Review

# Collaborative actions in anti-trypanosomatid chemotherapy with partners from disease endemic areas

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**The protozoan diseases leishmaniasis, human African trypanosomiasis and Chagas disease are responsible for substantial global morbidity, mortality and economic adversity in tropical and subtropical regions. In most countries, existing strategies for control and treatment are either failing or under serious threat. Environmental changes, drug resistance and immunosuppression contribute to the emergence and spread of these diseases. In the absence of safe and efficient vaccines, chemotherapy, together with vector control, remains the most important measures to control trypanosomatid diseases. Here, we review current limitations of anti-trypanosomatid chemotherapy and describe new efforts to safeguard existing treatments and to identify novel drug leads through the three multinational and interdisciplinary European Union Framework Programmes for Research and Technological Development (FP7) funded consortia KALADRUG-R, TRYPOBASE, and LEISHDRUG.**

## Trypanosomatid diseases are a major global health problem

Trypanosomatids are parasitic kinetoplastid protozoa that cause major diseases in humans [1], including human African trypanosomiasis (HAT or sleeping sickness) caused by *Trypanosoma brucei* sspp., Chagas disease caused by *Trypanosoma cruzi*, and various forms of leishmaniasis caused by species of the *Leishmania* genus (Table 1). The leishmaniases are characterized by a spectrum of clinical manifestations, including visceral, cutaneous and mucocutaneous infections. In this review, we will focus on visceral leishmaniasis (VL), as this is the most severe form of the disease, being lethal if untreated. VL, also known as kala-azar, is caused by the protozoan parasites *Leishmania donovani* and *L. infantum* (syn. *L. chagasi)*. VL has been reported from 51 countries around the world with an annual incidence of 500 000 cases arising from recurrent epidemics each year in the rural areas of the Indian subcontinent (India, Nepal, Bangladesh), Brazil, Sudan and Ethiopia (Table 1). The real burden of VL is unknown, but it is estimated that only 20% of cases in India are reported [2]. Almost all VL patients die within months if untreated. *Trypanosoma brucei rhodesiense* and *T. brucei gambiense* cause human African trypanosomaisis (HAT), with active foci of disease in 36 sub-Saharan countries, within the area of distribution of the tsetse fly vector. HAT causes an estimated 50 000 cases annually with over 60 million people exposed to the etiological agent of the disease (Table 1) [3]. The disease occurs in two forms: (i) an acute form, caused by zoonotic *T. b. rhodesiense,* which occurs in eastern and southern Africa, and (ii) a chronic form caused by anthroponotic *T. b. gambiense,* which occurs in west and central Africa and provokes sleeping disorders, seizures, apathy and ultimately death. *Trypanosoma cruzi* causes Chagas disease, which currently affects 12 to 15 million people (Table 1) [4,5], some of which will develop chronic disease leading to cardiac, gastrointestinal or neurological damage.

## Current limitations of anti-trypanosomatid chemotherapy

Chemotherapy, together with vector control, remains one of the most important elements in the control of trypanosomatid disease as there are currently no vaccines to prevent either *Leishmania* or *Trypanosoma* infection [6]. The arsenal of available drugs is limited, and all current treatments suffer from significant drawbacks (Table 2) (i.e. parenteral route of administration, length of treatment, toxicity, and/or cost, which limits their utilization in disease endemic areas).

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**Table 1. Impact and distribution of the trypanosomal diseases**

| Diseases | Parasite | Vector | Impact | Geography |
|---|---|---|---|---|
| **Visceral Leishmaniasis (VL)** | *Leishmania donovani, L. infantum* | phlebotomine sand flies | 500 000 cases of VL; 51 000 deaths 2 357 000 DALYs[a] | Leishmaniasis affects populations in ~88 countries across Asia, East Africa, South America and the Mediterranean region. Over 90% of VL cases occur in India, Sudan, Bangladesh, Brazil and Nepal. |
| ***Human African Trypanosomiasis (HAT)*** | *Trypanosoma brucei gambiense, T.b. rhodesiense* | tsetse flies | 50 000–70 000 cases 48 000 deaths 1 525 000 DALYs | Endemic in 36 countries in Central Africa. The Democratic Republic of the Congo (DRC) accounts for 2/3 of cases. |
| ***Chagas Disease*** | *Trypanosoma cruzi* | 'kissing bugs' (e.g, *Triatoma infestans)* | 8 million cases 14 000 deaths 667 000 DALYs | Endemic in 21 countries across Latin America. |

[a]DALYs, Disability-adjusted life years

### *Leishmaniasis*

Pentavalent antimonials ($Sb^V$) remain the first-line treatment for VL in most parts of the world. They require long (20 to 30 days depending on geographic area) and toxic treatment (3 to 5% deaths due to treatment) [7]. In addition, *L. donovani* resistance to $Sb^V$ drugs is confirmed in Bihar State, India, and in Nepal, and it could develop in other regions of the Indian sub-continent and in Africa.

**Table 2. Drugs in use or in clinical trial for the treatment of trypanosomal diseases**

| Disease | Drug | Comments |
|---|---|---|
| **Visceral leishmaniasis** | sodium stibogluconate (Pentostam and SSG) | *Current status:* Generic sodium stibogluconate (SSG) from Albert David (India) has made treatment cheaper. Sanofi-aventis has also reduced the price of Glucantime. |
| | meglumine antimoniate (Glucantime) | *Limitations:* toxicity, parenteral administration, long courses, resistance in India. |
| ***First line drugs*** | amphotericin B (Fungizone) | *Limitations:* dose-limiting toxicity, intravenous administration, long course of treatment. |
| | liposomal amphotericin B (AmBisome) | *Current status:* This is the most effective and least toxic lipid formulation for VL. Single high dose effective in India. Reduced cost of $20 per 50 mg through WHO. |
| | miltefosine | *Current status:* Registered in India and available in private market. Cheaper price available via WHO. Only oral drug, used in elimination programme in southeast Asia. *Limitations:* teratogenicity, poor adherence, cost, potential for resistance. |
| | paromomycin | *Current status:* In phase IV trial in India and should be low cost. *Limitations:* long course intramuscular administration. |
| ***Clinical trials*** | sitamaquine | *Current status:* Completed Phase II trials in India and Kenya. Potential low cost oral drug. *Limitations:* As an 8-aminoquinoline, concerns about G6PDH deficient patients. |
| | Other lipid amphotericin B formulations | Several formulations (eg Abelect, Amphocil) have been on trial. Recent Phase II trial with lipid emulsion Amphomul showed it to be effective. Generic AmBisome available. |
| | Combination (co-administration) of AmBisome + miltefosine, AmBisome + paromomycin, miltefosine + paromomycin, SSG + paromomycin | *Current status:* Ongoing Phase III trials in India that aim to: (i) reduce length of treatment and (ii) prevent selection of resistant parasites. Ongoing Phase III trial in Sudan for SSG + paromomycin to reduce length of treatment. |
| **Human African trypanosomiasis** | | |
| ***Haemolymphatic stage*** | pentamidine | *Current status:* A 3-day short course of therapy is in clinical trial. *Limitations:* toxicities, parenteral administration |
| ***First line drugs*** | suramin | *Limitations:* toxicities, parenteral administration |
| ***CNS stage*** | melarsoprol | *Current status:* Concerns over increasing numbers of treatment failures. *Limitations:* toxicities and reactive encephalopathy in 10% patients. |
| ***First line drugs*** | eflornithine | *Current status:* supply guaranteed by WHO *Limitations:* High dose, frequent intravenous administration, side effects. |
| | nifurtimox + eflornithine co-administration | *Current status:* Following successful Phase III trials completed in 2008, this combination is now on WHO Essential drugs list. |
| **Clinical trials** | fexinidazole | *Current status:* In early clinical stage as potential drug candidate for CNS stage. |
| **Chagas disease** | | |
| ***Acute stage*** | nifurtimox | *Limitations:* long courses (oral), toxicities |
| ***First line drugs*** | benznidazole | *Current status:* Manufacture transferred to Brazil. Paediatric formulation under investigation. *Limitations:* long courses (oral), toxicities |
| ***Indeterminate stage*** | no treatment | |
| ***Chronic stage*** | | |
| ***Clinical trials*** | benznidazole | Phase III trial for chronic disease will be completed in 2010/11. |
| ***Clinical trials*** | posaconazole (Noxafil ®,) and other triazole derivatives | *Current status:* Posaconazole is approved as antifungal drug. Clinical trial for line extension to Chagas in preparation. |

Amphotericin B is currently used as the second line drug in India, but it requires careful administration and has known nephrotoxicity. AmBisome™, a liposomal formulation of amphotericin B, has remarkable activity even in a single dose in India, but its cost is very high ($20 per 50 mg vial from WHO), and it requires slow intravenous infusion. Two new therapeutic agents, miltefosine (MIL) and paromomycin (PMM), have recently been developed and have raised hopes for improved therapy. MIL was introduced in India in 2002 for VL and has completed Phase IV trials. It has been registered for cutaneous leishmaniasis (CL) in Colombia and is on trial against other forms of CL. A low cost parenteral formulation of PMM (aminosidine), completed Phase III clinical trials and was registered for VL in India in 2006; it is currently in Phase III clinical trials in East Africa. However, both agents have been shown to rapidly select for resistance in laboratory studies, indicating a risk for short clinical usage [8,9]. For MIL, widespread over-the-counter drug sales and uncontrolled drug use increase this risk. Extensive use of MIL is limited by its teratogenicity and the requirement for women of childbearing age to take contraceptives for up to two months after completion of treatment [10]. There is only one other drug in clinical trial for the treatment of VL, namely the 8-aminoquinoline sitamaquine, a potential oral drug, that completed Phase IIb trials in India in 2007.

### *Human African trypanosomiasis*

The chemotherapy of HAT relies on a few drugs which have adverse side effects and are unsatisfactory: (i) pentamidine for early-stage (haemo-lymphatic) *T. b. gambiense* sleeping sickness, (ii) suramin for early-stage *T. b. rhodesiense* sleeping sickness, and (iii) melarsoprol and eflornithine for late-stage disease of sleeping sickness, when the parasite is developing in the central nervous system (Table 2). Increasing numbers of patients do not respond to melarsoprol treatment, probably owing to resistance. Recently, a coadministration of nifurtimox, a drug registered for American trypanosomiasis, with eflornithine has been found to be as effective as the standard eflornithine therapy but in a shorter course of treatment and has been included as a WHO recommended treatment in 2009 [11]. The only promising drug candidate in current development is fexinidazole (www.dndi.org), a 5-nitroimidazole compound, which was originally designed as a broad-spectrum antiprotozoal. This compound has recently been reactivated as a potential drug candidate for stage 2 HAT, and has now entered clinical trials in collaboration between Sanofi-Aventis and the Drugs for Neglected Diseases initiative (DNDi) [12].

### *Chagas disease*

The treatment of Chagas disease relies largely on two drugs, nifurtimox and benznidazole, which are capable of curing at least 50% of acute stage infections (Table 2). These drugs require long (60 days) courses of treatment, and both have serious and frequent side-effects. Benznidazole has been shown effective in early stage chronic disease, and an extensive clinical trial is in progress. New and safer drugs for the treatment of Chagas disease, especially for the chronic phase, are urgently needed. Antifungal azoles that are highly effective in experimental models are under consideration [13]. These include the antifungal drug posaconazole (Schering-Plough) [14] as well as two other triazole derivatives, ravuconazole (Eisai) [15] and TAK187 (Takeda) [16] that have all shown encouraging *in vitro* and *in vivo* results.

In conclusion, despite some progress in treatment options for VL in India (but not East Africa), no ideal drugs are available against trypanosomatids that fulfil the major requirements of safety, high efficacy, ease of administration, low cost, and high patient compliance. Novel concepts towards improving and protecting current therapeutic approaches and the identification and optimization of drug leads are urgently needed to overcome the current situation and more efficiently combat and eventually eliminate these deadly diseases.

## Novel efforts to confront limitations in anti-trypanosomatid chemotherapy

The European Commission (EC) has supported research in parasitic diseases over the past 25 years through the successive Framework Programmes for Research and Technological Development (FP). Prior to the launch of the most recent Framework Programme (FP7, 2007-2013), the political support to increase European research in neglected infectious diseases mounted as a result of advocacy by the scientific community, international humanitarian organisations and stakeholders in disease-endemic countries. The European Parliament (EP) adopted a resolution in 2005 on 'Major and Neglected Diseases in Developing Countries,' and the EC has subsequently prioritised neglected infectious diseases in the health research programme of FP7. The increased attention to neglected infectious diseases has also been accompanied by additional funding, and during the first year of FP7, the EC has allocated more than 20 million Euros to support research in trypanosomatid diseases, including the development of new or improved chemotherapies. FP7 has an increased focus on applied research, with the aim of using new modern research methods and technologies to translate basic research findings into clinical practice. A first important step in fulfilling this agenda has been the creation of three consortia (Boxes 1–3) (Figure 1), which together cover three crucial aspects of anti-trypanosomatid chemotherapy: (i) screening for novel antiparasitic lead compounds, (ii) identification and characterization of novel parasite drug target molecules, and (iii) surveillance of drug efficacy and parasite susceptibility.

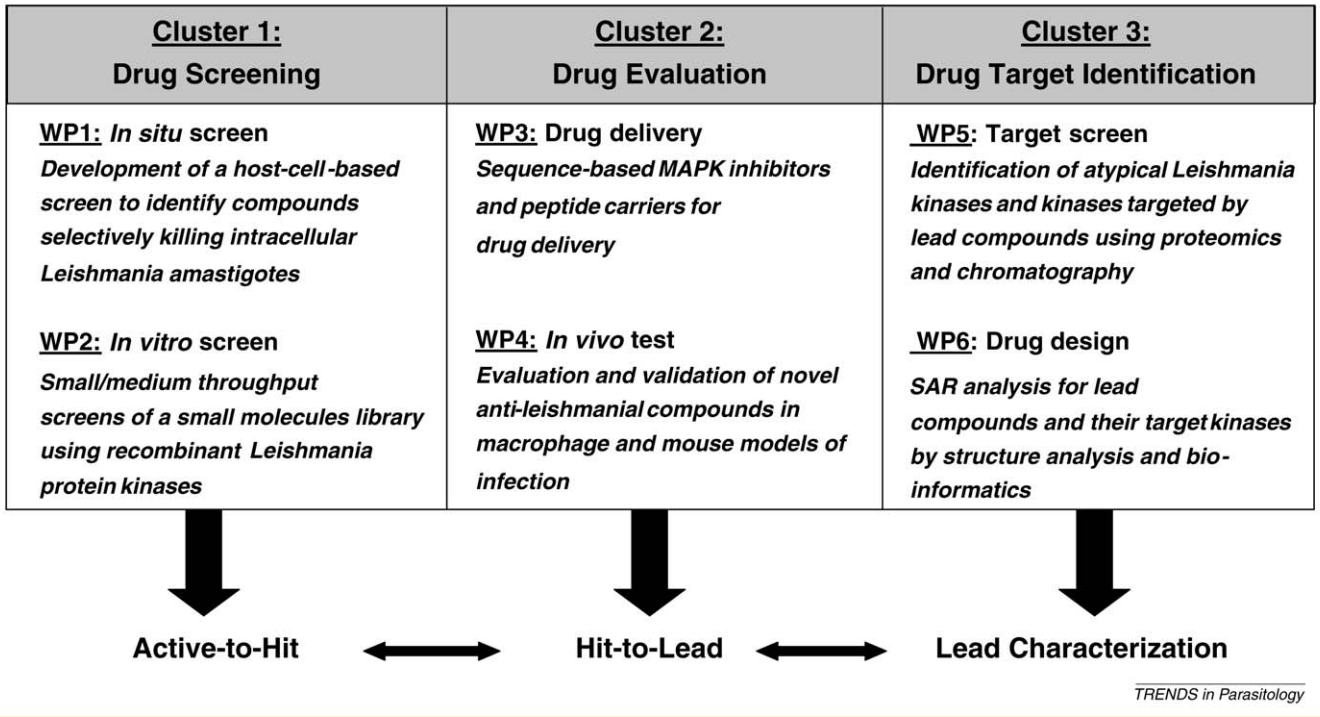
### *LEISHDRUG consortium: targeting the Leishmania kinome for drug development*

The LEISHDRUG consortium (www.leishdrug.org) (Box 1) proposes to explore *Leishmania*-specific signalling pathways with the aim to identify and validate *Leishmania* protein kinases as novel drug targets.

LEISHDRUG coordinates the action of 14 teams worldwide and builds upon complementary expertise in cutting edge bioimaging [17–20], *in silico* biology [21], phosphoproteomics [22,23], peptide chemistry [24–27], structure-based drug design [17,28–29], medium/high-throughput drug screening [30–32] and antimicrobial drug development [27,33–36], parasite virulence mechanisms and disease-associated signalling processes (Box 1) [37–39]. The

**Box 1. Overview of the LEISHDRUG consortium**

- **Title:** Targeting the *Leishmania* kinome for the development of novel antiparasitic strategies
- **EC contribution:** €2.9 million
- **Duration:** 36 months
- **Starting date:** 01/10/2008
- **Project web-site:** www.leishdrug.org
- **Coordinator:** Gerald Späth, Institut Pasteur, Paris, France, gerald.spaeth@pasteur.fr




The consortium is based on three clusters each with two work packages that follow the major stages of the drug development process. Consortium members utilize libraries of chemical compounds, small inhibitors, and peptides in cell-based phenotypic (WP1) as well as target-based (WP2) medium or high throughput screens. Novel target kinases will be identified by in-gel activity assays utilizing defined amastigote-specific phosphoproteins as substrates (WP5). Hit-to-lead validation will be achieved using mouse and macrophage infection models (WP4) together with peptide-based delivery strategies (WP3). Leads will be characterized by identification of compound targets through proteomic approaches (WP5), and SAR analysis using complementary bio-informatics and structural approaches (WP6).

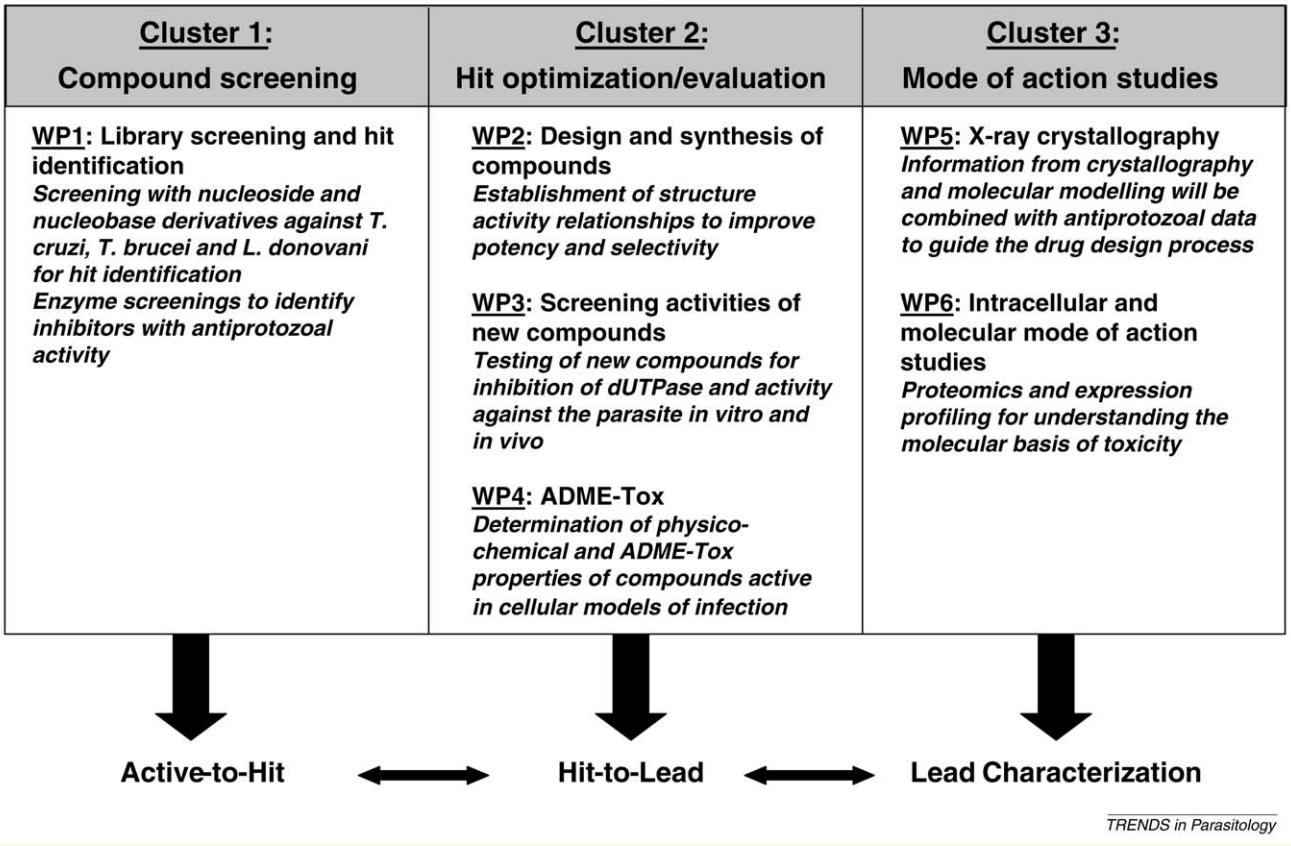
consortium uses two complementary innovative screening concepts not applied previously on parasitic systems. First, a visual screening will be applied to discover compounds capable of killing intracellular *Leishmania* amastigotes without damaging the macrophage host cell. This *in situ* screen utilizes infective fluorescent amastigotes in combination with primary macrophages and thus provides a physiological highly relevant setting. This cell-based assay will be developed into an efficient format through three stages, including: (i) optimization of the primary host cell-based screening assay using selected small compounds or peptides having kinase inhibitory activity, (ii) applying targeted strategies based on the screening of compounds from small molecule libraries including kinase inhibitor specific libraries, and (iii) hit-to-lead optimization using secondary screens based upon compound effects on isolated luciferase-expressing parasites in a plate reader-based assay [18]. This approach will enable the identification of inhibitors *in situ* under physiological conditions and simultaneously allows for the assessment of antiparasitic activity and host cell toxicity.

Second, a complementary target-based strategy will be employed using recombinant *Leishmania* protein kinases in combination with small molecule inhibitor libraries. LEISHDRUG uses an unconventional proteomics screen to identify atypical *Leishmania* kinases that escaped previous bioinformatics analysis. These kinases will be identified in an unbiased way by tracing phosphotransferase activities towards previously identified phosphoproteins in axenic amastigote extracts that were separated by 2D electrophoresis [22,23]. The discovery of new protein kinase families and their interaction partners, distinct or even absent from the host, will provide powerful targets for the LEISHDRUG drug screening pipeline.

Both screening approaches are supported by: (i) *in vitro* screens using optimized small molecular weight pharmacological compounds to identify inhibitors specific for validated target kinases identified by the consortium, (ii) *in silico* screens using bioinformatics tools developed by consortium partners to narrow down the list of potential compounds and identify the most promising targets among the *Leishmania* kinases, and (iii) high resolution, three-dimensional structural analysis of validated targets, which will enable structure-activity relationship (SAR) analysis and provide the consortium with potential drug leads. Finally, LEISHDRUG will exploit phosphoproteomic data

**Box 2. Overview of the TRYPOBASE consortium**

- **Title:** Nucleobase derivatives as drugs against trypanosomal diseases
- **EC contribution:** €2.5 million
- **Duration:** 36 months
- **Starting date:** 01/01/2009
- **Project web-site:** www.ipb.csic.es/trypobase.html
- **Coordinator:** Dolores González Pacanowska, Instituto de Parasitología y Biomedicina ''López-Neyra'', Granada, Spain, dgonzalez@ipb.csic.es




Activities are specifically designed to feed the compound design and synthesis work-package (WP2), which constitutes the central theme of this project and is aimed at identifying new nucleobase or nucleoside derivatives with antiprotozoal activity. Hits will be initially identified in WP1. Information emerging from WPs 3, 4 and 5 will be used in the optimization of compounds with drug-like properties and high activity *in vitro* and *in vivo*. Effort will be also devoted to understanding the mechanism of action of selected candidates in WP6.

obtained by the consortium to develop a peptide-based strategy to antiparasitic treatment. Cell penetrating peptides will be used to deliver phosphopeptides that are recognized by *Leishmania* kinases, thus competing with the endogenous substrate and interfering with signalling processes relevant for intracellular parasite survival, amastigote development and virulence.

*TRYPOBASE consortium: nucleobase derivatives as drugs against trypanosomal diseases*

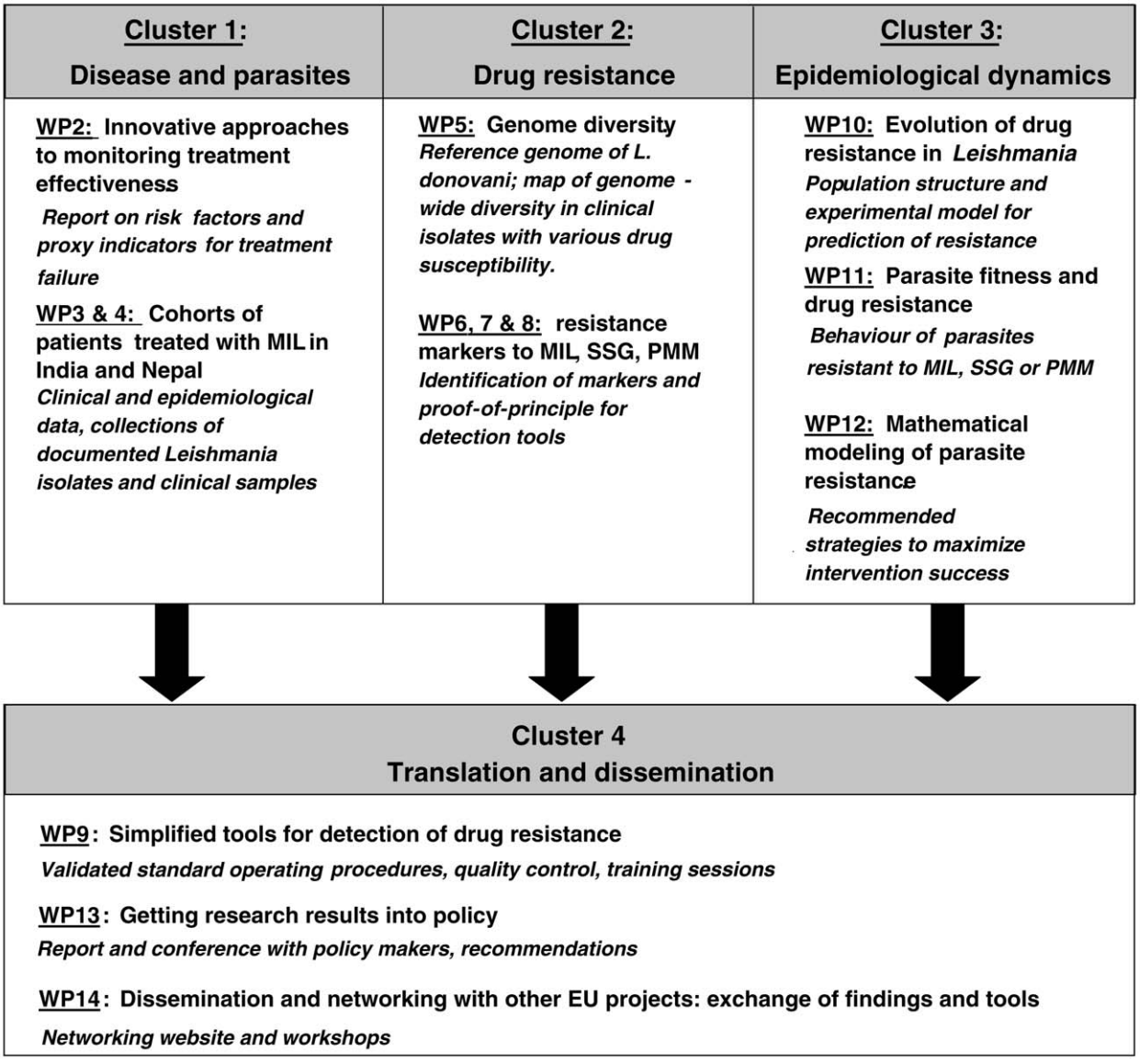
TRYPOBASE (http://www.ipb.csic.es/trypobase.html) (Box 2) exploits the unique features of the *Leishmania* and *Trypanosoma* nucleotide metabolism for drug development. Several parasite-specific enzymes involved in the salvage of purines and biosynthesis of pyrimidines are essential for viability [40–42] and emerge as ideal candidate targets for the design of new inhibitors with antitrypanosomal potential. Of special interest in pyrimidine metabolism is the existence of an enzyme specific only to trypanosomes: the dimeric deoxyuridine triphosphate nucleotidohydrolase (dUTPase) that catalyses the hydrolysis of dUTP to dUMP and pyrophosphatase (PPi) and has a dual role in pyrimidine nucleotide metabolism. Structure determinations of the dUDP-enzyme complex from *T. cruzi* have revealed a novel protein fold that displays no similarities to previously described dUTPases [43] and thus might be a prime target for drug development.

TRYPOBASE brings together a combination of expertise for drug discovery, and members of the consortium have been pioneers in identifying and characterizing these enzymes (Box 2) [43–48]. The TRYPOBASE activities are divided into six work packages and involves seven participating groups from different European and disease endemic countries with capabilities in the areas of protozoan biology [49,50], drug screening [34,51,52], medicinal chemistry [53–57], structural biology and drug metabolism and pharmacokinetics (DMPK) [48,58,59].

The consortium aims to identify new purine and pyrimidine derivatives for the treatment of the leishmaniases and trypanosomiases using a two pronged approach. First,

**Box 3. Overview of the KALADRUG-R consortium**

- **Title:** New tools for monitoring drug resistance and treatment response in visceral leishmaniasis in the Indian subcontinent.
- **EC contribution:** €3 million
- **Duration:** 48 months
- **Starting date:** 01/11/2008
- **Project web-site:** www.leishrisk.net/kaladrug
- **Coordinator:** Jean-Claude Dujardin, Institute of Tropical Medicine, Antwerp, Belgium jcdujardin@itg.be




The consortium is based on four clusters with each three to four work packages. Field-based activities will allow development and evaluation of pharmaco-vigilance tools (WP2) together with material for laboratory work (WP3 and 4). This material will be used for searching resistance markers through a combination of whole genomic (WP5) and targeted (WP6 to 8) approaches. Epidemiological dynamics of resistance will be assessed by experimental (WP10 and 11) and mathematical (WP12) approaches. Tools will be assembled and simplified (WP9) before dissemination to policy makers (WP13) and the scientific community (WP14). WP1 concerns the management of the consortium and is not indicated.

a target-based approach will be specifically centred on the development of inhibitors of the enzyme dUTPase. High throughput evaluation of several diverse libraries and molecular modelling will be used to identify drug-like molecule inhibitors of dUTPase from *T. cruzi, T. brucei* and *Leishmania* with antiprotozoan activity. Hits from these screens will be validated and medicinal chemistry will be used in iterative optimization efforts.

In a second, phenotypic approach, medium throughput screening of a library of nucleobase derivatives provided by partners from the pharmaceutical industry will allow for the identification of compounds that inhibit the growth of

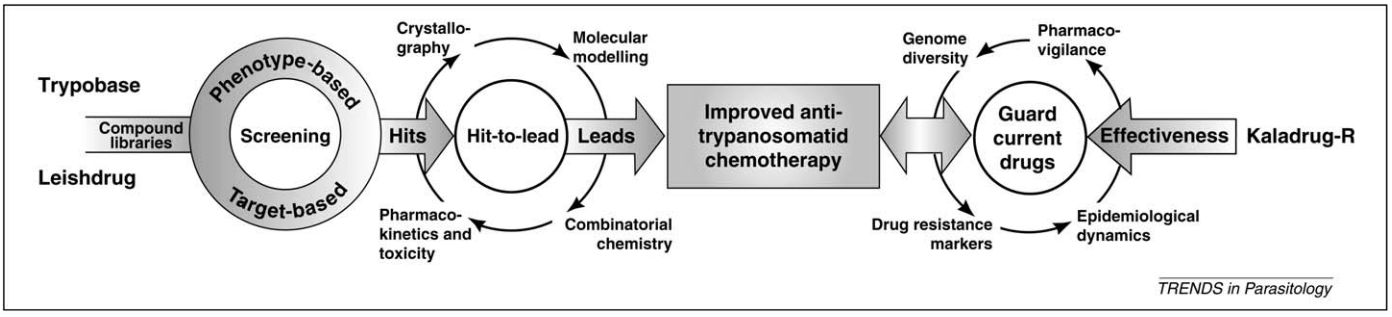



**Figure 1. EC-funded strategy to improve anti-trypanosomatid chemotherapy.** The strategy relies on two major axes that focus on the identification of novel lead compounds and drug targets, and on the securing of current drugs through the three multidisciplinary consortia LEISHDRUG, TRYPOBASE and KALADRUG-R. LEISHDRUG and TRYPOBASE utilize dedicated compound libraries together with phenotype- and target-based screening approaches with medium and high-throughput capacities to target protein kinase signalling and trypanosomatid nucleotide metabolism, respectively. Determination of SAR within a compound series by target structure analysis and molecular modelling will allow prioritizing selected lead compounds for combinatorial chemistry and pharmacokinetic studies, and aid the iterative process of compound design, synthesis, and testing. KALADRUG-R evaluates the effectiveness of available anti-trypanosomatid drugs, with the aim to safeguard and prolong their clinical application and to render future drugs less vulnerable against the development of drug resistant parasites. This is achieved through a number of actions, including monitoring treatment efficacy, determining parasite genome diversity in relation to drug susceptibility, and developing mathematical models on the epidemiological dynamics of drug resistance.

parasites *in vitro*. All three major kinetoplastid parasites will be included in this screen. Early optimization of hits will be performed using data emerging from enzyme and cell assays, crystallography and molecular modelling studies. Optimization at a later stage will be based additionally on *in vivo* data and ADME-tox (Absorption, Distribution, Metabolism, Excretion, toxicity) information. Biopharmaceutical properties of molecules will be investigated at an early period to assist the drug design and discovery process.

Finally, for active entities emerging from the screening activities, a number of approaches will be used to provide insight into the mode of action of selected compounds. Studies that involve comparative proteomics and array based gene expression profiling will aid the identification of the repertoire of parasite proteins specifically associated with inhibitory action. For dUTPase inhibitors, changes in the intracellular nucleotide pool will be monitored. The ultimate aim is to have a compound series with drug-like properties that shows efficacy in a relevant animal model of infection of at least one trypanosomal disease.

### *KALADRUG-R consortium: guarding current anti-leishmanial drugs*

The KALADRUG-R consortium (http://www.leishrisk.net/kaladrug) (Box 3) aims to develop, validate and disseminate new tools for evaluation of drug resistance in *L. donovani* as well as innovative methodologies for monitoring kala-azar treatment effectiveness in routine conditions. KALADRUG-R comprises ten partners, six from Europe and four from the Indian sub-continent. This multidisciplinary consortium combines clinical research and epidemiology [60,61] with basic research in cellular [62–64] and molecular biology [65–67], biochemistry [68], population genetics [69] and mathematical modelling [70] (Box 3). In addition, in order to guarantee the future implementation of our findings and tools, the consortium is anchored in the kala-azar elimination program through the participation of regional clinicians and scientists. KALADRUG-R will contribute to safeguard the few existing drugs by providing knowledge and tools relevant for monitoring their effectiveness and to establish the base line data for their long term and more rational use. KALADRUG-R will focus on three drugs: (i) the pentavalent antimonial (SSG), the previous first-line drug, (ii) MIL, the recently introduced drug, and (iii) PMM, a potential future drug. This approach will allow us to learn from past failures, to impact on current treatments and to render future treatments more efficient.

Our clinical and epidemiological work in India and Nepal will be undertaken in peripheral health services and in reference hospitals. The consortium aims to develop an innovative approach for monitoring the effectiveness of kala-azar drug treatments in routine conditions (Box 3). This should contribute to an early identification of the persons at higher risk of treatment failure and a more rational therapeutic attitude. Clinical work will also provide a unique collection of well-documented isolates presenting various degrees of susceptibility to SSG or MIL.

KALADRUG-R comprises three major research activities. First, the molecular and biological mechanisms leading to resistance will be identified in clinical resistant isolates (for SSG and MIL) or experimentally induced resistant parasites (for MIL and PMM). We will combine targeted analyses of specific genes or metabolic pathways together with a global, genome-wide approach; the latter will be strengthened by a metabolomic diversity study conducted by a spin-off project termed GeMInI (www.leishrisk.net/gemini).

The second activity aims to build models to understand the dynamics of the past spread of parasite SSG resistance in order to anticipate and avoid resistance to MIL or future drugs. This will involve a central work package of mathematical modelling that integrates the results of parasite population genetic studies and studies on the impact of drug resistance on the parasite fitness. Indeed, the multiple molecular adaptations of SSG resistant parasites present in India and Nepal might affect other drugs and challenge MIL efficacy or expedite the emergence of MIL resistance. Furthermore, the persistence of SSG resistant parasites, even after years of lower drug pressure, could limit the clinical utility of SSG if reintroduced as part of a combination or rotation therapeutic treatment program. Both these issues would have a major influence on shaping drug policies in other endemic regions that might abandon clinical use of SSG.

Finally, KALADRUG-R aims to gather, simplify, standardize and disseminate all the biological and molecular tools developed by the consortium for the detection of resistance to MIL, SSG and PMM. The aim is to translate research results to advise policy at the regional level and disseminate the generated knowledge and validated tools in other regions in the world endemic for leishmaniasis. Key stakeholders should be reached, i.e. the health authorities of the endemic countries (Ministry of Health, managers of the VL elimination programs, district public health services, or others) as well as the affected communities in the study areas. Scientific dissemination and collaboration with other research projects (such as LEISHDRUG and TRYPOBASE) is a major objective, and could enable, for example, the provision of drug-resistant isolates for testing the efficacy of new drugs.

## Future perspectives for a global trypanosomatid network

The establishment of the three consortia, LEISHDRUG, KALADRUG-R and TRYPOBASE, is the first important step in a new, concerted attempt to establish a global initiative to safeguard existing drugs and discover, develop and deliver new and improved drugs for neglected protozoal diseases. The three consortia presented in this review provide three distinct ways to tackle and overcome the challenges associated with discovery, development, delivery and guarding of new drug candidates for three protozoal diseases (Figure 1). Taken together, 27 different research teams from 14 countries on four continents are taking part in this new endeavour to confront protozoal diseases. The three projects have an initial duration of three or four years. Although this period is too short a time to bring new drug candidates or monitoring tools from the bench to the clinic, it is sufficient to establish the new initiative and equip it with significant scientific momentum for its long term sustainability. To actually deliver new drugs, a longer period, additional partners with expertise in drug development and significantly higher funding levels must be mobilized. At the same time, strengthening of clinical capacity in disease-endemic countries must be planned as new drug candidates approach the clinical stages. Close interaction and exchange of expertise between the three consortia will therefore be necessary with a view to consolidate the most promising elements from the three projects into a single collaborative initiative. The long term success of the initiative will require sufficient economic backing, but also the integration of scientific potential to undertake state-of-the-art discovery activities, and subsequently, with new partners, drive the new drug candidates through the developmental stages and implement the adequate platforms for surveillance of drug effectiveness. This would also include synergy with relevant existing networks involved in disease control. Such networks currently exist in leishmaniasis research (see www.leishrisk.net) and demonstrated their relevance in domains as variable as information, quality assurance, provision of recommendations or advocacy.

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