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Review

Current Status of the Sm14/GLA-SE Schistosomiasis Vaccine: Overcoming Barriers and Paradigms towards the First Anti-parasitic Human(itarian) Vaccine

Miriam Tendler[1,*], Marília S. Almeida[1], Monica M. Vilar[1], Patrícia M. Pinto[1] and Gabriel Limaverde-Sousa[1]

[1] Laboratório de Esquistossomose Experimental, Instituto Oswaldo Cruz, FIOCRUZ - Av. Brasil, 4365, Manguinhos, 21045-900, Rio de Janeiro, Brazil

* Correspondence: mtendler@ioc.fiocruz.br; Tel.: +55-21-2562-1320

Abstract:

Schistosomiasis, a disease historically associated with poverty, lack of sanitation and social inequalities, is a chronic, debilitating parasitic infection, affecting hundreds of millions of people in endemic countries. Although schistosomiasis control approach has shown that chemotherapy is capable of reducing morbidity in humans, rapid re-infection is a reminder that the impact of drug treatment on transmission control or elimination initiatives is marginal. In addition, and regardless of more than two decades of well-executed control activities based on large-scale chemotherapy, the disease is expanding in many areas including Brazil. The development of the Sm14/GLA-SE schistosomiasis vaccine is an emblematic open knowledge innovation that has successfully completed Phase I and Phase IIa clinical trials, with Phase II/III trials underway in the African continent and to be followed in Brazil. Discovery and experimental phases were long term achievements leading to a robust collection of data that are strongly supporting the presently ongoing Clinical Phase. This paper reviews the development of the Sm14 vaccine formulated with GLA-SE (Glucopyranosyl Lipid A), from the earlier experimental developments to clinical trials including the recent status of Phase II studies.

Keywords: Schistosomiasis; vaccine; Sm14; FABP

1. Introduction

Schistosomiasis is the second-most socioeconomically devastating parasitic disease after malaria. It is a chronic and debilitating endemy with an estimated 200 million people infected, out of which 120 million are symptomatic, with 20 million presenting severe disease symptoms, and most of them (85%) live in Africa [1]. These estimates may error on the low side as a meta-analysis has found the number of people at risk to be closer to 800 million [2]. The global Schistosomiasis remains as high as ever and the estimated number of Disability-Adjusted Life Years (DALYs) has increased with the inclusion of previously under-recognized morbidities not counted for the DALY index before (eg., growth stunting, anaemia associated to retarded intellectual development) in infants, toddlers and school age children, the part of the population whose physical health and intellectual capacity are fundamental to nation development and sustainability[3,4]. Under this more realistic scenario, the impact of schistosomiasis comes second in the list of the 18 World Health Organization (WHO) neglected tropical diseases (NTDs) [5]. In Brazil, the largest endemic country for schistosomiasis, 6 million individuals are estimated to be infected, 25 million are at risk of contracting the disease [6,7].

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Mass chemotherapy has been the strategy of choice in an attempt to control schistosomiasis and intestinal helminthiasis with the support of international health funding agencies. Estimates show that at least 206.5 million people required treatment in 2016 [8]. However, the strategy of large-scale treatment, based on chemotherapy also equivocally called "prophylactic treatment" failed to control transmission for more than thirty years. Approximately 300 millions of US dollars are still being spent annually in treatments to be applied to the same populations year after year, with no prospect to prevent reinfections and subsequent treatments, in addition to overloading children and young population of endemic countries with chemical drugs [9]. "Deworming" initiatives, originally applied to animal species only, were proposed as a tool for schistosomiasis control programs addressed to school children in endemic countries [10,11].

Contrastingly, under One Health policies for the control of veterinary helminth infections, such as *Fasciola hepatica* the major parasitic infection of livestock worldwide, there is a strong demand for the replacement of anti-helminthic drugs - that implies in significant amount of chemical residues detected in meat, milk and added-value products – for safe vaccines, considered as the most environment and human health friendly methods for the control of Fascioliasis in livestock [12].

The insertion of vaccines in the context of programs towards effective control of schistosomiasis brings hope to a future scenario for the poor. The Brazilian Sm14 Schistosomiasis Vaccine Platform was launched and strongly pushed in the context of a formal WHO program, specifically structured towards the Development of Anti Schistosomiasis Vaccine in the 1990's. The main outcome of this initiative was the selection of six priority antigen candidates out of which only Sm14, continued to be developed (Table 1, adapted from [13]).

With strategic support of WHO, this initiative is moving forward, emerging from an endemic country, to the final development of Sm14 Schistosomiasis Vaccine based on a recombinant protein. It is being developed under the most sophisticated and modern technological platforms and professionally conducted in a network of outstanding companies and collaborators. This was the result of long-term scientific developments carried under the coordination of FIOCRUZ, a public institution linked to the Brazilian Ministry of Health. Sm14 based vaccine is protected by strong patents owned by FIOCRUZ in all countries of interest worldwide.

Table 1. Schistosomiasis priority antigens selected by WHO for independent testing (adapted from [13]).

Antigen	Size (kDa)	Stage expressed	Description	Protection (%)	Place of Development
Glutathione S-transferase (P28/GST)	28	Adult/somula/ egg	Enzyme	30-60	Institut Pasteur, Lille, France
Paramyosin (Sm97)	97	Adult/somula	Muscle protein	30	Case Western Reserve University/ National Institute of Health/Cornell University, USA
IrV-5	62	Adult/somula/ egg	Muscle protein	50-70	Johns Hopkins School of Medicine, Baltimore, USA
Triose phosphate isomerase (TPI)	28	Adult/somula/ egg	Enzyme	30-60	Harvard School of Public Health, Boston, USA
Sm23	23	Adult/somula/ egg	Integrated membrane protein	40-50	Johns Hopkins School of Medicine/Harvard School of Public Health, USA
Sm14	14	Adult/somula	Fatty acid-binding protein	65	Instituto Oswaldo Cruz, Rio de Janeiro, Brazil

Recently, the Consultative Expert Working Group on Research and Development: Financing and Coordination (CEWG/WHO) selected the Sm14 Vaccine as one of the six Demonstration Projects, globally, under an extensive selection and several rounds of shortlists as it has demonstrated to be fully adherent and in accordance with the principles of CEWG, such as to demonstrate clear mechanisms of Delinkage of costs from investments in R&D from costs of final product, Accessibility, Affordability, Viability and to be an Open Knowledge Innovation. The CEWG recognized that the Sm14 vaccine may become a key tool for the implementation of effective programs, based not only on chemotherapy, but yet in infection reduction and transmission control of Schistosomiasis [14].

Over the last years it was possible to overcome important bottlenecks in the process of new product/vaccine development: scaling up production process from laboratory bench to production scale and successful conclusion of two Phase I human trials in healthy adults (man and woman) living in a Brazilian non-endemic area (2011-2014) [15] and the first Phase II trial in 30 male adults living in highly endemic area for both *Schistosoma mansoni* and *S. haematobium* at the Senegal River Basin (2015-2017). Safety was extensively confirmed and strong and long-lasting immunogenicity was also demonstrated (manuscript in preparation). Process development and master cell bank generation were recently completed and GMP manufacture of Sm14 lot is currently ongoing, under the coordination of the Infectious Disease Research Institute (IDRI, Seattle, US). This article is an overview of Sm14 Schistosomiasis vaccine development from the antigen discovery to the current human studies (Fig. 1).The need to overcome barriers for the rise of a truly humanitarian vaccine - to the needs of the developing world - will be discussed .

2. Innovative Strategies Adopted for Antigen Discovery and Early Development Phases

Biotechnological advances in various areas of vaccine research have contributed to the development of safer and more effective formulations. Efforts to develop anti-helminth vaccines lasted for many years and are continuing to progress in identification of candidate antigens, which have recently been aided with the generation of a number of helminth genomes [16]. The implementation of a vaccine against schistosomiasis represents an important step in a context of research and development in public health for poor populations infected and exposed to Schistosomes. There have been initiatives from research groups in different countries to develop a vaccine against schistosomiasis. The Brazilian Sm14-based anti-schistosomiasis vaccine is the sole technology that is at an advanced stage of development toward a safe highly innovative product [17].

In contrast to the current "OMICS" strategies, in which high-throughput screenings of potential antigens are being processed in parallel by automated "discovery protocols and platforms", the Sm14 project was borne by gathering observations from animal models of infection, and a sequential and specific rational thinking of development and experimental design based on evidences.

The first original approach was in how the worm extract was obtained for subsequent assessments of protection. Instead of lyophilized parasites, generally used by other groups, a saline extract containing secreted/excreted antigens derived from **living adult schistosomes** was obtained for the initial immunizations. The restricted number of potential protective antigens released in this saline extract, allowed a more direct identification of strong candidates for the following molecular phase of the development [18–22]. Innovative methods were also adopted in the experimental phase, when the use of outbred models, in contrast to the commonly employed inbred animals, allowed a better representation of the ultimate target population and provided a unique opportunity to develop an alternative strategy for the protection assessment, improving the overall understanding of the outcomes. The analyses were stratified based on the measurement of frequencies of worm burden distribution of vaccinated–challenged animal population over non-vaccinated infected controls, as opposed to evaluation of mean values of parasite loads, as usually adopted. A solid base of pre-clinical data was raised establishing the immunization protocols that would be adopted on the following steps of the development [23–31].

As molecular biology tools evolved and gene-cloning techniques became available, several antigens released in the saline culture medium of live schistosomes, were cloned and sequenced. Antiserum from rabbits immunized with the "saline extract" was used to screen an adult *S. mansoni* cDNA library and the most promising antigen was identified as a member of the Fatty Acid Binding Protein (FABP) family, termed Sm14 [32]. Molecular modeling studies from our group predicted the beta-barrel structure of the Sm14 tridimensional structure [33] that was later experimentally confirmed by crystallography [34] and Nuclear Magnetic Resonance [35]. Such analyses allowed the engineering of a stabilizing mutation that rendered this antigen a remarkable long-term stability, while maintaining its function and immunogenicity [36].

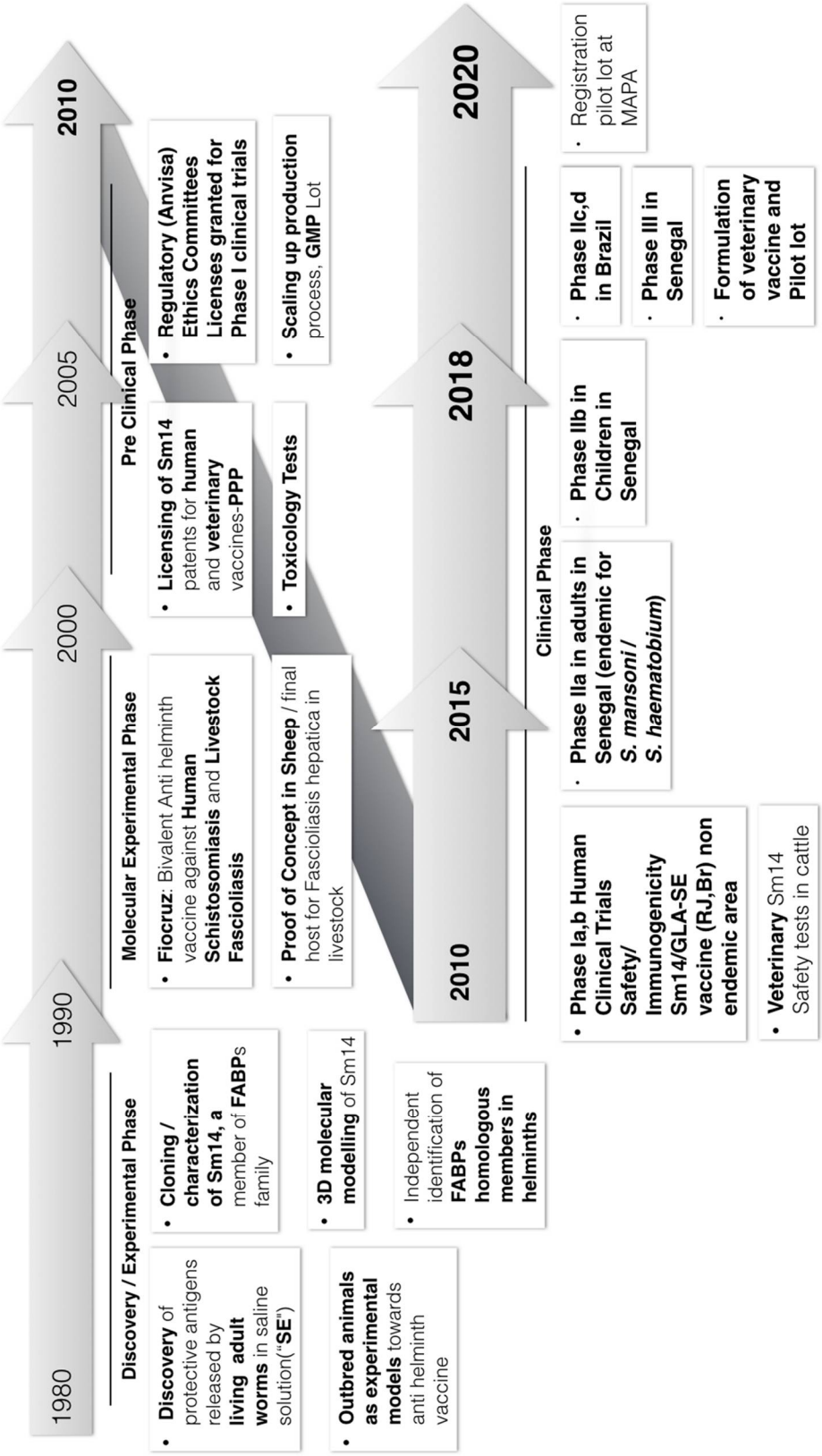

Figure 1. Timeline: Sm14/GLA-SE Anti Schistosomiasis Vaccine - From Discovery Phase to Final Product. MAPA: Brazilian Ministry of Agriculture.

Sm14 was shown to be particularly important to helminths, that are not capable of synthetizing fatty acids themselves, which ultimately are provided by the host species. Lipids, apart from being constituents of membranes, have important role in development of different lifecycle stages and evasion of immune responses by adults and larvae [37] After the first publication on the presence of a FABP family member in the *Schistosoma mansoni* [32], different groups published on the identification of homologous protein members from FABP family in many helminths of human and veterinary importance. The first one published after Sm14, was the *Fasciola hepatica* FABP, the main parasite of livestock worldwide [38].We have managed to successfully test with Sm14 vaccination against *F. hepatica* in mice followed by two independent experiments in sheep, one of the final host species for fascioliasis [39]. It was thus demonstrated that Sm14 is also protective against *Fasciola hepatica* infection and it is therefore being also developed in parallel as the molecular basis for a veterinary vaccine by FIOCRUZ in collaboration with the private Brazilian company Ourofino Animal Health.

3. Clinical Studies

The licensing of the Sm14 patents for veterinary use gave birth to a Public-Private Partnership (PPP) model of product development that rendered significant visibility to the Sm14 vaccine project. Such gain in momentum was followed by a strong support of the human vaccine development branch by the Brazilian government project financing agency (FINEP) that allowed the use of Contract Research Organizations (CROs) for antigen production, quality control and fill-finish in GMP world-class facilities based in the United States of America, under the coordination of the Infectious Disease Research Institute (IDRI, Seattle, USA).

In order to have a consistent, stable and defined final product for clinical human use, the Sm14 antigen was formulated with the synthetic adjuvant Glucopyranosyl Lipid A (GLA-SE), a clinically approved molecule already used in a number of commercially available human vaccines.

In December of 2010, the Brazilian Health Regulatory Agency (ANVISA) approved the Phase Ia Clinical Trial in 20 healthy male volunteers in non-endemic area (Rio de Janeiro, Brazil) to evaluate the safety of the investigational product. The study was conducted by the Brazilian National Institute of Infectious Diseases (INI/FIOCRUZ). The results of this first trial attested the safety of the vaccine on the studied population, showing no systemic reactogenicity and no adverse event was associated to the investigational product [15]. The Phase Ib Clinical Study, to evaluate the safety and immunogenicity of the vaccine preparation in 10 healthy women volunteers, was successfully concluded in 2012 (manuscript in preparation).

In 2015-2016, already under the scope of CEWG Demonstration Project, it was developed and concluded the first Phase II trial (Phase IIa) in 30 male adults living in highly endemic area for both *Schistosoma mansoni* and *S. haematobium* at Senegal River Basin, conducted by specialized team from Espoir Pour La Santé (EPLS), linked to the Pasteur Institute of Lille (IPL, France), headed by Dr. Gilles Riveau (IPL) in conjunction with Brazilian group guidance (ClinicalTrials.gov Identifier: NCT03041766) [40]. Main objectives of safety and immunogenicity in the context of vaccination with Sm14/GLA-SE vaccine were fully achieved in Phase II a trial. The investigational product rSm14 (50 µg) formulated with GLA-SE in two dosages (2.5 µg and 5 µg/dose, denominated groups 1 and 2, respectively) and administered IM was shown to be safe with no observed serious adverse events in either group. The most common reactions were local pain and heaviness of vaccinated arm, that were transitory and mild. Seroconversion of 92% of individuals after second dose was observed, in an analogous pattern as described in Phase I trials [15]. Immunogenicity based on additional cellular response, memory cells and T cell activation markers was analyzed at IDRI in an extensive panel focusing on the identification of vaccine related immune response.

After the closure of the Phase IIa, based on the good results of induced strong and lasting immune response, an extension study to assess the possible persistence and profile of this response beyond the initial schedule of the trial was duly authorized by Senegalese Ministry of Health (MoH) and carried out between August and December 2017 with the inclusion of two additional time points, 9 and 12 months, after the first vaccine injection. ELISA preliminary tests were performed at Centre

de Recherche Biomédicale/Espoir Pour La Santé (EPLS) and showed the persistence of significant specific antibody titers for until 12 months after first vaccination dose with Sm14/GLA-SE (manuscript in preparation).

Phase IIb trial design and protocol were defined in January 2018 based on results of the Phase IIa trial in adults. Organization of Phase IIb clinical trial with 95 school children from 07-11 years old living at the same endemic area for both *Schistosoma mansoni* and *S. hamatobium* of the Senegal River Basin Region was concluded, Ethical Committee approval and Regulatory License granted by Senegalese MoH. Trial is already ongoing under conduction of EPLS, at the same region of precedent Phase IIa trial in adults, using the same Lot of GMP Sm14 under a regimen of three IM doses of 50 µg/dose formulated with 2.5 µg of GLA-SE, 30 days apart. The Phase IIa and b trials are mainly being sponsored by the private Brazilian partner Orygen Biotechnology and governmental institution FIOCRUZ with support of CEWG-WHO platform and Financial Fund.

4. Sm14 +GLA-SE anti-Schistosomiasis Humanitarian Vaccine

During the process of analysis by member state representatives under WHO regional structure and posterior extensive questioning by technical experts and ad hoc committees, Sm14 Schistosomiasis vaccine, was selected as one of the present list of six Demonstration Projects, confirmed by WHO Executive Board. In June of 2015, a Stake Holders Meeting was organized by WHO at Genève headquarters, before the first installment was granted. During the process of selection and shortlisting of presented projects; discussions on the scientific merits, state of the art of the project and demanded full adherence to CEWG principles, much was learned on the mandatory need to assure the Accessibility and Affordability of this vaccine to the poor endemic countries to which it is ultimately addressed [14].

From its inception, the Sm14 based Schistosomiasis vaccine was conducted targeting an effective and low-cost product as the final objective. To achieve this goal, several innovations to vaccine development were implemented and a strong effort of choosing IP free components was prioritized [36] successfully leading to very low cost of a stable end product after recombinant protein purification process for large scale production.

Delinkage of final product price from the costs of the long R&D phases was privileged by Sm14 being developed at a governmental scientific foundation (FIOCRUZ) from the Brazilian MoH and strongly supported by funds from public Brazilian financial institutions (FINEP and FAPERJ).

After 2005, licensing of FIOCRUZ patent rights to a private company was ruled by specific contracts designed to protect the accessibility, affordability and supply strategies to lower- and middle-income countries (LMIC) that are the target areas to receive the anti-Schistosomiasis vaccine. Presently licensee company, Orygen Biotechnology, a startup owned by Brazilian giant players from Pharmaceutical sector, is highly contributing to final development of the Sm14 human vaccine under contracts based on cost plus strategy of pricing vaccines, as adopted by WHO [41].

In parallel, the veterinary anti-fasciola vaccine that is being developed in keeping with current European guidelines to reduce the presence of anti-helminthic drug chemical residues in milk and meat of livestock, is addressed to rich countries and markets and designed to contribute/support future potential large scale delivery programs.

We are not anymore at a time when anti-Schistosomiasis vaccine is to be discussed, attacked or delayed, as it was for decades along with all anti-parasite vaccines. Our knowledge about vaccines improved enormously, as well as technical resources available.

Vaccines represent the intervention strategy with the best cost-benefit ratio so far applied in public health. Likewise, transmission control of infectious/transmissible diseases has only been achieved through vaccination. Sanitation, chemotherapy and health education are not sufficient to eliminate parasitic diseases that affect disproportionally people living in endemic areas of poor countries. Immunization with a safe and effective vaccine, can contribute to a long-term reduction of egg excretion from the host, truly controlling transmission. So far, there are no vaccines against parasites that afflict countries fighting to emerge from poverty and reach better conditions of health and overall development.

The Sm14 vaccine against Schistosomiasis is being developed as a humanitarian vaccine to be included in effective Schistosomiasis transmission control programs and hopefully invert the paradigm for north-to-south route for technology generation, contributing to the broad use of the most safe, effective and environment- and human-friendly health-promoting strategy.

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