Review

EXPERT REVIEWS

Advancing a multivalent 'Pan-anthelmintic' vaccine against soil-transmitted nematode infections

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The Sabin Vaccine Institute Product Development Partnership is developing a Pan-anthelmintic vaccine that simultaneously targets the major soil-transmitted nematode infections, in other words, ascariasis, trichuriasis and hookworm infection. The approach builds off the current bivalent Human Hookworm Vaccine now in clinical development and would ultimately add both a larval *Ascaris lumbricoides* antigen and an adult-stage *Trichuris trichiura* antigen from the parasite stichosome. Each selected antigen would partially reproduce the protective immunity afforded by UV-attenuated *Ascaris* eggs and *Trichuris* stichosome extracts, respectively. Final antigen selection will apply a ranking system that includes the evaluation of expression yields and solubility, feasibility of process development and the absence of circulating antigen-specific IgE among populations living in helminth-endemic regions. Here we describe a five year roadmap for the antigen discovery, feasibility and antigen selection, which will ultimately lead to the scale-up expression, process development, manufacture, good laboratory practices toxicology and preclinical evaluation, ultimately leading to Phase 1 clinical testing.

KEYWORDS: *Ascaris lumbricoides* • *Ascaris suum*, deworming • geohelminth • hookworm • intestinal helminth • *Necator americanus* • Pan-anthelmintic vaccine • soil-transmitted helminth • soil-transmitted nematode • *Trichinella spiralis* • *Trichuris muris* • *Trichuris trichiura*

Rationale for a Pan-anthelmintic vaccine

The three major soil-transmitted nematode infections, in other words, ascariasis, trichuriasis and hookworm infections, are highly prevalent neglected tropical diseases that rank near the top of the list of most common human afflictions [1]. According to some estimates, approximately 800 million people are infected with the roundworm, *Ascaris lumbricoides*, and 600 million people with the whipworm, *Trichuris trichiura* or hookworms, mostly by *Necator americanus* [1,2]. There is widespread geographical overlap of these three soil-transmitted nematode infections (also referred to as soil-transmitted helminth, intestinal helminth, intestinal nematode or geohelminth infections) in impoverished areas of sub-Saharan Africa, East Asia and South Asia and tropical regions of Central and South America [3]. Coinfections with two or even all three soil-transmitted nematode infections are extremely common in children [2,3]. The WHO currently estimates that 874.5 million children are infected or exposed to *A. lumbricoides*, *T. trichiura* and hookworms, and therefore, require regular and periodic anthelmintic treatment ('deworming') (TABLE 1) [4]. Such children are often chronically infected and suffer from long-term disabling consequences including growth stunting, reductions in physical fitness, and cognitive and intellectual delays [2]. Moreover, there are millions of pregnant women in developing countries with soil-transmitted nematode infections, especially hookworm infection [5]. Recent estimates from the Global Burden of Disease Study 2010 indicate that soil-transmitted nematode infections are responsible for 5.18 million disability-adjusted life years, which leads all neglected tropical diseases [6]. In addition, ascariasis is responsible for 2,700 deaths annually [7].

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Table 1. Geographical distribution (according to WHO regions) of children who require regular deworming for their soil-transmitted nematode infections.

WHO region	Number of children requiring albendazole or mebendazole (million)	Worldwide population requiring treatment (%)
African (AFRO)	296.5	33.8
Americas (PAHO)	48.0	05.4
Southeast Asia (SEARO)	371.0	42.4
Europe	4.3	00.5
Eastern Mediterranean (EMRO)	80.5	09.2
Western Pacific (WPRO)	73.3	08.4
Globally	874.5	100

AFRO: Regional office for Africa; EMRO: Eastern Mediterranean regional office; PAHO: Pan American Health Organization; SEARO: Regional office for Southeast Asia; WPRO: Regional office for the Western Pacific.
Data taken from [4].

Global control of soil-transmitted nematode infections is based on annual (or sometimes twice-annually) mass drug administration with either albendazole or mebendazole for children between the ages of 1 and 14 years who live in areas where the prevalence of these infections exceeds 20% [4]. The WHO estimates that in 2011, 30.6% of the world's children who require mass treatment actually received their medication [4]. Currently, pregnant women do not consistently receive anthelmintic treatments even though they too might benefit from deworming in their second or third trimester [5].

Aside from the low treatment coverage for children, a situation that is being remedied through expanded donations of albendazole and mebendazole and new global policies including a recent World Health Assembly resolution [8], there are concerns that pediatric mass drug administration alone may not be sufficient to effect global control of soil-transmitted nematode infections and certainly not global elimination [9]. Among the major reasons why annual deworming may not be successful as an isolated intervention:

- High rates of post-treatment reinfection, especially in areas of intense transmission, which might require a higher frequency of deworming, in some cases every 4 months or more [10];
- Lack of efficacy for single-dose albendazole or mebendazole to effect cures or sufficient worm burden reductions against *T. trichiura* and *N. americanus* [9,11,12];
- The specter, as yet unproven, of emerging drug resistance;
- Failure to fully consider the transmission dynamics of soil-transmitted nematode infections, especially for hookworm [13], which affects both adult and pediatric populations and would require targeting of both populations for a truly effective elimination strategy [9];
- Failure to co-implement aggressive programs of sanitation and access to clean water in order to complement deworming [14].

A recent Cochrane analysis has questioned the benefits of mass treatments and deworming based on lack of consistent evidence for its beneficial impact on nutrition, hemoglobin and school attendance or performance [15]. It is likely that some of the factors outlined above, in other words, rapid reinfection and lack of drug efficacies, have a role in the Cochrane findings. However, the Cochrane analysis also did not differentiate between the effects of individual nematode species or their differential drug susceptibilities [8]. As an example, hookworm infection has been linked to anemia in children and adults in a systematic review, with demonstrated benefits of albendazole (but not mebendazole) on improving anemia [16]. The Cochrane analysis partly blurs these findings by treating all soil-transmitted nematode infections and their treatments as equivalent [8]. Nevertheless, the information to date indicates that there are urgencies to improve the effectiveness and efficiencies of global deworming in order to achieve key milestones and Millennium Development Goals.

Approaches to improve global deworming were recently suggested [9]. They include adding ivermectin to albendazole in areas where high levels of trichuriasis (and strongyloidiasis) transmission occur, and possibly adding new anthelmintics such as tribendimidine or a novel *Bacillus thuringiensis*-derived Cry5B crystal protein in order to target hookworms [9]. Still another approach to the global control and elimination of soil-transmitted nematode infections includes the development and distribution of anthelmintic vaccines [17]. Hookworm disease accounts for almost two-thirds of the global disease burden from soil-transmitted nematode infections [6], so this infection was selected for initial vaccine development. Currently, the Sabin Vaccine Institute Product Development Partnership is developing a bivalent recombinant protein-based human hookworm vaccine [17]. The vaccine comprises two *Necator americanus* antigens, *Na*-GST-1 and *Na*-APR-1, which are formulated on alum together with a second adjuvant [17]. The *Na*-GST-1 component is in Phase 1 clinical trials with the expectation that *Na*-APR-1 will follow [17].

Here we explore the concept of adding *Ascaris* and *Trichuris* antigens to the two *Necator* hookworm antigens under development in order to launch a multivalent 'Pan-anthelmintic'

Table 2. Summary of major *Ascaris suum* antigens discovered to date.

Antigen	MW (kDa)	Function	Parasite stage/localization	Vaccine/level of protective immunity	*Ascaris lumbricoides* homolog	Ref.
As14	14	Unknown	Larva, adult, ES	r-protein/64%	Yes	[22]
As16	16	Larval molting	Larva, adult, ES, Intestine, hypodermis	r-protein/58%	Yes	[23]
As24	24	Larval molting	Larva, adult, ES, Intestine, hypodermis	r-protein/58%	Yes	[41,42]
As37	37	Ig family	Larva, adult, surface, muscle	r-protein/69%	Yes	[43–45]
As-Enol-1	46	Enolase, larval development	Larva, adult, ES	DNA/61%	ND	[46–48]
As-GST-1	26	GST	Adult, intestine	ND	ND	[77]

GST: Glutathione *S*-transferases; ND: Not done; r-protein: Recombinant protein.

vaccine for soil-transmitted nematode infections [9]. We will briefly review the status of existing *Ascaris* and *Trichuris* antigens that have undergone preclinical testing and the steps that will be required to perform additional antigen discovery, selection and down-selection to just a single vaccine candidate antigen (one for each nematode) for further preclinical evaluation, process development, manufacture, good laboratory practices toxicology preclinical evaluation and early stage clinical testing.

Status of *Ascaris* antigen discovery

Most of the work on ascariasis vaccine antigens has focused on the larval stages of the pig roundworm *Ascaris suum*. *A. suum* is found in pigs worldwide and is closely related to *A. lumbricoides*. They are morphologically and antigenically indistinguishable and differ by only 4% in their mitochondrial genome sequence [18]. There is controversy in the literature about whether *A. lumbricoides* and *A. suum* should be considered separate species or as part of the same complex, and the extent to which either parasite can jump between humans and pigs [19]. The genome of *A. suum* has been completed [20], with *A. lumbricoides* to follow soon [19], which should help to illuminate their differences.

While *A. suum* can complete its life cycle in pigs, their large size and the costs and complexities of animal husbandry have limited the use of pigs for purposes of vaccine development. As a convenient alternative, incomplete *A. suum* infections can be established in mice and other rodent hosts [21–23]. Following oral administration of *A. suum* eggs, the released larvae migrate from the small intestine to the liver (where they create characteristic 'white spots') and lungs. As described below, experimental vaccines have been successfully developed, which can reduce the number of migrating larvae following challenge infections [21,24]. These animal studies complement epidemiological studies conducted in human populations indicating that *A. lumbricoides* worm burdens in endemic areas and areas of high transmission are lower in adults than children [25]. Such observations are suggestive of possible evidence for acquired immunity.

In the laboratory, pigs can be orally vaccinated with infective eggs (containing living larvae) attenuated by ultraviolet irradiation [26–28]. Following challenge, vaccinated pigs exhibit significant reductions in both the number of migrating larvae and in the number of adult worms in the small intestine [27–29]. Induction of acquired immunity depends on both host antibody and cellular responses including intestinal infiltration of effector mast cells and eosinophils [30].

To assess the potential feasibility of reproducing protective immunity through subunit vaccines, several efforts have been made to immunize pigs and mice with either crude parasite antigens or recombinant *A. suum* antigens. With respect to the former, pigs can be partially protected using larval excretory and secretory (ES) products [28], antigens from the worm surface cuticle [31], liposome-formulated adult-stage antigens [32] and antigens in the adult worm pseudocoelomic fluid [33]. Although it might be possible to mass produce eggs, larvae and adult *Ascaris* worms for purposes of vaccine development, the opportunity to use such crude antigen sources is tempered by the allergenic nature of these biologicals and their propensity to elicit high levels of IgE including antibodies to dust mite antigens [34]. Indeed, ascariasis itself is associated with asthma and other atopic conditions in disease endemic areas [35,36]. ABA-1, the most abundant protein in *Ascaris* pseudocoelomic fluid, has been identified as a major parasite allergen [34,37,38], but others are likely present.

Chemically defined antigens

In order to circumvent the allergenic properties of *Ascaris* biologicals, several investigators (including N Tsuji at the Japanese National Agricultural Research Organization) have cloned and expressed chemically defined *A. suum* antigens, which in some cases elicit high levels of protective immunity (Table 2).

As14 & As16

These 14 and 16-kDa larval antigens were discovered by immunoscreening a larval cDNA library with sera from rabbits immunized with infective eggs [22,23]. The antigens are found in both larval and adult *Ascaris* worms and are present in ES as well as defined parasite structures [22,23]. Mice vaccinated intranasally with recombinant As14 or As16 (expressed in *Escherichia coli*) and formulated with cholera toxin B subunit exhibited about

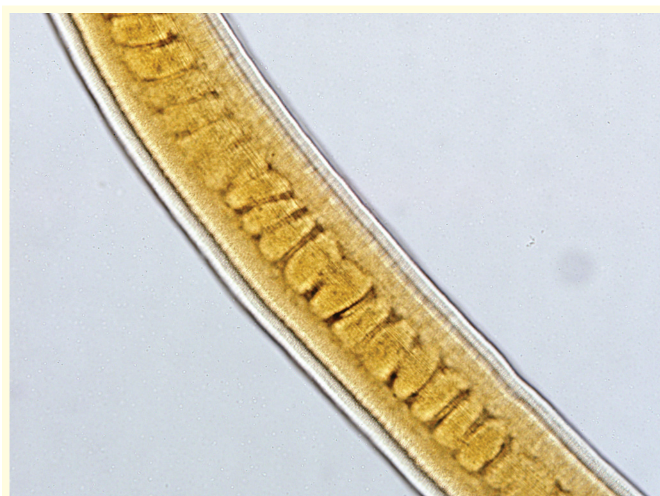

Figure 1. The stichosome of *Trichuris* spp. Single row of gland cells (stichocytes) in the esophagus of *Trichuris suis*; this characteristic structure of the esophagus is unique to enoplid nematodes.
Photo courtesy of Florian Röber, originally published in [85]
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60% reduction in the numbers of migrating larvae in mice after challenge compared with control mice [22,23]. Protection was associated with elevations in host antibody as well as IFN-γ and IL-10 in cultured supernatants of activated spleen cells, indicative of a mixed T_H1 and T_H2 response [22,23]. As14 has also been transgenically expressed in rice as a novel oral vaccine delivery approach [39], while As16 ortholog was cloned from *A. lumbricoides* and shown to be identical [40].

As24

This molecule was also cloned from *A. suum* larval cDNA, using immune sera from pigs infected repeatedly with *A. suum* eggs [41,42]. This nematode-specific protein was expressed in *E. coli* and found to elicit a 58% reduction in the recovery of *A. suum* lung-stage larvae. Protection was associated with high levels of anti-As24 IgG and elevated levels of both IFN-γ and IL-10, again indicating T_H1 and T_H2 mixed responses [41,42]. Anti-As24 IgG inhibited molting of *A. suum* lung stage indicating its function in the development of *Ascaris* larvae [42].

As37

As37 is an immunodominant *A. suum* larval surface antigen recognized by pig immune serum [43,44]. The molecule, which contains multiple immunoglobulin-like domains, is also found in adult worms. An *A. lumbricoides* ortholog has been cloned (Al37) and found to exhibit 91% amino acid homology with As37. The molecule elicited 69% reduction in larvae from the liver and lungs following *A. lumbricoides* egg challenge [45].

As-Enol-1

Ascaris suum utilizes exogenous glucose for energy generation through the glycolytic pathway, and enolase is one of the enzymes catalyzing the synthesis of phosphoenolpyruvate. *Ascaris suum* enolase (*As*-Enol-1) was first cloned from an *A. suum* infective larvae-specific cDNA library using a microarray analysis [46], and it was also identified as one of the major secreted proteins in *A. suum* adult ES products that can trigger nitric oxide production in macrophages [47]. Specific knockout of *As-enol-1* by RNAi resulted in the delay of larval development [48]. Mice immunized with DNA coding for *As*-Enol-1 exhibited 61% reduction in larvae recovery from lung compared with empty plasmid DNA control, with protection correlated to high levels of specific antibody and lymphoproliferative responses [48].

As-GST-1

Many parasitic nematode glutathione *S*-transferases belong to a unique Nu class of enzyme that is involved in heme scavenging and detoxification [49]. As noted above, *Na*-GST-1 from *N. americanus* is a protective antigen found in the Human Hookworm Vaccine [17], and the *A. suum* ortholog exhibits 50% amino acid homology to this enzyme [50].

Approaches to antigen discovery, scoring & ranking

Since five of these six antigens listed in Table 2 are the major antigens recognized by protective immune sera induced by living UV-attenuated eggs, we are now comparing them for protective efficacy while simultaneously determining the requirements for additional antigen discovery. In order to down-select an appropriate antigen(s) for further development, these recombinant antigens will be compared in a mouse challenge model. The recombinant proteins will be produced in an appropriate expression system (i.e., bacteria, yeast or baculovirus) and used as immunogens for immunogenicity studies and vaccine trials in a mouse model. In parallel, a proteomics approach will be undertaken to evaluate additional ES and surface proteins from *A. suum* for their immunoreactivity to immune serum from the pig model as a backup strategy for identification of vaccine candidates. The goal is to rank the top two candidate antigens with respect to their efficacy in a preclinical challenge model, together with the ability to express these antigens as soluble proteins in high yield (see below). The corresponding antigens from *A. lumbricoides* will also be cloned and expressed and examined for immunological cross-reactivity.

Status of *Trichuris* antigen discovery

Trichuris trichiura is a parasite of the superfamily Trichinelloidea, known as the common whipworm of humans and nonhuman primates. Due to its host specificity, there is no established laboratory animal model for this pathogen. Fortunately, the closely related *Trichuris muris* parasite, specific for rats and mice, has emerged as a useful surrogate for immunogenicity and efficacy models. Additionally, animal models for *Trichinella spiralis* (a cause of trichinellosi*s*), also a member of the Trichinelloidea superfamily, exhibit some immunological similarities for *Trichuris* sp. Both *Trichuris* sp. and *Trichinella* sp. possess a unique stichosome – a modified esophagus and esophageal gland that contains longitudinally arranged cells known as stichocytes – which is a rich source of ES

antigens (Figure 1). Such ES antigens are produced in the stichosome and released through the anterior ends of adult whipworms embedded in the colonic mucosa. The stichosome and its stichocytes are being explored through proteomics approaches.

Both *T. trichiura* and *T. muris* follow a similar oral–fecal life cycle. Once embryonated eggs are ingested, they hatch in the small intestine. The larvae then mature and develop to adult worms in the cecum and ascending colon [49]. Different mouse strains vary in their degree of resistance or susceptibility to *T. muris.* The most notable strains are BALB/c (resistant) and AKR (susceptible) mice. BALB/c mice exhibit a T_H2-skewed immune protective response against *T. muris*, associated with elevations in host IL-4 and IgG1 [49,51–53]. In addition to the *T. muris*-specific adaptive immune response, innate responses have also been identified including a multipotent progenitor type 2 cell population in the gut-associated lymphoid tissue that is induced by IL-25 and can differentiate into innate immune cells such as monocytes, macrophages, mast cells and basophils [54]. Furthermore, an interesting mechanism of parasite expulsion has been linked to the aforementioned cytokine profiles. This mechanism is known as the 'epithelial escalator' in which the epithelial cells migrate from the crypt area to the top of the epithelium while bringing the parasite along with it and ultimately shedding the parasite [55]. This phenomenon appeared to be more efficient in the resistant BALB/c model compared with the susceptible AKR model [55]. Additionally, this turnover was linked to the cytokine profiles where T_H2 responses were linked to the upregulation of turnover, and T_H1 responses were correlated with downregulation of turnover [55].

As the resistant strains are ideal for examining the natural protective immune response, the susceptible strains are invaluable for efficacy models in vaccine development. For example, AKR mice are attractive for purposes of vaccine development because they are susceptible to infection and yet acquire a protective T_H2 immune response when vaccinated with *T. muris* ES antigens [51,52]. *Trichuris muris* ES proteins and homogenates also elicit protective immunity in humanized hu-PBL-SCID mice [56].

Chemically defined antigens

To date, *T. muris* has not been studied as extensively as *A. suum* with respect to the cloning, expression and evaluation of recombinant antigens. Hence, the field is at an earlier stage in generating a vaccine development pipeline. Most of the successes with *T. muris* have been associated with parenteral injection of adult-stage worm antigens, especially those containing stichosome-derived proteins. In contrast, similar studies with *Trichuris suis* in pigs have not yet been advanced. Several promising *Trichinella* antigens have also been described (Table 3).

Worm extracts

Homogenized adult *T. muris* antigens, either emulsified in Freund's complete adjuvant for subcutaneous immunization, or combined with cholera toxin for oral administration, have been administered to several different mouse strains [57]. While the mucosal response was enhanced by oral vaccination, this was not sufficient for protection against infection in the low responders; in other words, protection was achieved only in the BALB/c high-responder mice [57], a result similarly found in oral vaccination for *Trichinella* leading to only protective immunity in high-responder mice [57]. In contrast, subcutaneous administration provided protective immunity in multiple strains [57,58]. In vaccinated humanized mice, an immunodominant 66 kDa *T. muris* antigen was identified from worm homogenates [15].

ES antigens

Stichosome-related adult-stage ES products have been identified as potential sources of protective antigens [59] although it has been noted that some stichosome proteins remain stored in the organ and are not actually secreted [60]. Partial protection has been observed following immunization with adult ES products [56], especially when emulsified with Freund's incomplete adjuvant [52]. The protection elicited in susceptible AKR mice included reductions in parasite egg shedding and was associated with peripheral lymph node responses [52].

Trichinella antigens

Findings of cross-reactivity and cross-immunity between *Trichuris* and *Trichinella* [61] have prompted the study of shared antigens. Using ELISA, immunoprecipitation and immunoblotting, it was demonstrated that *T. trichiura*-infected mice had cross-reactive antibodies to *T. spiralis* [61]. Shared stichocyte antigens have also been noted [51]. Among the defined candidate antigens identified from *T. spiralis* that provide partial protection are an aminopeptidase [62], serine proteases [63–65], parasite-derived cytokines [66], paramyosin [67], heat-shock protein [65] and 87, 53 and 43 kDa secreted antigens of unknown function [67–69].

Approaches to antigen discovery

As outlined above, there are no defined *T. trichiura*-specific antigens that have been specifically identified for further vaccine development. Thus, there is still a need to discover and develop specific *T. trichiura* antigens. One approach could utilize the established *T. muris* AKR murine model. Since the adult ES products of *T. muris* induced complete protection in immunized mice against infective egg challenge [52], the immune sera will be used to immunoscreen a *T. muris* adult stichosome cDNA library and identify secreted proteins that induce the protective immunity. Another approach to identify the vaccine antigen is to take advantage of studies already completed with the major *Trichinella* antigens highlighted previously. *Trichuris muris* orthologs of these antigens highlighted above can be cloned and expressed in platforms such as bacteria, yeast or baculovirus. These antigens can then be tested in subsequent immunogenicity studies. In parallel, proteomics approaches are useful to identify the full complement of *T. muris* stichosome antigens and their reactivity to immune serum from a mouse model. Once promising candidates are identified, protein expression on the *T. trichura* homologs of

Table 3. Summary of major *Trichuris muris* and *Trichinella spiralis* antigens discovered to date.

Antigen	MW (kDa)	Functions	Parasite stages	Protective efficacy		Ref.
				Adjuvant	*Worm reduction (HR, LR)*	
Trichuris muris						
Tm adult worm extracts	Various	Unknown	Adult	FCA Cholera toxin	HR (BALB/c) = 99.2% LR (C57BL/10) = 97.8% LR (B10.BR) = 41.4% HR (BALB/c) = 97.6% LR (C57BL/10) = 59.0% LR (B10.BR) = 17.8%	[57]
Tm adult ES	Various	ES products	Adult	IFA	LR (AKR) = 100%	[52]
Trichinella spiralis						
TsAP	54.7	Aminopeptidase	Larva	FCA/IFA	8.1% (adult worm) 59% (muscle larva)	[62]
TspSP-1.2	35.5	Serine protease Larva invasion	Adult, ES	None	34.92% (adult worm) 52.24% (muscle larva)	[65]
rTs-Adsp	47	Serine protease	Adult	Alum	46.5%	[63]
pVAX1-Tsmif-Tsmcd-1	59 68	Macrophage inhibitory factor and cysteine protease inhibitor	All	None Ubiquitin	23.17% 37.95%	[66]
rTs-Pmy	102	Binding to C8, C9 (complement)	Adult, Larvae	ISA50	21.8% (muscle larva) 33.4% (induced by epitope 88–107 amino acid)	[67]
Ts87	87	Surface antigen	Adult	DNA carried by *S. typhimurium*	29.8% (adult worm) 34.2% (muscle larva)	[68]
Ts53	53	Secreted glycoprotein	Adult, larva, ES	ND	ND	[69]
Ts-gp43, peptide 40–80	43	Immunodominant glycoprotein	Larval, ES	IFA	64.3% (adult worm)	[78]
Ts adult ES	Various	ES products	Adult	IFA	93.3% (adult worm)	[68,50,52]
Ts-Hsp70	70	Heat-shock protein	Adult	FCA	37%	[65]

ES: Excretory and secretory; FCA: Freund's complete adjuvant; HR: High responder; IFA: Incomplete Freund's adjuvant; ISA50: Incomplete Seppic adjuvants 50; LR: Low responder; MW: Molecular weight; ND: Not done.

these candidates can begin and further antigen down-selection can take place.

Prospects & challenges for vaccine product development

Avoiding allergic responses

A key observation made during the clinical testing of human hookworm candidate antigens was that human populations living in endemic areas acquire IgE responses to selected antigens, especially those from infective larvae [70]. Volunteers with pre-vaccination IgE can develop urticarial reactions, including generalized urticaria, upon receiving recombinant forms of such antigens [70]. Based on these findings, currently, evidence for prevaccination antigen-specific IgE antibodies among endemic populations will be used to discard or down-select potential candidate vaccines [17]. Larval *Ascaris* antigens in particular will be closely scrutinized. Therefore, a key step in developing a vaccine antigen pipeline will be preventing allergic responses by first examining sera from populations living in areas endemic for ascariasis and trichuriasis.

Selection/down-selection of vaccine antigens for process development

Selection of the lead candidate antigens will rely on ranking them through a scoring system that assigns a number ranging from 0 to 3, 4 or 5 for each of the critical categories described below. This approach was used previously to rank the leading candidate hookworm antigens used in the Human Hookworm

Vaccine [71]. For ascariasis and trichuriasis, the major criteria will include: level of protective immunity; amino acid homology between *A. suum* and *A. lumbricoides* or *T. muris* and *T. trichiura*; known or presumed mechanism of action; absence of prevaccination IgE among an endemic population; solubility and stability; and expression yield.

Scale-up expression & process development

The lead two candidate *Ascaris* and *Trichuris* antigens will then be subjected to protein expression at the 10–20L fermentation scale and large-scale protein purification. In anticipation of generating a target product profile that would focus on vulnerable populations living in helminth endemic areas of low- and middle-income countries, it is likely that only low-cost expression systems such as yeast (e.g., *Pichia pastoris* or *Hansenula polymorpha*) or bacteria (e.g., *E. coli*) would be selected [72]. In addition to production yield, solubility and stability, the expressed proteins would be evaluated for immunogenicity and ability to induce protective immunity in the animal models highlighted above. A process will be developed for technology transfer to a current good manufacturing practices manufacturer in the USA, Europe or possibly a developing country manufacturer. Following a good laboratory practices toxicology study, an investigational new drug application would be prepared and filed with the US FDA prior to Phase 1 clinical testing. These antigens would be evaluated initially as monovalent antigens formulated on alum, possibly together with a second adjuvant such as a toll-like receptor agonist used for other vaccines under development by the Sabin Vaccine Institute Product Development Partnership, such as a synthetic lipid A [73], E6020 [74] or a CpG oligodeoxynucleotide.

Coformulation

To develop a pan-helminthic vaccine, the identified antigens along with adjuvants will need to be initially evaluated via coadministration and later coformulated together into one vial. This will require several principles to be considered:

Protein stability

As proteins differ in their individual characteristics (e.g., size and pH), buffers and excipients that provide stability for one protein may not be suitable for another antigen of interest. As such, extensive buffer screenings may be required to identify a formulation that will be ideal for all of the vaccine antigens. Also to be considered are the necessary storage conditions of these formulations, which could affect longtime storage, shipment and short-term storage in the clinic.

Adjuvant/delivery system

T_H2-mediated immune responses appear to be the desired protective response common to all three soil-transmitted nematodes targeted by the vaccine. To ensure that the same adjuvant will be appropriate for all antigens, a well-established and relatively inexpensive option are to use an alum formulation, possibly with a second adjuvant as outlined above.

Immunogenicity/efficacy

A recurring concern with combining multiple antigens in a vaccine is the potential for immune interference. For example, the immune response to one antigen may dominate or interfere with the others in the vaccine. In endemic areas, many people suffering from helminth infections are polyparasitized [75], making a coinfection animal model critical to vaccine development. Currently, multiple animal models are used as surrogates for human *Ascaris* and *Trichuris* infections, including rodents, pigs and nonhuman primates, with rodents and pigs used most frequently [23,41,42,45–49,76–78]. Rodent models, in other words, mice and rats, are more attractive than pigs or nonhuman primates for initial immunogenicity and efficacy studies as their small size and ease of handling allow larger numbers of animals to be evaluated concurrently. Additionally, genetically defined inbred strains allow identification of genetic backgrounds responsible for susceptibility and resistance to parasites. Several strains, including AKR, SCID and Nude, have been shown to be susceptible to *T. muris*, developing patent infections [49]. In contrast, while *A. suum* larvae migrate through the liver and lungs of C57BL/6-infected mice, to date, mice cannot support the development of adult worms, nor patent *A. suum* infections [79]. Nonetheless, several *A. suum* antigens identified by screening with immune serum from pigs have been shown to reduce lung larval burdens in susceptible mice, suggesting that protective immune responses in susceptible mice correlate with protective response in pigs [41,42,44–48]. Since C57BL/6 mice infected with a low dose of *T. muris* develop a T_H1-skewed immune response and a susceptible phenotype [80], this mouse strain could potentially serve as an initial coinfection model for evaluating a pan-helminthic vaccine against *Ascaris* and *Trichuris* infections. Furthermore, mice would be invaluable in evaluating regulatory mandated immunogenicity testing of antigen/adjuvant combinations and future potency testing of a clinical vaccine [81]. Ultimately, to thoroughly evaluate a coformulated vaccine, both mice and pigs may be necessary to establish sufficient protection data to advance a potential vaccine candidate into product development. Pigs offer some promise as a laboratory vaccine model [82] although they have not yet been used extensively for evaluating *T. suis*. Further studies will be undertaken to assess whether studies confirming host protection in pigs will be on the critical path for vaccine development.

Modeling

It will be useful to build on previous mathematical models of helminth transmission dynamics [25] to assess the level of protective immunity necessary to achieve cost–effectiveness relative to current deworming approaches, as previously done for the Human Hookworm Vaccine [74]. Modeling would also help to assess the potential of the vaccine to achieve control and elimination targets by interrupting helminth transmission [13].

Expert commentary

A strategy for the discovery, expression, isolation and preclinical development is being advanced for lead candidate antigens to prevent both ascariasis and trichuriasis.

- For ascariasis, the six identified antigens listed in Table 2 will be targeted based on the previous evidence that they can reproduce some of the protective immunity afforded by living UV-attenuated eggs [22,23,39–48]. Some of these antigens are expressed in both larval stage and adult worms. In parallel, a proteomics approach will be advanced to identify additional ES and possibly surface antigens.
- For trichuriasis, antigens from the stichosome will be emphasized given the immunological protection afforded by extracts of this organ, which is a rich source of antigens from adult worms embedded in the colonic mucosa [52,56,59,60]. Some of these stichocyte/stichosome antigens may also be related to previously discovered *T. sprialis* antigens [15,61,63–69,83,84].

Ultimately, these antigens will be evaluated for protection alongside the two antigens comprising the Human Hookworm Vaccine. In so doing, it may be necessary to produce a tetravalent vaccine comprising two hookworm antigens together with an *Ascaris* and *Trichuris* antigen, respectively, or even possibly a pentavalent vaccine if a second *Ascaris* or *Trichuris* antigen is required to achieve adequate protective immunity. The costs of such vaccines will have to be carefully looked at, even if only inexpensive bacterial or yeast expression vectors are employed. A cost economic modeling exercise may be required to examine the comparative advantage of a Pan-anthelmintic vaccine over current control approaches that rely on anthelmintic drugs and deworming. Previously, the Human Hookworm Vaccine was shown to be cost-effective and even cost-saving under different scenarios [75].

Five-year view

The next 5 years will be focused on working to resolve the major gaps and key issues that could hinder or slow a pan-helminthic vaccine development program. One major goal will be spearheading a focused antigen discovery program for both *Ascaris and Trichuris* and their eventual down-selection as detailed above. Once the antigens are identified and expressed, they will enter a process development, characterization and initial stability assessment program. A main consideration will include designing a formulation that will support stability of not only the candidate *Ascaris* and *Trichuris* antigens but also the Human Hookworm Vaccine antigens. Another key endeavor will be to build on previous preclinical studies to establish a suitable animal model for the Pan-anthelmintic vaccine. This model would support both immunogenicity and preclinical efficacy studies of our vaccine candidates, as we move toward an investigational new drug filing and clinical trials. Such studies will be critical for determining if the protective immunity achieved for these helminth antigens in laboratory animals can translate to human medicine.

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Key issues

The major issues in the development cycle of the Pan-anthelmintic vaccine include the following:

- Reproducing the protective immunity afforded by UV-attenuated *Ascaris* eggs or *Trichuris* stichosome extracts using recombinant antigens.
- Avoiding the potential allergic responses by down-selecting antigens that elicit IgE among endemic populations.
- Scaling-up soluble and appropriately refolded recombinant proteins that could be manufactured under current good manufacturing practices and identifying a suitable manufacturing partner.
- Selection and evaluation of an appropriate adjuvant formulation and delivery.

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