Development of Class D CpG Odn (D35) as an Adjunct to Chemotherapy for Cutaneous Leishmaniasis and Post Kala-Azar Dermal Leishmaniasis (Pkdl)

United States Food and Drug Administration (US FDA), Osaka University, et al.

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Project summary:*

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1) Product synthesis and characterization: over 20 lots of D35 have been successfully produced in small scale at the core facility of the Food and Drug Administration. Its simple production is amenable to manufacturing at affordable cost and the technology to produce it is easily transferable. Two different contract manufacturers, Girindus in USA and Gene Design Inc in Japan have provided estimates of the cost to produce the 10 grams of the oligonucleotide needed for pre-clinical and phase I-II clinical studies. Their estimated time to deliverable for 10g of product including CMC characterization: 8 months.

2) Pre-clinical studies: Although there is vast positive experience with synthetic CpG ODN in clinical trials, this particular CpG ODN sequence has had limited testing in humans, therefore all preclinical requirements must be met. D35 stimulates Toll like receptor 9 (TLR9) of pigs and primates but is not active on TLR9 from small rodents. Previous studies using synthetic phosphorothioate ODN show that mice routinely tolerate single doses of CpG ODN of up to 400 ug, and repeated doses of 100ug (i.p. 4 days apart) as compared to reported therapeutic doses of 5-50 ug. The most common histopathological findings in safety toxicology studies were modest splenomegaly and lymphoid hyperplasia with an accompanying accumulation of mononuclear cells (B cells, monocytes). In macaques studies performed in Dr Verthelyi’s laboratory no significant changes in body weight, temperature, behavior, CBC or metabolic panel at effective doses were observed (Figure 12). Additional studies to establish NOAEL in mice and primates (Figure 13) will entail dose escalation studies. Planned studies include a single and a multi-dose study in mice using 30, 100 and 300 ug of D35 (SC dose/week x 5). Readouts will include clinical signs, body weight, food consumption, hematology and blood chemistry, PK and PD on 1st and 5th dose, and histopathology. Single and multi-dose studies in rhesus macaques will use doses of 1, or 3 mg/kg SC. Clinical signs, body weight, food consumption, hematology, urinalysis, and blood chemistry, immunogenicity, pharmacokinetics and histopathology on 1st or 4th dose. Estimated time to deliverable: 12 months.

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a) A Phase Ia, randomized, double blind, placebo-controlled single dose escalation study will be conducted to establish safety in humans for D35 in 35 treatment naïve patients with cutaneous leishmaniasis due to Leishmania braziliensis using a 4:1 design. The anticipated starting dose will be at 0.01mg/kg (1:100th of a known safe dose in macaques) and will escalate to 1 mg/kg. The site of the study has not been selected but would likely be in Peru, Brazil or Colombia.

b) A randomized, double blind, placebo-controlled, dose escalation, repeated administration Phase Ib study with PKDL patients receiving 3 doses of 0.3 and 1mg/kg 2 weeks apart (8:2 per dose cohort for a total of 20 patients). Safety monitoring will be performed in days 1, 3, 7, and 14 after each dose. Exploratory PK & PD data will be collected. Monitoring of immunological parameters will include serum levels of IFNa, CXCL-10 and IFNg. Results will be compared with pre-treatment samples for each subject. The site of the study has not been selected but Bangladesh
or Sudan, where the incidence of PKDL is higher, would be desirable. Estimated time to deliverable: 12 months.

c) c. A phase II studies to collect additional safety data, PK, PD and initial efficacy in CL patients (extended to include Leishmania amazonensis, L. guyanensis etc.)

4) Multicenter pivotal clinical trials to continue to collect safety data and establish efficacy in CL and PKDL populations and Licensure application in target countries under a concerted effort with National Medicines Regulatory authorities

*As taken from original proposal template, question 5.
1.* Title of the project: Development of Class D CpG ODN (D35) as an Adjunct to Chemotherapy for cutaneous leishmaniasis and Post Kala-Azar Dermal Leishmaniasis (PKDL)

2.* Submitted by:

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3.* Target disease or health condition:
Cutaneous Leishmaniasis (CL), also known as button d’orient, chiclero’s ulcer, Aleppo sore, Delhi’s boil, etc., is a neglected disease that is characterized by disfiguring skin lesions. CL has a wide distribution in the Indian subcontinent, Central and South Western Asia, the Mediterranean region, Africa and Central and South America. Worldwide, an estimated 1.5 million suffer from different forms of CL and Post Kala-azar dermal Leishmaniasis (PKDL) every year (1), but only a small percentage receives treatment. Currently there is no vaccine for Leishmaniasis and little R & D is aimed at alleviating the suffering of millions of CL cases, mostly children. In the Americas, leishmaniasis represents a significant public health problem due to its high morbidity and wide geographic distribution. It affects predominantly the poorest sections in society with reduced access to health services. Between 2001 and 2011, 638,702 cases of cutaneous and mucocutaneous leishmaniasis were reported in the region (95.7% corresponded to CL), with an annual average of 58,063 cases (2).

CL is caused by over 15 different species of the protozoan parasite *Leishmania*. Its complex transmission cycle includes different vectors and reservoirs that may be either infected humans (anthroponotic: ACL & PKDL) or mammals (Zoonotic, ZCL). CL can present in different forms ranging from uncomplicated self-healing skin lesions, to debilitating large chronic or recurring lesions, disfiguring mucosal or muco-cutaneous lesions or diffuse CL with severe social stigma. The sores typically progress from small papules to open sores with a raised border and central crater (ulcer), which can be covered with scales or crust. The lesions usually are painless but can be painful, particularly when open sores become infected with bacteria. PKDL may appear during visceral leishmaniasis (VL) but typically is developed months or years following successful treatment of VL. The estimated incidence of PKDL in patients with VL varies from 1-2% in India to about 20% in Bangladesh and over 40% in the Sudan. CL & PKDL are not life threatening, and this is an important factor in it being chronically neglected. However, CL & PKDL are disfiguring disease that result in stigma, economic loss and affects mainly unprivileged populations with limited resources. Scars from CL lesions last for a life time and particularly on the face severely affect the whole life of afflicted individuals, particularly girls and women. PKDL is strongly believed to act as a reservoir of visceral leishmaniasis (VL) which underscores the need to develop an effective treatment. CL and PKDL patients require weeks or months of daily antimonial injections (toxic, painful, expensive). The
objective for this demonstration project is to develop a short, safe, affordable and field-friendly treatment that are efficacious at least for CL caused by L. tropica and L. braziliensis (but which would ideally also work for CL caused by other organisms) and for PKDL using a collaborative approach governed by a WHO based consortia that will coordinate with national regulatory and health authorities from affected countries, donors and other relevant stakeholders convened by WHO.

4.* The suggested health technology that project seeks to develop:
The purpose of treatment in cutaneous leishmaniasis is to accelerate healing, reduce scarring, and prevent relapses. The proposed strategy is to combine the use of proven chemotherapy to accelerate the elimination of the parasite with an enhancer of the effector immune response to improve the immune response to the parasite and accelerate healing. D35 is a synthetic oligonucleotide designed to activate the innate immune system and enhance the T cell effector mechanism to control *Leishmania* infection. Synthetic oligonucleotide D35 could meet the requirements of the a target product profile for a disease such as CL/PKDL in terms of being field-friendly, affordable, and expected to be safe, as well as in leading to accelerated healing, with reduced incidence of mucocutaneous complications. Preclinical work with rodents and primates support this belief as studies in mice and non-human primates show that a single administration of CpG ODN D35 can accelerate the immune response to the parasite and improve the clinical outcome without inducing any detectable adverse effects. Importantly the effect was evident in animals that already had established lesions. In summary, administration of D35 would be combined with the currently used treatment, antimonials with the aim of reducing the dose and duration of antimonial treatment thus reducing cost and occurrence of side effects, as well as increasing compliance (often low due to painful antimonial injections). Accelerated clearance of the lesions would also reduce the risk of development of disseminated or mucosal leishmaniasis.

5.* Project summary:
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(Approximately 500 words)

6.* Public health need that the proposed project aims to address:
Kinetoplastid infections (including Chagas, Leishmaniasis and African trypanosomiasis) are among the diseases with the higher death toll in developing countries (3). Treatment of kinetoplastid infections is hampered by outdated drugs, and a lack of vaccines and effective standard diagnostic tools." (3). While not life threatening, CL & PKDL are disfiguring and result in stigma, and economic loss for unprivileged populations with limited resources. An estimated
1.5 million suffer from different forms of CL and Post Kala-azar dermal Leishmaniasis (PKDL) every year (1), but only a small percentage receives treatment. CL has a wide distribution with most (up to 90%) of the world’s cases of CL occurring in only a few countries: Afghanistan, Algeria, Iran, Iraq, Saudi Arabia, and Syria in the Old World; and Bolivia, Brazil, Colombia, Nicaragua, and Peru in the New World (CDC Yellow book, 2013) (1). Scars from CL lesions last for life time and prove particularly devastating, to girls and women. PKDL is strongly believed to act as a reservoir of visceral leishmaniasis (VL) which underscores the need to develop an effective treatment.

There are no satisfactory treatments for any forms of CL or PKDL. Available treatment include pentavalent antimonials: sodium stibogluconate (Pentostam, Stibanate) and meglumine antimoniate (Glucantime) which have been used since the 1940’s and are the main first-line drugs. Antimonials are toxic and their efficacy in many regions has diminished over time. Further, antimonials requires painful intra-muscular injections daily for 3-4 weeks for CL and for up to 3 months for PKDL. Over the last decade, new drugs (AmBisome, Miltefosine) have been used for CL and PKDL. Both are expensive, require refrigerated storage and lengthy interventions. AmBisome has not been fully evaluated for treatment of different forms of CL or PKDL primarily due to cost, required storage conditions and i.v. infusion, but it has been used for a limited number of patients that failed standard antimonial therapy. Trials with Miltefosine used for 6 or 8 weeks for PKDL have shown only a 75% cure rate at 12 months leaving a significant percentage without additional treatment options. Moreover, CL trials with miltefosine have yielded varying results, as it is clearly ineffective against CL caused by *L. tropica* and is potentially teratogenic. Optimally, this demonstration project will provide for an adjunct therapy that accelerates healing and reduces the frequency and doses of antimonials required for treating CL and PKDL.

7.* Explain which new and innovative approaches and mechanisms to supporting financing and coordination of R&D this project would demonstrate?

a) CL and PKLD largely affect poor populations in developing countries and therefore not an economic target for private industry. Further, since it is not a fatal disease it has received little attention from global health organizations despite affecting the lives of an estimated 1.5 million people every year.

b) This project will allow demonstrating the effective use of delinking of the price of R&D and the price of the product though equitable or humanitarian licensing for global access, which ensures a low price of the final product given that the US-FDA has no have recovering the investment in R&D as part of the Agency’s mission.

c) This project would bring together basic and translational scientists at the Food and Drug Administration and the University of Osaka with the WHO experience in product development and program implementation for neglected diseases in a unique collaborative effort. If this project is selected for a global demonstration initiative it will also allow for testing the benefits of relying on a public health based/global coordinating mechanism that directly involves developing countries that may benefit from the new product to foster and accelerate the developing of essential health technologies that are stalled due to market failure.
d) The clinical testing of the new therapeutic regimen would be paired with continuing research into the immune responses elicited by the disease and into the identification of useful biomarkers to track and predict treatment success. These will be particularly important for the population at risk of PKDL. The new scientific knowledge that this research will generate will be shared and available to the global community.

e) The technology required to manufacture D35, the synthetic oligonucleotide used to activate the innate immune system and accelerate the development of an effective adaptive response is easily transferable thus facilitating the availability of the product at the countries where it is needed.

f) Development will require pooled funding from member countries. The time from manufacture to license is estimated at 7-8 years. But a new coordinated and collaborative approach with involvement of target countries’ National Health authorities, National Regulatory Authorities, donors and other relevant international and national stakeholders will foster a more efficient and faster process for making this medicine available and affordable to populations in need in target countries.

8.* Evidence of market failure/research landscape:

The geographical and epidemiological distribution of CL and PKDL makes them a low priority for for-profit organizations and therefore there have been almost no efforts from that sector to develop new medicines that target these ailments over the last decade. The NIH remains the top funder for these group of diseases followed by the Gates foundation; private industry investment remains below 10% of the overall funding for these diseases and overall the funding has decreased between 2009 and 2011 (3). According to MSF and DNDi only 3.8 % of newly approved drugs between 2001 and 2011 were for neglected diseases and only 1.4 % of registered clinical trials are focused on neglected tropical diseases. For most neglected diseases the public sector provides the largest funding but CL & PKDL are not life threatening, and therefore have traditionally commanded fewer resources making them truly neglected diseases. Since CL and PKDL affect patients of low resources the cost of the treatment for CL & PKDL is usually affronted by local governments that implement programs to purchase and distribute the drug product the appropriate health care centers. Current treatment of CL in most South American countries involves the use of antimonials and requires daily painful intra-muscular injections for 3-4 weeks or 6-8 intralesional injections for up to 12 weeks, whereas treatment of PKLD patients typically involves daily injections of antimonials for 2-3 months. As a result few patients receive full treatment. Even with newer products such as AmBisome PKLD patients require 60 to 80 doses over 4 months or 12 weeks with Miltefosine(4). Recently, AmBisome was used by MSF in Bangladesh for treatment of PKDL at total dose of 30 mg/kg given in 6 intravenous infusions during 3-4 weeks at a cost of $480 at WHO- negotiated price; obviously beyond the means of most patients or health systems of endemic countries

9. The scientific and technical feasibility:
Leishmaniasis is a vector-borne disease initiated by the bite of an infected sand fly. The influx of parasites is followed by a rapid infiltration with neutrophils that take up but do not kill the parasites\(^5\). The parasites hide in the neutrophils and induce the neutrophil to present markers that prevent DC activation allowing the parasites to amplify unchallenged\(^6\). D35, a CpG ODN that triggers innate immune receptor TLR-9 expressed selectively by plasmacytoid dendritic cells, was selected based on its activity profile and evidence of safety in animal studies. Administration of D35 results in systemic activation of T cells and dendritic cells in skin enabling the cells to respond to the parasite to clear the infection. Treatment with D35 induces the immune cells embedded in the skin to produce high levels of IL-12, IFN\(\alpha\), IFN\(\gamma\), IP-10, and low levels of IL-10 which corresponds to the responses associated with parasite clearance and lesion healing (Figure 1-5)\(^7\). The use of selected cytokine levels has been proposed as biomarkers of local innate and adaptive immune activation.

The relevance of the rhesus macaque model is supported by the similar profile of immune activation in humans and rhesus macaques (Figure 2) as well as by the characteristics of the skin lesions in the macaque (Figure 6). Using the macaque model we have shown that D35 significantly reduces the severity of lesions in macaques challenged with L. amazonensis or L. major (Figures 7-8)\(^8\).

Crucially, the improved outcome was evident not only in macaques that were healthy, but in SIV-infected macaques as well (Figure 8) indicating that even patients with other underlying illnesses (co-infection of HIV and Leishmania is a problem in several countries, particularly in sub-Saharan Africa) or -potentially- with malnutrition, the use of D35 will be beneficial as well. Treatment of macaques was associated with systemic activation of genes linked to innate immune responses in skin (Figure 4-5) and with increased number of T cells producing IFN\(\gamma\) in response to Leishmania antigen \(^9,10\). The improved clinical outcome did not interfere with the development of effective memory to the parasite \(^11\). The protection was systemic (Figure 5, 9-10) as animals treated with D35 SC showed increased expression of immune related genes in skin distant to the site of treatment and macaques inoculated with D35 in the leg had improved outcome when challenged in the forehead.

D35 has been tested in primates quite extensively in our lab without any adverse events. There is a potential concern that the 3’ poly G tail of D35 could lead to the formation of G-tetrads and product aggregation. In our hands -with over 20 different lots manufactured- the degree of aggregation has not been significant (<5%), but upon large scale manufacturing this could change and therefore it will be important to perform formulation studies. Of note, our lab has developed an alternative chemistry for the backbone of D35 where the poly G tail is protected from forming G-tetrads by moieties that fall off in a temperature dependent manner \(^9\). Although the data shows that these Pro-ODN do not form G-tetrads and can improve the outcome of CL in a similar fashion as D35, the chemical backbone of these ODN require temperature control during storage making them a less desirable compound than D35 (Figure 11). D35 with its current chemical backbone made of phosphorothioate and phosphodiester bonds is expected to be very stable and the plan for clinical trials is to provided it as dry film (desiccated under a constant airflow) or lyophilized to be resuspended at the point of treatment.

10. Reasons for proposing:
Even with the advent of newer products, there are no satisfactory treatments for any forms of CL or PKDL. Developing an affordable, safe and thermostable product that can increase the speed to complete cure, reduce scarring, and prevent relapse will greatly improve treatment conditions and outcomes for affected populations in LMIC. D35 is a synthetic oligonucleotide that acts as
an enhancer of the effector immune response to improve healing and reduce recurrences of *Leishmania* infection and could meet the requirements of desirable target product profile for CL/PKDL in terms of being field-friendly, affordable and safe.

It has long been known that the underlying mechanism for controlling *Leishmania* infection and self-healing of CL lesions is the cellular immune response, mediated by antigen-specific γ-interferon producing T-cells. The need for a Th replen type 1 response is supported by studies from Convit et al in Venezuela using heat killed Leishmania together with live BCG vaccine and studies in Brazil showing BCG (which stimulated Toll receptor-9 like D35) improved the response to low dose antimonials (12,13). These data suggest that immune response modulators (IRM) can reduce the dose and duration of chemotherapies that at present require prolonged, toxic, painful and often unaffordable treatments.

The use of defined IRM is a new approach for leishmaniasis treatment and unlike anti-parasitic drugs that target the parasite directly; the IRM’s involve the host’s immune machinery and its intricate regulations. Several other innate immune modifiers were considered including IC-31 (TLR-9 agonist, Intercell, Vienna), MPL (IDRI), Imiquimod, double stem loop (Mologen), Phentoxysphilline and Poly I:C. but these were discarded due to absence of efficacy in Leishmaniasis (e.g. Imiquimod) or lack of interest of the manufacturer due to the relatively small/low profit market. D35 a CpG ODN Class A/D acting on TLR-9 developed at the FDA was considered the best candidate based on its activity profile, the evidence of efficacy in animal studies, and the likelihood of safety since the molecule selectively activates TLR9 on plasmacytoid dendritic cells and thus should not have significant off-target effects. Studies in primates support the expectation of a good safety profile. Importantly, the improved clinical outcome was evident when using 2 different strains of Leishmania (*L.*major and *L.* amazonensis), and even when the treatment was delayed for days-weeks after challenge, or when the animals are co-infected with SIV (Figures 8-9). Lastly, its relatively simple manufacturing requirements amenable to large scale manufacturing at affordable cost and its stability in the absence of cold chain assurance are also important.

11. **Who could potentially develop the technology/carry out the research?**

The technology could be developed under an agreement between FDA and a WHO-based consortia that includes representatives from the countries affected by Leishmania. DNDi, a non-profit organization interested in developing products for neglected diseases has reviewed the plan with interest and is considered a potential partner for development of the product under a CRADA agreement. The manufacture of the GMP lots of D35 for clinical trials can be carried out at one of several fee-for-service contract manufacturing facilities including Girindus in the USA or Gene Design, Inc in Japan. Daniela Verthelyi at FDA and Ken Ishii at Osaka University could provide technical expertise in oligonucleotide manufacture and qualification, however the FDA would not play a role in the design or implementation of the clinical trials as this would be construed as a conflict of interest. The laboratory of Daniela Verthelyi would be available to perform head to head functional study comparisons of the GMP product with D35 manufactured at the FDA to provide a link between the data collected using the product manufactured at FDA with that produced by the contract manufacturer. The WHO-based consortia would supervise the selection of the manufacturing site as well as coordinate and audit the pre-clinical and clinical trials following current Good Pre-clinical and Clinical Practices guidelines. Further, the expectation is that WHO consortia would include representatives from the countries affected by CL and involve the corresponding regulatory authorities early in the process thus facilitating regulatory approval, adoption and deployment of the D35 by health systems. Following licensure, WHO would provide support by pre-
qualifying the manufacturing sites, ensure affordability of the product for countries that require it and/or implement a UN /donor based medicine procurement mechanism that ensures access to the product.

12. **Who could potentially manufacture the final product?**

The manufacture of D35 drug substance employs standard solid phase techniques to build the oligonucleotide sequentially, and the final commercial product is likely to be a lyophilized formulation. There are a number of potential companies and contract manufacturing organizations worldwide that can fulfill the necessary technical and quality requirements. A WHO consortia will evaluate potential commercial partners during the clinical development phase that will become responsible for the industrial development, registration, manufacturing and distribution of the product. DNDi has expressed an interest to support the process of identifying partners to manufacture the final product and develop strategies to ensure its access. This includes negotiation of suitable agreements to ensure sustainable production and patient access.

13. **What could be the role of WHO, if any, in this demonstration project to bring this venture to fruition?**

Participation of WHO in this demonstration project would be instrumental in the development of the product, at early stages through the performance of quality assurance assessments and monitoring and auditing of the trials. WHO will become the convening entity for a coordinating collaborative consortia that includes national health and regulatory authorities, donors and other relevant stakeholders. The involvement of the member countries as part of a WHO-based consortia would allow engagement of national health authorities from countries that may benefit from the product and for the implementation of larger clinical trials that assess the safety and efficacy of the new treatment regimen in patients infected with different strains of leishmaniasis. It will also enable data sharing early in the development process and facilitate market authorizations. At later stages we hope WHO would play a role in pre-qualifying manufacturing sites, monitoring clinical trials, and producing recommendations to implement the new therapeutic approach if indicated. Lastly, since accelerating the induction of a strong Th1 type response may apply to other chronic infectious diseases, WHO might play a role in assessing whether the use of innate immune modulators for other indications is warranted.

14. **Please outline a timeframe and projected milestones for the project covering the first 5 years. This should also highlight the immediate actions that need to be taken?**


Year 3: Phase Ia and Ib in CL patients studies completed. Phase II studies in CL and PKDL plans developed and submitted to all pertinent regulatory authorities. Phase II studies initiated.
Year 4: Phase II studies completed and analyzed. Selection of sites for manufacture of Phase III clinical material and of Phase III clinical studies. Phase III studies submitted to regulatory authorities. Initiation of commercial product design and development; manufacture of clinical supplies for Phase III. Validation of assays for product release and characterization. Pre-NDA meeting with corresponding regulatory authorities.
Year 5-6: Phase III studies at multiple clinical sites in South America, Asia and Africa. Commercial product scale-up, validation and registration activities initiated.

15. What is the intellectual property (IP) landscape relative to this project? Is there any IP, e.g. patents that need to be licensed in to be able to develop and market the product in developing countries? How would IP and related intellectual assets, including knowhow, proposed to be managed in this project?
FDA holds the patents for both D35 and PRO-D35, the alternative chemistry of the oligonucleotide backbone discussed above with similar intentions of development. To facilitate this project, FDA could establish a Cooperative Research and Development Agreement (CRADA) with WHO for the further research and development of this product. It also may include the University of Osaka or DNDi in this CRADA or establish a separate agreement with them for this project. Under the standard terms of the CRADA, the FDA’s partner would have access to the patented material as part of the FDA’s background inventions, and would not need to license the patented products to proceed under this agreement. Further, for any inventions that are made by FDA alone or jointly with the CRADA partner during the CRADA, FDA would grant the Collaborator access by “an exclusive option to elect an exclusive or nonexclusive commercialization license.” If the Collaborator does not exercise its option to elect an exclusive license or decides to elect a nonexclusive license, FDA can license the CRADA subject invention to others. Discussions about the licenses, fees, and ability to license the technology more broadly will occur at a later date, however FDA, like NIH, has Non-Profit License Agreement available for patented inventions and non-patented biological materials from NIH and FDA intramural laboratories. The available license scope includes vaccines, drugs, therapeutics and diagnostics (or enabling technologies to produce such products) to prevent, diagnose or treat neglected tropical diseases (“NTDs”, as defined by WHO), HIV, TB and malaria in humans or animals. The Agreement establishes reasonable terms, including patent cost reimbursement and royalties that would be acceptable to most non-profit institutions. The license is available to non-profits with a demonstrated commitment to diligence in providing broad global access to technologies, products and services consistent with the submission of an acceptable product development plan to bring the technology to practical application. The model license usually has a $2,000 up front fee and modest royalties on sales of 1.5% for exclusive licenses and 0.75% for non-exclusive licenses, excluding sales to public sector institutions or institutions using public-sector funds (such as PEPFAR or Global Fund). If the licensee sublicenses the technology to another institution to bring it to market, the sublicense fee for exclusive licenses will be 15% of the value received if NIH or FDA has provided in vivo model data and 10% if such data have not been provided. For non-exclusive licenses, the sublicensing fees are half these amounts.

16.* What would be the strategy to ensure access to the product once it is developed?
Treatment of chronic diseases in the countries affected by CL and PKLD relies heavily on local governments, which often depend on programs to purchase and distribute the drug product to the appropriate health care centers. The plan is that the organization of a WHO-lead consortia that includes representatives from the countries where CL and PKLD have high impact will allow for 1) the early involvement of local public health and regulatory authorities to identify and access the target populations; 2) the implementation of purchasing mechanisms that enable local governments to access the medication; and 3) implementation of a program of pre-qualification of manufacturing facilities in the target countries to supply the region. Alternatively, the consortia will look for manufacturing partners that could produce the product under conditions that allow for facilitated access to the product. These objectives may be facilitated by organizations such as DNDi, or MSF, which have a commitment to make sure that the needed products reach those who need them. Of note, the parties involved in the development of the program are not for-profit and therefore there is no impetus to recover the investment in product discovery.

17. How could the project be financed paying particular attention to the need to demonstrate new and innovative forms of financing? Also provide an estimated cost of the project.

The estimated cost of the project is as follows:
Production of GMP quality D35 in contract facility: 250,000
Comparability study with product manufactured at FDA core facility: 50,000
Preclinical studies: 250,000
Formulation development 200,000
Clinical drug product stability 100,000
Assay development and validation for product characterization (to be conducted during Phase I clinical studies (estimation based on 55 patients): $357,500
Includes:
  Cost per patient (including diagnosis, labs for enrollment, outcome measurement, compensation): 1500/per patient
  Clinical monitoring: $80,000
  Training and data management: $75,000
  Study Insurance: $15,000
  Supervision (staff and travel costs): $105,000
Phase II clinical studies (estimation based on 150 patients): $580,000
Includes:
  Cost per patient (including diagnosis, labs for enrollment, outcome measurement, compensation): 1500/per patient
  Clinical monitoring: $120,000
Training and data management: $100,000  
Study Insurance: $30,000  
Supervision (staff and travel costs): $105,000  
Total needed to take the project to phase III: $1,737,500  

Additional costs to run the WHO based consortia and to further develop the product need to be determined.  

18. **How could the project be governed and coordinated paying particular attention to the need to demonstrate better way of coordination?**  
WHO can facilitate the establishment of a global consortia to oversee the project. This should be a dynamic ad-hoc group in charge of governance and designated to oversee coordination and implementation of the project but would ideally delegate day-to-day operation to an entity such as DNDi or similar. The consortia should include representatives from national health authorities from countries than can benefit from the technology, national regulatory authorities and experts that can oversee the project. The benefits of such a model are multifold: it will help optimize allocation of funds and other resources, facilitate information sharing, support active engagement of countries that will benefit from the product, improve transparency and dispel conflict of interest.

19. **Have any donor agencies/governments already indicated interest in supporting the project?**  
DNDi reviewed and is interested in the development of this project but does not have the necessary funds to see it through at this time. A joint letter of intent to submit a proposal has been sent by DNDI, Ken Ishii and Daniela Verthelyi to a Japanese funding agency to finance the manufacture and preclinical testing of the ODN. In addition, Daniela Verthelyi has approached scientists at the Center for Human Immunology at the US-NIH to provide scientific support in the characterization of the immune response of the patients. Their involvement would significantly add to the project in that they have expertise in performing in depth analysis of the local (skin) and systemic immune response of the patients to the parasite in the presence and absence of treatment using flow cytometry, message RNA arrays and bioinformatics. This represents a unique opportunity to gain a new understanding of the pathophysiology of the disease, particularly PKDL, where there are serious gaps in our knowledge.

Reference List


Individual innate immune response modifiers induce distinct responses in human PBMC.

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Similar responses recorded on >100 PBMC samples stimulated in vitro with 1-3 uM of oligonucleotide.
Sequence specific response to TLR9 agonists is conserved among primates

Response of PBMC from healthy blood donors or rhesus macaque stimulated in vitro with 103 uM of D ODN. D35 and D29 have similar sequences and backbone structure, while K ODN have CpG motifs, but different framing sequence and backbone. Results show mean + SD of 6 samples.
In vivo D35 induces increase levels of IFN\(\alpha\) and IFN\(\gamma\) in serum

Macaque treated *in vivo* with D35 (SC 0.5mg/kg on days 1, 3 & 7). 5 macaques per group. Graphs show mRNA expression *ex vivo* over time.
Fold Change in expression of key immune genes in the skin of rhesus macaques injected s.c. with D-type CpG (500ng/kg)

Mean fold increase in gene expression of 3 macaques 24hrs after single injection of D35 relative to skin from the same macaques prior to treatment. Note local increase in gene related to inflammation (yellow), chemokines (orange), antigen presentation (green), type I interferons (pink), and IFNg (light blue).
Fold increase in gene expression in skin over time in macaques injected with D35 or L. major (note response in hours vs. days). Mean change in expression levels over saline treated macaques. 3 macaque/group.

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Model: Leishmania amazonensis

Challenged w/L. major or amazonensis (10^6 metacyclic promastigotes) ID

Typical course of Leishmania lesion.
Juvenile rhesus macaques were challenged with L. amazonensis (10^6 metacyclic promastigotes) and treated ID with CpG ODN D35 or K3 3 days before and after challenge (500ug/macaque). Note: Lesion size of D ODN treated animals was significantly reduced when compared to saline treated controls or macaques treated with a different ODN sequence (p < .03, N=6/group).
Figure Local or systemic treatment with D ODN reduces the severity of *L. major* lesions

Healthy macaques

SIV-infected macaques

- 4-5 monkeys/group
- 500 μg ODN on days -3 and +3. IM and SC ODN were administered in the leg.
- *L. Major*, 10^7 metacyclic promastigotes id on forehead
- Note that SIV infected macaques do not heal spontaneously. There was no increase in VL during study with D ODN in SIV infected macaques
D35 reduces the severity of lesions in macaques with established infections

- 4-6 macaques per group challenged with $10^6$ metacyclic promastigotes
- Treated 10-15 days after challenge with CpG ODN D35 ID (500ug id) or SC (0.5mg/kg in the leg).
Examples of lesions in macaques challenged with L. major and left untreated or treated with D35 or Pro-D35 (SC 0.5mg/kg)

Lesions ~3 weeks post challenge
Systemic Pro-D35 ODN protects rhesus from cutaneous leishmaniasis and accelerates the induction of antigen specific IFN\(\gamma\) producing cells

Note that macaques treated with CpG ODN develop faster antigen-specific IFN\(\gamma\) producing cells than untreated macaques.
CBC and chem. panels for rhesus macaque challenged with L. major and treated with D-ODN

No significant differences observed for most parameters. Exceptions are marked with asterisks and data is provided.

- WBC
- RBC
- hemo
- Hematocrit*
- MCV
- plat
- Polys A
- Lymphs
- Monos
- eos
- Bas
- Polys
- Lymphs A
- Monos A
- Eos A
- Basop A
- Neut
- Neut A
- Na
- K
- Cl
- Ca
- Mg
- P
- glucose
- BUN
- Creat
- UA
- Alb
- Tot protein
- Cholest
- Trigly
- Alk Phos
- ALT
- AST*
- AST A
- amylase
- CK
- LD

* p<0.05 Statistical differences only evident in individual T tests, unlikely to be clinically meaningful.
### Proposed pre-clinical studies

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<td>Clinical signs, body weight, food consumption, hematology and blood chemistry, Pathology macro + micro</td>
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<td>Multi-dose Mice (6-12 wk old at start) 6 F + 6 M</td>
<td>100ug/ wk SQ x 5 doses</td>
<td>Clinical signs, body weight, food consumption, hematology and blood chemistry, toxicokinetics on 1st and 5th dose. Pathology macro + micro ssDNA abs.</td>
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<tr>
<td><strong>Single dose</strong> Rhesus macaques 1F+1M /dose</td>
<td>0.3mg/kg, &amp; 3mg/kg SQ (10 times the maximal dose planned in humans)</td>
<td>Clinical signs, body weight, food consumption, urinalysis, hematology and blood chemistry, toxicokinetics</td>
</tr>
<tr>
<td><strong>Multi-dose</strong> Rhesus macaques 3 M +3 F</td>
<td>3mg/kg q2wk SQ x 4 doses</td>
<td>Clinical signs, body weight, food consumption, hematology and blood chemistry, toxicokinetics on 1st and 4th dose. Pathology macro + micro ssDNA abs.</td>
</tr>
</tbody>
</table>
## Proposed Phase Ia clinical trial: CL

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>#doses</th>
<th>Design</th>
<th>Dose/pt (assuming 70kg body weight)</th>
<th>Total D35 needed for Phase I study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.01</td>
<td>1</td>
<td>4:1</td>
<td>0.7 mg</td>
<td>2.8 mg</td>
</tr>
<tr>
<td>2</td>
<td>0.03</td>
<td>1</td>
<td>4:1</td>
<td>2.1 mg</td>
<td>8.4 mg</td>
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<tr>
<td>3</td>
<td>0.1</td>
<td>1</td>
<td>4:1</td>
<td>7 mg</td>
<td>28mg</td>
</tr>
<tr>
<td>4</td>
<td>0.3</td>
<td>1</td>
<td>8:2</td>
<td>21 mg</td>
<td>210mg</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>1</td>
<td>8:2</td>
<td>70mg</td>
<td>700 mg</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>949.2 mg</td>
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</table>

## Proposed Phase Ib clinical trial: PKDL

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>#doses</th>
<th>Design</th>
<th>Dose/pt (assuming 70kg body weight)</th>
<th>Total D35 needed for Phase I study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.3</td>
<td>3</td>
<td>8:2</td>
<td>63 mg</td>
<td>504mg</td>
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<tr>
<td>2</td>
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<td>3</td>
<td>8:2</td>
<td>210mg</td>
<td>1680 mg</td>
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<tr>
<td>Total</td>
<td></td>
<td>3</td>
<td>8:2</td>
<td>2184 mg</td>
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Proposed Single dose Phase II clinical trial:

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Patients</th>
<th>#doses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antimonials</td>
<td>50</td>
<td>1</td>
</tr>
<tr>
<td>Antimonials+D35</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>150</td>
<td></td>
</tr>
</tbody>
</table>
ADDENDUM

Development of Class D CpG ODN (D35) as an Adjunct to Chemotherapy for cutaneous leishmaniasis and Post Kala-Azar Dermal Leishmaniasis (PKDL)

1. Intends to delink the price of the final product from the cost of the R&D.

Delinking is the fundamental principle on which this proposal is based. FDA publishes the results of the basic scientific research in peer reviewed journals so they can be accessed by the public. The US-FDA does not contemplate recovering the investment in R&D as part of the Agency’s mission. One of the agency’s goals is to foster fundamental creative discoveries, innovative research strategies, and their applications to protect and improve human health. Indeed, for licenses on inventions coming from the Health and Human services (HHS) intramural program, HHS promotes commercial development of technologies in a way that provides broad accessibility. This project will put in practice the effective use of equitable or humanitarian licensing as a means to improve global access to essential products by ensuring affordability of the final product. The US-FDA developed and holds the patents for D35 and Pro-D35 and can license them to a WHO consortium. Thus, the R&D costs and the price of the product will be fully delinked under this proposal.

2. Utilizes collaborative approaches, including open knowledge innovation approaches.

The objective for this demonstration project is to develop a short, safe, affordable and field-friendly treatment that are efficacious for CL and PKDL using a collaborative approach governed by a WHO based consortium that will coordinate Member States representatives (in particular, national regulatory and health authorities from developing countries where leishmaniasis is endemic and that may benefit from the R&D), scientific institutions, donors and other relevant stakeholders convened by WHO. While necessary R&D may be conducted in an array of institutions both in developed (US-FDA, Osaka University for example) and developing countries (additional testing and clinical trials should be conducted in developing countries), the consortium will ensure that results and knowledge produced during the course of the project is open and transferable. This approach will enable any result that emanates from this project to reach the public domain and where information can be used as the basis of more ideas and new developments. The establishment of such a development path may enable future joint development efforts that follow the same principles. For example, similar approaches might be useful for treating similar entities and results from these trials may open the door to new applications of the proposed technology.

3. Utilizes licensing approaches that secure access to your research outputs and final products.

The US-FDA holds the patents for both D35 and PRO-D35, the alternative chemistry of the oligonucleotide backbone discussed above with similar intentions of development. FDA, like NIH, has Non-Profit License Agreements available for patented inventions and non-patented biological materials from intramural laboratories. The available license scope includes vaccines, drugs, therapeutics and diagnostics (or enabling technologies to produce such products) to prevent, diagnose or treat neglected tropical diseases (“NTDs”, as defined by WHO), HIV, TB and malaria in humans or animals. The Agreement establishes reasonable terms, including patent cost reimbursement and royalties that would be acceptable to most non-profit institutions. The license is available to non-profits with a demonstrated commitment to diligence in providing broad global access to technologies, products and services consistent with the submission of an acceptable product development plan to bring the technology to practical application. For example a license could have a $2,000 up front fee and modest royalties on sales of 1.5% for exclusive licenses and 0.75% for non-exclusive licenses, excluding sales to public sector institutions or institutions using public-sector funds (such as PEPFAR or Global Fund). If the
licensee sublicenses the technology to another institution to bring it to market, the sublicense fee for exclusive licenses will be 15% of the value received if NIH or FDA has provided in vivo model data and 10% if such data have not been provided. For non-exclusive licenses, the sublicensing fees would be half these amounts. Additional research on the patented products performed by the FDA is made available without restrictions.

4. Proposes and fosters financing mechanisms including innovative, sustainable and pooled funding.

The proposal includes the development of a WHO-based consortium that will be in charge of managing and coordinating the project and will include stakeholders from key institutions and Member States. The funds that sustain the cost of implementing the necessary R&D could come from pooling monies within the global health community. The resources needed for this project include not only funds but expertise (clinical trials, laboratory testing, manufacturing capacities among others) for a sustainable and efficient implementation. The mechanism chosen to pooled these resources is beyond the scope of this proposal and should be the subject of an in-depth analysis of Member States and/or the coordinating consortium

5. Fosters effective and efficient coordination mechanisms amongst existing organizations/initiatives.

This project would be coordinated by a WHO-based consortium that includes representatives from the Member States and including those countries affected by Leishmaniasis. Other stakeholders that can bring expertise and knowledge to the project should also be including in the coordinating mechanism and are by no means limited to the authors of the proposal. This multi-stakeholder body will ensure proper coordination and an open knowledge approach of all relevant institutions. Importantly, because the technology to produce the product is easily transferable and manufacturing could eventually take place at facilities in the affected regions, the consortium could oversee that technology transfer does effectively take place. Scientists form FDA and Osaka University could provide technical expertise at different points in the R&D process, however the FDA would not play a role in the design or implementation of the clinical trials as this would be construed as a conflict of interest. If clinical trials are deemed successful, the WHO-based consortium would supervise the selection of the manufacturing site as well as coordinate and audit the pre-clinical and clinical trials following current Good Pre-clinical and Clinical Practices guidelines. Further, the expectation is that WHO consortium will not only include public health representatives from the countries affected by CL but also involve the corresponding regulatory authorities of these countries early in the process thus facilitating regulatory approval, adoption and deployment of the D35 by health systems.

6. Strengthens capacity for research, development and production, including through technology transfer, in developing countries.

The manufacturing platform for D35 is easily transferable and the expectation is that one or more manufacturing sites would be chosen in the affected regions to produce the material for clinical trials and then after licensure WHO would provide support by pre-qualifying additional manufacturing sites to ensure affordability of the product for countries that require it and/or implement a UN /donor based medicine procurement mechanism that ensures access to the product. As mention before, the US-FDA licensing arrangement allows sublicensing and the coordinating consortium will be responsible for selecting and overseeing these agreements in a way to ensure that maximizes access to an affordable, safe, quality and effective product.