

Vaccine for leishmaniasis: New era of CRISPR generated Live attenuated dermatropic Leishmania

Kamaleshwar Singh, Swarnendu Kaviraj, Subir Karmakar, Sanjay Singh*

Vaccine Formulation and Research
Center, Genova Biopharmaceuticals
Ltd., Pune, Maharashtra 411057, India

*Author for correspondence:
Email: Sanjay.Singh@genova.co.in

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Abstract

Using CRISPR gene-editing technology, researchers have generated a live attenuated *centrin* gene deleted *Leishmania major* parasites (*LmCen*^{-/-}) which are able to protect against visceral leishmaniasis caused by *Leishmania donovani* parasite. Since *LmCen*^{-/-} vaccine is antibiotic resistant marker free, safe and prevents mortality, scientists are planning to use it in Phase I human clinical trials.

Keywords: *Leishmania*, Live attenuated Vaccine, *centrin* gene, Clinical trial, CRISPR

Leishmaniasis, is a neglected parasitic disease, transmitted by the bites of *Leishmania*-infected female sand flies, affecting millions of peoples in 98 countries worldwide. According to clinical manifestation, leishmaniasis is broadly classified as tegumentary leishmaniasis (comprises of localized-cutaneous, diffuse-cutaneous and mucosal forms) and visceral leishmaniasis (VL; affecting liver, spleen, lymph nodes and bone marrow) which is most fatal and systemic, if left untreated. Depending on geographic distribution of species, VL is anthroponotic in South Asia and East Africa, caused by *L. donovani*, whereas the disease is zoonotic in the Central and South America, caused by *L. infantum* and/or *L. chagasi* [1-3]. More than ninety percent of VL cases occur in six countries, namely India, Bangladesh, Nepal, Sudan, Ethiopia and Brazil [4].

Current treatment against any form of leishmaniasis is mostly dependent on available drugs like, pentavalent antimonials, amphotericin B, paromomycin, and miltefosine. However, these drugs are expensive, have prolong treatment duration, severe side effects and often leading to drug resistance in humans [5-8], suggested vaccine would be the best alternative of drug therapy. Recovery from primary infection including VL develop lifelong immunity against subsequent infection, suggesting that a vaccine is feasible [8-10] and also the most cost-effective ways to eliminate the disease than vector control and treatment strategies. According to a computer modelling study, a vaccine with a minimum 50% efficacy and as little as 5 years' duration of protection, would be more cost-effective than the current available chemotherapies for both Cutaneous Leishmaniasis (CL) and Visceral leishmaniasis (VL)- signify its importance for low- and middle- income countries (LMIC) [11,12]. This suggest the need for a pan *Leishmania* vaccine which would be effective against all form of leishmaniasis around the globe. Over the past decades, several strategies have been applied to develop a vaccine against various species of *Leishmania*. However, currently there is no licensed human vaccine available for any form of leishmaniasis.

Catering to develop this unmet need of a safe and effective vaccine, Dr. HL Nakhasi group initially developed *centrin* gene deleted live attenuated *L. donovani* parasites which was protective against *L. donovani* challenge in various preclinical animal models of VL [13-15]. *Centrin* is a calcium-binding cytoskeletal protein involved in the duplication of centrosomes in higher eukaryotes including *Leishmania* [16]. To overcome the regulatory barrier of using a visceral strain as a vaccine candidate, researchers applied modernize approach of CRISPR technology to develop *centrin* gene deleted live-attenuated vaccine from demotrophic *L. major* strain (*LmCen*^{-/-}) for the most effective century-old Leishmanization (LZ) practice where live parasite was used for human inoculation, leading to self-healing lesions and subsequent life-long immunity against future cutaneous infection

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[9,16-18]. Importantly, using CRISPR, generation of antibiotic resistance marker free *LmCen*^{-/-} makes it compliant for human vaccine trials. In the murine model, researchers have shown that *LmCen*^{-/-} is safe, immunogenic and induces robust host protection against sand fly transmitted cutaneous infection [16].

In the study by Karmakar S et al., researchers have taken an important step forward in defining the protective efficacy of dermatotropic *LmCen*^{-/-} vaccine against VL [19] which was supported by epidemiological evidence where pre-exposure to wild-type *L. major* parasites confer cross-protection against VL in various animal models and humans [20-22]. They have confirmed that the mutant parasite is safe and did not cause any signs of disease pathology even in immune-suppressed animals. Furthermore, they have explained that no viable *LmCen*^{-/-} parasite were recovered from immunized animals even though they persist in sub-clinical condition for long enough to generate acquired immunity against future infection. However, the major concern of using live attenuated parasites as vaccine is the probability of reversion to virulent form. Test in sand flies through xenodiagnoses, possible source of genetic exchange with wild type parasites to regain virulence [23] and in various immune deficient animal models [16] including immune-suppressed hamsters [19], they have confirmed that the mutant parasites didn't revert back to wild type form, so safe as vaccine candidate for human trial.

In this study, they have used hamsters as the gold standard animal model for VL, because of its similarity to humans in regard to disease symptoms, pathogenesis and immune responses [24]. Furthermore, vaccinated hamsters showed robust control of parasitemia in visceral organs, challenged with *L. donovani* either through needle injection or by natural mode of infected sand fly bite. Importantly, this vector-challenge result validated the efficacy of *LmCen*^{-/-} as a vaccine candidate during pre-clinical testing and provide stringent evaluation of their performance under natural conditions with respect to needle-initiated infection [25]. This protection was associated with significant higher expression of protective humoral as well as cell mediated immune response, similar to cured VL patients for controlling infection [26]. Moreover, induction of distinct upstream regulators compared to wild type infection predicted by IPA analysis, suggests *LmCen*^{-/-} as a potent immune-modulatory agents and biomarkers of immunogenicity in human clinical trials.

Beyond pre-clinical studies, to evaluate the vaccine potential of a live attenuated vaccine for human clinical trial, several prerequisite need to be satisfied for approval by the regulatory agencies. This include the vaccine strain should be manufactured under current Good Manufacturing Practice (cGMP). Towards achieving this goal, researchers manufactured *LmCen*^{-/-} parasites under Good Laboratory Practice (GLP) conditions, in a cGMP compliant facility in collaboration with their industry partner, Genova Biopharmaceuticals Ltd., India. GLP-grade *LmCen*^{-/-} parasites produced in large scale are also safe, induced significant host protection and prevents mortality against natural mode of sand fly challenge. Importantly, their long-lasting protective efficacy further satisfied the essential characteristics of an efficacious vaccine. In addition, an IFN- γ dominated immune response in PBMCs of healthy human volunteers living in non-endemic regions (United States of America, USA) suggested that *LmCen*^{-/-} is ready to be tested in human's clinical trial, corroborated by Osman et al. study [27]. However, the immunological response from non-endemic region human PBMCs needs to be further validated by the GMP-grade

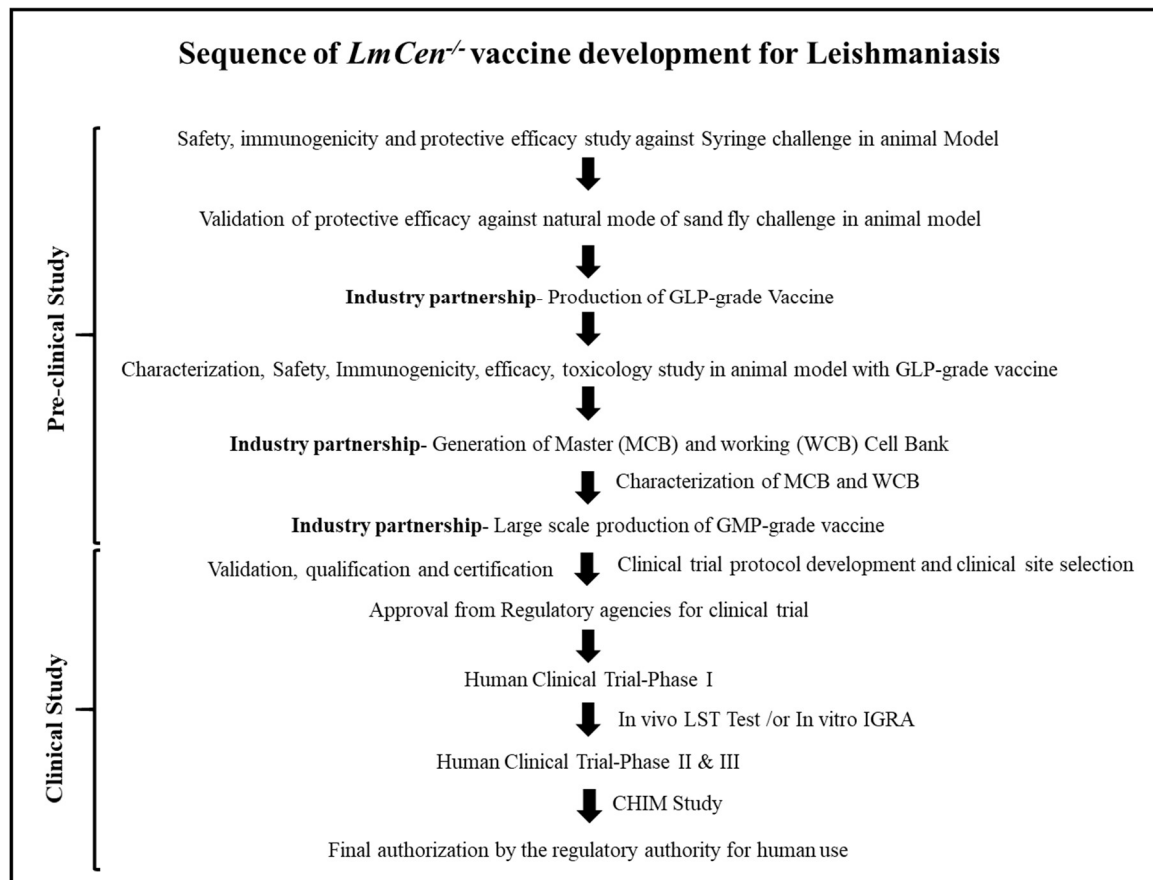
parasites in future studies.

Another major challenge associated with *Leishmania* vaccine development, is the clinical evaluation of the safety, immunogenicity and efficacy of a vaccine. The most traditional way to determine the efficacy of a vaccine is the development of disease [https://www.who.int/biologicals/expert_committee/Clinical_changes_IK_final.pdf], which needs large number of participants and may take long duration to complete the study due to variation of incidence of cases in the endemic areas [28]. Therefore, alternative approaches should be applied which can be invaluable in expediting time to check the safety and efficacy of a vaccines. One approach is the use of sand fly-initiated controlled human infection model (CHIM) to determine the vaccine efficacy against CL during phase II trial [29], previously studied in many other diseases [30-33]. However, the use of such CHIMs model to determine vaccine efficacy against VL remains to be solved. As per epidemiological evidence, exposure to wild-type *L. major* parasites confer cross-protection against VL in various animal models including humans [20-22,34], suggested that an effective vaccine against CL in CHIM study would provide immunity against VL. Another *in vivo* approach to define the immune correlates of protection is the re-introduction of Leishmanin Skin Test (LST) as a surrogate biomarker of immunogenicity during phase I study. LST has been used for decades to determine long-lasting protective immunity for both CL and VL [35-38]. Currently GMP-grade leishmanin antigen is not available for clinical purpose anywhere in the world, forcing the practice of LST has to decline. Moreover, advancing the development of leishmanin antigen under GMP condition, strongly suggest re-introduction of LST for the surveillance programs as well as vaccine trials to eliminate the disease. However, the sensitivity of LST is known to vary depending upon the appropriate dose, geographic regions or phase of visceral infection [22,38], suggesting the development of better markers for the assessment of infection and cellular immune response in endemic regions of VL. These findings indicate that an *in vitro* approach of IFN- γ release assay (IGRA), could be useful to detect *Leishmania* infection and to assess the immune response of a vaccine candidate during human studies in highly endemic regions of VL [39,40].

In addition, other challenges need to be overcome to develop a vaccine for human trial which includes: development of assays to determine batch to batch consistency, storage condition, evaluation of potential toxicities due to interactions of the components present in the final formulation, complete genome sequence of the GMP-grade parasite to check any off target deletion/mutation, absence of any adventitious agents like *Leishmania* RNA virus that can exacerbate the disease progression [41], well defined media formulation that is either animal component (serum) free or free from serum containing Bovine Spongiform Encephalopathy (BSE) [4].

Conclusion

Leishmaniasis is a serious threat globally and the development of a vaccine is a major public health priority. Current understanding of *Leishmania* pathogenesis and host protective immunity suggested that a single vaccine has the potential to protect against one or more than one species and prevent the disease. Despite substantial efforts by many laboratories, no vaccine is available for human use still to date. The major impediments in *Leishmania* vaccine research is mostly related to their biosafety, efficacy against natural exposure to infected sand fly bites that can correlates the vaccine success in



real field and finally their production. The most advances of new CRISPR gene editing technology, together with surrogate practice (leishmanization) results in new avenues for *Leishmania* vaccine research that evolved the generation of live attenuated *LmCen*^{-/-} vaccine with precise deletion of the desired gene. Pre-clinical studies showed that the vaccine has overcome all biosafety issues and single intradermal injection can significantly induce long-lasting host protective immunity to prevent the disease. Importantly, robust and durable protective efficacy of *LmCen*^{-/-} against both homologous and heterologous challenge through natural sand fly mode, suggests the feasibility of *LmCen*^{-/-} as a pan-*Leishmania* vaccine for the first time. Currently, researchers are planning for toxicology study with GLP-grade *LmCen*^{-/-} vaccine in India. Moreover, they have developed and characterized the Master Cell Bank (MCB) as well as Working Cell Bank (WCB) for the manufacturing of GMP-grade *LmCen*^{-/-} parasites. Since the site of clinical trial is of critically important, researchers are now planning for Phase 1 study in the non-endemic regions (United states) as well as in the endemic Regions (India, Ethiopia, Sudan, Brazil, Iran etc.) of VL to assess the safety and immunogenicity of this vaccine. They are also in a process of exploring the efficacy of *LmCen*^{-/-} vaccine in CHIM model.

Conflicts of Interests

The authors have no conflicts of interest to declare.

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