreters will be used where necessary. The recruitment will be done in the private patient area in the clinic to enable potential participants to ask questions without embarrassment. In case we need to inform a patient about increasing viral load despite treatment, we will contact the clinic involved, identify patient records and phone the patient to come to clinic for evaluation by their doctor. Patient phone numbers and that of a close relative will be collected to ensure we can reach patients for follow up calls and as needed.

No patient identifiers will be on electronic devices. Only patient numbers with corresponding viral loads, CD4, age and sex will be kept on electronic devices. Paper consents with patient codes will be kept in a locked cabinet in the PI's office. All computers and jump drives used in the study will be encrypted and password enabled. CDs or DVDs will not be used. As compensation, participants will be paid their National Health Insurance premium during the study and be paid 50 Ghana Cedis per visit for transport. They will also receive more regular medical checkups as per study protocol. Participants that will be recruited for study AIM 3, to donate 100ml of blood, will additionally receive nutritional support (400g sachet of Milo and 400g of Milk) to replenish blood drawn.

**Expected Outcome/Results**

(1) *Data on rates on HIV suppression from a large cohort in Ghana*. Given that most patients in Ghana are on first generation antiretroviral drugs, this is a recipe for development of resistance that could erode the

gains made if not urgently addressed. Two small cross-sectional studies that measured viral load and resistance in patients (total of 64 patients) found high levels resistance among the patients. This study will follow 300 patients on treatment and measure their viral loads to assess virologic suppression. It will be the largest such study in Ghana. Such information will be critical in informing policy changes in Ghana and other African countries.

(2) *Information on the true picture of HIV resistance in Ghana*. As a spin-off from (1) above, future studies will also determine the resistance levels, and what type of resistance patients in Ghana have. Again, all the studies done on this subject in Ghana are very small probably due to lack of funds. This information will be critical in designing the next generation of antiretrovirals for patients in Ghana and beyond.

(3) *Finding the attitude of patients towards HIV clinical research and what sacrifices they are willing to make to support trials.* Very few studies have been done on this topic mostly in the developed countries. Information obtained from this part of the study will enable researchers and policy makers to assess what kinds of therapies in the cure pipeline are acceptable to patients in Africa. This will enable researchers to concentrate on areas that are likely to be embraced by patients for studies. We may also find that patients are willing to take more risks than we think. This is critical if we are to avoid paternalism in HIV research, and give patients in Africa more say in what kinds of trials we bring to them.

(4) *Strengthening HIV basic research infrastructure in Ghana*. HIV basic and clinical research are highly intertwined. For instance if we have a patient with resistance virus, we need to have the capacity not only to do genotypic resistance testing (which we do at NMIMR), but also be able to do phenotypic testing which requires HIV cell biology studies, something we currently do not do in Ghana. The infrastructure we will set up for the drug screening studies will enable us to train students, and technical support staff in these procedures, thus enabling us to take better care of our patients. This same infrastructure and capacity building will also enable us to evaluate herbal extracts and other compounds synthesized in our chemistry labs for their effect on HIV replication.

(5) *Discovery of new therapies for HIV treatment and cure and intellectual property acquisition.* Our screen of 150 compounds is likely to yield at least few compounds that are able to reactivate HIV from latency. Pharmaceutical companies will be interested in evaluating these compounds further. Thus this study may generate novel intellectual property for the University of Ghana and more importantly may bring novel HIV cure therapies. The PI has two patents on HIV cure therapies and have experience talking with Merck about one of the compounds discovered in his lab at Washington University.

(6) *Readiness of a well-characterized patient cohort for clinical trials.* The patients we follow and characterize in this study will be an excellent resource for future clinical trials for HIV cure or remission studies. It is critical that most of the future HIV cure clinical trials are performed in Africa since the continent bears the brunt of the epidemic. This is most important when it comes to permanent remission or cure where genetic differences in patients and viruses will determine success of failure.

(7) *Training of students in HIV translational research.* Another key area that this proposal will be impactful is in capacity building. In addition to the applicant training and progressing to become an established independent research leader, he will also train two graduate students and a postdoctoral fellow. Indeed, the

project has room for more students especially as we build a biobank for future studies.

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## NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH (NMIMR)
## COLLEGE OF HEALTH SCIENCES, UNIVERSITY OF GHANA, LEGON

### INSTITUTIONAL REVIEW BOARD
### Work Plan

| Activity | Identification of Novel agents for HIV Cure or Remission | Year 1 | | | | Year 2 | | | | Year 3 | | | | Year 4 | | | | Year 5 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | **Aim 1: To characterize a cohort of HIV patients and evaluate their knowledge and attitude towards participation in HIV cure research** | | | | | | | | | | | | | | | | | | | | |
| | 1. Protocol finalization and ethics approvals | x | | | | | | | | | | | | | | | | | | | |
| | 2. Hiring of WP staff – project nurse and community health personnel | | x | | | | | | | | | | | | | | | | | | |
| | 3. Recruitment of HIV patients on treatment from three hospitals in Accra, measurements of viral loads and other parameters specified in proposal | | x | x | x | x | x | x | x | x | x | | | | | | | | | | |
| | 4. Data analysis and publication of interim results from first 200 patients | | | | | | | | | x | x | | | | | | | | | | |
| | 5. Study closeout data analyses, manuscript preparation and publication | | | | | | | | | | | | | | | | | | | x | x |
| 2 | **Aim 2: To screen a panel of 150 epigenetic modifying compounds for ability to reactivate HIV from latency in a cell line and a primary cell model of latency** | | | | | | | | | | | | | | | | | | | | |
| | 1. Protocol finalization, environmental health and safety approval and lab set up | x | x | | | | | | | | | | | | | | | | | | |
| | 2. Laboratory screen of epigenetic modifying compounds for HIV reactivation | | x | x | x | x | x | x | x | x | x | x | x | | | | | | | | |
| | 3. Completion of screen and publication | | | | | | | | | | | | x | x | | | | | | | |
| 3 | **Aim 3: To evaluate top 10 latency reversing agents ex-vivo in resting CD4 T cells isolated from patients suppressed on combination antiretroviral therapy for more than 6 months** | | | | | | | | | | | | | | | | | | | | |
| | 1. Institutional review board and ethics approvals | x | x | | | | | | | | | | | | | | | | | | |
| | 2. Collection of blood from patients, isolation of resting CD4 T cells and treatment with selected compounds ex-vivo for HIV reactivation | | | | | | | x | x | x | x | x | x | x | x | x | x | x | x | | |
| | 3. Interim and preliminary data analysis and report | | | | | | | | | | | | x | | | | | | | | |
| | 4. Study close out, data analyses, manuscript preparation | | | | | | | | | | | | | | | | | | | x | x |
| 4 | **Aim 4: To create a biobank of characterized samples from HIV patients on cART for future research and training purposes** | | | | | | | | | | | | | | | | | | | | |
| | 1. Development and vetting of standard operating procedures for the biobank and storage space acquisition | | x | | | | | | | | | | | | | | | | | | |
| | 2. Deposition of specimen into biobank throughout the study | | | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | | |

**NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH (NMIMR)**
**COLLEGE OF HEALTH SCIENCES, UNIVERSITY OF GHANA, LEGON**

**INSTITUTIONAL REVIEW BOARD**
**Budget and Budget Justification**

| **A.1. Personnel** | | Year 1 | Year 2 | Year 3 | Year 4 | Year 5 | Total (€) |
|---|---|---|---|---|---|---|---|
| PI | (15%) | 3000 | 3200 | 3200 | 3500 | 3500 | 16,400.00 |
| Postdoc | (100%) | 8600 | 9600 | 10600 | 11600 | 12500 | 52,900.00 |
| PhD student | Fees | | 3000 | 3500 | 4000 | 4500 | 15,000.00 |
| | Stipend | 1800 | 7200 | 8500 | 9700 | 10900 | 38,100.00 |
| M.Phil. student | Fees | 2500 | 2500 | | | | 5,000.00 |
| | Stipend | 7200 | 7500 | | | | 14,700.00 |
| Project Nurse | (100%) | 7800 | 8800 | 10000 | 10500 | 12000 | 49,100.00 |
| Community Health Worker | (100%) | 4800 | 5200 | 6200 | 7500 | 8000 | 31,700.00 |
| **Total Personnel Cost** | | | | | | | **222,900.00** |
| D. Other direct Costs | | | | | | | |
| D.1. Travel | | | 4800 | 7200 | 7200 | 7200 | 26,400.00 |
| **Total travel cost** | | | | | | | **26,400.00** |
| | | | | | | | |
| **D.2. Equipment** | Five mid-level computers for project personnel: 2 staff and three trainees | | | | | | 10,000.00 |
| **D.3. Other goods and services** | | | | | | | |
| Viral load testing | For 30 euros per test for 300 patients, each measured 8 times | | | | | | 72,000.00 |
| Resting CD4 T cell isolation kits | Twenty kits at 950 euros each | | | | | | 19,000.00 |
| Laboratory consumables | | 4000 | 15000 | 10000 | 10000 | 6000 | 45,000.00 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Patient compensation | Transport and health insurance | | | | | | 35,500.00 |
| Sundry expenses | | | | | | | 4000 |
| | | | | | | | |
| **Total other direct cost** | | | | | | | **211,900.00** |
| **Total direct cost** | | | | | | | **434,800.00** |
| **Total Institutional Charges (13%)** | | | | | | | **65,200** |

**Budget Justification**

**Personnel:** We have requested salary support for the applicant (15% effort), a high level research nurse with MPhil degree (100%) and a community health worker (100%), all for the duration of the project. The research nurse will manage the project office and also supervise and help the community health worker to recruit patients at the three locations in Accra: Korle-Bu Teaching Hospital, La Polyclinic and Manna Mission Hospital. Patient recruitment and follow up is expected to last throughout the study (Aim 1). For Aim 3, they will also help recruit patients who are suppressed on therapy to donate 100 mls of blood at least on two occasions for ex-vivo reactivation studies.

**Postdoctoral scientist and students:** We have requested funding for a postdoc (100%) for the length of the study, an M.Phil. student (100% two years), PhD student (100% 4-5 years) based on recommendations from University guidelines for salaries and stipends. In addition, we requested funding for tuition fees for the M.Phil. and PhD students. The postdoctoral fellow will oversee day-to-day management of the project, will lead the analysis in Aim 1 and co-supervise the graduate students. He/she will also work on part of Aim 3 to test select compounds ex-vivo on patient cells. The M.Phil. student will work on Aim 1, the survey part and analyze the first 150 patients for viral suppression as his M.Phil. thesis. The PhD student will work on Aim 2 and 3, screening the compounds for HIV reactivation and testing the best candidates in patient CD4 T cells ex-vivo.

**Viral load testing and resting CD4 cell isolation**: We have requested funding for viral load testing on 300 patients, 4 times per year for 2 years. The hardware is available at the reference laboratory at the Korle-Bu Teaching Hospital, but we will have to provide funding to purchase the kits for testing. We conservatively estimated 30 Euros per testing based on assurances from the National AIDS Control Program to donate some kits for the study as well. Viral load testing for 300 patients at 30 euros each: 30x300x8 = € 72,000.00. For resting CD4 cell isolation, we will use a kit from Stem Cell Technologies, which is uses negative selection technique. This technique prevents cells from being activated, a state that is critical for our study. Each kit

costs about 950 euros and can perform 5 isolation reactions. We expect to need 20 kits for this study for parts of Aim 2 and all of Aim 3 costing €19,000.00.

**Equipment:** We have requested funds to purchase 5 mid-level computers for the three students and 2 staff involved in the study.

**Travel to meetings:** We have requested funding for travel to international meetings, starting from year 2. Each year 2 or 3 of personnel will travel based on data they have available for presentation. This is important for trainees to present their work at international fora. We have made provision for airline ticket and room and board for 5 days for each international travel. Estimation of travel cost was done as follows: 1200 euros for airline ticket and 1200 euros for conference registration hotel stay per person per travel. Trainees will be encouraged to apply for travel scholarships as well.

**Consumables:** We have requested funds for laboratory and other consumables such as qRTPCR kits, RNA and DNA isolation kits, consumables for testing blood counts and CD4, cell culture media etc. These kits will be needed for accomplishing Aim 2 and 3, as we will need to perform cell culture, do measurements on FACS and perform multiple quantitative real time PCR assays. We anticipate about 9,000 Euros per year for consumables. This is based on the fact that several of these are already available at the NMIMR.

**Sundry expenses:** We have requested funds for items such as IRB submission fees, fees charged for using equipment such as qRTPCR machines and FACS machines, BSL3 user maintenance fees

**Consent Form** (Attached)

**Assent Form and Parental Consent Form** (Not applicable)

**Data Collection Instruments** (Attached)

## SECTION C – SIGNATURE

As the **Principal Investigator / Co-investigator** on this project, my signature confirms that:

1. I will ensure that all procedures performed under the study will be conducted in accordance with all relevant policies and regulations that govern research involving human participants.
2. I understand that if there is any change from the project as originally approved I must submit an amendment to the NMIMR- IRB for review and approval prior to its implementation. Where I fail to do so, the amended aspect of the study is invalid.
3. I **understand** that I will report all serious adverse events associated with the study within seven days verbally and fourteen days in writing.
4. I understand that I will submit progress reports each year for review and renewal. Where I fail to do so, the NMIMR-IRB is mandated to terminate the study upon expiry.
5. I agree that I will submit a final report to the NMIMR-IRB at the end of the study.

Name & Signature of Principal Investigator / Co-investigator: DR. EVELYN YAYRA BONNEY

Date: 10/10/2018