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### WHO/TDR SCIENTIFIC WORKING GROUP ON 'RNA INTERFERENCE AS A MEANS OF IDENTIFYING DRUG TARGETS FOR FILARIASIS' REPORT

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#### Executive Summary

River blindness (onchocerciasis) and lymphatic filariasis, caused by infections with filarial nematodes, are important tropical diseases causing considerable morbidity, estimated at 951,000 DALYs for river blindness and 5.5 million DALYs for lymphatic filariasis.

Control and treatment of these infections is difficult: no vaccines are available, vector control programs have ceased or are threatened by insecticide resistance, and the repertoire of effective drugs is very limited. Onchocerciasis is currently treated with ivermectin; the drugs used for lymphatic filariasis are albendazole, diethylcarbamazine (DEC) and ivermectin. None of these is effective in killing the long-lived adult worms (macrofilariae) and the treatments are therefore aimed at reducing transmission and pathology.

New drugs that affect new molecular targets are required to improve treatment and control by killing macrofilariae, and to replace the currently-used drugs when resistance to them starts to become evident.

Two recent scientific advances provide new opportunities for discovering and validating antifilarial drug targets. These are (i) the accumulating gene sequence resources for filarial nematodes, in particular the draft *Brugia malayi* genome sequence due for publication later in 2004, and (ii) the development of the gene silencing method, RNA interference (RNAi), as an experimental tool for investigating gene function in model nematodes such as *Caenorhabditis elegans* and in parasitic nematodes, including *B. malayi*.

A Scientific Working Group, chaired by Dr Carolyn Behm, was convened to evaluate opportunities for establishing a systematic approach to identification and validation of antifilarial drug targets and to assess how RNAi in *C. elegans* and filariae might contribute to this process. The Working Group prepared a practical strategy for identifying and validating new molecular targets, sets of criteria for prioritizing candidate drug targets, and a framework for their exploitation for drug discovery.

The Recommendations of the Working Group are as follows:

- (i) *The B. malayi genome sequence should be exploited for identification of candidate drug targets. We recommend that TDR support a Target Selection Team which will meet from time to time to assess and prioritize candidate targets. The targets will be identified*

initially by a combination of bioinformatic analyses in *B. malayi* and, where available, *Onchocerca volvulus*, and information from RNAi experiments in *C. elegans* and other nematodes.

- (ii) *A center for target validation using RNAi analyses in B. malayi should be established.* We recommend that TDR provide funds to (a) develop the current RNAi technology for *B. malayi* and (b) fund an established laboratory to validate high priority candidate targets by RNAi experiments in *B. malayi*.
- (iii) *Completion of the O. volvulus genome sequence should be encouraged.* We recommend that TDR actively encourage efforts to support an *O. volvulus* genome sequencing project.

The Working Group also considered mechanisms for enhancing the post target validation phases of antifilarial drug discovery. Four major areas were identified as warranting attention:

- (iv) *Implementation of high-throughput screens (HTS) based on existing validated targets.* We recommend that TDR publish the target validation and selection criteria outlined in this Report, or modifications thereof, and call for nominations of candidate targets, preferably HTS-ready, from the scientific community. These could be evaluated by the Target Selection Team and forwarded to collaborating HTS facilities.
- (v) *Evaluation of dormant anthelmintic candidates in industry chemical files.* We support TDR pursuing discussions with animal health and pharmaceutical companies possessing files of compounds with anti-nematode activity that have not been developed, with the aim of providing candidates for testing for potential antifilarial activity.
- (vi) *Selection of secondary assays and drug testing models.* We encourage TDR to maintain and improve the capacity to test compounds from HTS by critically evaluating the preclinical screening models.
- (vii) *Advancement of nematode-specific resources for drug development.* In order to improve antifilarial drug discovery we encourage TDR to consider supporting research specifically to investigate drug uptake and metabolism in filariae, and the development of nematode cell lines.

## I. Needs in anti-filarial drug discovery

River blindness and lymphatic filariasis, caused by filarial nematodes, are among the most important tropical diseases. Nearly 140 million cases exist and over 1 billion people are at risk for infection. River blindness is caused by the microscopic larvae (microfilariae) of the filarial nematode worm *Onchocerca volvulus*, transmitted by blackflies. Adult worms can live for over a decade in nodules under the skin and release millions of microfilariae. Circulating microfilariae cause persistent, debilitating itching, severe dermatitis, and ocular lesions resulting in blindness. According to World Health Organization (WHO) estimates, 17.7 million people are infected in 37 tropical countries of Africa and Latin America [1]. Of these, 270,000 individuals are blind and an additional 500,000 are visually impaired, making onchocerciasis the second leading cause of infectious blindness worldwide. Morbidity is estimated at 951,000 disability adjusted life-years (DALYs). Mosquito-transmitted filarial worms, including *Wuchereria bancrofti* and *Brugia malayi*, cause lymphatic filariasis by colonizing the lymphatic system. Worms live up to 8 years and release millions of microfilariae into the blood. Symptoms include blockage of lymph ducts by adult worms leading to dramatic swelling of appendages (lymphedema, elephantiasis) or genitals (hydrocele) [1]. Disease causes disability, loss of work and marriage opportunity, and social stigmatization. 120 million people are infected in 80 countries, according to WHO estimates. 15 million people have clinically significant lymphedema or elephantiasis and 25 million men have hydrocele. Lymphatic filariasis is the world's second leading cause of long-term disability, with morbidity estimated at 5.5M DALYs.

For both diseases, coordinated global efforts at control are ongoing. Onchocerciasis is treated with ivermectin, which can reverse skin itching and prevent further damage to the eyes. Annual treatments can reduce circulating microfilariae, thereby disrupting disease transmission, but these treatments do not kill adult worms [2]. Since 1987, the Mectizan Donation Program has provided annual doses of ivermectin in Africa and Latin America [3]. From 1974 – 2002, the Onchocerciasis Control Program also worked to control onchocerciasis in West Africa by aerial spraying of insecticides to kill blackfly larvae. While this effort succeeded in opening millions of hectares of arable river valley farmland to settlement and cultivation, closure of the program places the entire burden of disease control on use of drugs. Drug treatments for lymphatic filariasis also attempt to reduce the incidence of circulating microfilariae in the human population to disrupt disease transmission. Drugs include annual doses of albendazole plus diethylcarbamazine (DEC), albendazole plus ivermectin, or use of DEC fortified salt [2]. While effective on larval stages, these treatments are fairly ineffective at killing adult worms and provide only partial benefit to infected patients. Efforts to alleviate suffering and disability for infected patients focus on hygiene aimed at decreasing secondary bacterial and fungal infection. In 2000, control efforts were formalized as the Global Alliance to Eliminate Lymphatic Filariasis, a coalition with the ambitious goal of eliminating the disease by 2020 through the distribution of drugs [4]. Control efforts are scaling up with 54 million people in 32 countries treated in 2002, an increase from just 3 million in 2000. Despite these admirable global efforts, eradication of filarial diseases will be extremely challenging with current technology. No vaccines are available, vector control programs have ended or are facing insect resistance, and drugs are largely ineffective against the worm's adult stage. Current drugs can effectively eliminate the worm's larval stages, but their broad use also increases the likelihood of accelerated drug resistance. The requirement for ongoing annual dosing to prevent the build-up of new larvae from the surviving adult worm is a significant operational challenge in endemic regions subject to poverty and civil unrest.

Anti-nematode drug discovery, lacking a comprehensive molecular or mechanistic understanding of nematode parasitism as a basis for rational drug development, has traditionally relied either on direct screening of compounds against whole target organisms or on chemical modifications of existing anthelmintics. These conventional approaches have resulted in relatively few classes of agents acting on a limited number of known biological targets. Organophosphates and carbamates target a single, biologically conserved enzyme, acetylcholinesterase. Imidazole derivatives such as albendazole exert their antiparasitic effects by binding tubulin. Levamisole and the avermectins agonize the nicotinic acetylcholine receptor and glutamate-gated chloride channels, respectively [2].

These existing agents are effective against some significant nematode infections, but as noted above face a number of limiting concerns. To build on the success of the current treatment regimen and to ensure that the path towards reduction and elimination of these diseases continues, new chemical classes of compounds with strong macrofilaricidal activity are urgently needed. Such drugs could:

- accelerate programs to eradicate lymphatic filariasis
- make elimination of onchocerciasis possible
- increase patient benefit and compliance
- decrease the likelihood of drug resistance and program failure.

## **II. Strategies for new anti-filarial drug discovery**

The pharmaceutical industry has not prioritized nematode diseases of humans, as these infections predominantly afflict populations in poor and developing countries that currently lack the economic resources to support a profitable drug market. However, parasitic nematode infections are a major problem for livestock and companion animals worldwide, and anti-parasitic agents constitute a significant portion of the animal health market. The major anti-nematode drugs available for treatment of human disease were all first developed for the animal health market. Testing new anti-parasitic agents that emerge from animal health drug discovery efforts in assays predictive for efficacy against lymphatic filariasis and onchocerciasis will continue to be one viable strategy for identifying new anti-filarial drug candidates.

Anti-nematode drug discovery in the modern animal health industry utilizes an integrated combination of:

- direct *in vitro* screening of compounds on target parasites,
- compound screening on surrogate organism models predictive for efficacy against target parasites (when target parasites are not available in sufficient numbers to support a screening effort), and
- biochemical or cell-based screening against validated molecular targets important for parasite viability.

For filarial parasites of humans, the utilization of direct *in vitro* screening is limited by restricted availability of parasites and limitations in parasite culture technology. The historical adoption of animal health drugs for treatment of nematode infections of humans essentially represents exploitation of surrogate drug discovery models. High-throughput compound screening against molecular targets, which has become the dominant paradigm in pharmaceutical discovery, has not yet been widely pursued for nematode diseases of humans [5].

Assay development for molecular target-based compound discovery and subsequent high-throughput screening are major undertakings requiring considerable investments of time and money. The identification of biologically validated targets amenable to miniaturized assays is thus of critical importance. Molecular genetic methods for uncovering the biological functions of individual genes and proteins in an organism, a prerequisite for full target validation, have been essentially non-existent for filarial nematode species. However, two recent scientific advances have opened up new opportunities for target validation for anti-filarial drug discovery:

- The large-scale production of gene sequence information for filarial nematodes [6], including the pending release of a draft of the *Brugia malayi* genome sequence, allows the identification of genes shared with other nematodes. Such comparative sequence analyses can point to conserved biological functions, establishing a basis for exploitation of the experimentally tractable and well-characterized free-living nematode *Caenorhabditis elegans* as a surrogate for anthelmintic target validation with a stronger focus on filarial targets than has previously been possible.
- The development of RNA interference (RNAi) as a tool for probing the biological functions of genes known only by DNA sequence has accelerated the elucidation of genetic functions in *C. elegans* [e.g. 7, 8-10]. Recent successes in utilizing RNAi to probe gene functions in parasitic nematodes [11, 12], including *Brugia malayi*, offer the promise of direct elucidation of gene function for at least some aspects of filarial nematode biology.

### **III. WHO Scientific Working Group on RNAi as a means of identifying drug targets for filariasis (15-17 October 2003, Glion sur Montreux, Switzerland)**

This Scientific Working Group was convened to evaluate current opportunities for implementing a systematic approach to molecular drug target identification and validation for anti-filarial drug discovery, and to assess the contribution that RNAi analyses in *C. elegans* and other nematodes might make to this process. The meeting was held on 15-17 Oct. 2003. The primary goal of the discussions was to formulate a set of practical recommendations to TDR on how it could stimulate drug target identification and validation for the filarial diseases. In addition, the group sought to outline some more general guidance regarding strategies for forwarding validated targets through subsequent stages of the drug discovery process.

### **IV. List of participants**

#### **Chair:**

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## **V. Framework for target identification, validation, and exploitation for drug discovery**

The Working Group discussed at length the utility of the *C. elegans* resource and RNAi of *C. elegans*, *Brugia* and *Onchocerca* for antifilarial drug discovery, strategies for target identification and validation, issues and strategies in advancing validated targets through high-throughput screening to identification of suitable antifilarial compounds. The group considered practical and collaborative strategies for future research to discover and validate new antifilarial targets. The outcomes of the discussions include a strategy for drug target identification and validation, plus sets of criteria to be further developed and then applied in the recommended target prioritization processes. These major outcomes are summarized in the set of Tables 1 and 2, and Figures 1 and 2, described below.

### ***(1) Strengths and Weaknesses of RNAi-based Target Discovery***

RNAi is a process of post-transcriptional silencing of gene expression that occurs in the cells of most, if not all, eukaryotes. The phenomenon was observed in plants and the fungus *Neurospora* some years before it was first described in an animal system, *C. elegans*, in 1998 [13]. Since then, RNAi has been observed in a variety of animal taxa, including mammals, and is transforming the field of functional genomics in a number of animal, plant, protist and fungal experimental systems. RNAi was voted 'Breakthrough of the Year' by *Science* magazine in 2002 [14] and its exploitation in drug target validation in mammalian and other biological systems, including parasitic nematodes, has great potential to bring about rapid advances in drug discovery.

The process of silencing gene expression by RNAi in cells can be described briefly as follows. Intracellular double-stranded RNA (dsRNA) – of exogenous or endogenous origin – is bound by the Dicer protein complex. The dsRNA is cleaved by RNase activity of the Dicer complex to yield small interfering dsRNA products (siRNAs, approx. 21 nucleotides in length) which bind to another protein complex, the RISC, in which the two strands of the siRNA are unwound to expose the antisense strand which hybridizes to a complementary mRNA molecule. The mRNA is then cleaved at a site corresponding to the centre of the siRNA sequence and the resulting mRNA fragments are degraded. The RNAi effect is very specific: only transcripts complementary to the siRNA are cleaved and degraded, and thus the knockdown of mRNA expression is gene-specific. The gene silencing effect of dsRNA within a cell is quite long-lasting and under appropriate experimental conditions can lead to a decline in abundance of the targetted transcript of 80-90% or more, with consequent decline in levels of the corresponding protein.

The Working Group recognized that a target discovery and validation program based on RNAi and bioinformatic filters has considerable potential to make rapid progress in antifilarial drug discovery. The advantages of using RNAi in such a program for filarial worms include the following:

- (i) RNAi provides, for the first time, a means of determining gene function in filarial worms. By establishing the importance of a gene to the life cycle, valuable resources in target development can be focused on those gene products known to be crucial for parasite survival.
- (ii) RNAi targets mRNA complementary to the sequence of the applied dsRNA. Thus only the sequence of the target mRNA needs to be known, libraries of chemicals are not required in the target discovery and validation stages, and the genome sequences of *Brugia* and other nematodes can be utilized.
- (iii) RNAi is specific to the targeted mRNA and the effects of silencing expression of the targeted gene are thus specific to that target.
- (iv) Target discovery and validation is carried out *in vivo* using intact nematodes.
- (v) RNAi is effective to some extent in both *Brugia* and *Onchocerca* and allows researchers to circumvent in part the challenge presented by the limited technology (e.g. genetic) currently available for investigating biological function in filariae.

The Working Group also recognized that an RNAi-based program presents some limitations, which are outlined below. We would expect that additional targets will emerge from other discoveries outside the scope of that proposed here, and these should be given consideration by WHO/TDR on a case-by-case basis. New methods that arise to further open up target opportunities should be incorporated into a flexible strategy. Examples of targets that may not be revealed by an RNAi-based program include the following:

- (i) Targets that exhibit 'gain of function' phenotypes in the presence of agonists such as channel openers. Two major classes of current anthelmintics are agonists and their targets would probably not have been discovered by an RNAi screen.
- (ii) Targets expressed in tissues with less sensitivity to exogenously applied dsRNA. In *C. elegans*, nervous system mRNAs are not susceptible to RNAi by soaking. The sensitivity of filarial nervous system transcripts is not known.
- (iii) Targets that have no or subtle effects *in vitro* but which could have major effects if disrupted *in vivo*, such as genes critical for immune evasion, or behavior in the host.
- (iv) Targets eliminated in the selection process because of high conservation to human gene products but for which differential exposure, uptake, binding, or sensitivity could allow selective targeting.
- (v) Targets that cannot readily be assessed by RNAi in *Brugia* or *Onchocerca* because of the limitations of the current RNAi and *in vitro* culture technology for filariae: for example *Brugia* is currently accessible to RNAi only at the adult stage.

The Working Group concluded that the use of RNAi and the bioinformatic/curation filters recommended (Figure 1) is very likely to generate an abundance of viable and validated targets on which drug discovery and development work can begin.

## **(2) Target Product Profile**

The ideal candidate compounds for human antiparasitics should possess the properties summarized in Table 1.

## **(3) Target Selection and Validation Criteria**

The most important criteria for selecting appropriate drug targets for further investigation are summarized in Table 2. Critical to the prioritization and decision path for validation of candidate targets is assessment of ‘druggability’ and ‘assayability’ of the candidate target molecules. The major criteria recognized as important in these assessments are included in Table 2.

## **(4) Strategy for Identification and Validation of Antiparasitic Drug Targets**

A strategy for identification and validation of antiparasitic drug targets, employing the criteria outlined in Tables 1 and 2, was designed by the Working Group. A flow chart outlining the recommended strategy is presented in Figure 1. The growing resource provided by the imminent *Brugia* genome sequence and the availability of RNAi techniques for adult *B. malayi* make it the filarial species of choice for the initial phases of the strategy. The Working Group expects that relevant *Onchocerca* information will be utilized wherever available or obtainable, and considers it highly desirable that any target be present and shown to be essential in *Onchocerca* as well as *Brugia* and, where possible, *Wuchereria*, before it can enter the later stages of the target decision pathway.

## **(5) Downstream Drug Discovery Filters**

Consideration was also given by the Working Group to appropriate downstream drug discovery processes required to follow the successful validation of targets, including in particular the choice of appropriate animal models. Appropriate counterscreens to deselect compounds acting via mechanisms with little anticipated clinical utility, and robust secondary assays for identifying the most promising lead candidates are essential to the success of a high-throughput screening program. The use of *in vitro* and *in vivo* secondary screens predictive of clinical efficacy is of particular importance. A flow chart describing the major stages in the identification of suitable compounds is presented in Figure 2. In order to maintain a focus on macrofilaricidal activity, it will be important to avoid over-interpretation of data generated on other species and life-cycle stages. For instance, compounds might fail *in vitro* larval assays yet still have potent macrofilaricidal activity.

## **VI. Recommendations for WHO/TDR actions to enhance anti-filarial drug discovery**

The Working Group discussions converged on two sets of recommendations. The first and most immediate of these focuses on specific actions through which “seed investment” by TDR could facilitate filarial target identification and validation. The second comprises related considerations for enhancing the anti-filarial drug discovery phases subsequent to target validation.

## **A. Recommendations for specific initiatives in target identification and validation:**

(1) Exploit the *Brugia malayi* genome sequence for the identification of candidate drug targets. A systematic analysis of the *Brugia* genome sequence, including comparison with the *C. elegans* genome sequence and RNAi phenotype database, and utilization of any relevant available information for *Onchocerca*, should be carried out for the explicit identification of desirable candidate drug targets, based on the criteria outlined above (see Figure 1 and Tables 1 and 2). The initial analysis can be performed in two phases, only one of which would require expenditures by TDR:

(a) The first phase of the analysis is already in progress beginning with the *B. malayi* genome annotation “jamboree” in January 2004, organized by The Institute for Genome Research (TIGR), where the genome sequence has been generated and assembled. Dr. Jim McCarter and Dr. Aziz Aboobaker, along with Dr. Clotilde Carlow of New England Biolabs in collaboration with Dr Elodie Ghedin of TIGR, have indicated a willingness to undertake a preliminary target identification as part of the *Brugia* genome analysis.

(b) Following the formal public release of the *B. malayi* genome sequence by TIGR, anticipated before the end of 2004 concurrent with publication, TDR should convene a meeting of a Target Selection Team to extend the preliminary analysis and produce a prioritized list of more thoroughly evaluated candidate drug targets. This meeting will need to be held at a venue able to provide access to the necessary computing infrastructure. The Target Selection Team should include established investigators with expertise in bioinformatics and functional analyses of nematode gene sequences. In addition, the team will benefit from the participation of industry representatives cognizant of the specific considerations important for assay development and drug screening. Furthermore, the inclusion of advanced trainees from bioinformatics programs in endemic countries could lay the foundation for establishing an ongoing target analysis project with significant contributing effort from these programs.

(c) Subsequent meetings of the Target Selection Team can be convened as needed to take advantage of emerging new data and/or to refine the working priority list of candidate drug targets. For instance, while use of RNAi knockout data will result in a focus of initial selection work on targets with strong loss-of-function effects, a later review might concentrate on targets with potentially strong gain-of-function phenotypes (i.e. channel agonists).

(2) Establish a center for target validation using RNAi analysis in *Brugia malayi*. Biological validation of selected candidate drug targets will be needed to reap the full benefit of the genome analyses. Genetic and RNAi screens by the *C. elegans* research community have yielded and will continue to yield results that provide valuable guidance in the selection of biologically relevant anti-nematode targets, and these efforts are in general well-supported by the mainstream funding agencies. The selection of targets for *anti-filarial* drug discovery would be strengthened by similar biological validation in a filarial nematode. While recent advances in applying RNAi technology to filarial nematodes, in particular to *B. malayi*, have opened the possibility of obtaining such validation, the resources and infrastructure for exploiting and improving this technology remain quite limited, and investment by TDR could make a significant contribution toward filling this particular gap in anti-filarial drug discovery.

The current status of *Brugia* RNAi is as follows. The process, though shown to work in *Brugia* adults, requires further optimization and study. In particular questions about the longevity of effect, which parasite stages can be affected, and the amount of dsRNA required (the significant cost in the procedure) remain unanswered. The existing protocol is labor intensive as it was designed to show proof of principle rather than to screen a large number of genes. Dr Aziz Aboobaker, who was responsible for adapting the technique to *Brugia*, recommends that a further 12-18 months by 1.5 full time researchers (full time postdoc, part time technical aid) be

spent on optimizing the technology. As with any subsequent target validation screen using the technique, Dr Aboobaker considers the most important factor for this work to be the onsite availability of the *B. malayi* lifecycle. Although Dr Aboobaker is no longer working directly on this research he has offered to give technical advice as requested to any laboratory that undertakes RNAi in *Brugia*.

The Working Group thus recommends that TDR fund a center with the function to (a) improve the technology for optimal RNAi in *B. malayi*, and (b) to carry out systematic RNAi analysis of candidate drug targets in *B. malayi*. The identification of a laboratory should be based on a competitive award process involving proposal solicitation, peer review and defined milestones.

(3) Advocate completion of the *Onchocerca volvulus* genome sequence. While the *B. malayi* genome sequence will be an invaluable resource for selection of candidate anti-filarial drug targets, target identification and validation would be further enhanced by availability of the *O. volvulus* genome sequence. Development of assays and drug screens predictive of drug efficacy for onchocerciasis would also be facilitated by having the *O. volvulus* genome sequence available as an information resource. Although full funding of a genome sequencing effort is currently outside the scope of TDR's program, the Working Group recommends that TDR actively encourage other potential efforts to support an *O. volvulus* genome sequencing project.

## **B. Considerations for enhancing other phases of anti-filarial drug discovery:**

(1) Implementation of high-throughput screens (HTS) based on existing validated targets. A variety of candidate filarial drug targets are the subjects of ongoing investigations in a number of laboratories. However, most academic laboratories lack the infrastructure and resources to advance a validated target into high-throughput compound screening. TDR is actively seeking access to high throughput screening capabilities through a variety of approaches, some of which involve setting up collaborations with biopharma companies, and others that involve working with dedicated screening centers being set up within academic research institutes or universities. For example, TDR has recently funded a program at a new HTS facility that has been established at the Walter and Eliza Hall Institute in Australia. The target validation and selection criteria outlined above (Table 2) provide a basis for prioritizing existing validated targets that could be provided to the collaborating HTS facilities for compound screening. Ideally, HTS-ready assays will be available for targets forwarded to the screening facilities. To identify such targets, the working group recommends that TDR publish the validation and selection criteria in a call for letters of interest, asking responding researchers (a) to nominate candidate targets that meet the criteria, (b) to summarize the status of assay development for nominated targets, and (c) work to select targets up front that are amenable to an HTS format assay. The subsequent review process would evaluate and rank the nominated targets to select the most promising for compound screening.

(2) Evaluation of dormant anthelmintic candidates in industry chemical files. Animal health and pharmaceutical companies with anthelmintic discovery programs may have files of compounds exhibiting anti-nematode activity in early-stage screens that were not forwarded to late-stage development due to lack of broad spectrum activity or other limitations. However, some of these compounds could provide productive leads for anti-filarial drugs. TDR has pursued discussions with some companies having such files about the possibility of testing these compounds in assays predictive of anti-filarial efficacy. The Working Group was supportive of this strategy for identifying a pool of compounds enriched for potential anti-filarial candidates.

(3) Selection of secondary assays and drug testing models. For anti-filarial drug discovery, reliable secondary *in vitro* and *in vivo* nematode screening models predictive of clinical efficacy are of critical importance, and will be necessary for effective utilization of the target validation

process that was the primary focus of this Working Group. Thus the Working Group would encourage TDR to maintain and improve the capability to test compounds from HTS in meaningful models, perhaps by convening a similar Working Group to consider the preclinical screening models.

*(4) Advancement of nematode-specific resources for drug development.* The successful development of an active drug from a potent HTS hit is an enormous challenge. Antifilarial drug development would benefit from a better knowledge of drug metabolism and uptake in filariae, and from the development of nematode cell lines. These are long-term goals which the Working Group encourages TDR to support.

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## VIII. Acknowledgements

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## **Table 1: Target Product Profile for a Suitable New Antifilarial Drug**

### *(i) Primary Desired Characteristics*

- oral use
- microfilaricidal
- macrofilaricidal or cause permanent sterilization of adult female (and ideally male) worms, for *Onchocerca*, *Brugia* and *Wuchereria*
- lack of side effects directly following from worm death (i.e. cause slow rather than rapid killing of worms)
- effective with one treatment per year for several years
- stable for 2 years under disease endemic area conditions
- safe to administer without prescription or health worker supervision
- safety profile comparable with ivermectin
- no evidence of cross resistance to existing anthelmintics

### *(ii) Secondary Desired Characteristics*

- oral use for all ages, including children as young as two years
- safe to use during pregnancy and lactation
- single dose
- new mode of action
- molecularly defined mode of action (allows for improvements, resistance monitoring, etc)
- efficacy and safety in combination with existing anthelmintics in use for filariasis
- cost effective at costing levels appropriate to disease endemic areas

**Table 2: Criteria for Selecting Suitable Antifilarial Drug Targets**

*1. Selectivity*

- target is absent from mammals, or
- target has molecular properties which distinguish it from related mammalian molecules, and/or
- evidence that the target can be selectively chemically inhibited or agonized relative to other members of the same protein family

*2. Validation*

- evidence (RNAi, knockouts, inhibitors, etc) that the target is essential for growth, survival, or fertility

*3. 'Druggability'*

Priority is given to:

(i) Molecules with a small molecule ligand binding pocket, for example

- channels
- receptors
- transporters
- enzymes

(ii) Molecules that have precedents, i.e. existing drugs or ligands

*4. Structure*

- amino acid sequence of the target known
- desirable, but not essential: crystal or NMR structure known or obtainable, preferably with bound cofactors, inhibitors or agonists/antagonists

*5. 'Assayability'*

(i) Important features:

- expression precedent available
- existing biochemistry/enzymology
- single subunit is desirable
- specific readout that can be predicted, especially optical, that is compatible with HTS
- chemistry available and/or structural approach possible via crystallization or NMR

(ii) Other desirable features include:

- focused chemical library already available for the class of molecule
- cell-based assays
- assays with functional endpoints
- assays with fewer steps (e.g. washes)

*6. Potential for development of resistance*

- absence of isoforms of the target with varying susceptibility within a species
- absence of biochemical 'bypass' reactions to circumvent function of the target

**Figure 1: Flow Chart for Identification and Validation of Antifilarial Drug Targets**

