Status of Vaccine Research and Development of Vaccines for Leishmaniasis
Prepared for WHO PD-VAC

I. About the Disease and Pathogen

Basic information on pathogen, including transmission, estimated global disease burden for those at risk, for morbidity and for mortality, including uncertainties/data gaps, geographical distribution, economic burden if available, age groups affected and target groups for vaccination. Existing preventive, diagnostic and treatment measures and their limitations.

Leishmaniasis is a vector borne disease that currently threatens approximately 350 million people living in endemic areas, with an estimated 1.3 million new cases annually, and a total of 10-12 million cases occurring globally [1,2,3]. The most recent estimates from the Global Burden of Disease Study 2010 indicate that leishmaniasis results in more than 50,000 annual deaths and 3.3 million disability adjusted life years (DALYs) ranking leishmaniasis, along with schistosomiasis and hookworm infection, the highest disease burden among the neglected tropical diseases [4]. There are more than 20 species of the protozoan parasite of the genus Leishmania known to be pathogenic in humans, with a broad range of clinical manifestations. Various forms of Leishmaniasis are classified according to the clinical syndromes caused by different species, which range from disfiguring skin ulcers resulting from Cutaneous Leishmaniasis (CL), caused by *Leishmania mexicana* complex in Mesoamerica and Leishmania major and Leishmania tropica in the Middle East and Central Asia; more severe and chronic mucocutaneous infections of Mucosal/Mucocutaneous Leishmaniasis (ML), caused by *Leishmania braziliensis*; to the severe, life-threatening Visceral Leishmaniasis (VL), caused by the *Leishmania donovani* complex and *Leishmania infantum* and *Leishmania chagasi*, and its sequelae Post-Kala-azar Dermal Leishmaniasis (PKDL).

VL is the second most common parasitic cause of death, after malaria and infantile cryptosporidiosis. Infected individuals present with fever, wasting, anemia, hepatosplenomegaly, and a depressed immune response, leading to pancytopenia and superimposed infections. The disease has a high case-fatality rate in the absence of treatment. Its sequelae, Post-Kala-azar Dermal Leishmaniasis (PKDL), is a chronic, disfiguring cutaneous condition associated with considerable morbidity and social stigma, in addition to likely contributing to Leishmania transmission. Leishmaniasis affects the poorest people living in the Mediterranean Basin, South-East Asia, East Africa, Afro-Eurasia, and The Americas [3], and periodic epidemics are known to occur in these areas (Ethiopia [2005–2006], Kenya [2008], and Sudan [2009–2011]). CL, while not typically a life-threatening infection is a cause of stigma and disfigurement, especially for girls and women. The disease has reached epidemic proportions because of the conflicts and public health breakdowns in Syria, Iraq, Afghanistan and elsewhere in the Middle East and Central Asia, and it is widespread in the Americas [5].

Chemotherapy is usually effective and is the mainstay of treatment for all three major forms – VL, CL, MCL. However, factors such as high costs, toxicity, and long-term and complicated regimens compromise most chemotherapeutic options. Conventional control measures such as insecticide spraying or bednets are difficult to sustain as there are an extensive number of vectors, and potential reservoirs for parasite transmission [6, 7]. Controlling one of the main reservoirs of this infection, dogs, has been identified as a crucial epidemiological area of intervention but presents logistical concerns [3]. Although treatments have improved in recent years, and tests of new therapies aimed at alleviating some of the therapeutic concerns with safer and more effective options are on-going [3], it is likely that elimination will only be achieved through vaccination [6]. It has been estimated that if the population within seven countries of Latin America: Bolivia, Brazil, Colombia, Ecuador, Mexico, Peru and Venezuela were vaccinated using a vaccine formulation that would provide at least 10 years of protection, an estimated 41,000-144,000 cases of CL could be averted, and that each of these would be at a cost that is lower than the cost of current recommended treatments [8]. Even a vaccine that provides as little as 50% efficacy and only five years of protection would still remain cost effective as compared to current treatments [8]. A similar study for VL in Bihar State has also confirmed the cost-effectiveness of a VL vaccine [9].
II. Overview of Current Efforts

A. Biological feasibility for vaccine development

Evidence that vaccine development is biologically feasible including from development of naturally acquired immunity, from vaccine development for related pathogens, from animal models or in vitro data

Most individuals who have been infected, and who recover from the infection, become resistant to later clinical infection [7,10], supporting the possibility of vaccine development. Further proof of biological feasibility of vaccine development (at least for CL) is provided by an ancient practice that predates modern vaccinology, “leishmanization” [6,7]. Widely practiced in the Middle East and Central Asia, individuals were “vaccinated” using thorns or sharp instruments to introduce live parasites and artificially simulate a cutaneous infection, in an unexposed site on the body to prevent the risk of facial lesions through natural infection. Leishmanization vaccines have been developed and were in use in various countries (former Soviet Union, Central Asia, Israel, Iran) and remain the only group of leishmaniasis vaccines to show efficacy. However, issues pertaining to control of dose and strain and the occurrence of persistent lesions limit the actual deployment of such an approach.

Resistance to infection has been associated with a T_H1-mediated immune response. However, increasing evidence suggests that true protection from disease will require the involvement of both a T_H1-mediated and a T_H2-mediated immune response from vaccination [10,11]. An ideal vaccine will allow for long-lasting immunity to Leishmania infection, thereby limiting the need for the use of chemotherapy. However, so far no vaccine has gone on to licensure and most have not progressed beyond early clinical trials [7,10].

B. General approaches to vaccine development for this disease for low and middle income country markets

What are the scientific approaches and indications and target/age/geographic groups being pursued? What public health needs will these vaccines meet if successfully developed? Where there are several different possible indications/target groups, how much consensus is there as to prioritization between these for vaccine development in LMIC.

As Leishmanization has largely been abandoned due to safety concerns, vaccine development approaches have evolved through live, whole parasite vaccines and killed or inactivated whole parasite vaccines. Attempts at the introduction of suicidal cassettes into the Leishmania genome have also been explored [6,7,10]. These early vaccines focused on cutaneous and visceral Leishmaniasis, and clinical results from first generation Leishmania vaccines have been inconsistent. There are concerns that many of these preparations cannot be appropriately standardized to be a viable option for licensure [6,7]. Currently, the emphasis of the so-called second generation vaccines is on the subunit/recombinant protein approach using a range of adjuvants to augment the immunogenicity of the selected antigens [6,7]. As mentioned earlier, the evidence that parasite control and protective immunity is dependent on T_H1 immune responses has been helpful for research in this area. Leishmania antigens that induce protective immune responses have been identified, that induce protection in mice, hamsters, dogs, and non-human primates, and various combination of these antigens are being tested as recombinant proteins. Although vaccines for targeting T cell-dependent immunity for protection have been more difficult to develop compared to those targeting antibody-mediated immunity, progress in the development of safe and effective adjuvants present novel options for adjuvanted formulations of these approaches. Alternatively, antigens delivered as DNA formulations are also being tested. Another vaccine approach targets the parasite vector, the sand fly saliva, which can exacerbate Leishmania infection.

III. Technical and Regulatory Assessment

Highlight perceived positive/negative aspects in clinical/regulatory pathways e.g. well established product development and regulatory pathway to licensure, accepted immune correlates and/or functional assays, accepted surrogate efficacy endpoints, existence of well accepted animal or challenge models, agreed trial designs and endpoints. Possibilities to develop case for correlates/surrogates should be included.
The work currently being done in the development of second generation vaccines against Leishmaniasis involves preparations that are more refined than their first generation counterparts and rely on the use of recombinant DNA technologies for the production of proteins, which are then adjuvanted in the final vaccine formulation [6]. It is believed that these new strategies will be easier to scale up for large scale dissemination in a cost effective reproducible manner and will be able to meet the current regulatory requirements for vaccines [6,7].

A murine CL model has been developed and is used extensively for assessing vaccines and adjuvants for Leishmaniasis. This model provides a wealth of information related to cellular immunity and has facilitated in vivo studies of the mechanism of action of numerous adjuvants [12].

IV. Status of vaccine R&D activities
Summarize status of vaccine design, pre-clinical and clinical trial activity, including platforms, vectors, and adjuvants. Note academic, government, biotech and industry entities engaged. Summarize antigenic targets (if subunit approaches). Section on major advances in last 3-5 years, including key opportunities highlighted by recent science developments in the area.

Many different recombinant proteins have been investigated for their use as preventive and therapeutic vaccine candidates. The first candidate of this type to make it into phase I and II clinical trials was LEISH-F1 from The Infectious Disease Research Institute (IDRI, Seattle, WA) [7,13]. LEISH-F1 is comprised of three proteins that are conserved across various Leishmania species; which includes L. donovani, and L. chagasi, causative agents of New World visceral Leishmaniasis; and L. braziliensis, a causative agent of both mucosal and cutaneous Leishmaniasis in The New World [7,13]. Multiple phase I trials have been conducted with the LEISH-F1 vaccine in the United States, Colombia, Brazil, Peru and India, targeting VL and CL, and have shown the vaccine to be immunogenic, safe, and well tolerated in populations both with and without a sero-positive subpopulation [7,13]. Additionally, the LEISH-F1 vaccine has also demonstrated some therapeutic significance in patients with ML when used with chemotherapy [7,14]. With the great preliminary successes of the LEISH-F1 vaccine, IDRI redesigned this vaccine candidate and has taken their new construct (LEISH-F2) through both a phase I and a phase II trial. The new candidate includes a construct without the histidine tag on the N-terminus, as well as the replacement of Lys274 with Gln, in an effort to overcome possible regulatory concerns and to aid in the manufacturing process [7,15].

Positive findings related to immunogenicity and safety from the Phase I trial lead to a phase II trial where the efficacy, safety, and immunogenicity of the vaccine was studied after three administrations of LEISH-F2 (10µg) + MPL-SE (25µg) to treat adults and adolescents with cutaneous Leishmaniasis, when compared to treatment with standard chemotherapy [7,15]. IDRI is another new LEISH-F3 vaccine, for use against VL, in a phase I trial of healthy adult volunteers [7,16]. The LEISH-F3 vaccine is a fusion polypeptide made by linking in tandem two Leishmania proteins: residues 1-314 of the Leishmania infantum/donovani nonspecific nucleoside hydrolase (NH) protein and residues 2-353 of Leishmania infantum sterol 24-c-methyltransferase (SMT) protein [7,16]. The LEISH-F3 vaccine is given with GLA-SE, a novel TLR-4-based adjuvant, and is compared to administration of unadjuvanted LEISH-F3 [5,13]. This first phase I trial will enroll 36 adult volunteers in Washington State. A second phase I trial will then take place in India, where IDRI has plans to transfer its vaccine manufacturing process to Gennova Biopharmaceuticals [8,7,17].

As mentioned earlier, in addition to these advances by IDRI in recent years, other groups including the Sabin Vaccine Institute Product Development Partnership (Sabin PDP) are investigating third generation vaccines, comprised of recombinant proteins targeting CL caused by L. mexicana which have shown great promise in animal models but have yet to be brought to the clinic. It is also believed that immunomodulatory salivary proteins of the sand fly vectors (Phlebotomus and Lutzomyia spp) known to transmit Leishmania parasites may make good vaccine candidates, as many transmission blocking vaccines based on this idea are in early pre-clinical testing. Efforts are in progress by the Sabin PDP to evaluate combination vaccines comprised of Leishmania-derived recombinant protein(s) including LdNH36 together with one or more sandfly salivary gland antigens.
Table 1: Development Status of Current Vaccine Candidates (POC = Proof of concept trial)

<table>
<thead>
<tr>
<th>Candidate Name/Identifier</th>
<th>Preclinical</th>
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References