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Meeting Report

Visceral Leishmaniasis Control and Elimination: Is There a Role for Vaccines in Achieving Regional and Global Goals?

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Abstract. In September 2015, the National Institute of Allergy and Infectious Diseases organized a workshop to address the roles of vaccines in achieving regional and global goals for visceral leishmaniasis (VL) control and elimination, a critical step in determining desired product characteristics as well as research and development needs and opportunities. Although current regional programs and strategies are making progress to control and perhaps eliminate the disease in some endemic areas, such as India, Bangladesh, and Nepal, workshop participants concluded that vaccines would still be necessary to sustain elimination efforts and ultimately block and reduce transmission. In addition, vaccines would be valuable and even critical tools for other areas of the world, such as east Africa, where treatment options are more limited and control programs for VL are less effective. Because different disease foci present different epidemiological features, product characteristics should be carefully designed to reflect vaccines that either target common antigens for all forms of VL or are tailored to fit regional needs.

INTRODUCTION

Leishmaniasis is a neglected tropical disease (NTD) responsible for up to 3.32 million disability-adjusted life years annually.[1] There are 21 parasite species that can cause human infection, and the clinical presentation of leishmaniasis is dependent on the parasite species and the host's immune responses. For example, *Leishmania* (*Leishmania*) *donovani* and *Leishmania infantum* (known *as Leishmania chagasi* in South America) will lead to pathology of internal organs, that is, visceral leishmaniasis (VL, also known as kala-azar), whereas *Leishmania major*, *Leishmania mexicana*, *Leishmania amazonesisi*, and *Leishmania braziliensis* mainly cause cutaneous lesion, that is, cutaneous leishmaniasis (CL). Worldwide, there are 314 million people at risk and 1.3 million new cases each year with 200–400,000 new cases of VL.[2] VL can also evolve into a skin disease known as post-kala-azar dermal leishmaniasis (PKDL). VL mainly affects regions of World Health Organization (WHO)–defined southeast Asia (SEA), Africa (primarily east Africa [EA]), eastern Mediterranean, South America, and Europe. The parasite species associated with VL are mainly *L. donovani* and *L. infantum* in the Eastern Hemisphere and *L. infantum* in the Western Hemisphere. Although the prevalence of VL is relatively low compared with CL, VL could lead to fatality as high as 95% if left untreated, whereas CL would only cause skin sores and ultimately scars and serious disability. The high fatality rate and the increasing incidence of coinfection with human immunodeficiency virus (HIV) or other infectious agents of VL are of concern. Complete disease elimination using the existing tools and development of new tools to combat VL are both difficult given the uniquely clustered disease distribution, complex life cycle of the parasites, diversity of vectors, and possible remaining sources of parasites for transmission within the community (e.g., asymptomatic infections or PKDL cases). In addition, compared with traditional vaccines, developing VL vaccines for ultimate use in lower- and middle-income countries is particularly challenging because of a weak research and development (R&D) pipeline, limited financial support, and sparse commercial interest.

The National Institute of Allergy and Infectious Diseases is the lead federal agency for the support of basic and applied research in the areas of immunology and infectious diseases. This workshop was organized to seek input from members of the VL research community and obtain their perspective on the potential roles of vaccines and strategic planning for vaccine R&D, to identify opportunities for synergy among available and newly emerging tools for VL control and elimination, and to forge collaboration and encourage partnership among interested stakeholders. Meeting participants 1) reviewed the WHO NTD roadmap for leishmaniasis control and current strategies; 2) discussed the current toolbox of interventions, knowledge gaps, and new interventions needed to achieve regional and global control goals; and 3) defined the roles of VL vaccine(s) and proposed basic features of VL vaccines(s) to help ensure optimal development strategies. Below is a summary of the collective views from the workshop participants.

CHALLENGES IN VL CONTROL AND ELIMINATION

The major global foci of VL are the SEA, EA, and eastern Mediterranean regions, and Latin America. In the SEA region (SEAR), the VL incidence rate has significantly decreased and elimination, defined as one case in 10,000 at subdistrict level of the SEAR, has become potentially feasible. This is mainly due to the tight geographic localization of the VL cases, the fact that human beings are the only known and confirmed reservoir, the availability of effective drugs and rapid diagnostic tests, and the integrated vector management control research program. There has also been an improvement in health-care infrastructure and improved cooperation at district level and training programs. In Nepal, the elimination target has been achieved and sustained for 2 years under the WHO/SEAR program. Other countries in

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the regions are near to achieving their elimination targets, for example, over 70% of endemic subdistrict areas in India and 90% in Bangladesh have hit their targets.[3]

In 2014, five countries from SEAR signed a memorandum of understanding to achieve the elimination target by 2017, and a regional task force was formed to provide advice on practical measures and to facilitate elimination activities.[4] As countries have approached the elimination target, many issues and knowledge gaps have also been identified. For example, recent studies indicated that PKDL remains a real threat in India and that *L. donovani*–infected, asymptomatic individuals with a high antibody titer are at greater risk for disease progression.[5] Whether a parasitological cure can be achieved in VL patients and the extent to which PKDL patients contribute to disease transmission, are still unknown. Similarly, much remains to be learned about asymptomatic infected individuals, such as: Does a large endemic population of asymptomatic infected individuals exist? Do they serve as a reservoir for transmission? What determines disease development in asymptomatic infected cases? As such, it will be important to identify biomarkers, such as markers to measure parasite load indicative of disease progression and development or the ability to assess transmission in asymptomatic cases. Also of particular concern are the possibility of an animal reservoir (not confirmed yet) which could help sustain *L. donovani* in the SEAR, new species of parasites, for example, *L. siamensis* diagnosed in VL cases in Thailand,[6] and new cases emerging in nonendemic areas. Workshop participants concluded that while the current control and elimination efforts should be strengthened, development of a vaccine to reduce or block transmission and/or to treat PKDL patients, would ultimately be very important and should be a priority for the SEAR.

VL and its related diseases in EA, on the other hand, are particularly complex and do not share the same promising disease control situation as in SEAR. In contrast to the situation in Nepal, Bangladesh, and Bhutan, some EA countries (Ethiopia, South Sudan, and Sudan) are reporting increasing numbers of cases annually, and high levels of HIV–VL coinfection have been observed (WHO Global Health Observatory). South Sudan has the most reported cases in Africa due to continuous conflict and migration causing sustained transmission, followed by Sudan, Ethiopia, and Somalia.[2] Cases in Somalia have increased 20% in the last 2–3 years. For the eastern Mediterranean Region, the number of new cases does not seem to be declining; however, underreporting of cases may be significant due to the difficulty in disease surveillance in the areas.

In EA, current disease control options are not as effective as in SEAR. Widespread asymptomatic infection, active VL, and new foci are of increasing concern, and the transmission is anthroponotic and occurs almost exclusively outdoors. Two local ecotypes of vectors are susceptible to *L. donovani* infection: *Phlebotomus* (*P.*) *orientalis and P. martini/celiae*. However, newly changed transmission patterns from man to man have also been recently reported and zoonotic transmission also remains to be confirmed. Factors most likely contributing to the spread of disease include weak or absent national control programs and health systems, suboptimal diagnostic assays, concomitant infections, malnutrition, and other population or social factors (such as conflicts, population growth or displacement, seasonal migration/resettlement, famine, and food insecurity). Increased agricultural development/deforestation could also create favorable breeding sites for sandflies. Although active surveillance and prompt treatment are options for disease control, given the caveats mentioned above, workshop participants concluded that vaccines capable of inducing long-lasting protective immunity against infection likely represent the best tools for VL control in EA.

In Latin America, Brazil has more than 96% of the VL cases, with sporadic disease in Paraguay, Argentina, Colombia, Mexico, Guatemala, Honduras, El Salvador, and Nicaragua. VL occurs mainly in children, with male children over 10 years of age bearing the greatest risk. The disease is characterized by higher case fatality rate than in other regions of the world as well as cross-border transmission, and an increase in comorbidity in coinfected individuals with HIV and neoplastic disorders. The role of dogs as a significant reservoir of VL has been a concern in Brazil, since several studies reported a correlation between VL incidence in dogs and humans, and a field trial of insecticide-treated dog collars as a population-based intervention suggested an impact of the intervention on VL incidence in humans (Selma Jeronimo, unpublished data). Asymptomatic infections are numerous in areas where there are reports of both human and canine VL, although the role of the asymptomatic infection in maintaining endemicity is unknown. With regard to diagnostics, a cross-sectional study suggested that anti-*Leishmania* serological assays were good predictors of leishmanial infection (Selma Jeronimo, unpublished data), but specificity could be further improved by adding a molecular assay (e.g., quantitative polymerase chain reaction).[7]

Overall, progress to control and perhaps eliminate VL disease is promising in SEAR, but sustainment of disease control and elimination efforts and ultimately transmission elimination remain as concerns. The WHO definition of VL disease elimination is to reduce the number of cases to ≤ 1 per 10,000 persons and to sustain that level for 3 years.[4] Currently, disease surveillance is being used to assess the impact of elimination efforts. However, such surveillance may not reflect a reduction in transmission. Without sustainable interruption of transmission, the disease will never be eliminated. Indeed, there seemed to be endemic cycles in South Sudan with the number of cases peaking every 12–15 years during the period of 1977–2014, and another peak is anticipated in 2022. In addition, PKDL and asymptomatic infection could possibly serve as potential reservoirs for transmission, and would continue to contribute to potential rebound or future disease outbreaks. As such, although surveillance of transmission is challenging and would require additional monitoring infrastructure, workshop participants still suggested that the term "elimination" as applied to VL be redefined to take transmission into account.

Many challenges to the current control and elimination programs were identified, including inconsistent performance of rapid diagnostic tests in Africa, regional differences in access to treatment and in treatment outcome, lack of drug resistance monitoring and test of cure, and the impact of ecological and climatic changes, particularly in regions where new cases are increasing in previously nonendemic areas. Areas described as needing improvement included program management, such as health-related human resource training, improved monitoring and evaluation systems, and

appropriate project prioritization as well as surveillance and control strategies. In the view of the workshop participants, to consolidate progress in eliminating VL and protecting against future outbreaks and cases of PKDL, existing interventions need to be successfully implemented, programs need to be tailored to meet the specific regional conditions, and new tools for vector and reservoir control as well as vaccines for VL should be priorities.

EXISTING MODALITIES AND GAPS IN VL CONTROL AND ELIMINATION

Currently, VL treatment options are limited and vary geographically. In SEA, miltefosine demonstrated initial efficacy of 95%; up to 20% of patients, however, relapsed within 12 months.[8] Several field studies in India were conducted to determine the safety and effectiveness of new treatments, and interim analyses indicated good efficacy of single-dose liposomal amphotericin (AmBisome®), which was subsequently rolled out as primary treatment in many areas of India and Bangladesh.[9] Combination therapies with miltefosine or paromomycin (PM)[10] are being considered in areas where access to AmBisome is limited.

In EA, studies using single-dose AmBisome have been disappointing with cure rates as low as 30%. Sodium stibogluconate (SSG) is still efficacious with a cure rate of 93%, but has a high degree of toxicity, and the 30-day injection regimen is difficult to administer. Treatment with a combination of SSG and PM has been shown to be as efficacious as treatment with SSG alone, and importantly, reduce duration of treatment without imposing additional safety concerns. Therefore, the combination has been recommended by WHO for treatment in the region.[11,12] In a pharmacovigilance study, the SSG + PM combination confirmed results from a Phase III clinical trial and showed 95% efficacy at the end of treatment and an overall 0.9% mortality rate; efficacy, however, was lower in specific populations such as patients over 50 and those coinfected with HIV (Fabiana Alves, unpublished data).

In Latin America, meglumine antimoniate (Glucantime) is recommended as the first-line therapy, with the lipid formulation of amphotericin B (e.g., AmBisome) and amphotericin B deoxycholate as the second- and third-line treatments, respectively. Various compounds are still in development and are expected to transition from preclinical to clinical development in 2017. In addition, Drugs for Neglected Diseases initiative (DNDi) is in the process of developing treatment strategies and determining efficacy of treatment of PKDL. In summary, the workshop participants noted that it is systematically more difficult to treat patients in EA than in SEAR, that intraregion variability in efficacy rates is likely related to differences in parasite strains, and that it is important to monitor parasite resistance to antileishmanial drugs over time.

Vector control has become an important strategy for elimination in India, Nepal, and Bangladesh. The choices of vector control strategies have evolved over time, and effectiveness varies by country. Indoor residual spraying (IRS) of synthetic pyrethroids has been found to be more cost-effective than environmental and personal protection. However, there was slow reduction in vector density and development of resistance occurred in the targeted sandfly species due to substandard IRS spraying.[13] Personal protection methods, such as long-lasting insecticide-treated nets, N,N-diethyl-1, 3-methylbenzamide/neem oil, and chemical inhibition of feeding, resulted in up to 90% reduction in sandfly density. Control methods based on environmental management strategies included reduction in sandfly breeding sites and interruption of breeding using pheromone. Biological control has also been investigated. For example, paratransgenic manipulation of *Phlebotomus argentipes* was shown to be feasible in very early studies to render adult sandflies refractory to *L. donovani* infection.[14] As of now, the Government of India has adopted new tools and improved program strategies, including in the areas of insecticide spray, data management, and quality and quantity assessment, and has recently demonstrated significant improvement in the effectiveness of the previous vector control program.

A table was developed by the participants outlining tools that would be needed for VL control and elimination specific to the endemic regions (Table 1). Concerning regional specific treatment needs, low toxicity, and affordable treatment

TABLE 1

Knowledge gaps and tools of existing modalities for VL control and elimination

Regions	Diagnosis	Vector control	Treatment
All	Simple, reliable methods for detecting parasite load such as an ELISA-based assay	Understanding of vector biology for transmission-blocking vaccine development	Effective immune therapy
	Diagnostic biomarkers for asymptomatic individuals such as LST		Treatment of HIV–VL coinfection
	Methods to address reservoirs in asymptomatic individuals		Monitoring of drug resistance
Asian	Diagnosis and biomarkers of PKDL	Source reduction Vector surveillance Vector susceptibility to insecticides; specifics of insecticide-administration parameters: wall lining vs. others	Treatment of PKDL with low toxicity and low cost; establishment of parameters for PKDL identification and follow-up
Brazil	Better rapid diagnostics (including rK28) Diagnostic tools for canine VL	Better tools such as insecticide-treated dog collars	Shorter treatment duration and less toxicity option
East Africa	Diagnosis and biomarkers for VL and PKDL	Development of better tools	New treatment approaches for VL
	Better rapid diagnostics (including rK28)	Access to insecticide-treated bed nets and spray	Treatment of PKDL with low toxicity and low cost; Establishment of parameters for PKDL identification follow-up.

ELISA = enzyme-linked immunosorbent assay; HIV = human immunodeficiency virus; LST = leishmanin skin test; PKDL = post-kala-azar dermal leishmaniasis; VL = visceral leishmaniasis.

as well as establishing parameters for follow-up and identification of PKDL cases are required in SEAR and EA. Shorter duration and less toxic treatment options for VL are needed in Brazil. New treatment approaches for VL, including therapeutic vaccines or immunotherapy, would be of great value to EA. For vector control, improved vector surveillance, insecticide efficacy, and better defined specific insecticide administration parameters would benefit SEAR. Enhanced tools, such as dog collars, to reduce the canine reservoir are needed in Brazil. Additional efforts to develop effective tools for vector control as well as expanded access to insecticide-treated bed nets and insecticide spray are needed in EA. Diagnostic tools required to meet region-specific needs include tools to diagnose or tests for biomarkers of PKDL for SEAR and EA, improved rapid diagnostics for Brazil and EA, and diagnostic tools for canine VL for Brazil.

Other than regional specific needs, it also became very clear that the regions had many of the same needs, including monitoring of drug resistance, treatments for HIV–VL coinfected individuals, reliable methods for detecting parasite load such as enzyme-linked immunosorbent assay–based assay, diagnostic biomarkers, and means for identifying reservoirs including identification of asymptomatic infected individuals. In addition, vaccination for VL prevention and effective immune therapy treatment of PKDL as well as increased understanding of vector biology for developing transmission-blocking vaccines, were proposed as necessary for control and elimination in all major foci regions. Although the need for a vaccine is proposed for all regions, however, the priority of new intervention development (e.g., vaccines) applicable to control and elimination programs is expected to differ by region in accordance with the prevailing epidemiology and availability of tools and resources for public health efforts.

VL VACCINE RESEARCH AND DEVELOPMENT

Results from historical leishmaniasis vaccine trials, for example, a Bacillus Calmette–Guérin–adjuvanted, killed whole *L. major* parasite vaccine in alum,[15,16] have shed light on the scientific feasibility of developing a VL vaccine, even though an attempt to refine the crude vaccine with an adjuvanted recombinant vaccine later showed that the adjuvanted vaccine was safe and immunogenic in a Phase I trial but did not seem to shorten the time to cure when used in combination with standard chemotherapy for PKDL (R. Coler, personal communication, unpublished data). The successful development of vaccines for dogs against leishmaniasis also added to the promising aspect of a VL vaccine. Currently, two canine VL vaccines are commercially available to control spread of the parasite and possibly reduce the incidence in humans (Leishmune [Fort Dodge, Brazil] and Leish-Tech [Hertape Calier, Brazil]), and more are in field trials. Despite all the promising aspects of developing a VL vaccine, workshop participants did note the importance of careful vaccine design to avoid potential immunological defects–mediated pathology as evidenced by the altered levels of interleukin-10 production in patients with active disease[17,18] and certain human leukocyte antigen II regions associated with the disease susceptibility.[19]

Several human vaccine candidates intended to elicit various protective immunological mechanisms are currently in early development (Table 2). For example, a recombinantly expressed, adjuvanted fusion protein Leish-F3 with adjuvant GLA-SE (glucopyranosyl lipid adjuvant in stabel emulsion), has been shown both to prime $CD4^+$ T cell and to induce antibody responses, and demonstrated significant protection against two parasite species that cause VL in mice. Furthermore, when tested in a Phase I clinical trial, this vaccine was shown to be immunogenic and had an acceptable safety and reactogenicity profile.[20] Other types of vaccine candidates have, by design, targeted elicitation of $CD8^+$T cells as a mechanism to induce protection. Examples include a modular multiantigen T cell epitope–enriched DNA vaccine, LEISHDNAVAX (Germany), which was shown to induce $CD8^+$ and $CD4^+$ T cells in preclinical settings,[21] and a prime/boost strategy using an adenovirus vector expressing A2 antigen combined with the recombinant A2 protein which has demonstrated significant protective efficacy in a *L. infantum*-macaque infection model.[22] In addition, preclinical studies in a murine VL model have demonstrated the therapeutic efficacy of a single-dose adenoviral vaccine expressing kinetoplastid membrane protein-11 and hydrophilic acylated surface protein B1.[23] Following this work, a chimp adenovirus-vectored vaccine, ChAd63-KH (United Kingdom), was developed and has exhibited a good safety

TABLE 2
Examples of recent VL vaccine candidates in development

Vaccines	Antigen	Proposed targeted immune compartment	Development stage	References
Adjuvanted Leish-F3	Recombinant fusion protein of NH and SMT	CD4 T cells and Abs	Completed Phase I studies	20
LEISHDNAVAX	Modular multiantigen T cell epitope–enriched DNA vaccine	CD8 and CD4 T cells	Promising preclinical efficacy and safety results, Phase 1 study in preparation	21
Ad5-A2/rA2 Prime/Boost	Amastigote antigen A2	CD8 and CD4 T cells	Promising safety and efficacy results in NHPs	22
ChAd63-KH	Kinetoplastid membrane protein (KMP11) and hydrophilic acylated surface protein B (HASPB)	CD8 T cell biased	Completed Phase I study	ISRCTN07766359
Whole parasite	Attenuated *Leishmania* parasite with centrin gene deletion	Immunity mimicking natural infection	Promising safety and immunological profile in animals	25, 26
	Photoinactivated, attenuated promastigotes	Immunity mimicking natural infection	Safe and protective in small animals; promising immunotherapy for canine leishmaniasis	30

VL = visceral leishmaniasis.

profile and the ability to induce interferon-γ spot-forming cells in peripheral blood mononuclear cells in a Phase I trial in the United Kingdom (ISRCTN07766359), and a Phase II study in Sudan is slated for 2016 to examine immunogenicity and efficacy of ChAd63-KH as a therapeutic vaccine against PKDL. The broad applicability of ChAd63-KH beyond PKDL, and its potential to be combined with other partners, will be important for future studies. Furthermore, some salivary proteins from sandflies were recently and surprisingly identified as promising candidates for vaccine development, although the underlying protective host immune component(s) need further investigation. For example, several such proteins such as LJM19 or PdSP15 showed protection or increased efficacy of existing vaccine candidates in animals when challenged by bites from *Leishmania*-infected sandflies or by intradermal needle challenge.[24–26]

Lastly, vaccine development via genetically modified, live-attenuated whole parasites were also summarized in the workshop.[27] These vaccines are intended to mimic the natural course of infection to facilitate a strong, long-lasting, and protective immune response. One candidate vaccine currently being evaluated is an attenuated *L. donovani* parasite *LdCen*$^{-/-}$ (in which centrin, a Ca^{2+}-binding protein critical for cell division is deleted); thus far, it has been shown to have an adequate safety profile and induce immune responses in mice, hamsters, and dogs,[28,29] and to protect against infection from multiple parasite species in animal models.[30] Another approach uses oxidatively photoinactivated, attenuated promastigotes, which were shown to be safe[31] and protective against VL[32] in small animals, and demonstrated promising results as immunotherapy for canine leishmaniasis in an open-label trial in Naples, Italy.[33]

Despite progress made in early development, there is at present no effective human vaccine available against these leishmanial diseases (VL and PKDL), and correlates of protection for these devastating diseases remain to be fully defined and validated in humans. Given that the strategies above have shown that all immune components have provided some degree of protection, it was then suggested that all of the abovementioned approaches and vaccine design should continue to be investigated for VL vaccine development.

Several critical issues were raised with regard to the preclinical development process for a VL vaccine. There was consensus that the dog model is not appropriate for *L. donovani*, but its value remains to be confirmed for *L. infantum*–associated VL vaccine evaluation. Since dogs have very different immune responses and localization of the parasite than humans do, using dogs for modeling human disease may not be superior to using hamsters, and may in fact impose additional costs. The role of sandfly proteins that are able to modulate the environment surrounding bite sites leading to increased parasite survival, to modify the immune response of the host, and to exacerbate manifestation of the disease itself, have led to heated debates about the utility of a sandfly challenge model and the role of preexisting immunity in field setting. Sandfly challenge rather than needle inoculation may enable a better prediction of vaccine efficacy; however, the resources needed for and the feasibility of scaling up sandfly testing to meet the demands of vaccine and drug development should be carefully considered. It may be judicious to consider the sandfly challenge model for advanced testing of candidate vaccines so as to avoid creating a bottleneck that may impede progress. In addition, "clean" and inbred animals to evaluate vaccine efficacy may not be as relevant and valuable as compared with animals in the field of which some may have been preexposed to parasite infection. It was also noted that the role of preexisting vector immunity and the extent to which a vaccine can provide postexposure prophylaxis in the field should be carefully considered. Nevertheless, while there is a need to identify appropriate models to facilitate development effort, the group emphasized that the community should be cautious to avoid constraints early in the development pathway that could stifle progress.

VL VACCINE DEVELOPMENT CONSIDERATIONS

With multiple promising VL vaccine candidates on the horizon, several important aspects pertaining to VL vaccine development were discussed in the workshop (Table 3). It was suggested that the community as a whole needs to consider carefully the desired product characteristics, that is, target product profiles (TPPs). Modeling could help to guide the process of defining TPP,[34] such as establishing targets by determining the impact of different types of vaccination in combination with various interventions. Because an understanding of VL transmission dynamics is just emerging, however, VL modeling is still in early stages. Future modeling should consider different possible roles of vaccines (e.g., prevention of infection, disease postinfection, or PKDL), allow for relevant individual heterogeneities, include suboptimal performance of diagnostic tests, add animal reservoirs for zoonotic transmission, and include social and/or geographical stratification. In addition, modeling to investigate the economic value of a VL vaccine could help both to refine product development target profiles and to estimate the potential impact of a VL vaccine for decision makers with the intent of matching market demands. Different estimates for parameters regarding disease outcome, costs, and efficacy of an intervention can be incorporated to determine different scenarios and help guide development and ultimately implementation. For example, an initial model determined that a VL vaccine would be highly cost-effective, regardless of duration of protection if the cost per dose is $5 or less and efficacy is at least 70%.[35]

Some basic preferred product characteristics, which could then be further developed into a full TPP in the future,

TABLE 3
VL vaccine development consideration

TPP should consider a prophylactic vaccine against VL and a therapeutic vaccine as adjunctive therapy for PKDL.
Increased understanding of vector biology is needed for transmission-blocking vaccine development.
Some basic product characteristics need to be defined early during product development, and modeling would help to guide the process.
Clinical development remains a great challenge.
Effective diagnostic tools are needed for early detection of clinical endpoint.
Human leishmanization should be explored further to gain insight for a human challenge model for VL vaccine evaluation.
Creative development strategies and unique partnerships are needed to drive the entire development process.

PKDL = post-kala-azar dermal leishmaniasis; VL = visceral leishmaniasis.

should be considered in the early stages of product development. These would encompass what would be included in a product package insert, such as the indication, target population, efficacy, and clinical endpoint. It was proposed that efficacy of at least 70% should be a minimally acceptable feature for a prophylactic vaccine to prevent *L. donovani*–associated VL in children. A prophylactic vaccine with a broader geographic coverage, for example, using a common target antigen across different continents, would be an ideal design. In addition, a therapeutic vaccine to be used as adjunctive therapy to current standard of care for PKDL was also proposed. Such a therapeutic vaccine would be for both children and adults, and need to be at least 90% efficacious. Although a vaccine for dogs was proposed as one type of transmission-blocking vaccine to prevent transmission from dog to human, there is currently no sign of any other type of transmission-blocking vaccine candidate to block transmission from human to vectors or other intermediate hosts or vice versa, and more research is needed in this area. At the present time, workshop participants concluded that the VL vaccine R&D community should carefully define a TPP for the proposed vaccine(s), with broader or desirable parameters with respect to defining product-related characteristics more rigorously, a broader target population, and meaningful and measurable clinical efficacy and endpoint.

Clinical assessment of VL vaccine poses great challenges. For example, clinical presentations of VL may be very heterogeneous due to variability in parasite, host, and vector factors, and exhibit considerable regional variability. In addition, seasonal migrants and refugees would add to the significant challenging settings for clinical evaluation in the field. Effective diagnostic tools to allow early detection of clinical endpoints are important. The current WHO clinical case definition lacks specificity, and diagnostic aspiration for microscopic examination for amastigote parasites is an invasive procedure. Development of more sensitive serological tests shows promise. As an example, the rK39 immunochromatographic test showed excellent diagnostic performance in India and Nepal,[36] and further development and standardization of such an assay should be encouraged. Finally, the possibility to establish a controlled human infection model to accelerate clinical evaluation of VL vaccines was raised. It was pointed out that the ancient practice of leishmanization has historically been the best protection against CL, and has now been further improved for use as a live challenge to test candidate vaccines against CL.[37,38] Further development, characterization, and optimization of human leishmanization that could potentially lead to insight for a human challenge model for VL vaccine evaluation were recommended.

Vaccine development requires a substantial investment over a long period of time. The development of VL vaccines for poor and displaced populations has little commercial potential to attract R&D investment from private industry. Creative development strategies and unique partnerships are needed to drive the development effort to final commercialization. Under certain circumstances, the private sector does get involved, for example, when there are apparently added benefits to validate proprietary platform vaccine technology at low cost and provide proof of concept for novel vaccine design together with potential but limited revenue. This was the case when the development of a preventive and immunotherapeutic vaccine that targets CL as well as VL using a DNA technology platform was carried out by a biotech firm in Germany with a grant from the European Commission and in collaboration with DNDi and several academic groups. In addition, there appears that a pharmaceutical company in India, residing in the most important epidemiologic focus in the world for VL, is able to share the recognition of the public health challenge in the region. Through partnership and collaboration, the company would be willing to participate in the development and commercialization of a cost-effective vaccine that would have the potential to decrease both the direct and indirect health cost burden in affected developing countries.

After considering the resources required for VL vaccine R&D, workshop participants concluded that several strategies may be needed to facilitate progress. These include jointly clarifying R&D requirements and TPPs for VL vaccines with the WHO and endemic countries, encouraging public–private product development partnership to provide expertise and support, derisking potential early development failure, trading public funding for rights to affordable and accessible products, and streamlining the development process with milestone-driven funding throughout the entire development cycle.

In summary, there exist great challenges to VL control and elimination. Development of new tools to achieve and sustain the final elimination outcome is essential, but the prioritization of efforts will likely vary depending on the endemic regions. Better treatment options, improved vector control, more specific and sensitive diagnostics, and vaccines to prevent or treat diseases as well as to reduce or block transmission are all recommended. New VL vaccine candidates are on the horizon and will require careful consideration in development and product characteristics, as well as strategic planning to avoid potential pitfalls. Carefully designed development strategies and unique partnerships could help to facilitate development of VL vaccines for this complex disease afflicting the most neglected and poorest populations.

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REFERENCES

1. Hotez PJ, Alvarado M, Basáñez MG, Bolliger I, Bourne R, Boussinesq M, Brooker SJ, Brown AS, Buckle G, Budke CM, Carabin H, Coffeng LE, Fèvre EM, Fürst T, Halasa YA, Jasrasaria R, Johns NE, Keiser J, King CH, Lozano R, Murdoch ME, O'Hanlon S, Pion SD, Pullan RL, Ramaiah KD, Roberts T, Shepard DS, Smith JL, Stolk WA, Undurraga EA, Utzinger J, Wang M, Murray CJ, Naghavi M, 2014. The global burden of disease study 2010: interpretation and implications for the neglected tropical diseases. *PLoS Negl Trop Dis 8:* e2865.
2. Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, Jannin J, den Boer M, WHO Leishmaniasis Control Team, 2012. Leishmaniasis worldwide and global estimates of its incidence. *PLoS One 7:* e35671.
3. World Health Organization, 2015. *Kala-Azar Elimination Programme: Report of a WHO Consultation of Partners*. Geneva, Switzerland: World Health Organization.
4. WHO Regional Office for South-East Asia, 2012. *Regional Strategic Framework for Elimination of Kala-Azar from the South-East Asia Region, 2011–2015*. Geneva, Switzerland: World Health Organization.
5. Hasker E, Malaviya P, Gidwani K, Picado A, Ostyn B, Kansal S, Singh RP, Singh OP, Chourasia A, Kumar Singh A, Shankar R, Wilson ME, Khanal B, Rijal S, Boelaert M, Sundar S, 2014. Strong association between serological status and probability of progression to clinical visceral leishmaniasis in prospective cohort studies in India and Nepal. *PLoS Negl Trop Dis 8:* e2657.
6. Bualert L, Charungkiattikul W, Thongsuksai P, Mungthin M, Siripattanapipong S, Khositnithikul R, Naaglor T, Ravel C, El Baidouri F, Leelayoova S, 2012. Autochthonous disseminated dermal and visceral leishmaniasis in an AIDS patient, southern Thailand, caused by *Leishmania siamensis*. *Am J Trop Med Hyg 86:* 821–824.
7. Weirather JL, Jeronimo SM, Gautam S, Sundar S, Kang M, Kurtz MA, Haque R, Schriefer A, Talhari S, Carvalho EM, Donelson JE, Wilson ME, 2011. Serial quantitative PCR assay for detection, species discrimination, and quantification of *Leishmania* spp. in human samples. *J Clin Microbiol 49:* 3892–3904.
8. Rijal S, Ostyn B, Uranw S, Rai K, Bhattarai NR, Dorlo TP, Beijnen JH, Vanaerschot M, Decuypere S, Dhakal SS, Das ML, Karki P, Singh R, Boelaert M, Dujardin JC, 2013. Increasing failure of miltefosine in the treatment of kala-azar in Nepal and the potential role of parasite drug resistance, reinfection, or noncompliance. *Clin Infect Dis 56:* 1530–1538.
9. Sundar S, Chakravarty J, 2010. Antimony toxicity. *Int J Environ Res Public Health 7:* 4267–4277.
10. Sundar S, Sinha PK, Rai M, Verma DK, Nawin K, Alam S, Chakravarty J, Vaillant M, Verma N, Pandey K, Kumari P, Lal CS, Arora R, Sharma B, Ellis S, Strub-Wourgaft N, Balasegaram M, Olliaro P, Das P, Modabber F, 2011. Comparison of short-course multidrug treatment with standard therapy for visceral leishmaniasis in India: an open-label, non-inferiority, randomised controlled trial. *Lancet 377:* 477–486.
11. Melaku Y, Collin SM, Keus K, Gatluak F, Ritmeijer K, Davidson RN, 2007. Treatment of kala-azar in southern Sudan using a 17-day regimen of sodium stibogluconate combined with paromomycin: a retrospective comparison with 30-day sodium stibogluconate monotherapy. *Am J Trop Med Hyg 77:* 89–94.
12. Musa A, Khalil E, Hailu A, Olobo J, Balasegaram M, Omollo R, Edwards T, Rashid J, Mbui J, Musa B, Abuzaid AA, Ahmed O, Fadlalla A, El-Hassan A, Mueller M, Mucee G, Njoroge S, Manduku V, Mutuma G, Apadet L, Lodenyo H, Mutea D, Kirigi G, Yifru S, Mengistu G, Hurissa Z, Hailu W, Weldegebreal T, Tafes H, Mekonnen Y, Makonnen E, Ndegwa S, Sagaki P, Kimutai R, Kesusu J, Owiti R, Ellis S, Wasunna M, 2012. Sodium stibogluconate (SSG) and paromomycin combination compared to SSG for visceral leishmaniasis in east Africa: a randomised controlled trial. *PLoS Negl Trop Dis 6:* e1674.
13. Coleman M, Foster GM, Deb R, Pratap Singh R, Ismail HM, Shivam P, Ghosh AK, Dunkley S, Kumar V, Coleman M, Hemingway J, Paine MJ, Das P, 2015. DDT-based indoor residual spraying suboptimal for visceral leishmaniasis elimination in India. *Proc Natl Acad Sci USA 112:* 8573–8578.
14. Hurwitz I, Hillesland H, Fieck A, Das P, Durvasula R, 2011. The paratransgenic sand fly: a platform for control of *Leishmania* transmission. *Parasit Vectors 4:* 82.
15. Khalil EA, Musa AM, Modabber F, El-Hassan AM, 2006. Safety and immunogenicity of a candidate vaccine for visceral leishmaniasis (alum-precipitated autoclaved *Leishmania major* + BCG) in children: an extended phase II study. *Ann Trop Paediatr 26:* 357–361.
16. Musa AM, Khalil EA, Mahgoub FA, Elgawi SH, Modabber F, Elkadaru AE, Aboud MH, Noazin S, Ghalib HW, El-Hassan AM, 2008. Leishmaniasis research group/Sudan. Immunochemotherapy of persistent post-kala-azar dermal leishmaniasis: a novel approach to treatment. *Trans R Soc Trop Med Hyg 102:* 58–63.
17. Gautam S, Kumar R, Maurya R, Nylén S, Ansari N, Rai M, Sundar S, Sacks D, 2011. IL-10 neutralization promotes parasite clearance in splenic aspirate cells from patients with visceral leishmaniasis. *J Infect Dis 204:* 1134–1137.
18. Nylén S, Maurya R, Eidsmo L, Manandhar KD, Sundar S, Sacks DS, 2007. Plenic accumulation of IL-10 mRNA in T cells distinct from CD4 + CD25 + (Foxp3) regulatory T cells in human visceral leishmaniasis. *J Exp Med 204:* 805–817.
19. LeishGEN Consortium; Wellcome Trust Case Control Consortium 2, Fakiola M, Strange A, Cordell HJ, Miller EN, Pirinen M, Su Z, Mishra A, Mehrotra S, Monteiro GR, Band G, Bellenguez C, Dronov S, Edkins S, Freeman C, Giannoulatou E, Gray E, Hunt SE, Lacerda HG, Langford C, Pearson R, Pontes NN, Rai M, Singh SP, Smith L, Sousa O, Vukcevic D, Bramon E, Brown MA, Casas JP, Corvin A, Duncanson A, Jankowski J, Markus HS, Mathew CG, Palmer CN, Plomin R, Rautanen A, Sawcer SJ, Trembath RC, Viswanathan AC, Wood NW, Wilson ME, Deloukas P, Peltonen L, Christiansen F, Witt C, Jeronimo SM, Sundar S, Spencer CC, Blackwell JM, Donnelly P, 2013. Common variants in the HLA-DRB1-HLA-DQA1 HLA class II region are associated with susceptibility to visceral leishmaniasis. *Nat Genet 45:* 208–213.
20. Coler RN, Duthie MS, Hofmeyer KA, Guderian J, Jayashankar L, Vergara J, Rolf T, Misquith A, Laurance JD, Raman VS, Bailor HR, Cauwelaert ND, Reed SJ, Vallur A, Favila M, Orr MT, Ashman J, Ghosh P, Mondal D, Reed SG, 2015. From mouse to man: safety, immunogenicity and efficacy of a candidate leishmaniasis vaccine LEISH-F3 + GLA-SE. *Clin Transl Immunology 4:* e35.
21. Das S, Freier A, Boussoffara T, Das S, Oswald D, Losch FO, Selka M, Sacerdoti-Sierra N, Schönian G, Wiesmüller KH, Seifert K, Schroff M, Juhls C, Jaffe CL, Roy S, Das P, Louzir H, Croft SL, Modabber F, Walden P, 2014. Modular multiantigen T cell epitope-enriched DNA vaccine against human leishmaniasis. *Science Transl Med 6:* 234ra56.

22. Grimaldi G Jr, Teva A, Porrozzi R, Pinto MA, Marchevsky RS, Rocha MG, Dutra MS, Bruña-Romero O, Fernandes AP, Gazzinelli RT, 2014. Clinical and parasitological protection in a *Leishmania infantum*-macaque model vaccinated with adenovirus and the recombinant A2 antigen. *PLoS Negl Trop Dis 8:* e2853.
23. Maroof A, Brown N, Smith B, Hodgkinson MR, Maxwell A, Losch FO, Fritz U, Walden P, Lacey CN, Smith DF, Aebischer T, Kaye PM, 2012. Therapeutic vaccination with recombinant adenovirus reduces splenic parasite burden in experimental visceral leishmaniasis. *J Infect Dis 205:* 853–863.
24. Gomes R, Oliveira F, Teixeira C, Meneses C, Gilmore DC, Elnaiem DE, Kamhawi S, Valenzuela JG, 2012. Immunity to sand fly salivary protein LJM11 modulates host response to vector-transmitted *Leishmania* conferring ulcer-free protection. *J Invest Dermatol 132:* 2735–2743.
25. Zahedifard F, Gholami E, Taheri T, Taslimi Y, Doustdari F, Seyed N, Torkashvand F, Meneses C, Papadopoulou B, Kamhawi S, Valenzuela JG, Rafati S, 2014. Enhanced protective efficacy of nonpathogenic recombinant *Leishmania tarentolae* expressing cysteine proteinases combined with a sand fly salivary antigen. *PLoS Negl Trop Dis 8:* e2751.
26. Oliveira F, Rowton E, Aslan H, Gomes R, Castrovinci PA, Alvarenga PH, Abdeladhim M, Teixeira C, Meneses C, Kleeman LT, Guimarães-Costa AB, Rowland TE, Gilmore D, Doumbia S, Reed SG, Lawyer PG, Andersen JF, Kamhawi S, Valenzuela JG, 2015. A sand fly salivary protein vaccine shows efficacy against vector-transmitted cutaneous leishmaniasis in nonhuman primates. *Sci Transl Med 7:* 290.
27. Selvapandiyan A, Dey R, Gannavaram S, Lakhal-Naouar I, Duncan R, Salotra P, Nakhasi HL, 2012. Immunity to visceral leishmaniasis using genetically defined live-attenuated parasites. *J Trop Med 2012:* 631460.
28. Selvapandiyan A, Dey R, Nylen S, Duncan R, Sacks D, Nakhasi HL, 2009. Intracellular replication-deficient *Leishmania donovani* induces long lasting protective immunity against visceral leishmaniasis. *J Immunol 183:* 1813–1820.
29. Fiuza JA, Gannavaram S, Santiago Hda C, Selvapandiyan A, Souza DM, Passos LS, de Mendonça LZ, Lemos-Giunchetti Dda S, Ricci ND, Bartholomeu DC, Giunchetti RC, Bueno LL, Correa-Oliveira R, Nakhasi HL, Fujiwara RT, 2015. Vaccination using live attenuated *Leishmania donovani* centrin deleted parasites induces protection in dogs against *Leishmania infantum*. *Vaccine 33:* 280–288.
30. Dey R, Natarajan G, Bhattacharya P, Cummings H, Dagur PK, Terrazas C, Selvapandiyan A, McCoy JP Jr, Duncan R, Satoskar AR, Nakhasi HL, 2014. Characterization of cross-protection by genetically modified live-attenuated *Leishmania donovani* parasites against *Leishmania mexicana*. *J Immunol 193:* 3513–3527.
31. Dutta S, Waki K, Chang KP, 2012. Combinational sensitization of *Leishmania* with uroporphyrin and aluminum phthalocyanine synergistically enhances their photodynamic inactivation in vitro and in vivo. *Photochem Photobiol 88:* 620–625.
32. Kumari S, Samant M, Khare P, Misra P, Dutta S, Kolli BK, Sharma S, Chang KP, Dube A, 2009. Photodynamic vaccination of hamsters with inducible suicidal mutants of *Leishmania amazonensis* elicits immunity against visceral leishmaniasis. *Eur J Immunol 39:* 178–191.
33. Lun ZR, Wu MS, Chen YF, Wang JY, Zhou XN, Liao LF, Chen JP, Chow LM, Chang KP, 2015. Visceral leishmaniasis in China: an endemic disease under control. *Clin Microbiol Rev 28:* 987–1004.
34. Lee BY, Burke DS, 2010. Constructing target product profiles (TPPs) to help vaccines overcome post-approval obstacles. *Vaccine 28:* 2806–2809.
35. Lee BY, Bacon KM, Shah M, Kitchen SB, Connor DL, Slayton RB, 2012. The economic value of a visceral leishmaniasis vaccine in Bihar state, India. *Am J Trop Med Hyg 86:* 417–425.
36. Singh D, Pandey K, Das VN, Das S, Verma N, Ranjan A, Lal SC, Topno KR, Singh SK, Verma RB, Kumar A, Sardar AH, Purkait B, Das P, 2013. Evaluation of rK-39 strip test using urine for diagnosis of visceral leishmaniasis in an endemic region of India. *Am J Trop Med Hyg 88:* 222–226.
37. Saljoughian N, Taheri T, Rafati S, 2014. Live vaccination tactics: possible approaches for controlling visceral leishmaniasis. *Front Immunol 5:* 134.
38. Khamesipour A, Abbasi A, Firooz A, Mohammadi AM, Eskandari SE, Jaafari MR, 2012. Treatment of cutaneous lesion of 20 years' duration caused by leishmanization. *Indian J Dermatol 57:* 123–125.