Status of Vaccine Research and Development of Vaccines for Human Hookworm Infection
Prepared for WHO PD-VAC

I. About the Disease and Pathogen

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

Human hookworm infection is a neglected tropical disease caused predominantly by the nematode parasite *Necator americanus* [1]. New estimates indicate that approximately 440 million people are infected with hookworm worldwide with the majority of cases found in the developing regions of South Asia (140 million cases), Sub-Saharan Africa (118 million), Southeast Asia (77 million), East Asia (64.5 million), and the Latin American and Caribbean region (30 million) [2,3]. In these areas, hookworm infection is a leading cause of iron-deficiency anemia, caused by the ability of adult hookworms to extract blood from the intestinal mucosa and submucosa [4].

At 3.2 million disability adjusted life years (DALYs), the Global Burden of Disease Study 2010 has found that hookworm (together with Leishmaniasis and Schistosomiasis) is the most important neglected tropical disease [2,3].

Hookworm infection is transmitted when the larval stages infect through the skin. Because children and pregnant women have the lowest iron reserves these two populations are considered the most vulnerable to hookworm blood loss [5,6]. Children with chronic hookworm infection experience anemia and cognitive and developmental delays; hookworm has been shown to cause reductions in school performance and attendance, this potentially translating into reductions in future wage earnings. It has been further estimated that approximately 7 million pregnant women in Africa – almost one third of pregnancies in Africa - are also infected with hookworm, making this disease one of the most common complications of pregnancy [6]. Moreover, hookworm and malaria co-infections are common thereby adding to the anemia for both of these populations [7].

The current approach to the control of hookworm is mass drug administration with a single annual tablet of either albendazole or mebendazole. However, the cure rates with these drugs have been surprisingly low and for mebendazole, children often re-acquire helminth infections several months after and there is no improvement in anemia following treatment [5,8]. The development of anti-hookworm vaccines is considered to provide an alternative, cost-effective control measure to complement conventional chemotherapy [9].

II. Overview of Current Efforts

A. Biological feasibility for vaccine development

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

Natural immune response to hookworm infection does not appear to provide the human host with adequate protection and therefore does not provide a model to mimic for vaccine development. Human hookworms are strong immunomodulators from the onset of infection, which enables infections to persist for years. Even with chemotherapy, rapid reinfection is the rule in both the pediatric and adult populations. Furthermore, the prevalence and intensity of hookworm infections appear to increase with
age, which suggests a failure to develop effective acquired immunity [10].

However, biological feasibility for vaccine development has convincingly been demonstrated by the relative success of a radiation-attenuated infective larva stage (L3) commercial canine hookworm vaccine (marketed in the United States in the 1970s), which achieved high levels of protection against disease due to *A. caninum*. Although the vaccine was discontinued due to the high cost of production and complex storage and distribution issues, vaccinated canines were protected from disease and anemia. Passive transfer of sera from vaccinated dogs protected non-vaccinated dogs in challenge studies, demonstrating the primary role of antibodies in this protection [11].

Given the obvious challenges of the radiation attenuated approach for human vaccines, the strategy shifted to identifying and testing antigens from the L3 stage that can reproduce the protection seen by the live attenuated vaccine and the determining antigens from the parasite that could provide protection. A parallel approach sought to identify macromolecules required for parasite human blood feeding, and thus essential to worm survival [12]. Both strategies have produced recombinant antigens, with animal proof-of-concept showing protection against challenge infections. However, development of the leading L3 stage candidate against *Necator americanus*, *Na*-ASP-2 hookworm vaccine, was halted following results from clinical trials in Brazil where a subset of chronically infected subjects with high pre-vaccination IgE titers to larval antigens experienced generalized urticaria following vaccination [13].

For the lead approach targeting parasite blood feeding, (Fig. 2) [6, 14], several enzymes have been identified as promising protective antigens, including the aspartic protease-hemoglobinase APR-1 and the glutathione S-transferase (GST). Recombinant candidates of *Na*-APR-1 (modified by site directed mutagenesis to abolish the protease catalytic activity) and *Na*-GST-1 have both shown proof-of-concept laboratory dogs, and in the case of *Na*-APR-1, induction of neutralizing antibodies against multiple heterologous strains of the parasite [15].

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

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

One of the reasons cited for the canine vaccine’s commercial failure was the fact that sterile immunity was not induced. It is important to note that sterilizing immunity is not required for a human hookworm vaccine to deliver clinical benefit. This is because the clinical pathology of human hookworm infections is related to intestinal blood and protein loss, which is proportional to the worm burden. The overall goal
of a vaccine that would reduce the likelihood of vaccinated individuals developing severe hookworm infections and reduce blood and nutrient loss to a level not associated with clinical disease would still have a significant impact on reducing the burden of disease.

As described previously, vaccine discovery and design approaches are mostly focused on subunit approaches for delivering potentially protective targets. The bulk of development is currently supported through academic centers and public-private product development partnerships, the Human Hookworm Vaccine Initiative (HHVI) of the Sabin Vaccine Institute Product Development Partnership (Sabin PDP) [14, 16], which is also linked to a European Commission-supported Framework Program 7 (FP7) project known as HOOKVAC (http://hookvac.eu/).

The antigen selection strategy for the human hookworm vaccines (HHV) have been based on several key criteria: (1) efficacy in animal trials (2) immuno-epidemiological observations in individuals residing in endemic areas (3) feasibility of protein expression and scaled-up manufacture using low-cost expression systems such as yeast, bacteria, or plants and (4) a plausible mechanism of protection associated with them [13]. From approximately two-dozen proteins that are putatively involved in the hookworm blood-feeding process, the two lead candidate antigens, Na-APR-1 and Na-GST-1, have been selected for clinical development. The eventual goal is to license a vaccine that contains, Na-GST-1 and Na-APR-1 recombinant antigens, formulated on an aluminum hydroxide adjuvant (Alhydrogel®). Clinical testing will also evaluate whether an additional adjuvant will be required to achieve acceptable immunogenicity. Such adjuvants are synthetic Toll-like receptor (TLR) agonists, for example, glucopyranosyl lipid A (GLA) or CpG oligodeoxynucleotide. Following phase 1 testing of each individual antigen in adults and children, they will be combined into a single co-formulated product.

The target product profile of the HHV includes the following important features [16]:

• Intended for at risk children under the age of 10.
• Administered by intramuscular injection up to two doses and stored at between 2 °C and 8 °C.
• Administered concurrently with other childhood vaccines such as the measles vaccine.
• Efficacy of at least 80% in preventing moderate and heavy hookworm infections caused by *N. americanus* and the resulting intestinal blood loss and anemia

Widespread use of an effective HHV would significantly improve global public health averting up to 3.2 million DALYs annually and greatly reducing a leading cause of global anemia [17]. As outlined below, it could also become a critical technology for the eventual elimination of hookworm infection in low- and middle-income countries. Such a vaccine has been described as an ‘antipoverty vaccine’ because of its potential to improve the economic development of affected populations. Also, due to the synergistic effect of concurrent infection with malaria and hookworm on incidence of anemia, using the vaccine in sub-Saharan Africa could potentially also reduce the burden of disease due to *Plasmodium falciparum*.

III. Technical and Regulatory Assessment

*Highlight perceived positive/negative aspects in clinical/regulatory pathways e.g. well established product development and regulatory pathway to licensure, accepted immune correlates and/or functional assays, accepted surrogate efficacy endpoints, existence of well accepted animal or challenge models, agreed trial designs and endpoints. Possibilities to develop case for correlates/surrogates should be included.*

The monovalent Na-GST-1 and Na-APR-1 vaccines are currently in phase 1 clinical trials in Brazil and the US, with plans to advance clinical testing in Gabon. Three possible different regulatory strategies have been proposed: (1) registration in an endemic country where the vaccine will be manufactured at industrial scale (e.g. Brazil) followed by WHO prequalification (2) article 58 of the European Agency or (3) the US FDA. Clinical endpoints of the HHV are being developed in parallel with parasitological
endpoints including number of worms, fecal egg counts, and fecal blood loss. Neutralizing anti-enzyme antibodies are also being developed as potential surrogate correlates of immunity.

IV. Status of vaccine R&D activities

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

Both Na-GST-1 and Na-APR-1 have been manufactured at pilot scale according to current Good Manufacturing Practices. A major aspect of the HHV will continue under HOOKVAC (http://hookvac.eu/). The HOOKVAC Consortium is led by the Amsterdam Institute of Global Health and Development in collaboration with the Sabin PDP. Other members include the Amsterdam Medical Center, Q Biologicals, Universitat Tubingen, CERMEL (Centre de Recherche Médicales de Lambarene), Pharmidex, Leiden University, Baylor College of Medicine-Texas Children’s Hospital, and George Washington University. The major objectives of HOOKVAC include: (1) establish safety and immunogenicity of the vaccine candidate in an endemic population, (2) improve the manufacturing processes (3) provide clinical proof of concept through testing in Gabon, and (4) improve accessibility of the vaccine in endemic areas.

Table 1: Development Status of Current Vaccine Candidates (POC = Proof of concept trial)

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