Towards a Strategic Plan for Research to Support the Global Program to Eliminate Lymphatic Filariasis

Summary of Immediate Needs and Opportunities for Research on Lymphatic Filariasis

Identified by the Filariasis Community of Scientists in Association with an 'LF Research Forum"

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2.2 ESSENTIAL TOOLS—DRUGS AND CLINICAL DRUG TRIALS

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Summary of Prioritized Research Needs

1) Create a framework for monitoring for potential development of drug resistance, including
   a) defining *criteria* for the phenotype of reduced responsiveness and resistance,
   b) establishing a repository of mf and/or adult worms to provide base-line data on the occurrence of drug-resistant genotypes,
   c) initiating a surveillance system for diminished responsiveness to anti-filarial drugs,
2) Initiate clinical trials of available anti-filarial drugs to
   a) enhance mf reduction (through alternative dosages, frequency regimens, etc.),
   b) enhance adulticidal effectiveness,
   c) define ways to minimize patient treatment reactions at a population level,
   d) define a standard treatment for individuals with LF,
3) Ensure the safety of coordinated administration of drugs in linked public health programs (e.g., albendazole, ivermectin, azithromycin, praziquantel, etc.) or in other medical settings (e.g., used with human immunodeficiency virus/acquired immunodeficiency syndrome [HIV/AIDS] or tuberculosis [TB] multidrug therapy regimens),
4) Establish a framework for seeking a macrofilaricide,
5) Pursue discovery of an anti-*Wolbachia* agent suitable for MDAs and/or individual treatment,
6) Develop broadly applicable implementation strategies for use of DEC-fortified salt (with or without concurrent albendazole administration),
7) Evaluate use of moxidectin for LF.

2.2.1 Overview

Regimens currently in use.

A series of advances during the past decade transformed LF from a neglected disease of poor countries into a disease now recognized as potentially eradicable. Principal among the reasons for these advances were the identification of ivermectin and albendazole as new, effective anti-filarial agents and the discovery of new virtues for an old anti-filarial drug, DEC (i.e., its single-dose efficacy and macrofilaricidal action). These discoveries were essential for the subsequent creation of the Global Program to Eliminate LF,[1] whose very basis is the large-scale use of DEC, ivermectin (Mectizan®; Merck and Co., Inc., Rahway, NJ) and albendazole. Indeed, in many endemic countries literally millions of tablets of these drugs are distributed over a few short days each year (often on a single day), making GPELF the largest chemotherapy program ever undertaken.[2]

Currently it is just these three drugs that are available for use in single-dose, annual MDA programs. Albendazole is co-administered with ivermectin in areas of Africa and Ye-men where LF and onchocerciasis are co-endemic. For all other LF-endemic regions, albendazole is co-administered with DEC. At the recommended dose levels, none of the drugs is completely macrofilaricidal, although all appear to inflict some lasting damage to adult worms. Both DEC and ivermectin kill mf efficiently; albendazole, on the other hand, has no direct effect on mf, but rather appears to suppress embryogenesis in the adult female worm. An alternative treatment strategy involving the use of DEC as a fortificant in table/cooking salt for a period of 1–2 years is currently used in just one country, but was a mainstay of the earlier, successful LF elimination program in China.

Modes of action of available drugs.

The mechanisms by which ivermectin and albendazole achieve parasite destruction have been well studied in other, non-filarial organisms. Briefly, ivermectin binds selectively and with high affinity to glutamate-gated chloride ion channels in invertebrate nerve and muscle cells. The molecule increases permeability of the cell membrane to chloride ions, resulting in hyperpolarization of nerve or muscle cells, causing parasite paralysis and death. The drug is also observed to affect other ligand-gated chloride channels, particularly those gated by gamma-aminobutyric acid.[3] However, it is still not certain why ivermectin shows activity only against mf, and not adult worms.

The primary target for albendazole is tubulin, and the drug is observed to have a higher affinity for parasite tubulin than for that of the host.[4] By blocking tubulin polymerization and microtubule formation, the drug inhibits mitosis, and therefore embryonation and egg hatching. It is very likely that albendazole exerts an effect on adult filariae in this manner.

In contrast, the understanding of how DEC achieves a lethal effect on either mf or adult worms is very poorly developed. There is some evidence that the drug may require cooperation with a functional host immune system to be optimally effective.[5,6]

How these drugs act in combination on different lifecycle stages is not at all understood, or even well studied.

Resistance mechanisms.

All three of these anti-filarial drugs have been used extensively in humans, and to date there has been no unequivocal identification of resistance in LF to any of the drugs. While this is clearly most encouraging, it is also not entirely surprising since currently there is no phenotypic definition of such drug resistance and no comprehensive system for monitoring and evaluating the phenomenon in humans.

The mechanisms by which resistance to albendazole is conferred are well understood in nematode parasites of other animals, and PCR-based methods for detection and analysis of the expression of the beta-tubulin genes TUB1 and TUB2 are available. Resistance to albendazole appears to be associated with a loss of high affinity receptors, resulting from neucleotide changes, predominantly in the TUB1 gene.[7,8]

Mechanisms controlling parasite sensitivity to ivermectin appear to be considerably more complex. Studies on the nematode *Caenorhabditis elegans* show that simultaneous mutation of three genes (*avr-14*, *avr-15*, and *glc-1*) that encode glutamate-gated chloride channel (GluCl) α-type subunits confers a high-level resistance to the drug.[9,10]

A decrease in sensitivity of filariae to DEC has been suggested, particularly where the drug has been used for many years. However, whether these observations reflect a genetically conferred resistance, deficiencies at a programmatic level, or some other cause is as yet not certain.[10,11] These uncertainties are currently compounded by a lack of suitable targets to facilitate the evaluation of DEC resistance in humans.

Safety of one- and two-drug regimens.

The safety of each of the three individual drugs used in the GPELF has been well established. Diethylcarbamazine, in use for nearly half a century, has proven to be extremely safe and effective both in the clinic and the field without supervision; hundreds of millions of doses of ivermectin have been safely distributed since the drug was introduced for the treatment of onchocerciasis; and albendazole has been widely used for several decades, again in hundreds of millions of people, with a remarkable safety record. When co-administration of these drugs was initiated through GPELF, active surveillance programs were undertaken in all countries where MDAs were begun. These documented thoroughly the safety of the combinations in the field.[12–14] (Only in areas of Africa endemic for loiasis, where severe adverse reactions may occur with the administration of ivermectin, are MDAs for LF elimination now contra-indicated because of safety concerns [see below].)

2.2.2 Research Needs: Drug Development

Macrofilaricide.

While DEC and albendazole clearly have macrofilaricidal effects,[15,16] there is still recognition of the potential value a more potent macrofilaricide would bring to efforts to achieve LF elimination, not only in rapidly diminishing microfilaremia but also in halting progression of the initial morbidity associated with the infection and induced largely by the adult-stage worms. Since drug development is a long and costly exercise, with the pharmaceutical industry being generally reluctant to invest in ventures that have little or no direct financial return, any meaningful advance in the development of a macrofilaricide will likely be feasible only if an effective partnership between development agencies, academia, the pharmaceutical industry, and funding organizations can be established. Therefore,

- despite the complexity of the challenge, a working group should be established to explore the creation of an anti-filarial (and particularly, macrofilaricidal) drug development partnership, and
- this partnership should focus not only on novel drugs but also take leads from currently active drug classes and from active compounds identified in 'traditional' medicine.

Anti-Wolbachia drugs.

Over recent years, alternative approaches to classical chemotherapy have emerged as the *Wolbachia* endosymbionts of filariae have been recognized as potential drug targets.[17] Indeed, treatment with the antibiotic doxycycline for six weeks depletes *Wolbachia* from *W. bancrofti*, yielding an almost complete absence of microfilaremia one year later and suggesting effective, long-term block of embryogenesis[18] and/or macrofilaricidal activity. Since treatment of filariasis with such protracted regimens of antibiotics is not compatible with the principles of mass chemotherapy, an important challenge today is

- to identify or develop alternative antibiotics or regimens effective against *Wolbachia* that could be used in MDA programs and/or offer specific treatment to infected individuals.

Moxidectin.

Studies to evaluate moxidectin as an alternative to ivermectin for the treatment of onchocerciasis have shown moxidectin to be more effective than ivermectin in most animal models.[19] The drug has potent effects on mf and results in long-term sterilization of female adult worms, but there is no evidence as yet showing that moxidectin is macrofilaricidal. Moxidectin is currently being evaluated as a treatment for onchocerciasis through a partnership involving WHO and the owner (American Home Products [Wyeth]). If the evaluation of moxidectin for human onchocerciasis produces promising results, then

- moxidectin should be evaluated for effectiveness against LF parasites as soon as possible.

2.2.3 Research Needs: Clinical Drug Trials

Standardization of clinical trial techniques.

Clinical trials with existing and newer anti-filarial drugs are needed to address many of the issues that have arisen during implementation of the GPELF and to provide insights that will strengthen the Program's evidence base. Therefore,

- standard operating procedures need to be developed for controlled clinical trials to assess the efficacy of anti-filarial drugs and to define the methodologies used
  a) to estimate mf density,
  b) to calculate clearance of microfilaremia,
  c) to define the time points at which determinations will be made,
  d) to provide evidence of the parasite adulticidal effects of drug treatment (i.e., development of nodules post-treatment, ultrasound follow-up of identified nests of worms, and antigen detection assays at appropriate intervals),
  e) to document the side effects that may be less commonly recognized (such as proteinurea or hematuria).

Optimizing clearance of mf.

While the currently recommended two-drug regimens effectively clear microfilaremia,[16,20–22] if ways to improve them

could be found, clear programmatic benefits might result. Therefore, efforts to optimize the regimens need to be undertaken in different regions and with different strains or species of parasites

- by exploring alternative frequencies of treatment (e.g., six-monthly),
- by defining the total number of treatments required in areas with different prevalences of infection,
- by evaluating the effects of higher dosages of ivermectin (i.e., > 200 μg/kg) and/or albendazole (i.e., > 400 mg) in the two-drug co-administration regimens.

Recrudescence of microfilaremia post treatment is well recognized,[23,24] but he factors that govern it are not well understood. Therefore,

- controlled trials that follow microfilaremic individuals over extended periods of time should be undertaken to identify and define the factors that determine the reappearance of microfilaremia in treated individuals.

Diethylcarbamazine-fortified salt.

The use of DEC-fortified salt is an alternative to repeated single-dose regimens for interrupting transmission[25] and might be especially useful in those situations where reaching the entire at risk population is particularly difficult (as in urban populations). While DEC-fortified salt has documented effects on adult worms, decreased frequency of side effects, and potential prophylactic benefits, it is unclear if the presence of DEC, even at the low concentrations in fortified salt, would make it unsafe for use in urban or other areas of Africa where onchocerciasis might coexist. Also, current recommendations are for use of DEC-salt for a period of 1–2 years to achieve LF elimination, but studies to define the optimal duration of treatment have not yet been carried out. Therefore, additional studies are needed

- to evaluate the duration of use of DEC-salt yielding an optimal anti-filarial outcome,
- to determine the lowest LF-effective dose of DEC-fortified salt that could be safe for use in onchocerciasis-endemic areas, either urban or where previous treatment with ivermectin had been adminstered,
- to explore the potential for enhanced efficacy of DEC-salt when intermittent (e.g., once a year) albendazole is co-administered.

Decreasing parasitologic response to therapy.

Success of the LF elimination program could be jeopardized if a decrease in the efficacy of available drugs developed. Early identification of decreased susceptibility to currently available anti-filarial drugs is undoubtedly a most cost-effective means for ensuring the continued, long-term success of the LF elimination program. While analysis of changes in the parasite genotype has the potential for providing both baseline and situational information on the distribution and frequency of resistant genotypes,[10] a definition for resistant phenotype has yet to be agreed, and the challenge is made more difficult (particularly with respect to DEC) by the variability of responses in different individuals. For example,

many observations document the persistence of microfilaremia even after individuals or populations have received several courses of DEC in single or multiple doses;[23] and, just as concerning is that only a proportion (~70%) of adult *W. bancrofti* is usually killed by a single dose of DEC, with no additional killing of the remaining worms even with additional doses of the drug (documented by ultrasound).[26] Therefore, while to date there has been no documentation of the existence of resistance to DEC, ivermectin or albendazole in LF parasites, there is now an urgent need

- to establish a working group to create a phenotypic definition of reduced responsiveness and drug resistance and to identify the defining criteria that must be assessed,
- to estimate the frequency of occurrence of such reduced responsiveness (e.g., by examining program data from around the world) and to document the existence of the problem in identified individuals who can be further studied,
- to develop a central repository of genetic material from mf and adult worms in different endemic areas to facilitate
  1) collection of base-line data on the occurrence of potential drug resistant genotypes,
  2) implementation of an effective surveillance system for drug resistance,
- to model drug resistance in LF so that the implications of the possible emergence of resistance can be predicted and deficiencies in relevant knowledge can be identified.

New drugs and drug combinations.

Endosymbiotic bacteria (*Wolbachia spp.*) within filarial parasites influence the life cycle of the parasites to such a degree that killing these bacteria produces adulticidal effects. Recent trials in onchocerciasis and *W. bancrofti* infections suggest that long-term (six-week) administration of tetracycline may have profound anti-parasitic effects on adult filarial parasites.[27] Therefore, to find drug regimens that are more practicable for public health programs,

- the adulticidal effects of various regimens of tetracyclines, rifampicin, and combinations of antibiotics and conventional anti-filarial drugs (such as DEC) should be evaluated in controlled clinical trials.

Moxidectin is currently in early clinical stages of evaluation for onchocerciasis. If this compound progresses satisfactorily in clinical development,

- moxidectin should undergo clinical trials for evaluation of adulticidal and/or microfilaricidal activity in LF.

While the safety and pharmacokinetics (PK) of the two-drug co-administration regimens used for the LF MDA programs have been well established,[12–14] increasingly the desirability of linking LF programs with other public health initiatives also based on MDA is being appreciated. Similarly, the increasingly widespread and long-term use of drugs to treat populations with HIV/AIDS or TB means that many individuals could inadvertently receive their LF MDA drugs at the same time as they are receiving other drugs as well. There is a concern that excessive changes in the metabolism of al-

bendazole and/or ivermectin might occur when these LF drugs are administered to patients on anti-retroviral or anti-TB therapy. For all these reasons,

- PK and safety studies should be conducted with the LF MDA regimens and with those drugs used in other public health initiatives that might be linked with the GPELF (e.g., praziquantel [schistosomiasis], azithromycin [trachoma]),
- surveillance should be promoted for potential pharmacokinetic interactions between LF therapy and highly active retroviral therapy for HIV/AIDS and combination therapy for TB; if evidence for such potential interactions is found, formal PK and safety studies should be undertaken.

Treating LF infection in individual patients.

Physicians and other health providers in endemic areas must care for individual patients with individual treatment regimens, but since these health providers in most countries are only peripherally involved in the GPELF, many are unaware of the recent advances in the chemotherapy of LF (particularly the efficacy of single-dose treatment in curing infection). Current physician guidelines (including the major textbooks of medicine) do not refer to these recent advances; indeed, even the WHO guidelines (last issued in 1992) do not refer to the dramatic changes that have taken place in the chemotherapy of LF. Consequently, tens of thousands of medical students today are completely unaware of the changed concepts of dosing and optimal regimens used to treat LF.

The guidelines for program managers, focused on interrupting LF transmission, are based on studies of the effects of single-dose drugs and drug combinations on decreasing microfilaremia in individuals and populations. The principal goal for treating individual patients, however, is to kill the adult parasite. Guidelines for treating individual patients and populations in endemic areas must be harmonized, and while available information suggests that the same drug regimens used in MDAs may also be optimal for the treatment of individual patients,

- clinical trials to define the optimal regimen of drugs and drug combinations for treating individuals with LF are urgently needed,
- in addition to defining the most effective drugs, clinical trials must also resolve questions about
  1) the optimal frequency, duration, and end point of treatment,
  2) the best tools for monitoring successful therapy.

Preventing LF infection (chemoprophylaxis).

The current global strategy based on annual single doses of combinations of anti-filarial drugs aims to clear mf from infected individuals. Healthy individuals in these areas may see little value in consuming the drugs in the absence of any direct personal benefits or long-term protection against the infection. Identification of agents or regimens that would afford protective benefits to exposed individuals would likely enhance the interest of people in participating and, thus, the success and sustainability of programs. Earlier studies in animal models and epidemiologic observations in human populations suggest that DEC (as individual doses or in fortified table salt) may be prophylactic in LF. Therefore, it would be valuable

- to develop a trial design that can identify prophylactic properties of LF drugs in human populations,
- to assess the prophylactic effects of DEC, ivermectin, albendazole and other newer drugs (such as moxidectin) in human populations (as well as in animal models).

Minimizing treatment reactions.

A major programmatic challenge in LF control/elimination programs based on drug administration has been the occurrence of post-treatment reactions in endemic populations, as they sometimes threaten program implementation or expansion. The advent of single dose chemotherapy has greatly improved the compliance of populations, and while post-treatment reactions are usually mild, self-limited and in most situations require only symptomatic therapy, efforts to reduce the occurrence of even these mild reactions could very much improve compliance in control programs. Therefore, trials should be undertaken

- to test novel approaches to minimizing the occurrence and intensity of post-treatment reactions (e.g., by administering the drugs in divided dose, with or without food, at different times of the day, etc.),
- to compare the frequency and intensity of post-treatment reactions following anti-Wolbachia treatment with those following conventional anti-filarial treatment.

Treating LF in L. loa-*endemic regions*

Since the current recommendation for treating populations in most LF-endemic countries of Africa calls for use of ivermectin (Mectizan®) and albendazole, and since the risk-benefit assessment of Mectizan® administration in areas where loaisis and LF coexist without onchocerciasis currently is judged to weigh against MDA, large areas of central and west Africa are excluded from the MDAs using the drug regimens now available.[28] Therefore, there is an urgent need

- to conduct clinical trials that examine the safety and efficacy of a variety of anti-filarial regimens in areas were loaisis and LF coexist, with or without concurrent onchocerciasis, including the recently initiated trials of albendazole pre-treatment to reduce *Loa* mf densities prior to treatment with ivermectin.

Lymphatic filariasis and traditional, indigenous medicine.

In many endemic countries numerous traditional and indigenous therapies are currently available and used for the treatment of LF. In almost all instances these drugs and interventions have not been evaluated in controlled clinical trials. Therefore,

- efforts must be made to evaluate the efficacy and safety of these traditional and indigenous therapies; if any is found helpful, their incorporation into the GPELF should be encouraged.

2.2.4 References

1. Ottesen EA, Duke BO, Karam M, Behbehani K, 1997. Strategies and tools for the control/elimination of lymphatic filariasis. *Bull World Health Organ 75:* 491–503.

2. Molyneux DH, Zagaria N, 2002. Lymphatic filariasis elimination: progress in global programme development. *Ann Trop Med Parasitol 96 (Suppl 2):* S15–S40.

3. Campbell WC, 1991. Ivermectin as an antiparasitic agent for use in humans. *Annu Rev Microbiol 45:* 445–474.

4. Lacey E, 1990. Mode of action of benzimidazoles. *Parasitol Today 6:* 112–115.

5. Chandasekaran B, Ghirnikar SN, Harinath BC, 1980. Effects of diethylcarbamazine and diethylcarbamazine-N-oxide on microfilariae *in vitro* in the presence of immune sera and leukocytes. *Indian J Exp Biol 18:* 1197–1180.

6. King C, Greene B, Spagnuolo P, 1983. Diethylcarbamazine citrate, an antifilarial drug, stimulates human granulocyte adherence. *Antimicrob Agents Chemother 24:* 453–456.

7. Kwa M, Veenstra J, Roos M, 1993. Molecular characterization of beta-tubulin genes present in benzimidazole-resistant populations of *Haemonchus contortus. Mol Biochem Parasitol 60:* 133–144.

8. Lubega G, Prichard R, 1990. Specific interactions of benzimidazole anthelmintics with tubulin: high affinity binding and benzimidazole resistance in *Haemonchus contortus. Mol Biochem Parasitol 38:* 221–232.

9. Dent JA, Smith MM, Vassilatis DK, Avery L, 2000. The genetics of ivermectin resistance in *Caenorhabditis elegans. Proc Natl Acad Sci U S A 97:* 2674–2679.

10. Prichard R, 2001. Genetic variability following selection of *Haemonchus contortus* with anthelmintics. *Trends Parasitol 17:* 445–453.

11. Esterre P, Plichart C, Sechan Y, *Nguyen N, 2001. The impact of 34 years of massive DEC chemotherapy on Wuchereria* bancrofti infection and transmission: the Maupiti cohort. *Trop Med Int Health 6:* 190–195.

12. Horton J, Witt C, Ottesen EA, Lazdins JK, Addiss DG, Awadzi K, Beach MJ, Belizario VY, Dunyo SK, Espinel M, Gyapong JO, Hossain M, Ismail MM, Jayakody RL, Lammie PJ, Makunde W, Richard-Lenoble D, Selve B, Shenoy RK, Simonsen PE, Wamae CN, Weerasooriya MV, 2000. An analysis of the safety of the single dose, two drug regimens used in programmes to eliminate lymphatic filariasis. *Parasitology 121:* S147–S160.

13. Awadzi K, Edwards G, Duke BO, Opoku NO, Attah SK, Addy ET, Ardrey AE, Quartey B, 2003. The co-administration of ivermectin and albendazole: safety, pharmacokinetics and efficacy against *Onchocerca volvulus. Ann Trop Med Parasitol 97:* 165–178.

14. Shenoy R, Suma T, John A, Arun S, Kumaraswami V, Fleckenstein L, Na-Bangchang K, 2002. The pharmacokinetics, safety and tolerability of the co-administration of diethylcarbamazine and albendazole. *Ann Trop Med Parasitol 96:* 603–614.

15. Dreyer G, Amaral F, Noroes J, Medeiros Z, Addiss D, 1995. A new tool to assess the adulticidal efficacy *in vivo* of antifilarial drugs for bancroftian filariasis. *Trans R Soc Trop Med Hyg 89:* 225–226.

16. Ismail MM, Jayakody RL, Weil GJ, Fernando D, de Silva MS, de Silva GA, Balasooriya WK, 2001. Long-term efficacy of single-dose combinations of albendazole, ivermectin and diethylcarbamazine for the treatment of bancroftian filariasis. *Trans R Soc Trop Med Hyg 95:* 332–335.

17. Hoerauf A, Volkmann L, Hamelmann C, Adjei O, Autenrieth IB, Fleischer B, Buttner DW, 2000. Endosymbiotic bacteria in worms as targets for a novel chemotherapy in filariasis. *Lancet 355:* 1242–1243.

18. Hoerauf A, Adjei O, Buttner D, 2002. Antibiotics for the treatment of onchocerciasis and other filarial infections. *Curr Opin Investig Drugs 3:* 533–537.

19. Tagboto SK, Townson S, 1996. *Onchocerca volvulus* and *O. lienalis*: the microfilaricidal activity of moxidectin compared with that of ivermectin *in vitro* and *in vivo. Ann Trop Med Parasitol 90:* 497–505.

20. Beach MJ, Streit TG, Addiss DG, Prospere R, Roberts JM, Lammie PJ, 1999. Assessment of combined ivermectin and albendazole for treatment of intestinal helminth and *Wuchereria bancrofti* infections in Haitian schoolchildren. *Am J Trop Med Hyg 60:* 479–486.

21. Dunyo SK, Nkrumah FK, Simonsen PE, 2000. Single-dose treatment of *Wuchereria bancrofti* infections with ivermectin and albendazole alone or in combination: evaluation of the potential for control at 12 months after treatment. *Trans R Soc Trop Med Hyg 94:* 437–443.

22. Pani S, Subramanyam Reddy G, Das PK, Vanamail P, Hoti S, Ramesh J, Das P, 2002. Tolerability and efficacy of single dose albendazole, diethylcarbamazine citrate (DEC) or co-administration of albendazole with DEC in the clearance of *Wuchereria bancrofti* in asymptomatic microfilaraemic volunteers in Pondicherry, South India: a hospital-based study. *Filaria J 1: 1.*

23. Eberhard ML, Lammie PJ, Dickinson CM, Roberts JM, 1991. Evidence of nonsusceptibility to diethylcarbamazine in *Wuchereria bancrofti. J Infect Dis 163:* 1157–1160.

24. Andrade LD, Medeiros Z, Pires ML, Pimentel A, Rocha A, Figueredo-Silva J, Coutinho A, Dreyer G, 1995. Comparative efficacy of three different diethylcarbamazine regimens in lymphatic filariasis. *Trans R Soc Trop Med Hyg 89:* 319–321.

25. Houston R, 2000. Salt fortified with diethylcarbamazine (DEC) as an effective intervention for lymphatic filariasis, with lessons learned from salt iodization programmes. *Parasitology 121 (suppl):* 161–173.

26. Noroes J, Dreyer G, Santos A, Mendes VG, Medeiros Z, Addiss D, 1997. Assessment of the efficacy of diethylcarbamazine on adult *Wuchereria bancrofti in vivo. Trans R Soc Trop Med Hyg 91*: 78–81.

27. Hoerauf A, Mand S, Volkmann L, Buttner M, Marfo-Debrekyei Y, Taylor M, Adjei O, Buttner DW, 2003. Doxycycline as a novel strategy against bancroftian filariasis-depletion of *Wolbachia* endosymbionts from *Wuchereria bancrofti* and stop of microfilaria production. *Med Microbiol Immunol (Berl) 192:* 211–216.

28. Joint Action Forum, Fifth Session, 1999. *African Programme for Onchocerciasis Control.* Ouagadougou, Burkina Faso, 2.