is fortuitous that high-throughput methods have arrived for identifying the sequences of edited mRNAs, and determining whether translatable products exist for each cryptogene [6]. New analytical methods to reconstruct the likely fully-edited canonical product from a subpopulation of individual reads take advantage of existing high-throughput RNA sequencing technologies. Once products are known, tools are available to explore the progression (or lack of progression) of U-indel editing of each transcript in more detail. High-throughput analyses are particularly important for another reason. The incidence of functional mRNAs resulting from noncanonical U insertions and deletions (i.e., 'alternative editing') remains ambiguous [6]. These may even encode something other than ETC subunits. Such mRNAs are best detected by unbiased deep-sequencing methods. Messenger RNA high-throughput sequencing, combined with guide RNA population information (e.g., [10]) can be used in comparative studies to learn what products are made, and what their relative abundances are, under what circumstances. Of course, mass spectrometry methodologies are also improving apace, and we must additionally take every opportunity to apply these to the analysis of mitochondrion-encoded proteins.

A major argument in favor of multispecies analyses of mitochondrial genome expression is that differences in the composition and functionality of the trypanosomatid ETC are known to exist among species. One study specifically surveyed mitochondrial respiration in one-host species and found a plethora of differences that were, interestingly, unrelated to apparent phylogeny [11]. The origin of many of those differences will be the nuclear genome and its expression, but some may be of mitochondrial origin. Furthermore, the unidentified mitochondrial genes of the trypanosomatids are also likely to be part of, or influence, the ETC, and they are found in most trypanosomatid genomes. Finally, ongoing investigations into various possible biological roles of complex I, seemingly different among species, would benefit from a broader analysis [3] (Box 1). Ultimately, in characterizing products of the mitochondrial genome in diverse trypanosomatids, we will attain a more representative view. The explosion of newly sequenced genomes and available U-indel transcriptome sequencing analysis tools argues that the time for this approach is now.

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Forum

Advancing the Development of a Human Schistosomiasis Vaccine

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Three vaccines against human schistosomiasis are in different phases of clinical development, and a fourth is expected to enter the clinic soon. Successful introduction of an efficacious preventive human schistosomiasis vaccine will require integration into existing health systems such as those that deliver childhood vaccines or mass drug administration programs.

Schistosomiasis has one of the highest disease burdens of the neglected tropical diseases (NTDs), especially in Africa, where more than 90% of infections occur. The Global Burden of Disease Study 2016 estimates that 190 million people are infected with schistosomes, with more than 70 million new infections and thousands of deaths occurring annually [1]. Alternative estimates suggest that the prevalence and mortality attributable to this NTD may be much higher, with an estimated 300 000 people dying each year in Africa alone due to the chronic sequelae of infection [2]. In addition to the morbidity and mortality directly associated with schistosome infection, accumulating evidence points to the role of urogenital schistosomiasis caused by *Schistosoma haematobium* in promoting susceptibility to HIV in

girls and women [3]. Furthermore, interactions between intestinal schistosomiasis caused by *Schistosoma mansoni* and malaria may promote transmission of the latter [4]. Together *S. haematobium* and *S. mansoni* account for approximately 99% of the global human schistosomiasis cases.

Over the past decade, the global community has expanded annual mass drug administration (MDA) with praziquantel (PZQ) as the major approach to schistosomiasis control, targeting school-age children (and occasionally adolescents), who have been shown to have the highest prevalence and intensity of infection. MDA has produced a number of clinical benefits, including a decline in schistosomiasis-associated pediatric malnutrition and its associated cognitive delays [5]. Additional studies have shown the collateral benefits of MDA in preventing HIV and malaria transmission in Africa [3,4]. Indeed, between 2006 and 2016, the disability-adjusted life years (DALYs) lost from schistosomiasis have decreased by one-quarter to one-third [6].

However, the NTD scientific community remains divided on whether MDA alone will be sufficient to eliminate schistosomiasis in resource-poor settings of Africa, South America, and the Middle East or whether additional technologies will be required. The arguments against MDA as a single-dimension elimination strategy include observations that, in areas of high transmission, post-treatment reinfection rates are high and require frequent and repeated dosing with PZQ, which is not always feasible using the current MDA delivery model. Moreover, there is insufficient evidence that MDA alone, even after several years with high rates of coverage, can interrupt transmission in areas of high transmission in Africa. Finally, PZQ does not reverse the genital lesions thought to promote HIV transmission [7].

Recent mathematical modeling of the potential impact of a schistosomiasis vaccine indicates that a vaccine which reduces mean egg output by 60%, with a duration of efficacy of between 5 and 10 years, would interrupt transmission in low to moderate transmission settings, although higher efficacies (~80% egg reduction) may be required for areas of higher transmission [8]. A new generation of -OMICS, systems biology, and adjuvant technologies, have now made it possible for the selection and testing of new schistosomiasis vaccine candidates that meet the requirements of an ideal target product profile (TPP) that would make such a novel product a vital tool for the elimination of schistosomiasis [9].

TPP

An ideal TPP for a human schistosomiasis vaccine includes several key characteristics. First, it should preferably target both of the two major human schistosomes, *S. haematobium* and *S. mansoni*, which are often coendemic in Africa and the Middle East. *S. mansoni* is the sole species of the parasite present in Latin America and the Caribbean. Second, an ideal vaccine should prevent the major immunopathology of schistosomiasis by greatly reducing the number of parasite eggs deposited in the intestines and liver for *S. mansoni* and/or in the bladder and female genital tract for *S. haematobium*. Finally, it should be a prophylactic vaccine, which preferably would be superior to MDA with PZQ by preventing reinfection, which is critical to controlling this infection in endemic areas and reducing transmission of the parasites.

The Vaccines

The first human schistosomiasis vaccine to enter clinical trials consisted of a recombinant glutathione-*S*-transferase protein from *S. haematobium* (rSh28GST) adsorbed to aluminum hydroxide adjuvant, which was developed at INSERM and Institut Pasteur de Lille [10]. While the vaccine was observed to be safe and immunogenic in phase 1 [10] and phase 2 trials, it has not progressed to an application for licensure despite completion of a phase 3 efficacy trial in Senegalese school-age children in 2012, the results of which have not yet been published[i].

Traditional methods, and more recently immunomic, proteomic, or vaccinomic approaches, have identified several parasite surface and tegumental macromolecules involved in the schistosome host–parasite relationship. These approaches conducted in concert with profiling human sera from individuals with putative immunity (also known as endemic normals) [11] have led to the identification of the three lead vaccine candidates for human schistosomiasis, *Sm*-TSP-2 (*S. mansoni* tetraspanin 2), *Sm*-p80 (*S. mansoni* calpain), and *Sm*-14. As shown in Table 1, *Sm*-TSP-2 was identified as a tegumental protein, while calpain is a component of the apical membrane of the schistosome, and *Sm*-14 a cytoplasmic fatty acid-binding protein.

Sm-TSP-2 is a tetraspanin with domains that are located on the surface of the adult *S. mansoni* worm tegument. It is required for tegument biogenesis and integrity [11,12]. An orthologous *Sh*-TSP-2 has also been identified for *S. haematobium* [12]. In an experimental murine model, the 9 kDa recombinant extracellular domain of *Sm*-TSP-2 conferred high levels of protective immunity upon cercarial challenge even when formulated with only Alhydrogel® (aluminum hydroxide adjuvant). The *Sm*-TSP-2 schistosomiasis vaccine was selected for process development and scaled-up manufacture according to current good manufacturing practices (cGMPs) in the *Pichia Pink* expression platform [13]. Through a product development partnership (PDP) led by Texas Children's Hospital Center for Vaccine Development (Texas Children's CVD)

Table 1. Schistosomiasis Vaccine Candidates Currently Advancing through Clinical Development[a]

Macromolecule (parasite location)	Expression system	Adjuvant(s)	Current stage	Major partners
Sm-TSP-2 (Tetraspanin in schistosome tegument)	*Pichia Pink* (Yeast)	Alhydrogel® +/− AP 10-701	Phase 1 trial (nonendemic) complete; phase 1 trial (endemic) ongoing; phase 2 trial planned	Texas Children's CVD; VTEU Baylor College of Medicine; George Washington University; FIOCRUZ; James Cook University; IDRI
Sm-p80 (Calpain in schistosome apical membrane)	*Escherichia coli* (bacteria)	GLA-SE CpG ODN	Phase 1 trial to commence in 2019	Texas Tech University; IDRI; PAI
Sm-14 (Cytoplasmic fatty acid-binding protein)	*Pichia pastoris* (yeast)	GLA-SE	Phase 1 trial (nonendemic) complete, phase 1 and 2 trials planned	FIOCRUZ; IDRI

[a]Abbreviations: AP 10-701, glucopyranosyl lipid A (aqueous formulation); CVD, Center for Vaccine Development; FIOCRUZ, Fundação Oswaldo Cruz; GLA-SE, glucopyranosyl lipid A (stable emulsion); IDRI, Infectious Disease Research Institute; ODN, oligodeoxynucleotide; PAI, PAI Life Sciences; VTEU, Vaccine and Treatment Evaluation Unit.

and The George Washington University, yeast-expressed and purified recombinant *Sm*-TSP-2 was formulated on Alhydrogel® for administration together with an aqueous formulation of the Toll-like receptor-4 agonist known as AP 10-701 (glucopyranosyl lipid A) developed by the Infectious Disease Research Institute (IDRI) based in Seattle, WA, USA. Several clinical trials have either been completed or are in progress in nonendemic and endemic populations.

A phase 1 trial testing the safety and immunogenicity of *Sm*-TSP-2/Alhydrogel® with or without AP 10-701 was recently concluded in Houston at the NIAID, NIH-funded Vaccine and Treatment Evaluation Unit (VTEU) of the Baylor College of Medicine[ii]. Another NIAID/NIH-sponsored phase 1 trial of this vaccine is underway in an endemic area of Brazil[iii], while a phase 2 trial of this experimental product is currently being planned in Uganda.

A second *S. mansoni* surface molecule, *Sm*-p80 is also advancing into the clinic, with projections that it will be in a phase 1 trial by 2019 through a consortium led by Texas Tech University, IDRI, and PAI Life Sciences (a Seattle-based biotechnology company) [14]. *Sm*-p80 is a calcium-activated cysteine protease known as calpain, which is found on all stages of the parasite (including eggs). Recombinant *Sm*-p80 has been shown to elicit high levels of protection when administered to baboons subsequently challenged with *S. mansoni*, and hamsters challenged with *S. haematobium*, with evidence that the vaccine induces persistent levels of antibody in the former for several years [15]. The vaccine is scheduled to enter phase 1 trials as the recombinant protein formulated with a stable oil-in-water emulsion of glucopyranosyl lipid A (GLA-SE) or with a CpG oligodeoxynucleotide adjuvant [15].

The Fundação Oswaldo Cruz has led the development of a recombinant 14 kDa fatty acid-binding protein from *S. mansoni* known as r*Sm*14, which was expressed in yeast (*Pichia pastoris*) and shown to be safe and immunogenic in healthy, male adults from a nonendemic area when formulated with GLA-SE in a phase 1 trial [16]. A second trial was recently concluded in adult males in an endemic region of Senegal[iv]. Finally, it should be noted that *Schistosoma japonicum* vaccines are also under development for use in endemic areas of China and the Philippines. Because *S. japonicum* is a zoonotic schistosome of water buffalo and other mammals, efforts are concentrated on developing a veterinary vaccine that would interrupt transmission to humans.

Additional schistosome surface molecules located in the tegument or apical surface of the parasite have been identified and represent potential alternative vaccine candidates, although none of these are currently being developed for clinical trials.

Next Steps and Bottlenecks

As the three major *S. mansoni*-derived macromolecules advance through clinical development, they can be expected to face both scientific and economic challenges.

Among the scientific challenges is a problem characteristic of most recombinant protein vaccines: namely, that the low molecular weight, inert, recombinant antigens in these products rarely achieve protective levels of antibody unless formulated with potent adjuvants. Furthermore, it is to be seen whether a single vaccine antigen will be adequate to confer protection or if a cocktail of antigens will be required. Therefore, an optimal vaccine development strategy could be to evaluate the three existing candidates (*Sm*-TSP-2, *Sm*-p80, and Sm14) alone and in combination,

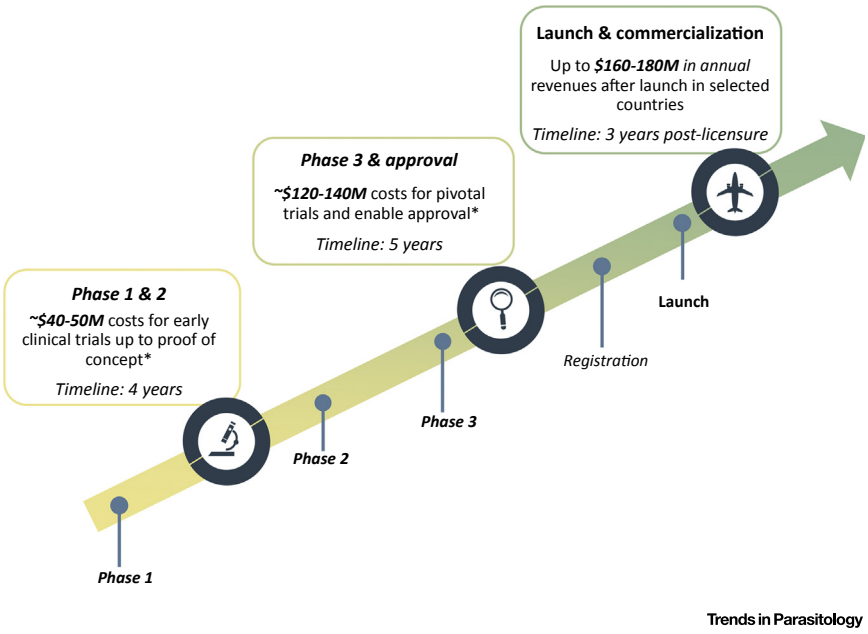

Figure 1. Timeline, Costs, and Revenues Projected for a Schistosomiasis Vaccine. The time needed for the development is depicted in years for each clinical phase. The expected costs (adapted from data obtained by L.E.K. Consulting) are itemized for each clinical phase and include the costs of scale-up and manufacturing (noted by the *). Finally, the revenues (adapted from data obtained by L.E.K. Consulting) are projected in annual revenues after launch in at least four initial selected countries.

together with several different adjuvants and immunostimulants.

A second issue centers on the coordination and financing of schistosomiasis vaccine clinical development. Currently, the three experimental vaccines are advancing through parallel product and clinical development pathways, even though both scientific and financial synergies could be realized by evaluating them together in a single program. A greater challenge is the expected cost of phase 2 and 3 trials to evaluate vaccine efficacy in areas of high transmission in Africa or Brazil. Figure 1 depicts a projection of the costs to bring a vaccine candidate through its clinical development up to licensure and the revenues that could generate upon launch and commercialization (adapted from data obtained from L.E.K. Consulting). One option that has been suggested to 'de-risk' such investments is through the development of a controlled human infection model (CHIM) using schistosome cercariae. Although such efforts are underway, there are still concerns about the safety and feasibility of this approach, as well as the relevance of the information that might be gained given that it is based on a single sex infection to avoid egg-induced pathology.

Finally, there are geopolitical hurdles that must also be overcome to integrate a human schistosomiasis vaccine into existing health systems. Programs that combine the regular administration of various anthelmintic drugs have been in place since 2006 through large-scale funding from the USA (USAID) and UK (DFID) aid agencies. So far, no vaccine has been incorporated into these drug-delivery systems, which are now functioning in many nations in sub-Saharan Africa. Therefore, operational studies will be required to evaluate how to best introduce a schistosomiasis vaccine through these mechanisms or whether it would be better to integrate such a vaccine into programs that routinely deliver measles or other childhood vaccines such as the Expanded Program on Immunization (EPI). The health economics of either pathway require further evaluation as will the regulatory strategy needed to obtain country approvals for importation and administration of a schistosomiasis vaccine.

Many of these issues are not unique to a schistosomiasis vaccine but are also being discussed for next-generation malaria and other parasitic disease vaccines. In conclusion, several exciting human schistosomiasis vaccine candidates are currently under product and clinical development; however, if eventually licensed, their appropriate use will require a high level of innovative thought and international cooperation.

Disclaimer Statement

The authors are patent holders and investigators on the *Sm*-TSP-2/Alhydrogel® vaccine described in this paper.

Resources

[i]https://clinicaltrials.gov/ct2/show/NCT01512277
[ii]https://clinicaltrials.gov/ct2/show/NCT02337855
[iii]https://clinicaltrials.gov/ct2/show/NCT03110757
[iv]https://clinicaltrials.gov/ct2/show/NCT03041766

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