Leishmanization trial, Ardestan: A cutaneous leishmaniasis lesion on the arm of a volunteer in a leishmanization trial in Iran. Use of leishmanization is accelerating the completion of trials of vaccine candidates. Photo: WHO/TDR/Crump
New and improved tools
The new or improved tools (area B of the TDR strategy) described below include not only drugs, vaccines and diagnostic procedures, but also products such as recommendations on regulatory procedures that govern the introduction of new drugs. During the biennium, TDR’s activities in the diagnostics area increased, with a major emphasis on tuberculosis (TB). TDR’s vaccine-related activities were focused on malaria and leishmaniasis. Among the important steps forward during the biennium were: registration of a new drug treatment for visceral leishmaniasis; progress towards registration of a new antimalarial formulation and a new antimalarial drug; impending entry into clinical trials of new recombinant vaccine candidates for malaria and leishmaniasis; and development of infrastructure to support laboratory and field testing of diagnostic kits for TB and some sexually transmitted diseases.

Drug development and discovery

AFRICAN TRYPANOSOMIASIS

African trypanosomiasis case numbers are increasing for various reasons, including the difficulty of controlling the tsetse fly vector of the disease in areas that are remote or insecure. Treatment is problematic, especially for patients with advanced disease and central nervous system involvement (‘sleeping sickness’), because current drugs are expensive or generally toxic, and because there is increasing evidence of treatment failures with patients demonstrating refractoriness to certain drugs. To compound this, a drug shortage seemed to be looming in 2000. However, this was averted by the signing of an agreement with Aventis in 2001. Aventis is now supplying three key anti-trypanosomal drugs – pentamidine, melarsoprol and eflornithine – as well as funding disease management and control measures, over a five-year period. As part of the agreement with Aventis, TDR is undertaking clinical studies on an oral formulation of eflornithine with the aim of optimizing the dose regimen. TDR and MSF, with Aventis, are also encouraging the search for a new, less expensive route of synthesis of eflornithine. For nifurtimox, a decision was taken, together with WHO, Bayer and MSF, to undertake further preclinical toxicology studies to update the regulatory dossier. A review of the available clinical data was commissioned by TDR. Additional studies are being undertaken to assess the efficacy of combinations of nifurtimox with the other anti-trypanosomal drugs mentioned above. For berenil, a review of the safety dossier was commissioned, and studies to better assess its bio-availability have been undertaken.

Two other compounds were considered as possible anti-trypanosomal agents during the biennium. The activity of a diamidine pro-drug, DB-289, was confirmed in animal models with TDR support. The Gates Foundation is now funding this project directly, independent of TDR. The further development of megazole was stopped, with the agreement of all parties concerned, after a detailed study of its genotoxicity.

**LEISHMANIASIS**

Miltefosine, the first oral treatment for visceral leishmaniasis (kala-azar), was successfully registered in India by the German company Zentaris in May 2002, and is now being prepared for registration in Germany. The European Agency for the Evaluation of Medicinal Products has already granted it 'orphan drug status' – the first example of such status being awarded for a tropical disease. Registration in other countries is planned. The drug was developed in close collaboration with TDR and the Indian Government, in an impressively short five years since the decision was taken to proceed with a drug development programme. This followed from earlier confirmation in TDR-supported laboratories of its anti-leishmanial activity in vitro. Phase IV studies have now started in India, Nepal and Bangladesh to establish, with additional implementation research, how best to use the drug in control programmes and as part of national drug policy. Miltefosine's potential for treating post-kala-azar dermal leishmaniasis is also being assessed. The whole miltefosine development programme has been notable for its strong and successful capacity-building element.2

The development of paromomycin, a low-cost injectable drug, as a back-up or alternative to miltefosine is currently being undertaken in partnership with the Institute of One World Health, supported by the Gates Foundation. The compound may prove safe for use by pregnant women, for whom miltefosine is not recommended. A Phase II clinical trial was completed in 2000, but was not followed up immediately due to lack of funds. Phase III studies are now ready to begin. On the other hand, development of PX-6518, a natural product that had shown promise in animal models of leishmaniasis, was stopped after extensive preclinical toxicology testing indicated liabilities, with the agreement of all partners concerned.

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Malaria is particularly threatening in cases of severe disease, when the patient – often a young child – may be unconscious and unable to take medicine by mouth, and cannot reach a hospital in time for intravenous drug infusion. Rectal artesunate, developed by TDR as an interim treatment while patients can be transferred to hospital, reached an important milestone. A ‘letter of approvability’ of registration for this new formulation was received from the US Food and Drug Administration under its fast-track approval process. Registration is still dependent on provision of further data, but studies are under way to provide these, and extended Phase IV clinical trials have already commenced. Additional submissions to regulatory authorities of malaria-endemic countries are planned.

The ongoing development by malaria parasites of resistance to current drugs poses another major threat. The development of resistance can be slowed by the strategy of combining two compounds in one formulation to prevent or treat the disease. For the last five years, TDR has worked together with GlaxoSmithKline (GSK) to develop a low-cost combination of chlorproguanil and dapsone as an alternative to sulfadoxine/pyrimethamine, which is already failing in some areas of Africa due to the appearance of resistant parasites. The key Phase III trials of the chlorproguanil/dapsone combination3 were completed in 2001. The dossier was submitted by GSK to the United Kingdom regulatory authorities in late 2002, and a decision is anticipated in the first half of 2003. If regulatory approval is obtained, development of a co-formulation of chlorproguanil/dapsone with artesunate is planned in partnership with GSK and the Medicines for Malaria Venture (MMV), in the hope that resistance to this three-drug, fixed-dose combination will be slow to spread. Phase I clinical trials have already been initiated for this combination.

TDR is also a partner in the development of various other fixed-ratio drug combinations, all of which contain artesunate, or a related artemisinin derivative, as one of the components. Studies that would allow the commercially available combination of lumezantrine and artemether to be recommended for use in young children or infants were undertaken in partnership with Novartis, Roll Back Malaria and MMV. A dossier on the label extension to young children is expected to be submitted to regulatory authorities in 2003. Pyronaridine/artesunate is being co-developed with Shin Poong of Korea and MMV. A decision on whether this should enter Phase I trials is expected in early 2003. Preclinical work aimed at evaluating the potential of three other combinations – amodiaquine/artesunate, mefloquine/artesunate and piperaquine/dihydroartemisinin – is progressing well, in collaboration with a variety of partners including the European Commission, Médecins sans Frontières (MSF), Far Manguinhos (Brazil), the commercial enterprise Holleykin, and others with whom discussions are ongoing.

Two other malaria drug development projects were not considered by TDR to be of sufficiently high priority, or did not receive adequate funding, during the biennium, so are currently on hold in its portfolio: pharmacokinetic studies of malarone (atovaquone/proguanil) in pregnant women, and clinical studies of fosmidomycin as an antimalarial drug. These projects were being undertaken in partnership with GSK and Jomaa Pharmaka respectively.

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**TUBERCULOSIS**

The current treatment regimes for TB are long and complex, typically involving a combination of four drugs over a six-month period. TDR is working together with partners to develop a standardized four-drug, fixed-dose combination that would simplify or shorten treatment. TDR, the Global Alliance for TB Drug Development (GATB) and partners also plan a project to help develop a standardized regulatory policy framework for new TB agents, with the aim of streamlining the process of developing and registering new chemical entities for TB.

**CHAGAS DISEASE**

Control measures have been successful in reducing the incidence of Chagas disease in the ‘Southern cone’ of South America. However, infection by *T. cruzi*, the causative agent, remains a problem in countries such as Venezuela and Colombia, particularly if the infection becomes chronic, when it can involve damage to the heart or intestines. A comparative study of the ability of several anti-fungal azoles to cure established *T. cruzi* infections in mice or dogs was supported by TDR, and led to the identification of one particular azole as a suitable candidate for further development. Discussions towards this end are under way with a pharmaceutical company.

**LYMPHATIC FILARIASIS**

TDR also provided technical support to clinical trials of combinations of albendazole with ivermectin or diethylcarbamazine for treatment of lymphatic filariasis. Results from the albendazole/ivermectin trial showed that the two drugs could safely be used in combination for treating the disease.

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Ivermectin is widely and successfully used in onchocerciasis control programmes. However, it does not kill adult worms, or sterilize female worms. At least one of these outcomes is needed to interrupt transmission of the disease in a reasonable time period. Moxidectin has now been evaluated as a promising potential improvement on, or backup to, ivermectin in preclinical tests. With Wyeth as the industrial partner, a Phase I clinical trial was completed in 2002, and discussions are currently under way with the company on the planning of Phase II studies for 2003.

Clinical trials were carried out to assess the macrofilaricidal effect (ability to kill adult worms) of combinations of albendazole with either ivermectin or levamisole. Neither combination proved more effective than ivermectin alone in these studies.

Drug discovery activities

TDR continued to support a network of centres that can screen and evaluate the activity of compounds against different parasites in laboratory models. During the biennium, the centres tested tens of thousands of compounds for activity against one or more of the parasites causing TDR’s target diseases. Thousands of selected compounds were also tested for activity against purified enzymes (kinases) from the malarial parasite. New compounds giving positive results in the parasite or enzyme assays included analogues of known drugs, as well as compounds with novel structures and natural products not previously known to have antimalarial activity. The positive results are now being followed up by confirmatory tests against cultured parasites and in animal models of parasite infection.

Concern about the poor pharmacokinetics and oral bioavailability of artemisinin derivatives, such as those mentioned above, have encouraged a broad search for other antimalarial peroxides with improved properties. Over the past five years, TDR funded the testing in vitro of hundreds of different antimalarial peroxides. During the biennium, the activity of dozens of these compounds against mouse malaria parasites was evaluated, and the pharmacokinetic properties of the best were determined in preclinical studies. MMV has now taken responsibility for further studies on four of the most interesting of these compounds, and one of these has recently led to a compound being selected for full-scale development.
Vaccine development and discovery

TDR’s vaccine development and discovery activities are now carried out as part of the WHO/UNAIDS Initiative for Vaccine Research (IVR), which was established in 2000 as a coordinating body for WHO’s various vaccine development activities. In this way, new vaccines of all sorts should be able to profit more rapidly from the introduction of general vaccine-related technologies such as new adjuvant formulations or delivery systems. TDR and IVR have closely collaborated, for example, in organizing the third symposium of a series on Novel Adjuvants Currently In or Close To Clinical Testing, held at Annecy in January 2002. They also collaborated in organizing a workshop on the Ethics of Vaccine Clinical Trials in Pediatric Populations (Accra, November 2002); a guidance document resulting from the workshop will appear in 2003.

TDR continues to maintain a strong engagement in vaccine development for both malaria and leishmaniasis. A review of its involvement in schistosomiasis vaccine research, coupled with lack of funding, led to reduction of TDR’s activities in this area in 2001. TDR and IVR collaborated to publish Guidelines for the Evaluation of Dengue Vaccines in Populations Exposed to Natural Infection in 2002.

After six decades of research efforts to develop a vaccine, recently there has been significant progress in developing strategies for attenuated and molecular-based vaccines. In December 2001, a major meeting on Accelerating the Development and Introduction of a Dengue Vaccine was convened in Ho Chi Minh City by the Rockefeller Foundation and the International Vaccine Initiative (IVI), co-sponsored by Aventis, GlaxoSmithKline and WHO/TDR. The meeting was very successful in bringing together vaccine researchers and developers with potential stakeholders, and in developing a consensus and blueprint for developing a paediatric dengue vaccine. 

Several candidate vaccines (live attenuated and second generation infectious clones/chimeric versions) are currently under clinical trial in USA and Thailand. TDR, together with the new WHO Initiative for Vaccine Research (IVR), has produced guidelines for evaluating dengue vaccines in populations exposed to natural infection. This document is addressed to national health authorities, in particular to those of dengue-endemic countries interested in the potential use of dengue vaccines for controlling dengue, and to research scientists interested in development and field evaluation of such vaccines.

5 Engers H et al. 3rd meeting on novel adjuvants currently in or close to clinical testing, World Health Organization, Fondation Mérieux, Annecy, France, 7-9 January 2002. Vaccine (in press).
7 Guidelines for the evaluation of dengue vaccines in populations exposed to natural infection. TDR/IVR/DEN/02.1
Much evidence, including the long-established but potentially risky practice of leishmanization (immunization by scarification with low doses of live parasites), supports the hope that it should be possible to develop a safe, cost-effective vaccine against leishmaniasis. The results of several clinical trials of ‘first-generation’ vaccines against leishmaniasis (vaccines containing killed parasites), carried out with TDR support, have now been analysed. The most immunogenic vaccines contained alum as adjuvant. More data on the potential efficacy of the \textit{L. major}/\textit{alum} and \textit{L. amazonensis}/\textit{alum} vaccines are being collected.

In 2002, TDR signed a collaborative agreement with the Infectious Disease Research Institute (IDRI), Seattle, and the commercial enterprise Corixa, to develop a ‘second-generation’ vaccine based on purified, recombinant Leishmania proteins, with funding from the Gates Foundation. A hybrid construct containing portions of three different proteins has been expressed in and purified from the bacterium \textit{E. coli}, and formulated with the adjuvant mono-phosphoryl lipid A. Its potential as a prophylactic vaccine against visceral leishmaniasis, and as a therapeutic vaccine against the visceral and cutaneous forms of the disease, will be assessed in clinical trials. Phase I studies of the safety and tolerability of the construct started in late 2002.

The number of malaria antigens that deserve study as vaccine candidates continues to increase. In 2000, TDR was supporting development of seven different malaria vaccine candidates – two targeting the parasite’s pre-erythrocytic stages, which enter and develop inside liver cells, and five targeting its asexual blood stages, which invade and multiply in red blood cells. During the biennium, these activities progressed along different paths. The two pre-erythrocytic vaccine candidates (RTS\textsubscript{S}/SBAS2 and CS-102) have since advanced further in clinical trials, and their development is now receiving independent financial support with only limited TDR input. The efficacy of RTS\textsubscript{S}/SBAS2, the most advanced candidate, is now being examined in field trials with different populations, including children, and the sponsors (GSK) are in discussion with IVR and TDR about future steps in its development.

Of the asexual blood-stage candidates, \textit{PfCP-2} was selected for further support by TDR. This is a hybrid protein, produced at high yield in yeast cells, and consisting of portions of two blood-stage proteins (MSP-1 and AMA-1) which play important roles in the invasion of red blood cells by the parasite. The construct was developed by Dr Weicheng Pan, Shanghai. Under a memorandum of understanding with Wanxing Pharmaceuticals, Shanghai, the \textit{PfCP-2} vaccine (protein plus an adjuvant) is
anticipated to enter Phase I clinical studies in early 2003. Development of an alternative vaccine candidate based on the AMA-1 protein alone was stopped due to difficulties encountered in scaling up production technology.

Building on past TDR support and ‘leverage’, groups developing three other asexual blood-stage antigens could obtain sufficient funds for the further progression of their candidates. The development of two of these, both based on the carboxy-terminal portion of the MSP-1 protein, has now gained substantial additional support from the US National Institutes of Health and the European Union respectively. Progression of EBA-175, a candidate based on an erythrocyte-binding antigen from the parasite and developed at the International Centre for Genetic Engineering and Biotechnology (ICGEB), India, is funded by the Gates Foundation through the Malaria Vaccine Initiative (MVI). TDR remains engaged in all of these projects.

Diagnostics activities, which have historically been under-emphasized in TDR, expanded greatly during the biennium. Following the launch of the TB Diagnostic Initiative (TBDI) in 2000, the main emphasis has been on tuberculosis, for which the standard diagnostic method (microscopic analysis of sputum) dates from the nineteenth century. Rapid, simple and inexpensive alternatives to this are a pressing need. Other diagnostic activities based in TDR include the work of the Sexually Transmitted Diseases Initiative (SDI), funded mainly by USAID, and the development of new diagnostic tools for onchocerciasis (funded by APOC and OCP), malaria, leishmaniasis and schistosomiasis.

Diagnostics development and discovery

Diagnosis of the disease at the district level or in refugees or other medically under-served patients is difficult, as current best practice involves bone marrow biopsy or splenic aspirate. Recently, possible alternatives to these procedures, based on serum or urine assays, have been developed. A multicentred field trial is under way in three African countries (Ethiopia, Kenya, Sudan) to assess the cost-effectiveness and overall performance of three of the new candidate test kits.

LEISHMANIASIS

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MALARIA

Rising drug costs, the poor accuracy of symptom-based diagnosis, and the difficulty of providing rapid microscopy-based diagnosis in remote areas, have increased interest in the use of rapid diagnostic tests for malaria. In conjunction with the WHO Regional Office for the Western Pacific in Manila, TDR has helped to develop consensus guidelines for field studies of these tests. Development of a quality assurance mechanism, so that users can be assured of the initial and continued accuracy of the tests in the field, is under way.
Funding from the Gates Foundation allowed TBDI to start developing the complex infrastructure necessary to evaluate new TB diagnostic tools in laboratory and field tests. A Tuberculosis Specimen Bank of reference clinical materials has been assembled and now includes more than 14 000 aliquots of sputum, saliva, urine, and serum. TBDI is also developing a bank of pedigreed M. tuberculosis isolates, including strains with different combinations of resistance to isoniazid, rifampin, ethambutol, and streptomycin. In addition, TBDI maintains a list of trial sites where high-quality, supervised field trials of new diagnostic procedures have been carried out, and performs a liaison function, putting test developers in direct contact with clinical investigators. A large comparative trial of four new drug resistance testing methods was initiated in several trial sites in late 2002. Since there is very limited laboratory capacity in most TB-endemic countries to evaluate new diagnostic tools, TBDI has established a programme, with additional funding from USAID, to increase operational research capacity in laboratories of selected high-burden countries.

In parallel, an active programme was undertaken to clarify the medical need for specific types of TB diagnostic tools, and facilitate their discovery, development and evaluation. It became clear from a TBDI-conducted survey that, although many small and medium-sized biotechnology companies had active programmes in TB diagnostics, there was little interest among the major diagnostic companies in meeting TB diagnostics needs of endemic countries. TBDI hosted a series of meetings involving researchers, diagnostics companies, regulatory agencies, and health officials, through which the medical need for TB diagnostics of specific type was clarified and communicated to industry. To prime the development pipeline, and attract new tool developers to the field, TBDI funded selected TB diagnostics R&D activities through competitive grants for the exploration of novel concepts or mechanisms with diagnostic potential.

In Ghana a male doctor examines stained sputum sample slides under a light microscope, a diagnostic technique that has changed little for almost a century. Photo: WHO/TDR/Crump

A TDR Scientific Working Group (SWG) meeting on Leprosy was held in December 2002, where the problems presented by the lack of fundamental knowledge about the epidemiology of leprosy, sources of infection, and precise mode of transmission, and the importance of contact patterns, were highlighted. The SWG emphasized that the need for specific diagnostic tools for use in the field was urgent, as did the subsequent Fifth WHO Technical Advisory Group (TAG) on Elimination of Leprosy in February 2003. TAG in fact recommended that all efforts should be made to ensure that such tests are available for use in the field programmes within the next five years.

Sequence data made available by the \textit{M. leprae} genome project\textsuperscript{10} and several other mycobacterial sequence databases\textsuperscript{11,12} should allow scientists to identify antigens specific for leprosy and so lead to the development of simple, rapid and specific diagnostic field tests for exposure and infection. A peptide-based test for exposure/infection with \textit{M. leprae} would go a long way to bettering our understanding about the transmission of leprosy and the impact of current treatment practices based on multidrug therapy on the global reservoir of \textit{M. leprae}. Once \textit{M. leprae}-specific peptides are identified and validated in preclinical studies, clinical trials conducted under GCP and using GMP grade antigens will be required to develop the test for initial regulatory approval and eventual use in leprosy research and control.

Currently, assessment of the success of control programmes includes measurement of the numbers of immature worms (microfilariae) in skin biopsies. A simpler procedure, involving the assessment of a patient’s reaction to a diethylcarbamate-impregnated skin patch, has advanced through preclinical development with a pharmaceutical partner and is now available for clinical evaluation. Dependence on a single drug – ivermectin – for control of the disease is undesirable, and the fear remains that resistance to ivermectin will appear in onchocercal helminths that infect humans, as it already has in veterinary parasites. A diagnostic nucleic acid amplification assay is being developed to detect resistant parasites.
Sexually transmitted infections

Most of the global burden of sexually-transmitted infections (STIs) occurs in the developing world where there is poor or no access to diagnostics. There is a particularly urgent need for improved diagnostics for STIs in HIV-endemic areas, as studies in sub-Saharan Africa have shown that STIs are important cofactors in the transmission of HIV infection. A meeting to establish a clear definition of priorities for rapid STI diagnostics was co-hosted with the Wellcome Trust. An inventory of relevant existing diagnostics technologies was drawn up, and a network of laboratory and field sites to perform evaluations of rapid STI diagnostics was identified. A laboratory-based evaluation of performance and ease of use of six rapid tests for syphilis was completed at eight sites worldwide. Limited co-funding of a nucleic acid amplification assay for gonorrhoea and chlamydia continued, pending identification of an industry partner. More strategic work included the development of a deterministic model to determine the performance characteristics required for a rapid test to be cost-effective at various settings and for different populations.