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Onchocerca volvulus: the road from basic biology to a vaccine

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Abstract

Human onchocerciasis—commonly known as river blindness—is one of the most devastating yet neglected tropical diseases, leaving many millions in Sub-Saharan Africa blind and/or with chronic disabilities. Attempts to eliminate onchocerciasis, primarily through the mass drug administration of ivermectin remains challenging and has been heightened by the recent news that drug-resistant parasites are developing in some populations after years of drug treatment. Needed, and needed now, in the fight to eliminate onchocerciasis are new tools, such as preventive and therapeutic vaccines. This review summarizes the progress made to advance the onchocerciasis vaccine from the research lab into the clinic.

Descriptive words

Onchocerca volvulus; vaccine; vaccine candidates; elimination

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Why a vaccine against *Onchocerca volvulus* is needed

Human onchocerciasis caused by *Onchocerca volvulus* and spread by the bite of infected *Simulium* black flies (Figure 1) remains one of the most important neglected tropical diseases (NTDs). Recent estimates from the Global Burden of Disease Study 2015 indicate that approximately 15.5 million people currently live with onchocerciasis, including 12.2 million people with *Onchocerca* skin disease (OSD) and 1.025 million with vision loss (river blindness) [1]. Almost everyone severely affected with OSD and river blindness lives in Sub-Saharan Africa or Yemen in the Middle East.

Through programs of mass drug administration (MDA) with ivermectin, tremendous strides have been made in reducing the global prevalence of onchocerciasis. Transmission has been nearly eliminated in Latin America, while globally there has been a 29 percent reduction in the prevalence of onchocerciasis since 2005 [1]. However, it remains unlikely that onchocerciasis can be eliminated as a public health problem entirely through ivermectin mass treatments. The reasons for this observation have been reviewed recently, and include the inability to implement large-scale treatment programs in areas that are co-endemic for loiasis, and the potential for emerging anthelminthic drug resistance [2]. Recent genome-wide analyses revealed significant genetic variation in *O. volvulus* parasites, differentiating between those that are good responders to ivermectin treatment and those that are sub-optimal responders. Those parasites that responded sub-optimally were taken from individuals in Ghana and Cameroon who experienced repopulation of the skin microfilariae earlier/more extensively than expected after ivermectin treatment [3].

In addition, disease modeling studies show that transmission interruption and elimination will require routine and regular quantum reductions in *O. volvulus* microfilariae in the skin and subcutaneous tissues following each round of MDA, but such targets are seldom achieved [2]. The African Programme for Onchocerciasis Control predicted in 2015 that to achieve elimination 1.15 billion treatments will have needed to be administered until 2045 [4]. Such estimates indicate that onchocerciasis may not be eliminated for decades using current approaches.

To accelerate elimination and advance towards the major targets of the 2012 London Declaration for NTDs (http://unitingtocombatntds.org/sites/default/files/document/london_declaration_on_ntds.pdf), there is an effort to develop new and improved control tools. These include better diagnostics, small-molecule drugs and vaccines that can improve surveillance and achieve longer and more sustained reductions in host microfilarial loads. There is also a need for better safety profiles for interventions used in loiasis co-endemic areas of Africa. Individuals who have high blood levels of *Loa loa* microfilariae, a filarial infection that usually does not cause clinical disease, and receive ivermectin as part of the MDA programs to eradicate lymphatic filariasis and onchocerciasis, may develop a severe inflammatory reaction that can result in encephalopathy, and rarely death. In 2015, an international consortium launched a new global initiative, known as TOVA – The Onchocerciasis Vaccine for Africa [2]. TOVA is evaluating and pursuing vaccine development as a complementary control tool. Briefly, TOVA is primarily using recombinant proteins and novel adjuvant platforms, with the goal to meet at least one of the desired target

product profiles (TPP). The TPP either relies on a preventive vaccine for children under the age of five who have not yet had access to MDA with ivermectin, or a therapeutic vaccine for both adults and children with onchocerciasis (Table 1) [2]. The efforts to develop an effective, safe, and logistically feasible vaccine against onchocerciasis builds on the evidence of protective immunity achieved using live attenuated vaccines. Immunization with irradiated larvae typically achieves ~70% protection in laboratory settings [5–9], but such vaccines are not feasible for mass human immunization on safety, logistical, and economic grounds. Current efforts to develop a subunit vaccine, such as confirmatory vaccine trials in large-animal models, modeling studies, and future clinical trials will build the necessary body of evidence to allow for the selection of the best TPP. The TPP presented in Table 1 was based in part on mathematical modelling that explored the potential influence of a prophylactic vaccination program on infection resurgence in areas where local elimination has been successfully achieved [10]. It assumed an initial prophylactic efficacy of 50% and an initial therapeutic efficacy of 90%, based on efficacy results in animal models. The vaccine was assumed to target 1 to 5 year olds based on the age range included in the Expanded Programme on Immunization. The modelling indicated that an onchocerciasis vaccine would have a beneficial impact in onchocerciasis-loiasis co-endemic areas, markedly reducing microfilarial load in the young (under 20 yr) age groups. The TPP for therapeutic vaccines is still hypothetical as it assumes that it will be safe to target immunologically residual microfilariae in young and adult populations living in endemic regions that went through many years of MDA with ivermectin.

Here, we provide a perspective of the importance of a rational design for the discovery and antigen selection process before embarking into advanced vaccine development of the onchocerciasis vaccine with a review of the current advancements and progress on the TOVA global initiative. Finally, we provide a prospective of how new technologies and artificial intelligence can catalyze and accelerate the evaluation and selection of suitable vaccine candidates leading to a greater chance of their translation into safe and efficacious human vaccines.

Discovery and evaluation of the first generation vaccine candidate antigens

Considerable effort has been expended in the 1990s on the identification of parasite molecules, primarily proteins, which induce a protective immune response in humans and in the available animal models of onchocerciasis. Anti-L3 protective immunity within the *O. volvulus* endemic population has been described in two populations: (1) immunity that impedes the development of a patent infection (microfilaria positive) in putatively immune (PI) individuals (i.e., individuals that had no clinical manifestations of the disease, even though they lived for at least 10 years within regions where onchocerciasis is endemic and were exposed to high transmission rates of infection); and (2) concomitant immunity, which develops in the patently infected individuals with increasing age and is independent of the immune responses that are induced by the adult worms and microfilaria associated with patent infection [11]. Protective immunity against the infective larvae was also shown in a mouse model employing *O. volvulus* L3 in diffusion chambers; a significant reduction of ~50% in the survival of larvae was obtained in mice immunized with normal, irradiated or freeze-thaw-killed L3 [5].

Two basic strategies were used to identify and clone *O. volvulus* target vaccine antigens: **(1)** Exploitation of the potential involvement of antibodies in protective immunity by immunoscreening various *O. volvulus* cDNA libraries to identify target proteins. The success of the immunoscreening effort relied mostly on the source and specificity of the immune sera from human or animal hosts and, hence, was done mostly with serum samples from individuals identified as putatively immune. In addition, sera from vaccinated or immune animals (chimpanzees, mice or cows), polyclonal antibodies raised against *O. volvulus* infective stage larvae also called L3, or monoclonal antibodies developed against specific parasite-antigens, were used to screen the cDNA libraries. Initially cDNA libraries constructed from adult worm stages of *O. volvulus* were used and later cDNA libraries constructed from *O. volvulus* larval stages (L3, molting L3 and fourth-stage larvae or L4) were used. Altogether, out of 26 recombinant antigens that were identified by immunoscreening and tested in the *O. volvulus* mouse model, 12 induced partial but significant protection (39–69%) in the presence of block copolymer, alum or Freund's complete adjuvant [11–13]. **(2)** Identification and isolation of molecules thought to be essential during the infection process. These molecules would include proteins with vital metabolic functions or defense properties, which would permit the parasite to survive in immunocompetent hosts. Targeting such molecules as vaccine candidates, would block or interfere with the establishment of the parasite in the host. In addition, antigens that are not normally seen by the host, but that are nevertheless accessible to host immune-effector molecules and cells, the 'hidden antigens', were also thought to be potentially useful as vaccine targets [14]. The identification of the genes and isolation of the encoding proteins of interest was achieved by one or multiple of the following methods: a) screening cDNA libraries using a heterologous probes [15]; b) amplification by PCR using degenerate primers and cloning strategies [15]; c) purification of the proteins from secreted products of larval stages followed by partial amino acid sequencing and molecular cloning [16]; or d) identification of the genes of interest by searching the *O. volvulus* expressed sequence tag (EST) database or the EST databases generated by the Filarial Genome Project [17]. Out of 18 recombinant antigens that have been cloned using these strategies and that were tested in the *O. volvulus* mouse model, four (*Ov*-ALT-1, *Ov*-CHI-1, *Av*-ABC and *Av*-UBI) induced partial but significant protection. Of these, *Av*-ABC and *Av*-UBI were cloned from the rodent filarial parasite *Acanthocheilonema viteae* and were protective in the presence of alum or Freund's complete adjuvant, as was *Ov*-ALT-1. In addition, chitinase, *Ov*-CHI-1, effectively induced protection using DNA immunization [18]. The *Onchocerca* homologue of *Av*-ABC has not been studied yet, whereas the *Av*-UBI of *A. viteae* is completely identical to *Ov*-UBI.

The characteristics of the parasite proteins corresponding to the above protective recombinant *O. volvulus* antigens have been described in detail previously [12, 13, 19]. Eight of the proteins, *Ov*-ALT-1, *Ov*-B8, *Ov*-RAL-2, *Ov*-B20, OI5/OI3, *Ov*-CHI-1, *Ov*-RBP-1 and *Ov*-103 are parasite specific antigens, whereas *Ov*-ASP-1 is a member of the vespid venom allergen-like protein family [20]. Six of the protective proteins are homologues to recognized proteins of higher organisms. Thus, *Ov*-CPI-2 (onchocystatin), *Ov*-TMY-1 (tropomyosin), *Ov*-FBA-1 (aldolase), *Ov*-CAL-1 (calponin), *Av*-ABC (ATP binding cassette protein transporter) and *Av*-UBI (ubiquitin) have 32, 31, 69, 42, 71 and

98% amino-acid identity, respectively, with human proteins. An important concern associated with vaccine antigens belonging to conserved gene families (e.g. enzymes, muscle proteins) is the risk of cross-reactions with host or environmental antigens. Eight antigens were also cloned from a very close relative of *O. volvulus*, *O. ochengi*, and used together to vaccinate cattle in the only field trial of a recombinant onchocerciasis vaccine performed to date [21]. These eight antigens included representatives from the parasite-specific [*Oo*-ALT-1, *Oo*-B8, *Oo*-RAL-2, *Oo*-B20 and Oo-FAR-1 (homolog of *Ov*-RBP-1)] as well as the highly conserved (*Oo*-TMY-1 *Oo*-FBA-1, and *Oo*-CPI-2) protein groups. The multivalent vaccine induced statistically significant protection also against patency (microfilaridermia), but did not significantly reduce adult worm burden [22].

Since the above described studies, only one additional antigen with protective properties, *Ov*-GAPDH, which was cloned using immunoscreening, has been recently reported [23]. Thus, out of a total of 16 vaccine candidates, 12 were identified by immunoscreening and 4 were identified using other approaches as illustrated in Figure 2. Below we will describe the 8 vaccine candidates chosen to be studied in greater depth for their ability to insure protection against infection.

Evaluation and selection of the best vaccine candidates for a prophylactic vaccine using two small animal models

Humans are the only definitive hosts of *O. volvulus*. Therefore, one of the significant challenges towards the development of a vaccine against onchocerciasis has been the absence of suitable small animal models that support the life-cycle of the parasite (Figure 1). To overcome this obstacle, we adopted a dual-model screening system. In the first model, *O. volvulus* L3 are implanted in mice within diffusion chambers [24]. This model has the advantages of using the target human parasite and allows the unique analysis of the host molecules and cells found within the parasite microenvironment. In addition, dissection of the mechanism of immunity induced by the vaccine can be accomplished with the plethora of reagents and assays designed for murine studies. A significant disadvantage of the mouse diffusion chamber model is that the parasites will only develop for a limited time in mice and thus adult worms and microfilariae do not develop. To overcome this limitation, we tested in parallel a second system, the *Brugia malayi*-gerbil model of lymphatic filariasis, using homologues of promising *O. volvulus* antigens. Injection of L3 subcutaneously in this model allows for examination of vaccine efficacy following the natural migration of developing stages of parasites and their maturation to adult stages [25].

From the pipeline of potential candidate antigens (Figure 2), fifteen proteins were evaluated in previous studies using the mouse-*Onchocerca* model and identified as being able to induce partial protection following vaccination [13]. To select the most promising protective antigens for the early pre-clinical process development, a scoring system was developed that allowed ranking these 15 antigens based on their other known characteristics (reviewed in [13]), and to select eight vaccinate candidate for more extensive studies. All the 15 *O. volvulus* protective antigens in the *O. volvulus* - mouse model were given a score of 1.0 (Table 2). The added scoring was based on the following criteria: (1) score 0.2 was given to

those that are nematode or parasite specific with or without known function (for example *Ov*-CPI-2 (cystatin), *Ov*-RBP-1 (retinoid binding protein) or *Ov*-CHI-1 (chitinase); (2) score 0.2 was given to those in which localization of the corresponding native proteins in L3 and/or molting L3 (mL3) by immunoelectron microscopy was in one or more regions that are also recognized by antibodies from protected humans and/or also from xL3 immunized and protected mice [11]; (3) score 0.2 was given to those being recognized by antibodies from protected humans (PI and INF with concomitant immunity) and/or animal models after immunization with xL3 (cattle, chimpanzees, mice); (4) score 0.2 was given to those being abundantly expressed in L3 and/or mL3, which indirectly indicates that the corresponding translated proteins are important for the parasite during the initial phases of the *Ov* infection; and (5) score 0.2 was given to those where studies have shown the ability of antibodies targeting the parasite antigen to kill larvae *in vitro*.

In addition, we have added two more criteria [45] that are based on more recent published and unpublished studies and thus provide added support for the selection of these 8 antigens for our proposed preclinical studies. A score of 1.0 was given to those (for examples *Ov*-ALT-1, *Ov*-CPI-2, *Ov*-RAL-2, chitinase, *Ov*-RBP-1 and *Ov*-B20) whose homologues have been shown to also induce protection in other filariae host–parasite systems [26–36]. Moreover, A score of 1.0 was given to those (*Ov*-ASP-1, *Ov*-103, *Ov*-CPI-2, *Ov*-RAL-2) having homologues in other nematode host–parasite systems that have been shown to be able to induce reduction in worm burden or other protective measures against hookworm infection in dogs and *Ascaris* in pigs [37–44]. Based on this rational innovative scoring system we have selected the top ranking 8 *Ov* protective antigens (*Ov*-CPI-2, *Ov*-ALT-1, *Ov*-RAL-2, *Ov*-ASP-1, *Ov*-103, *Ov*-RBP-1, *Ov*-CHI-1 and *Ov*-B20) for which we propose to conduct extensive preclinical evaluation and further selection. Those selected are ranked between a total score of 4.0 to 2.6 (Table 2). Those of the original 15 r*Ov*Ags that were not selected were only ranked at a total score of 1.0 to 1.6.

The eight selected *O. volvulus* proteins and the *B. malayi* homologues were expressed in both bacterial (*Escherichia coli*) and eukaryotic (*Pichia*) expression systems. In the presence of the adjuvant alum, the recombinant *Ov*-103 and *Ov*-RAL-2 proteins, together with their *Bm*-103 and *Bm*-RAL-2 homologues emerged as the most promising candidates in each animal model, validating the robustness of our selection and prioritization process. Combination of these two antigens by either co-administration vaccine strategies or single injections using a recombinant fusion protein vaccine induced enhanced levels of protective immunity, demonstrating that the antigens could act synergistically in both systems [45, 46]. Furthermore, these co-administered molecules or the fusion proteins reduced embryogenesis in *B. malayi* females, suggesting a potential impact also on microfilaremia and transmission [46].

Various adjuvants were evaluated and compared for their ability to improve efficacy by enhancing the killing of *O. volvulus* in diffusion chambers implanted in mice. Only adjuvants that induced Th2 responses, as determined by cytokine profiles, were effective at enhancing the vaccine efficacy, consistent with reports showing that IL-4, IL-5, and functional eosinophils are necessary for the development of adaptive immunity in mice immunized with irradiated *O. volvulus* larvae [47–49], and the *Litomosoides sigmodontis*

murine model [50–54]. Co-administration of both of the *O. volvulus* antigens enhanced parasite killing as compared to single antigen immunizations, with all of the adjuvants inducing Th2 responses. Antigen specific IgG1 was the dominant antibody isotype that developed in protected immunized mice. Based on chemokine levels within the diffusion chambers, it appears that eosinophils, macrophages and neutrophils participate in the killing mechanism. These findings suggest that the mechanism of protective immunity induced by the two *O. volvulus* antigens is multifactorial with roles for cytokines, chemokines, antibody and specific effector cells [55]. This observation was confirmed in the *B. malayi*–gerbil model, where it was demonstrated that serum from gerbils immunized with the two *B. malayi* antigens on alum, killed the parasites *in vitro*, in collaboration with peritoneal exudate cells [46].

Thus, based on the two model systems, *O. volvulus* in mice and *B. malayi* in gerbils, an effective two-antigen vaccine against *O. volvulus* has been identified. It consists of the proteins *Ov*-103 and *Ov*-RAL-2, administered with an adjuvant that induces Th2 responses. Immunization with both antigens enhanced the protective immune response and the mechanism of protective immunity appears to be antibody and effector cell dependent, in both model systems.

As mentioned above, a third small-animal model, the *L. sigmodontis*-BALB/c mouse model, has been developed and used for studying anti-filarial immunity and vaccines [56, 57]. This model also allows full development of the infective larvae to adult worms producing circulating microfilariae. It will be worthwhile to incorporate this third model into future efficacy pipeline studies and validate the *L. sigmodontis* homologous of the *O. volvulus* vaccine candidates also in this filarial infection model in mice.

The need for a rational and efficient process to generate a robust pipeline of second generation vaccine candidate antigens

The disappointing results obtained many times during human proof of concept clinical trials, continue to highlight the challenges and limitations of how to best predict whether a vaccine candidate translates successfully from animal testing into humans [58, 59].

Many articles call for a change in paradigm from an empirical development strategy to a rational vaccine design [60–62]. Amongst the parameters driving decisions during the development of new vaccine targets, the current consensus is that antigen selection and optimization represents the foundation in vaccine design. In addition, it is essential to have available appropriate preclinical models, but it is also crucial to have optimal vaccine formulations, adjuvants and delivery strategies. These are essential elements to target the appropriate immune mechanisms of protection [63]. This is especially important when developing vaccines for infectious diseases, such as for onchocerciasis, because unfortunately scientific advances and tools are still trailing and there is also a need for safety and efficacy studies to be done more quickly, with more certainty and at lower costs.

For example, strategies to identify the ideal *Onchocerca* vaccine candidate antigens can rely on selection processes based on the knowledge of candidates inducing effective immune

responses, identifying antibody-based epitopes via computational prediction tools, down-selection of candidates based on predictions of sequences that could induce immunopathology or allergy, and continuous assessment of parasite molecules by structural biology and stability assessments. Hence, systems biology approaches continue to lead the efforts seeking better understanding of the mechanisms of protection and safety of vaccines [61].

Considerable efforts have also been done in the area of novel adjuvant development. Subunit vaccines need help with secondary molecules modulating the immune responses. TOVA Initiative is also incorporating into the development path the evaluation of other adjuvants besides the traditional phosphate or hydroxide salts of aluminum such as oil-in-water emulsions and synthetic toll-like receptor agonists [62]. The objective is to select adjuvants that facilitate the most effective response, while in parallel investigate their optimized use, route and molecular mechanism.

Selecting and evaluating the ideal delivery route and system also provides a benefit towards rational vaccine design. Investigating the mechanisms to overcome pre-existing immunity, an understanding of the basis for the stimulation of memory responses, and examining the interface between innate and adaptive immunity can also maximize the potential for vaccines to trigger long-lasting immunity and protection.

Using ‘omics to catalyze and accelerate the decision process for the discovery of second generation vaccine candidate antigens

Recent technology advancements of the 21st Century have allowed the use of new animal or computer-based predictive models, biomarkers for safety and efficacy, and clinical evaluation techniques to assist in the improvement of predictability and efficacy needed along the critical path to move discoveries from the laboratory bench to licensure. Ultimately, developing and identifying methods to establish correlate markers or surrogate endpoints for protection will be necessary and essential [60].

The current accumulation of molecular data and expansion of filarial parasite RNA and DNA databases, as well as proteomic datasets, has already provided a fresh start by permitting a more rational approach to vaccine candidate discovery [64]. For instance, the availability of genomes for *B. malayi*, *L. sigmodontis* and *O. ochengi* has facilitated numerous secretome studies across the parasite lifecycle [65–67]. One group of vaccine candidates that was identified by this unbiased, high-throughput approach was a ShK toxin domain family in which each individual member contains six ShK domains; a situation that is unique to filarial nematodes [30]. These abundant secreted proteins probably have an immunomodulatory role [66, 68] that could be targeted using antigens incorporating rational mutation of critical amino acid residue(s); an approach that has been used successfully with CPI-2 [56, 69]. In addition, the *O. volvulus* genome, as well as the transcriptome and proteome of each stage from the definitive host (L3, molting L3, L4, adult male, adult female, and nodule and skin microfilaria stages), has been published recently [70, 71]. These new datasets, when combined with immunomics [72–76], have provided an opportunity to identify the antigens that, either alone or in combination, function as targets of natural

acquired immunity against filariae. Recombinant protein or synthetic peptide arrays can be used to interrogate the genome-wide proteome of infectious pathogens consisting of the entire potential antigens using only small amounts of individual sera samples. This approach permits investigators to perform extensive longitudinal, epidemiological and surveillance analyses, as well as identifying immune responses at various stages of infections in the human host in a fashion not possible with other technologies [77, 78].

Using the immunomics approach with sera samples from putatively immune individuals from Cameroon and the Americas *versus* sera from infected individuals, six new potential vaccine antigens were identified. This was accomplished by screening for IgG1, IgG3 and IgE antibody responses against a protein array containing 362 *O. volvulus* recombinant proteins [71], and identifying those with a significant IgG1 and/or IgG3 reactivity with little-to-no IgE reactivity. Notably, four of these antigens (OVOC10819, OVOC5395, OVOC11598 and OVOC12235) are highly expressed during the development of the early stages of the infective stage larvae, L3, in the human host; these would be worthy candidates for testing their efficacy in a preventative vaccine model of infection. Interestingly, the two other proteins (OVOC8619 and OVOC7083) are highly expressed by the microfilariae and were mostly recognized by sera from the putatively immune individuals who never developed a patent infection with microfilaridermia; these would be worthy to be tested as vaccine candidates for a therapeutic vaccine [71].

The initial objective for the *Onchocerca* vaccine was to identify candidate antigens for a prophylactic vaccine to be administered to children under the age of five who have not yet had access to MDA with ivermectin (Table 1); the first generation of our vaccine candidates fulfilled this objective. However, the immunomics approach now opens new possibilities for also developing a safe anti-transmission or therapeutic vaccine. The immunomics studies reported by Bennuru et al. [71] were the first time in which the *O. volvulus* stage-specific genome-wide expression data was used to discover empirically novel vaccine candidates. It would be of great interest to test the novel vaccine candidates identified by the immunomics approach [71] in the *O. volvulus* diffusion chamber mouse model [45] and *B. malayi* – gerbil infection model to validate whether the immunomics approach actually have identified vaccine candidates that protect against L3 and/or microfilariae.

Other potential applications of immunomic approaches include unbiased characterization of the immune response at the site of infection. In the *O. ochengi* system in cattle, a recent secretome analysis of nodule fluid identified almost 500 host proteins that ‘bathe’ the adult worms *in vivo* [67]. Interestingly, these proteins were dominated by antimicrobial proteins, such as cathelicidins, which probably originate from the neutrophils that dominate the intranodular environment. A parallel approach could be used to explore the immunological changes that occur within nodules in animals displaying partial protection induced by vaccination. Such studies will be very valuable in the future for the machine learning approach described below.

Prospective: The potential for machine learning to accelerate the evaluation and selection of vaccine candidates

Decades of research on prototype anti-filarial vaccines in animal models, the application of transgenic knockout mouse strains, and immunological studies of onchocerciasis patients presenting different clinical phenotypes, has led to a broad consensus on the characteristics of protective immunity and some of the key factors that drive immunopathology. Thus, a Th2-biased immune response directed against incoming infective larvae, with a secondary (but important) role for a Th1 component and the modulating influence of T-regulatory cells, is associated with 'benign' protection [57, 79, 80]. Conversely, at least in humans, unregulated Th2 responses against microfilariae in conjunction with Th17-driven inflammation and profound eosinophilia lead to effective parasite killing, but at the price of a hyperreactive form of onchocerciasis exhibiting severe skin inflammation also called sowda if the inflammation is unilaterally predominant [81, 82]. This very rare condition is associated with certain genetic polymorphisms in immune-related genes [83, 84]. However, adverse reactions with a clear immunological component are possible in a wider range of patients, as is not uncommon with antifilarial chemotherapy [85, 86]. Consequently, accurately predicting whether a vaccine candidate is likely to be both safe and effective is very challenging using conventional approaches alone, especially as we lack animal models that recapitulate the pathology seen in human onchocerciasis.

Traditional statistical approaches can be powerful at disentangling these immunological events, but tend not to generalize well from model systems to humans. However, machine learning techniques have been developed to improve generalizability by tuning models to maximize prediction accuracy to independent test samples, and tend to deal with large numbers of variables better than traditional statistical approaches [87, 88]. Such methods have been used successfully to analyze immune responses to bacterial infection using whole blood transcriptional signatures [89], and to detect local pathogen-specific immune profiles in peritoneal dialysis patients [87]. In principle, by combining vaccinology read-outs from animal models and natural immunity in humans, it may therefore be possible to improve the selection of vaccine candidates earlier than currently possible. Thus, by identifying robust markers of immunity that generalize well, such approaches may help bridge the divide between development, preclinical, and clinical phases of vaccine development (Figure 3).

Concluding remarks

Although it was previously considered that *O. volvulus* infections can be controlled using only MDA with ivermectin, it is becoming increasingly clear that without additional modalities such as drugs which kill or permanently sterilize the adult worms and/or a vaccine, elimination of onchocerciasis from Sub Saharan Africa may remain an unfulfilled goal. Vaccines aimed at preventing infection (anti-L3), and/or reduce microfilariae in adults and children with onchocerciasis could be the essential complement for the successful control or elimination of both diseases.

The successful vaccines developed against taeniases and the major advances already made in development of human anthelminthic vaccines [90], show that it is indeed possible to

develop and test protective vaccines against multicellular parasites. In regard to *O. volvulus*, the human studies have suggested that protective immunity can develop in humans. The experimental and natural infections of calves have demonstrated that protective immunity does develop and that vaccines can protect animals from infection under natural conditions. Moreover, using the small animal models for antigen screening have already accomplished the identification of two lead vaccine candidates; now the challenge is to optimize and formulate these vaccines for human usage, which can take advantage of the procedures currently being developed for the human hookworm and schistosome vaccines [91, 92], making the process potently quicker than usually expected (see Outstanding Questions). Efforts to develop novel diagnostic assays that support the monitoring of current and future control measures are underway and are expected to soon provide diagnostic assays that can predict efficacy of the prophylactic and therapeutic vaccines in human clinical trials.

Outstanding Questions

- What additional tools are needed to support the elimination of onchocerciasis in Africa?
- Adjuvants are an important component for vaccine delivery; additional adjuvants that may increase efficacy. Should other adjuvants be tested versus the alum formulated vaccines?
- Should we optimize the *O. volvulus* vaccine in regard to dosage, number of immunization and ability to provide sufficient memory?
- Should we proceed to identify new vaccine candidates for prophylactic and/or therapeutic vaccines using more rational approaches?
- How can new technologies and artificial intelligence catalyze and accelerate the evaluation and selection of more effective vaccine candidates leading to a greater chance of their translation into safe and efficacious human vaccines?
- Can we develop diagnostic assays that can predict efficacy of the prophylactic and therapeutic vaccines in human clinical trials?

The task ahead is to assure continued pre-clinical development by convincing potential donors that *O. volvulus* vaccine production and testing is a realistic goal worth supporting. The potential development of drug resistance to the drugs used for MDA and the many years of MDA now being anticipated to control onchocerciasis might provide such impetus.

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Box 1

Key points that support the advancement and progress towards an onchocerciasis vaccine

- It remains unlikely that onchocerciasis can be eliminated entirely through ivermectin mass treatments
- An international consortium launched in 2015 a new global initiative, known as TOVA – The Onchocerciasis Vaccine for Africa – with the goal of evaluating and pursuing vaccine development as a complementary control tool
- A rational design for the antigen discovery and selection process before embarking into advanced vaccine development of the onchocerciasis vaccine resulted in the identification of two recombinant proteins – Ov-103 and Ov-RAL-2 – that individually or in combination induced significant protection against infection

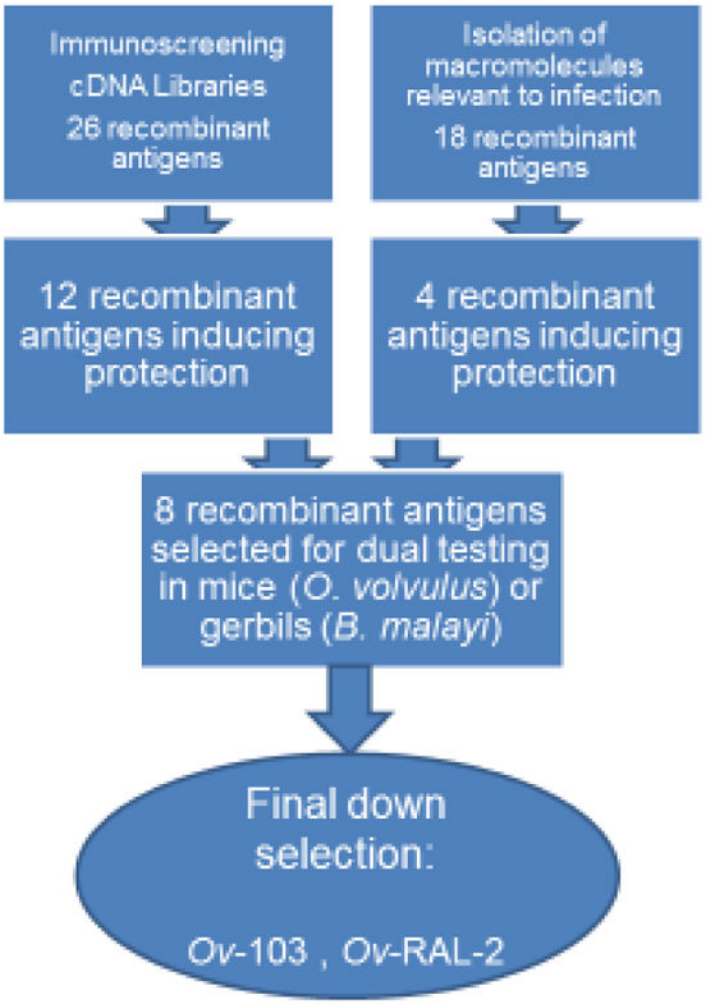

Figure 1.

The *Onchocerca volvulus* lifecyle. During a blood meal, an infected blackfly (genus *Simulium*) introduces third-stage filarial larvae onto the skin of the human host, where they penetrate into the bite wound ❶. In subcutaneous tissues the larvae ❷ develop into adult filariae, which commonly reside in nodules in subcutaneous connective tissues ❸. Adults can live in the nodules for approximately 15 years. Some nodules may contain numerous male and female worms. Females measure 33 to 50 cm in length and 270 to 400 μm in diameter, while males measure 19 to 42 mm by 130 to 210 μm. In the subcutaneous nodules, the female worms are capable of producing microfilariae for approximately 9 years. The microfilariae, measuring 220 to 360 μm by 5 to 9 μm and unsheathed, have a life span that may reach 2 years. They are occasionally found in peripheral blood, urine, and sputum but are typically found in the skin and in the lymphatics of connective tissues ❹. A blackfly ingests the microfilariae during a blood meal ❺. After ingestion, the microfilariae migrate from the blackfly's midgut through the hemocoel to the thoracic muscles ❻. There the microfilariae develop into first-stage larvae ❼ and subsequently into third-stage infective larvae ❽. The third-stage infective larvae migrate to the blackfly's proboscis ❾ and can

infect another human when the fly takes a blood meal ◐. Reproduced from the Center for Disease (https://www.cdc.gov/dpdx/onchocerciasis/index.html).

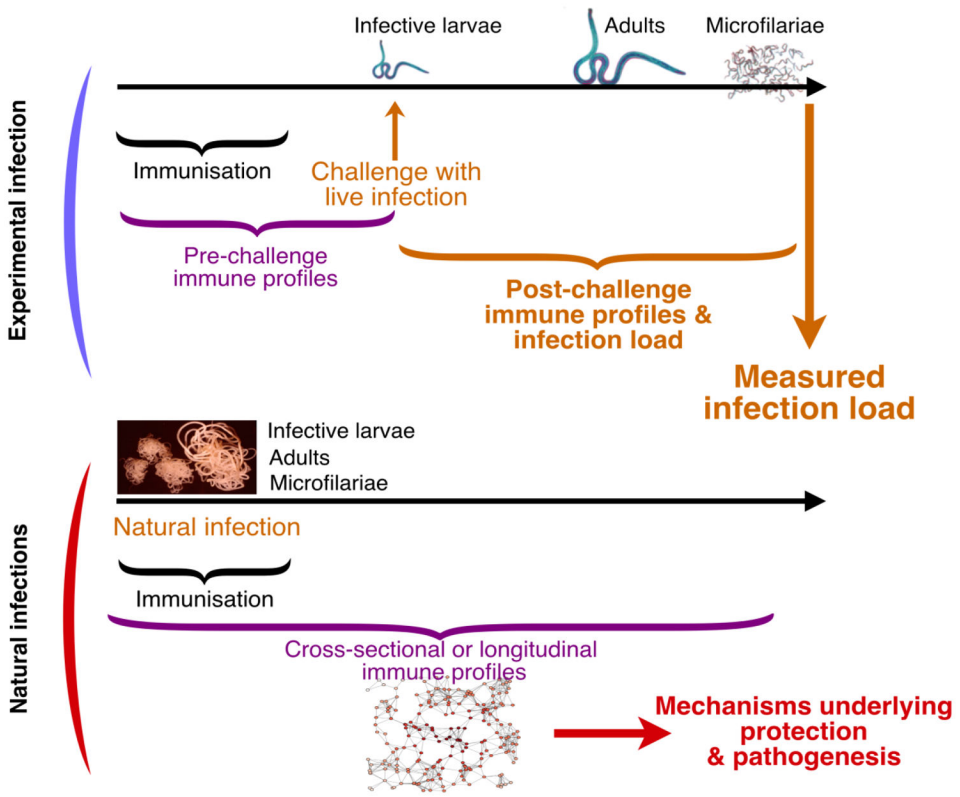

Figure 2.

Schematics that illustrates the down-selection process that resulted in the selection of the two most promising vaccine antigens for future clinical development.

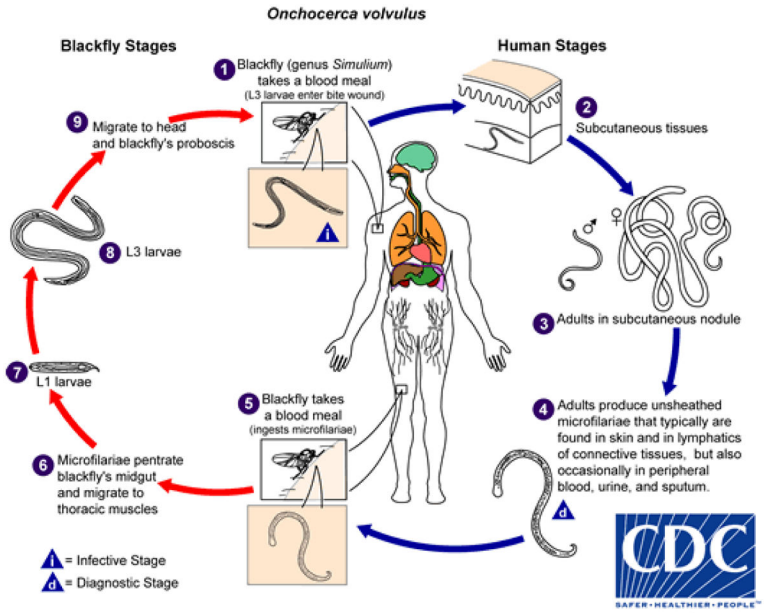

Figure 3.

Combining a systems analysis of response to vaccines and machine learning algorithms to help predict vaccine efficacy. (A) Applying machine learning to experimental infections across multiple model systems and species can help identify which immune variables throughout the time course of an infection most reliably predict infection load, while ensuring the trained models generalize well across biological systems. (B) These optimized models may then be useful in predicting vaccine efficacy in human trials in two ways: identifying what data to collect and predicting likely vaccine efficacy using incomplete data that are typical of human field studies.

Table 1

Target product profiles for prophylactic and therapeutic onchocerciasis vaccines

Characteristic	Desired target – prophylactic[a]	Desired target – therapeutic (if different)[b]
Indication	A vaccine to protect against infection with infective larvae and to reduce adult worm burden and microfiladermia for the purpose of reducing morbidity and transmission	A vaccine to reduce microfiladermia for the purpose of reducing morbidity and transmission
Target population	Children <5 years	older children and adults that already carry adult worms
Route of administration	Intramuscular injection	
Product presentation	Single-dose vials; <0.5 ml volume of delivery	
Dosage schedule	Maximum of 3 immunizations given 4 weeks apart	
Warnings and precautions/ pregnancy and lactation	Mild to moderate local injection site reactions such as erythema, oedema and pain, the character, frequency, and severity of which is similar to licensed recombinant protein vaccines. Less than 0.01% risk of urticaria and other systemic allergic reactions. Incidence of serious adverse reactions no more than licensed comparator vaccines	
Expected efficacy	>50% efficacy at preventing establishment of incoming worms; >90% reduction of microfilariae (based on current animal model results)	>99% reduction of microfilariae
Co-administration	All doses may be co-administered and/or used with other infant immunization programmes	
Shelf life	4 Years	
Storage	Refrigeration between 2 to 8 degrees Celsius. Cannot be frozen. Can be out of refrigeration (at temperatures up to 25 degrees) for up to 72 hours	
Product registration	Licensure by the Food and Drug Administration and/or the European Medicine Agency	
Target price	Less than $10 per dose for use in low- and middle-income countries.	

[a] adapted from [2].

[b] the assumptions for the blank cells are similar to those expected for the prophylactic vaccine

Table 2

The portfolio of eight lead *O. volvulus* protective larval proteins[a]

Characteristics of the *O. volvulus* protective protein						Protection in animal models			Total Score
Antigen (kDa)	**Identity (Function)**	**Localization[b]**	**Immunogenicity[c]**	**#ESTs[d] L3/mL3**	***In vitro* killing assays[e]**	**Protection in Ov mouse model (%)[e] [adjuvant]**	**Protection in lymphatic filariae models [animal model, adjuvant]**	**Protection in other helminth models (model, adjuvant)**	
Ov-CPI-2 (17)	Onchocystatin, (Cysteine protease inhibitor)	Hypodermis; basal layer of cuticle; separation of L3/L4 cuticles; secretory vesicles; ES	PI sera CI sera Chimpanzee anti-*Ov*-xL3	59/9	96–100% inhibition of *Ov* L3 molting	43–49% (alum)	Ls-cystatin; 50% reduction in patent infection[f] [Ls mouse model, alum and Pam3Cys]	Ac-cystatin; 22% reduction in worm burden [Ac dog model, AS03]	**4**
Score	**0.2**	**0.2**	**0.2**	**0.2**	**0.2**	**1**	**1**	**1**	
Ov-ASP-1 (25)	Novel, homologue of vespid venom allergen 5 and the PR-1 protein family	Granules of glandular esophagus; ES	PI sera CI sera Mice anti-*Ov*-xL3	50/1	Serum from jirds immunized with *Bm*-ASP-1 caused 62% cytotoxicity *in vitro* against *Bm* L3 and Mf	44 % (alum) 42% (FCA)	*Bm*-ASP-1; 62% reduction in survival of L3 in chamber [jird, alum] *Bm*-ASP-1+*Bm*-ALT-2 +; 79% reduction in worm burden [jird, alum]	Ac-ASP-2; 26% reduction in worm burden; 69% reduction in eggs output; serum from the vaccinated dogs induced *in vitro* 60% reduction in L3 migration [Ac dog model, AS03]	**4**
Score	**0.2**	**0.2**	**0.2**	**0.2**	**0.2**	**1**	**1**	**1**	
Ov-RAL-2 (17)	Novel, nematode specific	Hypodermis	PI sera CI sera Mouse anti-*Ov*-xL3	6/4	Anti-rAs16 from the protected pigs inhibit survival and molting of L3 *in vitro*	51–60 % (FCA)	rWb-SXP/*Bm*14; 30% reduction of L3 survival within chambers [mice, FCA] Wb-SXP; 19% reduction of L3 survival within the	rAs16; 64% reduction in *A. suum* L3 [mice, cholera Toxin (CT)]	**3.8**

Characteristics of the *O. volvulus* protective protein						Protection in animal models			Total Score
Antigen (kDa)	**Identity (Function)**	**Localization[b]**	**Immunogenicity[c]**	**#ESTs[d] L3/mL3**	***In vitro* killing assays[e]**	**Protection in Ov mouse model (%)[e] [adjuvant]**	**Protection in lymphatic filariae models [animal model, adjuvant]**	**Protection in other helminth models (model, adjuvant)**	
							chambers [mice, DNA] r*Bm*-SXP-1; >90% reduction in microfilaremia and 35% in adult worm burden [jirds, FCA or Alum]	rAs16; 58% reduction in *A. suum* lung – stage L3s [pigs, CT] rAc-16; 25% reduction in hookworm worm burden, 64% reduction in egg count and significant reduction of blood loss [dogs; AS03]	
Score	**0.2**	**0.2**	**0.2**	**0.2**		**1**	**1**	**1**	
Ov-ALT-1 (15)	Novel, filariae specific	Granules of glandular esophagus; cuticle; channels	PI sera CI sera	223/18	Serum from jirds immunized with *Bm*-ALT-2 caused 72% cytotoxicity against *Bm* L3 and Mf *in vitro*	39–62% (alum)	*Bm*-ALT-1; 76% reduction in worm burden [jird, FCA] *Bm*-ALT-2; 72% reduction in survival of L3 in chamber [jird, alum] *Bm*-ALT-2 + *Bm*-ASP-1; 79% reduction in worm burden [jird, alum]	ALT-1 is a filariae specific protein	**3**
Score	**0.2**	**0.2**	**0.2**	**0.2**	**0.2**	**1**	**1**		
Ov-103 (15)	Novel, nematode specific	In L3: Basal layer of the cuticle; hypodermis; basal lamina; channels; multivesicular bodies.	PI sera CI sera	5/0	Anti-*Ov*-103 killed Mf 79%	30–69 % (alum)	ND[g]	Ac-SAA-1; antibodies inhibited (46%) migration of L3 [Ac, FCA]	**3.0**

Characteristics of the *O. volvulus* protective protein						Protection in animal models			Total Score
Antigen (kDa)	**Identity (Function)**	**Localization[b]**	**Immunogenicity[c]**	**#ESTs[d] L3/mL3**	***In vitro* killing assays[e]**	**Protection in Ov mouse model (%)[e] [adjuvant]**	**Protection in lymphatic filariae models [animal model, adjuvant]**	**Protection in other helminth models (model, adjuvant)**	
		In Mf: surface							
Score	**0.2**	**0.2**	**0.2**	**0.2**	**0.2**	**1**		**1**	
Ov-B20 (52/65)	Novel, nematode specific	Cuticle; hypodermis; ES product	Cattle anti-*Ol*-xL3	3/2	ND	39 % [Alum];	49–60% [*Av*, FCA]	ND	**2.8**
Score	**0.2**	**0.2**	**0.2**	**0.2**		**1**	**1**		
Ov-RBP-1/*Ov*-FBP-1 (20/22)	Novel, nematode specific; Retinoid binding protein,	Body wall; ES product	PI sera CI sera	1/2	ND	42 % [BC]	36–55% [*Av*, FCA]	ND	**2.8**
Score	**0.2**	**0.2**	**0.2**	**0.2**		**1**	**1**		
Ov-CHI-1 (75)	Chitinase	Cuticle, granules of glandular esophagus	PI sera CI sera Jird anti-*Av*-xL3	0/0	ND	53% [DNA]	*Bm*-chitinase; induced 48% reduction in worm burden and >90% in Mf [jirds, FCA and alum]	ND	**2.6**
Score	**0.2**	**0.2**	**0.2**			**1**	**1**		

[a] This Table was adapted from [45].

[b] Localization based on the native protein in larval stages (L3 and mL3) as determined by IEM.;

[c] Immunogenicity based on data obtained from protected humans, putatively immune individuals (PI), infected individuals who developed concomitant immunity (CI), and/or antibodies from xL3 animal models (xL3 mouse model, cows or chimpanzees);

[d] The number of ESTs were determined by BLAST-searching the L3 and mL3 EST datasets (3,510 and 5,165 entries, respectively) using each individual gene sequence. A gene was considered up-regulated if the ESTs occurred at least 5 times in a particular stage.

[f] Using *in vitro* cytotoxicity assays few antigens were shown to be a target for antibodies raised against the recombinant antigens of ***Ov*-CPI-2** and ***Ov*-103**. Although antibodies against the other vaccine candidates were not tested *in vitro* for their ability to inhibit molting or kill larvae, it appeared that immunization with the recombinant antigens ***Ov*-CPI-2** and ***Ov*-ALT-1** also induced significant reduction in the molting of L3. Moreover, studies using antibodies from mice immunized with the *B. malayi* homologous recombinant proteins of ***Ov*-ALT-1 (*Bm*-ALT-2)** and ***Ov*-ASP-1** (***Bm*-ASP-1)** have shown that anti-***Bm*-ALT-2** antibodies elicited 71–72% cytotoxicity *in vitro* against both L3 and Mf, while anti-***Bm*-ASP-1** antibodies induced 61–62% cytotoxicity *in vitro* against both L3 and Mf. Interestingly, antibodies against the homologous proteins of ***Ov*-103** in hookworms (Ac-SAA-1) and ***Ov*-RAL-2** in Ascaris (rAs-16), when used *in vitro* inhibit invasion of L3 through dog skin or caused cytotoxicity *in vitro* against L3, respectively.

[g] Protection was determined in mice after two immunization with 25 μg of protein in the presence of an adjuvant or using a DNA vaccine, followed by challenge with 25 L3 within diffusion chambers, and is defined by a significant ($p<0.05$) % of reduction of L3 survival in the immunized mice vs. control mice.

[h] ND=Not determined.

Abbreviations:

Ov, *O. volvulus*; Ol, *O. lienalis*; Bm, *B. malayi*; Wb, *W. branconfti*; Ls, *L. sigmodontis*; Av, *A. viteae*; Ac, *A. ceylanicum*; As, *Ascaris Suum*; BC, block copolymer; ES, excretory–secretory product. XXX – protective in Ov and Bm; XXX- protective in Ov and other filaria animal model (Wb, Av or Lg); XXX protective in Ov and in other nematodes.