Minimising HIV deaths through rapid fungal diagnosis and better care in Guatemala

Table of Contents

Summary

At least 280,000 Guatemalans suffer from a serious fungal infection per year of which an estimated 4,790 are immediately life threatening. This proposal seeks to bring all the key tests into Guatemala, through the NGO "Asociación de Salud Integral (http://www.asi.org.gt)" and locally through the existing network of HIV comprehensive care units. Rapid, accurate fungal disease diagnosis will save many lives, as the treatment of fungal disease is well established. Support is sought for both provision of rapid diagnostics and clinical training, with a primary focus on AIDS, tuberculosis and 'endemic mycoses'.

In addition, this proposal has been designed to demonstrate that improving fungal diagnostic capability for HIV patients improves clinical outcome. By adopting a (small) country-wide approach, with a modest number of cases that tend to present late to care for all HIV comprehensive care units in Guatemala, data capture will be easier and impact easier to demonstrate. The first objective of a much better understanding of the incidence of the most common life threatening infections in Guatemala is crucially important, because it sets the stage both in and outside Guatemala. Patients with disseminated histoplasmosis, cryptococcal meningitis and *Pneumocystis* pneumonia do make full recoveries, if they respond to therapy, but early diagnosis and appropriate therapy is critical to achieving this.

The Global Fund for AIDS, Tuberculosis and Malaria supports about 100 low and middle-income countries with their AIDS programs. The 2014 shift in the working model has facilitated direct funding for opportunistic infections in AIDS and within year adjustments of allocations following additional requests based on data. Many other agencies also support AIDS treatment and care, notably PEPFAR and in remote locations, MSF and smaller agencies. Global Fund income and PEPFAR (and other) support flows from applications based on detailed incidence figures. Increased case detection will translate into increased support for treatment. Other countries that see a rise in Global Fund income in Guatemala will be encouraged to follow this model. Our detailed evaluations of the program using internationally accepted methodology and reporting, an intrinsic component of this proposal, will be useful for others countries, and widely shared. The findings will have profound impact for HIV programs

everywhere. Specifically, beyond Guatemala, the impacts will carry over to numerous other countries in Latin America and the Caribbean.

Introduction

In 2010, UNAIDS issued the aspirational target of Zero AIDS deaths by 2015. Yet still 1,500,000 people died of AIDS in 2013, a reduction of only 15% from 1,760,000 lives lost in 2010. In 2014, the new ambitious target of 90-90-90 for 2020 (90% of HIV infected patients know their infection status, 90% of all HIV patients receiving antiretrovirals and 90% viral load suppression) was announced with the aim to greatly expand access to diagnosis and antiretrovirals for all HIV infected patients. This important new target will result in a reduction of HIV transmission and will have some impact on deaths, but progress will be incomplete because coverage will not be 100% and antiretrovirals reduce the incidence of most co-infections but not all. Unless we do pay more attention to opportunistic infections, it will take many years to achieve the desired outcome of reduced deaths.

Opportunistic infections account for a significant burden of AIDS related morbidity and mortality. For example, about 25% of current AIDS deaths are attributed to tuberculosis. The other 75% are likely to be due to many causes, of which fungal disease is a major contributor, notably cryptococcal meningitis, disseminated histoplasmosis, *Pneumocystis* pneumonia and chronic pulmonary aspergillosis masquerading as tuberculosis. Available evidence suggests that these infections cause about 700,000 deaths annually, nearly half the total of deaths attributed to AIDS. Concrete steps to improve diagnosis and access to therapy, could realistically reduce deaths from 1,5000,000 to 457,000 per year by 2020, if 60-90% of patients are reached.

Cryptococcal meningitis, disseminated histoplasmosis and *Pneumocystis* pneumonia are universally fatal in AIDS if not diagnosed and treated early. The global burden of cryptococcal meningitis has been estimated as 1,000,000 patients in AIDS (range 371,700–1,544,000) with 600,000 deaths (range 125,000–1,124,900). A global estimate of disseminated histoplasmosis is 300,000 and in many locations in Latin America, up to 25% of patients presenting with AIDS have disseminated histoplasmosis. *Pneumocystis* pneumonia is a very common presenting infection in AIDS across the world, but grossly underdiagnosed; there are thought to be >400,000 cases annually worldwide. Cryptococcal meningitis and disseminated histoplasmosis both have excellent antigen tests recently developed, which is particularly important for disseminated histoplasmosis as the organism takes 10 – 20 days to only grows on fungal media. The cryptococcal meningitis antigen test performs extremely well, is very simple, takes 10 minutes and costs $2. *Pneumocystis* pneumonia diagnosis requires expert microscopy (immunofluorescence) or molecular methods. Neither is undertaken in most units looking after HIV patients in developing countries. Once these diseases have been diagnosed, the response rate to antifungal treatment is higher than 75% (figure 1); the sooner the diagnosis is made, the higher is the response rate.

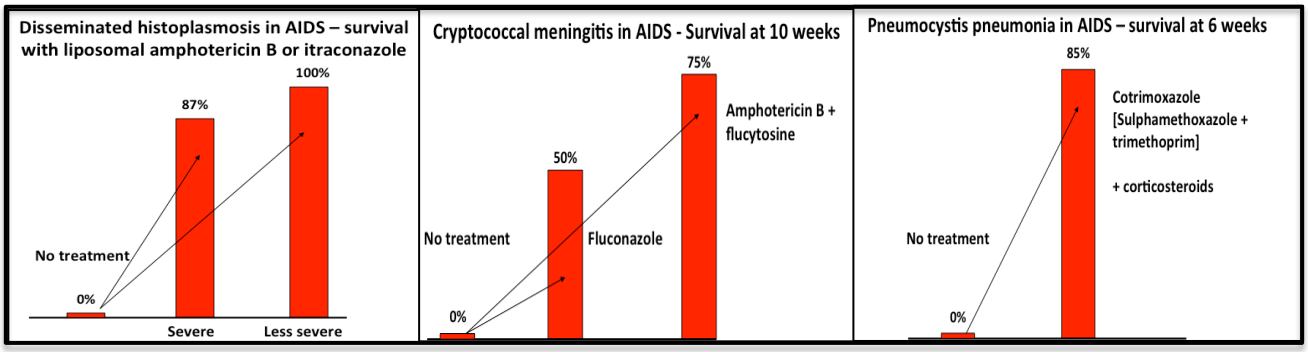


Figure 1. Response rate for three opportunistic fungal infections in AIDS patients

From 10 to 20% of patients treated for pulmonary tuberculosis will develop *Aspergillus* antibodies indicating infection and a large proportion of these patients develop chronic pulmonary aspergillosis. Chronic pulmonary aspergillosis is a slowly progressive lung fungal infection, which progresses to death over several years, unless arrested with treatment. The key diagnostic test, *Aspergillus* antibody detection, is not

available in most of the countries; so chronic pulmonary aspergillosis is not diagnosed or treated. After tuberculosis, modelling suggests that about 375,000 will go on to develop chronic pulmonary aspergillosis each year, with an annual 15% mortality. Therefore, about 1.2 million people probably have chronic pulmonary aspergillosis annually. A topic of real uncertainty currently is 'smear negative tuberculosis', which in many instances is not tuberculosis at all. In an unpublished study of 39 HIV positive, smear negative patients in Kampala, 26% had detectable *Aspergillus* antibodies and 40% of these patients died within 2 months. Based on their compatible radiology, symptoms and positive serology, it is highly likely these patients suffered from chronic pulmonary aspergillosis, or invasive aspergillosis. Other consistent unpublished data from Iran and Brazil support this finding. Patients with smear negative tuberculosis who fail to respond to therapy may be diagnosed with multi-drug resistant tuberculosis and treated with second line agents. In 2012, the WHO estimated that there are ~4 million multi-drug resistant tuberculosis cases with 1 million deaths; some of these patients probably have chronic pulmonary aspergillosis, or another fungal infection, instead of tuberculosis or as a co-infection.

Few international health agencies or funders have a fungal diseases program. WHO in Geneva has enabled, with external grant support, the formulation of HIV focused cryptococcal management guidelines (HIV Division) and supported the development of skin and oral health in HIV guidelines, through the maternal and child health division. Mycetoma has been accepted as a Neglected Tropical Disease, without any financial support. None of the regional WHO offices, including PAHO, have any fungal disease program. Médecins Sans Frontières run an access program endeavouring to enable access to amphotericin B and flucytosine for cryptococcal meningitis. PEPFAR provides fluconazole to many countries. The Pfizer donation program has also provided fluconazole to millions of patients worldwide.

Research grant funding is provided by numerous national agencies and by the European Commission. The vast majority of government funding is spent on pre-clinical, pathogenesis type work. An analysis of UK funding between 1997-2010 found 171 studies (2.8%) related to mycology (total investment for mycology £48.4 million, 1.9% of all infection research, with means annual funding £3.5M). Studies related to

global health represented 5.1% of this funding (£2.4M out of £48.4M) and preclinical work received 87% of grants. A similar situation exists in the USA with NIH funding.

In summary, despite its importance as a cause of morbidity and mortality in the world, especially in low and middle-income countries, fungal diseases remain neglected for international organizations, NGOs, and research agencies.

Global Action Fund for Fungal Infections (GAFFI), a Foundation based in Geneva, wants to ensure that 95% of people with serious fungal disease are diagnosed and 95% treated by 2025 (95-95) and therefore global action to address the current neglect of prevention and treatment of opportunistic fungal diseases is required.

The situation in Guatemala

In Guatemala in 2011, among a population of 14.7 million, an estimated 58,000 (36,000 -130,000) are HIV infected but only 15,136 patients currently attend the HIV comprehensive care units in the country (93% adults). 3,400 AIDS deaths were recorded in 2012. In Guatemala 27,000 should be receiving anti-retroviral therapy (only 53% coverage) but HIV infected patients tend to present very late because of denial and stigma and lack of access to the health system. This results in long hospitalisations and often death. Early death is the biggest problem. The estimated incidence for opportunistic fungal infections among the HIV population in Guatemala is: 477 cases of cryptococcal meningitis, 827 cases of disseminated histoplasmosis and 1,023 cases of *Pneumocystis* pneumonia. In contrast, the only Mycology Reference Laboratory (Asociación de Salud Integral, (http://www.asi.org.gt) recorded 21 cases of cryptococcal meningitis in HIV patients and 62 cases of disseminated histoplasmosis in 2013-4, indicating that only 4.4% of cryptococcal meningitis and 7.5% of disseminated histoplasmosis cases were diagnosed. There are not any diagnostic facilities for *Pneumocystis* pneumonia and chronic aspergillosis after tuberculosis in Guatemala.

Asociación de Salud Integral is an NGO operating one of the largest HIV comprehensive care units in Guatemala (http://www.asi.org.gt). It has a strong laboratory, which serves as a reference laboratory for HIV monitoring tests (viral load determinations, HIV-1 genotyping, CD4+ T cells counts), as well as specialized diagnosis of tuberculosis, for most of the HIV care units of the country. In the mycology area, the laboratory Asociación de Salud Integral has pioneered the development and implementation of

tests for the rapid diagnosis of *H. capsulatum*, in collaboration with the US Centers for Diseases Control and Prevention (CDC). In 2013, the laboratory processed 2,101 samples for fungal detection, from 12 country districts.

Guatemala is an excellent global location to both make big improvements in healthcare and to demonstrate the value of combined fungal diagnostic availability and enhanced clinical training. The specific features that will enable success are:

1) It has a single high quality mycology laboratory, partially integrated into the HIV care networks throughout the country; most HIV comprehensive care units across Guatemala already refer samples for different diagnostic tests to this laboratory.
2) Diagnostic test uptake across the country is limited and major improvements are possible in a short time frame;
3) Asociación de Salud Integral is led by a master AIDS clinician who will lead on clinician education across the country;
4) Histoplasmosis is endemic in the country, common in AIDS, and antigen testing greatly shortens time to diagnosis;
5) Asociación de Salud Integral has pioneered the evaluation of histoplasma antigen test already;
6) Roll out of local cryptococcal antigen testing has not yet happened, apart from 2 centres in Guatemala city;
7) *Pneumocystis* pneumonia is only diagnosed clinically, and so imprecisely, offering substantial scope for clinical improvements;
8) Asociación de Salud Integral has specialized diagnosis of tuberculosis, an essential tool to achieve the diagnosis of chronic pulmonary aspergilosis masquerading tuberculosis;
9) Most patients with AIDS present late, because of denial, stigma and lack of access to the health system;
10) Amphotericin B deoxycholate, fluconazole and itraconazole are all available in Guatemala and;
11) The geographic characteristics of Guatemala make it an ecologic niche for numerous fungal pathogens, namely *Histoplasma*, *Cryptococcus*, *Coccidioides*, *Aspergillus* and *Paracoccidioides*, among others, demanding an even higher level of mycology expertise than is necessary in other localities.

The proposal as a proof of concept for minimising HIV deaths through rapid fungal diagnosis and better care in Guatemala

This proposal will provide the foundation data on which to improve life-saving health care services for HIV patients in Guatemala and internationally. Estimating the burden of infection through provision of diagnostics and systematic data collection is a critical initial step. The interventions implemented through this project, especially the implementation of the mycology reference unit, will have a direct impact on the diagnosis, clinical management and treatment of the HIV patient co-infected with a fungal disease, which will likely translate into improved outcomes for Guatemalan HIV patients. Likewise provision of *Pneumocystis* real time PCR and *Aspergillus* antibody testing will enable the diagnosis of *Pneumocystis* pneumonia and chronic pulmonary aspergillosis, for the first time in the country. The specific parameters that determine outcome will be carefully studied to ensure that only those measures that really make a difference are developed further in AIDS programs.

The deliverables of this project will be:

1) **Implementation of rapid diagnosis of cryptococcal meningitis with rapid antigen test at each centre and for disseminated histoplasmosis, *Pneumocystis* pneumonia and chronic pulmonary aspergillosis, through strengthening of the Asociación de Salud Integral reference laboratory**. The strengthening of the fungal diagnostic capabilities of the Asociación de Salud Integral laboratory and local HIV comprehensive care units (n=15; 15,136 patients) is critical for diagnosis of fungal disease. Local centres will implement cryptococcal meningitis antigen testing using the lateral flow device (LFD) (Immy), with confirmation and titre at Asociación de Salud Integral. Only 2 centres in Guatemala City use this technique now. *Histoplasma* antigen detection on urine and serum and blood PCR will be offered for all centres through Asociación de Salud Integral. The core diagnostic modality for disseminated histoplasmosis is the antigen ELISA offered by CDC which will be phased out in favour a new EIA produced by Immy, with similar performance characteristics. *Pneumocystis* real-time PCR will be offered by Asociación de Salud Integral for respiratory samples (both sputum and bronchoscopy). For diagnosis of chronic pulmonary aspergillosis, *Aspergillus* IgG testing and PCR will be provided

for the first time in Guatemala. A more complete diagnostic offering is required to include fungal culture and serology for other prevalent fungal infections in Latin America such as those caused by *C. posadasii* and *P. brasiliensis*. An open source laboratory-based IT data system (BLIS; http://life-worldwide.org/media-centre/article/open-source-lab-it-system-spreads-its-wings-across-africa) will be adapted for mycological testing by the Asociación de Salud Integral mycology reference unit. Diagnosis of all fungal infections in AIDS, including coccidioidomycosis, aspergillosis, candidiasis and paracoccidioidomycosis will also be offered using microscopy (including cytospin), culture, serology and PCR.

The systematization of the diagnosis of fungal infections will allow, for the first time in the country, that the HIV comprehensive care units have access to sensitive, accurate and timely diagnostic methodologies, through the strengthening of the installed capabilities in the HIV care units and a national mycology reference laboratory, and provides a critical mass of expertise.

2) **Training program in fungal disease management for health workers in the HIV comprehensive care units of Guatemala**. Training in basic mycology will provide health workers at the HIV comprehensive care units with enough knowledge to recognise the signs and symptoms of infections potentially caused by fungal pathogens; proper sample collection, performing cryptococcal antigen lateral flow assays in serum and CSF, handling and shipping; clinical management and antifungal treatment, and follow up of HIV patients co-infected with a mycosis. Initial sessions will be face to face, supported by web-based lectures in real-time (to minimise time away from work and income loss), with opportunities to ask questions. Follow up sessions will feedback of diagnostic data from each centre and clinical updates will maintain awareness to ensure early suspicion of fungal infection. To further reinforce these efforts, standard operating procedures will be implemented in the HIV clinics. Training will be supported with online resources developed locally and in Spanish at www.LIFE-worldwide.org. Clinical care of especially complex patients will be supported by Dr Arathoon, the country's only clinical mycologist.
3) **Development of a national registry for fungal infections**. Clinical data collection will be supported by MANGUA, a national level cohort system, based on the

electronic registration of medical files that collect the follow up data of HIV patients. This application provides the national public health system with HIV data and allows for the decentralization of the HIV treatment, HIV health care services and patient follow up. Using low cost mobile phone data collection software (MAGPI; http://home.magpi.com), systematized data collection on HIV admissions and opportunistic infections will be collected from all HIV comprehensive care units, and integrated with Asociación de Salud Integral fungal diagnostic data, collected on BLIS. All patients meeting inclusion criteria who consent to participate in the study will receive symptom screening (any of the following: fever, any cough, night sweats, headaches, and weight loss) and a CD4 count and viral load. Each physician requesting fungal testing will complete a form on a mobile phone with the following data: symptoms, signs, vital signs, oxygenation prior medical, HIV and STI history: current medication including antiretrovirals and antifungals, body mass index. Subsequent data to be collected will be CD4 count and HIV viral load, complete blood count, renal and hepatic function, diagnoses made and therapy given (and timing), therapy dose and duration and outcomes. Timing of key events will be recorded. A unique study identifier number will be assigned, which will be used for identification of any specimens and data sent to the Asociación de Salud Integral. Specimens obtained as part of this evaluation may include some or all of the following: urine, blood, bone marrow, sputum, bronchoalveolar lavage, tissue, and cerebrospinal fluid. The results of other conventional mycology and microbiology tests and cryptococcal meningitis antigen will be collected. All Asociación de Salud Integral results will be communicated rapidly to the referring unit as well as recommendations for treatment, if required. A web page communication (or mobile phone) will be available for questions or medical consults.

4) **Documentation of the nation-wide incidence of and survival from these life-threatening infections**. The diagnoses and outcomes for all patients admitted to the HIV Comprehensive centres will be analysed using multiple statistical approaches, utilising key data such as CD4 cell count, age, sex, HIV diagnoses, comorbidities, time of admission, diagnosis, initiation of therapy (and the quality of that therapy in term of drug selection and dose), complications of therapy, time of

discharge or death, and fungal diagnostic test utilization, timing and performance. Both multiple logistic regression (for fixed follow-up points) and survival regression models (for variable follow-up between participants) will be constructed, and trends in survival will be assessed using the annual cohorts. Centre and disease specific effects will also be identified. The relationship between 'best' therapy and delay to treatment with survival outcome will also be investigated to address potential cohort bias. Baseline mortality data with demographics is being collected already, and this will be used in the baseline comparison of all interventions and as part of the confounding adjustment for subsequent cohort mortality.

5) **Program assessment in terms most useful for global and public health planning**. An Advisory Group comprising Dr Tom Chiller (Centers for Diseases Control, Atlanta) and Professor Imelda Bates (Liverpool School of Tropical Medicine, UK) expert in public health in the developing world will guide the whole program and in particular the analyses and assessments.

   Evaluation will be built into the project from the start, in order to:

   a. Further develop and improve the project as it proceeds and the network grows, through two key foci:
      i. Maximising impact - supporting and enhancing the project's "pathways to impact", following through the chain from inputs to activities to outputs to outcomes;
      ii. Achieving sustainability of the capacity developed through the project. This will address financial and human factors, including diversity of funding sources and the support of the networked organisations' management.
   b. Inform future scale up to other infections and host groups, and spread to other countries. The focus here will be on understanding key factors at play in the development/ implementation of the network, and how they interact with aspects of local organizational contexts. In view of the need for sustainability, we will also build individual and organisational capacity for evaluation, research and audit (e.g. through needs assessment and provision of online training), working in partnership with experienced evaluation specialists based in Guatemala/Latin America. It is also

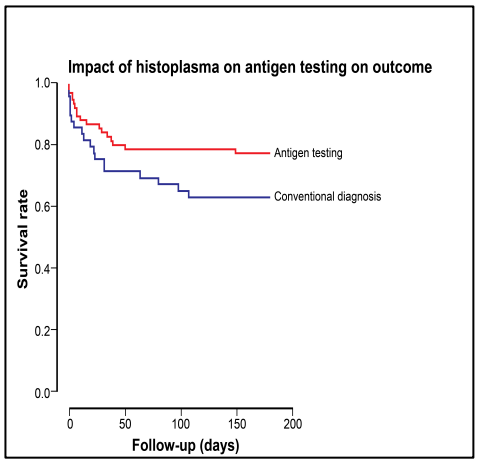
important that global health research for development is sensitive to equity issues, including women's access to diagnosis and treatment. We will take a systemic approach to evaluation, which includes stakeholder engagement, in order to understand what are desirable and feasible goals, and to mobilise resources/capabilities; development of suitable indicators to measure the achievement of the goals; and benchmarking with similar organisations/networks locally and internationally in order to promote learning and subsequent scale-up and spread. The specific model we will use will be the "prism" model for evaluating the capacity strengthening, as it goes through phases of awareness, experiential, expansion, and consolidation. The implementation of this model will incorporate principles from utilization-focused developmental evaluation, such as rapid feedback and learning cycles, in order to support ongoing project improvement and impact. A "programme theory" will be developed through stakeholder interviews, literature review and a workshop, to provide a shared understanding of the project, and a basis for appropriate indicator identification and the production of wider learning to inform scale up and spread. A baseline audit of capacity will be conducted, and initial capacity development needs assessed in relation to standards necessary for full and sustainable functioning of the network, as the basis for capacity strengthening. Process, output and outcome measures along the impact chain will include data on cases, tests, positive results, treatment given based on results, speed of reporting, hospitalisation duration and survival. Regular feedback on performance will be provided to individual HIV centres to support quality improvement, and overall disease/test specific feedback across the network as a whole. The process of developing and the maturity of the network will be evaluated through interviews, focus groups/workshops and document analysis (e.g. plans and minutes of meetings). This will also document key learning points to inform scale up and spread, which will be actively disseminated through the benchmarking network. The capacities of individuals, clinics, the laboratory and the network, and the sustainability of those capacities, will be assessed

annually using a mixture of hard and soft data, including feedback from internal and external stakeholders via a survey. As capacity develops over time, local actors and systems will take on more responsibility for monitoring and evaluating the project.

This grant will document for the first time:

1) Country-wide incidence of and survival after cryptococcal meningitis, disseminated histoplasmosis, *Pneumocystis* pneumonia in AIDS, and chronic pulmonary aspergillosis after tuberculosis through implementation of rapid diagnostics combined with clinical training using a new national registry for fungal infections in AIDS;
2) Assess the relative impact of better fungal diagnostic testing on mortality in AIDS patients, by centre and by time to diagnosis, and adjusted for other co-variables known to be associated with poor outcomes of opportunistic infections in AIDS. Figure 2 shows the impact of antigen testing on survival of disseminated histoplasmosis.

Figure 2. Impact of histoplasma antigen testing on outcome (reference)



Ethics and governance

The study will include all the HIV patients who require mycology diagnostic tests and whose samples are sent to the mycology reference unit and who give informed consent. Each patient will be registered in a database with a code number. Informed consent will be garnered from each patient before the study, this will be coordinated

by a collaborator in each HIV comprehensive care unit. This study will have the endorsement of the Guatemalan HIV and STDs National Program and will have approval from the Guatemalan National Ethics Committee and The University of Manchester. There are no contentious issues of magnitude in this program.

Data preservation and sharing

Data collection will be supported by 2 systems: 1) MANGUA, a national level cohort system, based on the electronic registration of medical files that collect the follow up data of HIV patients. This application provides the national public health system with HIV data and allows for the decentralization of the HIV treatment, HIV health care services and patient follow up; and 2) A laboratory-based IT data system (BLIS) located in the Asociación de Salud Integral mycology reference unit. All data will be archived long term on secure servers.

Pathways to impact on the international arena

This proposal has been designed to demonstrate that improving fungal diagnostic capability for HIV patients improves clinical outcome. By adopting a (small) country-wide approach, with a modest number of cases that tend to present late to care for all HIV comprehensive care units in Guatemala, data capture will be easier and impact easier to demonstrate. The first objective of a much better understanding of the incidence of the 3 most common life threatening infections in HIV patients in Guatemala is crucially important, because it sets the stage both in and outside Guatemala. Patients with disseminated histoplasmosis, cryptococcal meningitis and *Pneumocystis* pneumonia do make full recoveries, if they respond to therapy, but early diagnosis and appropriate therapy is critical to achieving this.

Diagnosis and treatment of these opportunistic fungal infections will make real progress towards the fulfilment of the 2.0 Treatment strategy recommendations and the Millennium Development Goals.

The Global Fund for AIDS, Tuberculosis and Malaria supports about 100 low and middle-income countries with their AIDS programs. The 2014 shift in the funding model has facilitated direct funding for opportunistic infections in AIDS and within year adjustments of allocations following additional requests based on data. The first point is restricted to funding for opportunistic problems for which there are WHO HIV

guidelines (cryptococcal meningitis, oral and oesophageal candidiasis, cutaneous fungal infections) and for which treatment is included on the WHO Essential Medicines List (fluconazole, amphotericin B and flucytosine). The second point allows new data on the incidence of fungal infections to be reflected in funding allocations for diagnosis, treatment cost and monitoring of 'approved' infections such as cryptococcal meningitis. Many other agencies also support AIDS treatment and care, notably PEPFAR and in remote locations, MSF and smaller agencies.

Key routes to substantial impact are:

1) Global Fund income and PEPFAR (and other) support flows from applications based on detailed incidence figures. Increased case detection will translate into increased support for treatment. Other countries that see a rise in Global Fund income will be encouraged to follow this model. Our detailed evaluations of the program using internationally accepted methodology and reporting, an intrinsic component of this proposal, will be useful for others, and widely shared;
2) This work will be written up and presented at multiple relevant national and international meetings in English and in Spanish, including the International AIDS Conference. Papers documenting the experience of improved diagnosis, with survival benefit documented, would be published in top-flight journals, as befits a major country program and significant investment. GAFFI will feed these data directly into UNAIDS and the Global Fund and support other countries in applying for and implementing fungal diagnostics for AIDS in a large number of countries in which GAFFI has partners
3) The findings will have profound impact for HIV programs in other countries. While the major thrust of AIDS work is, and must continue to be, rolling out antiretroviral therapy and prevention of transmission, many patients present with a life-threatening fungal infection before they benefit from antiretrovirals. They may succumb before antiretrovirals have had time to work, develop IRIS or have virological failure despite antiretrovirals. These patients need the expert diagnosis and care that mycology laboratories provide in concert with expert clinicians, and by demonstrating that this works in Guatemala, other major cities and countries will follow this example;

4) Specifically, beyond Guatemala, the impacts will carry over to numerous other countries in Latin America and the Caribbean. Patients at risk are primarily those with low CD4 cell counts not receiving medical care and antiretrovirals. We anticipate significant impacts in many other Central American and Caribbean countries (Latin America total requiring antiretrovirals = 790,000). At risk patients HIV patients in other countries include Mexico (100,000) Panama (9,200), El Salvador (13,000), Nicaragua (2,900), Honduras (14,000), Costa Rica (5,100) Belize (1,600), Dominican Republic (24,000), Haiti (57,000), Cuba (5,900), Equatorial Guinea (9,900), Surinam (2,000), Colombia (60,000), Venezuela (59,000) and Guyana (3,800).
5) At the end of the program, Guatemala will have the first national reference laboratory specialized in mycology providing diagnostic services to HIV patients in Central America. In the future, these diagnostic services could be extended to other vulnerable groups and general population (i.e. cancer and post-tuberculosis patients). Provision of timely, accurate and innovative diagnostic tools for the detection of critical fungal infections, such as the ELISA test for *Histoplasma* antigenuria and molecular tests for other fungal pathogens, will show the path for other laboratories still focusing on old culture technologies. For Guatemala, case detection through the mycology reference laboratory will provide statistical data that will be used to establish the prevalence of different fungal infections in the country. This knowledge will allow the Ministry of Public Health and Social Assistance to take decisions based on evidence, to set attainable goals and to design strategies oriented to improve the diagnosis, clinical management, treatment and follow up of fungal infections. One of these strategies should be to reduce the morbi-mortality of this type of infections. The information generated through the implementation of this project will help the Public Health Ministry to determine the impact of these infections in Guatemala. This evidence will justify the inclusion of fungal diseases in national health programs as a strategic component to comply with the 2.0 Treatment strategy recommendations and HIV Care Continuum Goals.
6) The standardization of case reporting and follow up of fungal infections through the mycology reference unit and the evaluation of the cases detected in the HIV

national reporting system (MANGUA) is a transferable resource. We intend to make it exportable.

7) One limitation to optimising care in Guatemala (as in many other countries) is the absence of flucytosine. A large research clinical trial in Vietnam convincingly demonstrated the superiority of combined amphotericin B and flucytosine for cryptococcal meningitis. Adoption of this proposed program in Guatemala will contribute to the multiple forces required to bring flucytosine into the market, where it is not. The enormous number of needy countries is shown here: http://www.gaffi.org/why/burden-of-disease-maps/. Bringing flucytosine to patients with AIDS is likely to be done through PEPFAR initially, and then market forces and Global Fund monies can sustain supply.
8) Another limitation to the management of patients with certain fungal infections in AIDS is the lack of a WHO guideline for management of disseminated histoplasmosis and *Pneumocystis* pneumonia. Discussions with key HIV personnel are not optimistic of inclusion of either of these diseases in a future guideline. A clear demonstration of the value of timely fungal disease diagnosis in ill AIDS patients could alter this position, which would be valuable for clinicians and national AIDS planners, as well releasing Global Fund support to national governments. An unequivocal demonstration of survival benefit with implementation of rapid fungal disease diagnostics and treatment would provide a major impetus for a fuller iteration of the WHO consolidated guidelines.
9) Itraconazole is not on the Essential Medicines List. It is not therefore included within Global Fund support. It is highly effective for histoplasmosis and other endemic fungal infections, and the only realistic drug for aspergillosis for low and middle-income countries because of price. Assuming this project documents improved outcome of disseminated histoplasmosis utilising antigen diagnosis and itraconazole therapy, these data will support an application to the WHO for inclusion of itraconazole on the EML. Other data to support this application will come from additional studies in aspergillosis complicating tuberculosis.
10) The information collected through this project will open the opportunity to develop more research in the mycology field, including determining the social and economic impact of these infections in Guatemala; aspects related to length of

survival related to adequate and timely provision of health care services, as well as the study of disease patterns such as, geographical delimitation of endemic areas, shifts in epidemiology related to global warming and human activities, potential vectors, and other factors associated with morbidity.

11) A major deficiency internationally in healthcare is the absence of public health mycology. Apart from the Centres for Disease Control, the Pasteur Institute and the collection of *Candida* bloodstream isolate numbers and resistance in the UK, there are no national programs assessing burden of fungal disease, antifungal resistance or impact. This program offers an example of how public health mycology could be utilised for the general health of a population. The careful assessment planned for this program of work, in terminology appropriate for a public/global health audience, will create an example for others.

References

1. Park BJ, et al. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. AIDS 2009; 2:525-30.
2. Colombo AL et al. Epidemiology of endemic systemic fungal infections in Latin America. Med Mycol 2011; 49:785-98.
3. Brown GD, et al. Human fungal infections: the hidden killers. Sci Transl Med 2012:4:165rv13.
4. Etard JF, Ndiaye I, Thierry-Mieg M, et al. Mortality and causes of death in adults receiving highly active antiretroviral therapy in Senegal: a 7-year cohort study. AIDS 2006; 20:1181-9.
5. Sodqi M, et al. Causes of death among 91 HIV-infected adults in the era of potent antiretroviral therapy. Presse Med 2012; 41:e386-90.
6. Walker AS, et al. Mortality in the year following ARV initiation in HIV-infected adults and children in Uganda and Zimbabwe. Clin Infect Dis 2012;
7. 55:1707-18.
8. Zachariah R, et al. Risk factors for high early mortality in patients on antiretroviral treatment in a rural district of Malawi. AIDS 2006; 20:2355-60.
9. Boulware DR, et al. Timing of antiretroviral therapy after diagnosis of cryptococcal meningitis. N Engl J Med 2014;370:2487-98.
10. Hage CA et al. A multicenter evaluation of tests for diagnosis of histoplasmosis. Clin Infect Dis 2011; 53:448–454.
11. Jarvis JN, et al. Evaluation of a novel point-of-care cryptococcal antigen test on serum, plasma, and urine from patients with HIV-associated cryptococcal meningitis. Clin Infect Dis 2011; 53:1019-23.
12. Binnicker MJ, et al. Comparison of four assays for the detection of cryptococcal antigen. Clin Vaccine Immunol 2012; 19:1988-90.
13. McTaggart LR, et al. Validation of the MycAssay Pneumocystis kit for detection of *Pneumocystis jirovecii* in bronchoalveolar lavage specimens by comparison to a laboratory standard of direct immunofluorescence microscopy, real-time PCR, or conventional PCR. J Clin Microbiol 2012; 50:1856-9.

14. Nowaseb V, et al. The frequency of Pneumocystis jirovecii in sputum samples of HIV and TB patients received at the Central Reference Laboratory in Windhoek, Namibia. J Infect Dev Ctries 2014; 8:349-57. 14.
15. Metersky ML,et al. A comparison of induced and expectorated sputum for the diagnosis of Pneumocystis carinii pneumonia. Chest 1998; 113:1555-1559. 15.
16. Instituto Nacional de Estadística: Casos de morbilidad 2012. Guatemala, INE 2012
17. Samayoa B, et al. Disseminated histoplasmosis (DH) before and after the implementation of urine antigen detection ELISA (UADE) in an HIV clinic in Guatemala. ICAAC October 2012 Poster M-1694.
18. Scheel CM, et al. Development and evaluation of an enzyme-linked immunosorbent assay to detect *Histoplasma capsulatum* antigenuria in immunocompromised patients. Clin Vac Immunol 2009; 16: 852-8.
19. Cáceres D, et al. Histoplasmosis and AIDS: Clinical and laboratory risk factors associated with the disease prognosis. Infection 2012; S16: 44-50.
20. Herrera R, et al. Burden of Serious Fungal Infections In Guatemala. ICAAC 2013, San Fran M 2163.
21. Samayoa V, et al. Preliminary results of a prospective cohort study of HIV positive patients in Guatemala: the Managua Project. Presented at the 2010 International AIDS Conference, Vienna 2010.
22. Basic Laboratory Information System (BLIS) is an initiative of the Computing For Good (C4G) program at Georgia Institute of Technology and the CDC. http://blis.cc.gatech.edu/index.php
23. Masanza MM, et al. Laboratory capacity building for the International Health Regulations (IHR [2005]) in resource-poor countries: the experience of the African
24. Field Epidemiology Network (AFENET). BMC Public Health 2010;10 Suppl 1:S8. 23.
25. www.magpi.com/login/auth [DataDyne]
26. http://goo.gl/PeQY8C 25. Savaya R. Predictors of sustainability of social programs. Am J Eval 2011;33:26-43.
27. Bates I, et al. A practical and systematic approach to organisational capacity strengthening for research in the health sector in Africa. Health Res Pol Syst 2014; 12:11.

28. Suarez-Balcazar Y, et al. Moving From Science to Practice in Evaluation Capacity Building. Am J Eval 2013:35:9-99. 28. Neufeld V, et al. (2014) Perspectives on Evaluating Global Health Research for Development: A Background Paper. Canadian Coalition for Global Health Research, Wakefield, Canada.
29. Boyd A, et al. Systemic evaluation: a participative, multi-method approach. J Op Res Soc 2007; 58:1306-20.
30. Patton MQ. (2011) Utilization-Focused Developmental Evaluation: Engagement Practices, Diverse Designs and Adaptive Methods. In: Developmental evaluation: Applying complexity concepts to enhance innovation and use. Guilford Press, New York.
31. Funnell, S.C. & Rogers, P.J. (2011) Purposeful program theory: effective use of theories of change and logic models. Jossey-Bass, San Francisco.