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Progress and Challenges in the Field of Snakebite Envenoming Therapeutics

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Abstract

Snakebite envenoming kills and maims hundreds of thousands of people every year, especially in the rural settings of tropical regions. Envenomings are still treated with animal-derived antivenoms, which have prevented many lives from being lost but which are also medicines in need of innovation. Strides are being made to improve envenoming therapies, with promising efforts made toward optimizing manufacturing and quality aspects of existing antivenoms, accelerating research and development of recombinant antivenoms based on monoclonal antibodies, and repurposing of small-molecule inhibitors that block key toxins. Here, we review the most recent advances in these fields and discuss therapeutic opportunities and limitations for different snakebite treatment modalities. Finally, we discuss challenges related to preclinical and clinical evaluation, regulatory pathways, large-scale manufacture, and distribution and access that need to be addressed to fulfill the goals of the World Health Organization's global strategy to prevent and control snakebite envenoming.

1. INTRODUCTION

Snakebite envenoming is a neglected tropical disease of high impact in terms of mortality and morbidity. It affects 1.8–2.7 million people annually, resulting in 80,000–140,000 deaths and leaving at least 400,000 people with permanent physical and psychological sequelae (1). Snakebite mainly affects those living in impoverished rural settings of sub-Saharan Africa, Asia, Latin America, and parts of Oceania (2, 3). The clinical manifestations of envenomings vary depending on the snake species causing the bite, owing to the great variation in venom composition and the multiple toxic effects that the venoms induce. Snakes of the family Elapidae typically cause neurotoxicity by blocking the neuromuscular junctions, although the venoms of some elapids also cause tissue necrosis (1). In turn, envenomings by snakes of the family Viperidae are characterized mostly by pronounced tissue damage, bleeding and coagulopathy, cardiovascular alterations, and kidney injury (1). The main toxins responsible for these effects are depicted in **Figure 1**. Attention to this public health problem has grown over the last decade, as reflected by the inclusion of snakebite envenoming in the World Health Organization's (WHO) list of neglected tropical diseases in 2017, the adoption of a resolution on this topic by the World Health Assembly in 2018, and the launch of the WHO strategy for the prevention and control of snakebite envenoming in 2019 (4).

The WHO strategy is based on four pillars, one of which is to ensure safe and effective therapies (5). Since 1894, the mainstay in the treatment of snakebite envenoming (as well as envenomings by other venomous animals, including scorpions and spiders) has been based on the parenteral administration of animal-derived antivenoms (i.e., preparations of immunoglobulins or immunoglobulin fragments purified from the plasma of large animals immunized with venoms) (6). While the principles of serotherapy remain similar, the technology behind antivenom manufacture has evolved over the years, and guidelines for production and control of these medicines have been issued by

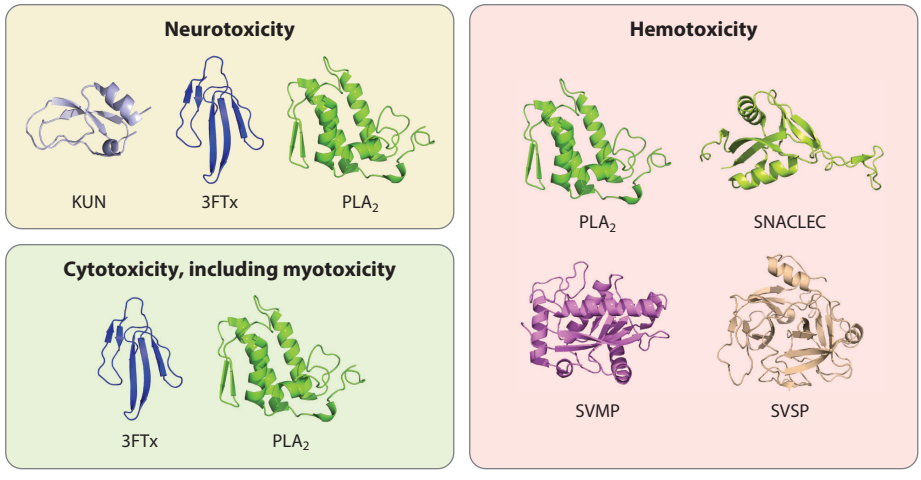

Figure 1

Overview of the main toxic components in snake venoms and their predominant toxic effects. The actual scenario of snake venom composition and toxic profiles is more complex, but the figure depicts the clinically most relevant components and effects. Elapid venoms are rich in 3FTx, PLA_2, and, in some venoms, KUN. Viperid venoms are predominantly rich in SVMP, PLA_2, SVSP, and SNACLEC. Protein Data Bank identifiers for toxins are as follows: KUN, 1DTK; SNACLEC, 1UOS; SVSP, 4GSO; SVMP, 1KUF; PLA_2, 1G2X; and 3FTx, 1QKD. Abbreviations: 3FTx, three-finger toxin; KUN, Kunitz-type protease inhibitor; PLA_2, phospholipase A_2; SNACLEC, C-type lectin-like protein; SVMP, snake venom metalloproteinase; SVSP, snake venom serine protease.

the WHO (7), including the description of target product profiles for antivenoms for sub-Saharan Africa (8). When manufactured with the use of appropriate mixtures of venoms and following good manufacturing practices, animal-derived antivenoms have good safety and efficacy profiles, especially for treating the systemic, life-threatening effects of envenoming. However, they also present limitations, such as their propensity to elicit adverse reactions owing to their heterologous nature; the fact that they should be administered only in health facilities by trained staff; their limited capacity to inhibit toxins that cause local tissue damage, which often results in long-term sequelae; and the restriction of their efficacy to venoms used in immunization (or very similar ones) (9). Hence, there is ample space for improving the therapy of snakebite envenoming.

Though several gaps remain, a large body of knowledge has been built on the characterization of snake venoms and their mechanisms of toxicity, clinical manifestations of envenomings, and the recognition of snake species causing the highest toll of death and morbidity (10). This information is being harnessed for developing and manufacturing antivenoms of higher efficacy and safety and for improving existing products. Moreover, technological platforms for generating therapeutic recombinant antibodies of various molecular formats have reached maturity in the pharmaceutical industry, and these developments are now being applied in the generation of toxin-neutralizing monoclonal antibodies. In addition, synthetic inhibitors capable of abrogating the toxicity of key venom components are being developed or repurposed as novel therapies for envenoming. These exciting developments constitute a fertile ground for improving current antivenoms and developing novel therapeutic options (**Figure 2**). This review summarizes the most relevant achievements reached in this field and highlights challenges and future directions in the global efforts to improve snakebite envenoming therapy.

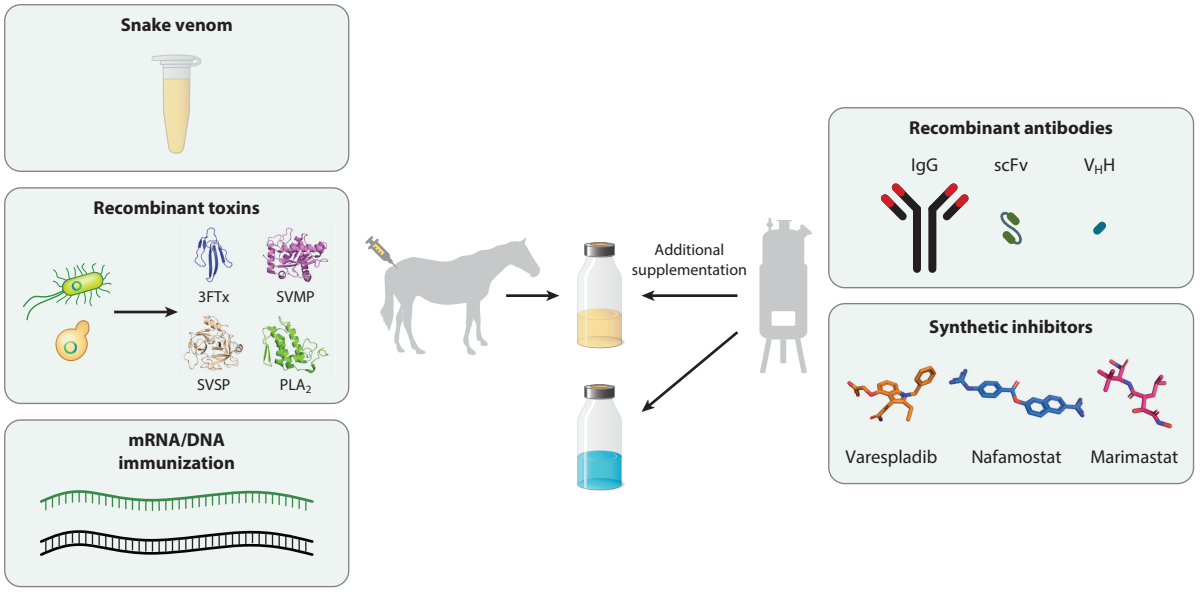

Figure 2

Schematic representation of the different approaches that can be used to optimize immunization strategies for plasma-derived antivenoms and the new types of molecules that might be of use for the development of new envenoming therapies and/or supplementing plasma-derived products. Protein Data Bank identifiers for antivenoms and toxins are as follows: varespladib, 8DND; nafamostat, 7VM7; marimastat, 3HY7; SVSP, 4GSO; SVMP, 1KUF; PLA_2, 1G2X; 3FTx, 1QKD. Abbreviations: 3FTx, three-finger toxin; IgG, immunoglobulin G; PLA_2, phospholipase A_2; scFv, single-chain fragment variable; SVMP, snake venom metalloproteinase; SVSP, snake venom serine protease; V_HH, high-affinity llama single-domain antibody.

2. ANTIVENOMS DERIVED FROM THE PLASMA OF IMMUNIZED ANIMALS

Antivenoms derived from the plasma/serum of large animals, usually horses and less frequently sheep, immunized with snake venoms have been the mainstay of envenoming therapeutics (6). A recent survey indicates that 127 antivenoms are available on the market (11). They show a high heterogeneity in terms of specificity, volume of production, and technological platforms used in their manufacture (12). Two types of plasma-derived antivenoms exist, but both rely on animals being immunized with venoms either from a single species (monospecific antivenoms) or multiple species (polyspecific antivenoms). After immunization, blood is collected, serum or plasma is separated, and the immunoglobulins or their $F(ab')_2$ or Fab fragments are purified and formulated as the final antivenom product (7, 13).

A challenge in the design of antivenoms is related to the inter- and intraspecies, as well as ontogenetic, variability in snake venom composition, making some antivenoms ineffective against venoms of species unrelated to those used in immunization (14). Thus, a key step is the selection of the most appropriate venoms for immunization. This has been traditionally done on a rather empirical basis, but this is changing due to the growing information gathered on the composition and immunological properties of venoms. This wealth of data, together with the identification of the medically most relevant snakes in various regions, allows for a knowledge-based selection of the best venom mixtures for immunization (15, 16). Ideally, an antivenom should possess a broad neutralization capacity to be effective not only against the venoms used in immunization but also against the venoms from related species. Studies of the paraspecificity of monospecific antivenoms are therefore useful to select the most appropriate immunizing mixtures (17), and various techniques can be used to assess this property. These techniques include animal-based and in vitro neutralization assays, as well as antivenomics, a translational approach that uses affinity chromatography to harness the information of venom proteomics for the identification of toxins recognized by antivenoms (18).

Another key aspect of antivenom manufacture is access to representative venoms. Procedures for maintaining snakes in captivity for venom collection should follow validated protocols, ensuring appropriate care of snakes and satisfactory quality of venoms (7, 13). Venom pools should be representative of the geographic and ontogenetic variability of venom composition, and their quality should be subjected to chemical and toxicological controls (7). One limiting factor in antivenom production is the low availability of venoms of some medically important species. This concern could be circumvented by identifying the most relevant toxins and expressing them as recombinant proteins (19) or as recombinant consensus proteins based on sequences of several related toxins to achieve broader coverage (20, 21). Another option is the in vitro production of venoms with the use of organoids (22), although the utility of this technology in antivenom production remains to be determined.

Innovation in the immunization of animals constitutes a fertile ground for improving antivenoms, and several experimental approaches have been explored (23). Usually, whole venoms mixed with adjuvants are injected into animals. Many manufacturers use Freund's adjuvants in the first immunization cycle and then other adjuvants such as bentonite or aluminum salts in the rest of the cycles (7, 13). Owing to the toxicity of Freund's adjuvants, there is a trend to search for alternatives, and for this, much can be learned from the field of vaccine development (24). An injection scheme known as low-dose, low-volume, multisite immunization has proven effective in eliciting an enhanced immune response (25). Other alternative immunization approaches include the use of recombinant virus-like particles displaying conserved toxin epitopes (26) and immunization with DNA constructs encoding relevant toxins or epitopes (27, 28). The latter approach could possibly

also be pursued by the use of more novel techniques involving toxin-/epitope-encoding mRNA delivered with lipid nanoparticles. Moreover, immunological interactions, including immunosuppressive effects, among venom components have been demonstrated, calling for the design of immunization schemes in which separate venoms are injected at different times (29). An area in need of research is the study of the impact of the genetic background of animals on their antivenom response (i.e., how the polymorphism of the major histocompatibility complex affects the immune response to venoms).

Another challenge is the low immunogenicity of key venom toxins, particularly neurotoxins and cytotoxins of low molecular mass, which makes the generation of antivenoms of high neutralizing efficacy difficult (30). This challenge can be confronted by enriching immunizing mixtures with purified toxins (either native or recombinant) or toxin mixtures (31), generating recombinant oligomers of toxins, or coupling these toxins to carriers to enhance their immunogenicity (21, 23). Since venoms also contain nontoxic proteins, it is relevant to identify the key toxins to be used in immunization. For this, the combination of proteomic analysis of venoms with the assessment of the toxicity of each component, a subfield known as toxicovenomics (32), enables the identification of priority toxins for immunization based on those exhibiting the highest toxicity score in each venom (33).

The veterinary care of animals immunized with venoms should be strengthened by introducing interventions that may help reduce the deleterious effects of venoms during immunization. Similarly, the protocols for animal bleeding should be optimized, including the introduction of automated plasmapheresis procedures. There are various plasma or serum fractionation protocols that are used to purify the active substance, either whole IgG molecules or IgG fragments [i.e., $F(ab')_2$ or Fab], obtained by digestion with pepsin or papain, respectively (7, 13). Antibody purification is performed by differential precipitation of proteins with ammonium salts or caprylic acid (7, 13). Some manufacturers use ion-exchange chromatography to further purify the active substance (7, 13). Other methods for IgG purification during antivenom manufacture have been described, such as the aqueous two-phase system (34). Furthermore, steps aimed at improving the safety of antivenoms include ion-exchange chromatography to eliminate bacterial endotoxins and procedures to remove or inactivate viruses (7, 35). Moreover, there is a need to improve the purification of toxin-neutralizing antibodies, since only a low percentage of them are directed toward the medically relevant toxins (36). This can be achieved by affinity chromatography, although few manufacturers use this procedure industrially (37) likely due to cost implications. Finally, the experience accumulated in the field of human plasma fractionation could be of benefit for antivenom production (38).

Officially, the shelf life of liquid antivenoms is 3 years, while freeze-dried antivenoms have a shelf life of 5 years (7). However, antivenoms can remain effective and safe beyond these periods (39, 40). Thus, manufacturers must evaluate the shelf lives of their products by carrying out stability studies, since their extension could improve antivenom availability. Moreover, the introduction of osmolytes and other protein stabilizers to antivenom formulations should be considered to expand the shelf life (41).

Owing to the high variability in venom composition and toxicity across and within species, and since some antivenoms are distributed to diverse geographical regions, the preclinical assessment of antivenom efficacy is of paramount relevance (42). A significant amount of work has already been done in this area (43, 44). Moreover, the WHO is developing a risk–benefit assessment of antivenoms and has issued guidelines, including a detailed account of the methods for preclinical evaluation (7). It is necessary to expand the systematic preclinical analyses of the efficacy of antivenoms, especially against the venoms exacting the highest toll of morbidity and mortality in various regions of the world, to determine which antivenoms are effective in which geographical

locations. Likewise, national and regional regulatory agencies should develop the technical capacity to evaluate the suitability of antivenoms that are offered, including the preclinical assessment of their efficacy and safety.

The quality control of antivenoms involves a set of chemical, physical, and biological tests to ensure their efficacy and safety (7). In this area, new methods should be introduced, in particular to substitute animal-based tests, in line with the 3R principles: replacement, reduction, and refinement. In vitro tests are needed to assess the efficacy of antivenoms. These can be immunochemical assays (i.e., enzyme-linked immunosorbent assays and antivenomics) or tests for evaluating enzymatic or toxic effects of venoms. These include procoagulant (45), protease, and phospholipase A_2 (PLA_2) activities (46); binding of neurotoxins to ion channels (47, 48); and other in vitro functional assays (49). Alternative in vivo assays include the use of fertilized hen eggs (50) and invertebrate animal models (51). Owing to the variability in venom composition and toxicological profile, the selection of alternative tests should be done on a case-by-case basis. Moreover, reduction in the use of animals in antivenom quality control can be achieved by screening antivenoms in vitro before progressing to in vivo models. Likewise, refinement of animal-based tests may involve the use of analgesia (52); the reduction in duration of envenoming required to complete the tests (53); and the development of models that better reflect clinical envenoming, such as implementing treatment delivery after venom challenge (54).

Another area in need of improvement in antivenom development and testing is the clinical evaluation of efficacy and safety (55). Despite the widespread clinical use of antivenoms, many of them have not been evaluated in clinical trials (56), and the lack of uniform criteria for these evaluations has been a limiting factor. A core outcome measurement set has been proposed that could guide future efforts in the clinical evaluation of antivenoms (57). It is necessary to develop novel clinical protocols that could be implemented in low-income settings with a high incidence of envenomings. Pharmacovigilance of antivenoms is another area that should be promoted, especially in the case of antivenoms that have not been robustly tested in clinical trials.

Despite ongoing efforts by the WHO and its regional offices, as well as by governments and public health advocates, there is a crisis in antivenom availability and accessibility in many regions (12). This crisis calls for a concerted and intersectorial global agenda aimed at tackling critical bottlenecks. Health economic analyses are required in order to understand the hurdles that antivenom manufacturers face; to promote investments aimed at improving current manufacturing facilities; and to bring new public and private investors, as well as pharmaceutical companies, to this field (11, 58). National policies for antivenom procurement and distribution should be strengthened, including the deployment of antivenoms to rural health posts, where most snakebites occur (59–61). In parallel, efforts should be directed toward training health staff on the management of snakebite envenomings and their complications, including the protocols for antivenom administration (5, 55).

3. RECOMBINANT ANTIVENOMS

While the use of monoclonal antibodies and antibody fragments as therapy against snakebite envenoming was envisioned more than four decades ago, attempts to develop such molecules have been limited, and so far, no antibody candidates have entered clinical development (62, 63). This has partly been due to the need for various antibody technologies to reach a more mature stage. However, now the discovery and manufacture of monoclonal antibodies has become a standardized operation. In addition, the many existing antibody scientists and research and development laboratories were not engaged in antivenom research. Conversely, many antivenom researchers were based in laboratories that lacked the necessary infrastructure and know-how to set up antibody discovery platforms. However, in the past decade, this has started to change, and several

research laboratories in various regions are now increasingly focusing their efforts on the discovery and development of monoclonal antibodies, antibody fragments, and antibody-like binding proteins that can neutralize snake toxins (64–71). The field of recombinant antivenoms is still in its infancy but is experiencing significant growth, which may pave the way for the future clinical use of recombinant antibodies in snakebite envenoming therapy.

Boulain et al. (72) first discovered a (murine) monoclonal antibody against a snake toxin in 1982, and since then, a growing number of studies have been reported (62, 63, 73). However, with the introduction of snake venom proteomics (i.e., venomics) (15, 74), a wealth of information has emerged, which has made it more feasible to apply systematic efforts to develop recombinant antivenoms (75). Venomics studies have helped unravel the complexity of snake venoms from a functional and evolutionary perspective and have pinpointed which toxins are most abundant and most important for venom function (i.e., toxicity) (14). In particular, toxicovenomics has emerged as a subfield that aims to use the aforementioned information to identify the specific toxins that are to be neutralized by antibodies and other types of inhibitors for an envenoming therapy to be efficacious (33, 76–78). Such studies now equip the recombinant antivenom developer with the information required to initiate rational antibody discovery campaigns using carefully selected snake toxins as target antigens (33).

Many different antibody discovery methodologies exist, including display technologies, hybridoma technology, B cell sorting, the use of transgenic animals, and emerging in silico approaches (79). Combined, these allow for the development of antibodies and antibody fragments that can bind and neutralize snake toxins, that are developable as therapeutics, and that are (more) compatible with the human immune system (80, 81). Most reported studies have utilized phage display technology to discover monoclonal antibodies and antibody fragments (62, 63, 73), possibly due to the ease of establishing phage display technology in molecular biology laboratories and the utility of this technique for working with toxic and low-immunogenic antigens (82–84). As a prominent example of the successful use of phage display technology, Richard et al. (85) reported the discovery of potent V_HH antibodies (a.k.a. nanobodies) that, at low doses, could neutralize the medically important α-cobratoxin from *Naja kaouthia* venom in vivo. The authors further showed that the V_HH antibodies could be reformatted into V_HH-Fc constructs (mimicking human immunoglobulins) while retaining neutralizing capacity. Later, the first examples of how phage display technology can be used to discover fully human monoclonal IgG antibodies were reported (64, 66). Here, the researchers used phage display libraries comprising genes encoding naive human antibody V_H and V_L and thus proved that neutralizing human IgG antibodies could be discovered without the need for immunization (and/or humanization), although several of the reported IgG antibodies possessed modest affinities and were relatively specific to only a few toxins (66). To address this latter challenge, recent work has focused on increasing both affinity and broadly neutralizing properties of the antibodies by using light-chain shuffling (48, 67) combined with stringent panning during a phage display campaign, which can be used to select antibodies with high affinity to the antigen, and by using cross-panning (i.e., panning against alternating antigens during a phage display campaign), which can favor the selection of antibodies with more broadly neutralizing capacities (67, 71, 86, 87). Additionally, efforts involving the use of IgG-encoding genes derived from a human individual who has been subjected to snake venoms are reportedly ongoing (65); similar approaches involving immunized horses are also being explored. Finally, an emerging approach involving the use of consensus toxins has been recently reported (88). These toxins represent an average toxin sequence (21), and they can be used to generate a broadly neutralizing polyclonal antibody response (20). However, the recent report on the use of consensus toxins as antigens in a phage display campaign has shown that they may also serve as a beneficial tool for the discovery of broadly neutralizing monoclonal antibodies (88).

Beyond the initial discovery process itself, the developability and safety profile of snake-toxin-targeting monoclonal antibodies has recently gained increased attention (67, 81). Part of the explanation for this relies on new findings that some monoclonal antibodies may under certain circumstances induce unwanted effects, such as antibody-dependent enhancement of toxicity (89). Such observations underline the importance of proper biophysical characterization and preclinical assessment of new toxin-neutralizing molecules, even of well-known formats such as human monoclonal IgG antibodies and Fab fragments. We therefore predict that, within the field of next-generation antivenoms, even more focus will be directed toward the preclinical evaluation of monoclonal antibodies (and other toxin inhibitors) and that more types of (in vitro) assays will be implemented in early discovery (42, 47, 90).

Further optimization and new methods for discovering monoclonal antibodies are expected to emerge over the next years and will likely include an increased use of recombinant toxins (21, 91), application of more advanced in silico methods (92), and the combination of existing methods in new ways (79). However, all in all, the discovery pipelines are now in place to enable researchers to identify efficacious toxin-neutralizing molecules (67), and the principles behind how to develop and design recombinant antivenoms have been formulated (73, 80, 93). One of the next challenges that needs to be tackled is within manufacturing to ensure that recombinant antivenoms can be produced cost-competitively, as this is key for their future deployment in low-income settings (94–96). To this end, ongoing efforts using oligoclonal cell cultivation techniques to generate oligoclonal human IgG antibodies in a single batch may catalyze increased (industrial) attention to the area of recombinant antivenoms.

Another avenue also related to the aspects of low-cost manufacturing that is gaining increased attention is the use of smaller, alternative antibody formats, such as single-domain antibodies (e.g., V_HH antibodies). Although the first publications on this topic are more than a decade old (62, 85), this avenue of research has gained renewed attention recently. As a first example, Bailon-Calderon et al. (68) demonstrated how an immune V_HH library, derived from a llama, in combination with phage display technology could be used to discover V_HH antibodies against hemorrhagic and myotoxic proteins present in the venom of *Bothrops atrox*. The authors showed how these V_HH antibodies could neutralize the hemorrhagic and myotoxic effects of this venom, although the V_HH antibodies were ultimately unable to prevent lethality (68). More recently, Benard-Valle et al. (97) followed a technologically similar approach, which further included the use of a consensus toxin, and reported the discovery of a panel of V_HH antibodies that could neutralize short-chain α-neurotoxins and PLA_2 from different coral snake venoms (*Micrurus* spp.) (97). Excitingly, these authors (97) also showed that by combining the V_HH antibodies in an oligoclonal cocktail, they could prevent lethality in mice challenged with *Micrurus* whole venoms. We foresee that more reports on the development of recombinant antivenoms based on simpler antibody formats are likely to be published within the next few years, but also that there remains a need to assess the pharmacokinetics of these formats in relation to the toxicokinetics of their venom targets. For example, some venom toxins of low molecular mass are readily distributed to the extravascular compartment, whereas whole IgG antibodies largely remain in circulation, thus creating a mismatch between venom toxicokinetics and antibody pharmacokinetics. This mismatch could possibly be addressed by using antibody formats of low molecular mass.

Further innovative approaches to discover more advanced types of antibodies are also being explored, such as the use of larger multimeric antibody-like constructs with high binding site density (98) and the use of acid-switched antibodies (99) that can potentially be used at lower doses. In this relation, having many binding sites per antibody-like construct allows the construct to neutralize more toxin molecules without the need to increase its dose, whereas the acid-switched antibodies work by a more complex mechanism and exploit the natural recycling mechanism for antibodies

in the body mediated by the neonatal Fc receptor (FcRn). More specifically, acid-switched antibodies possess pH-dependent antigen-binding properties that allow them to bind antigens in circulation (pH ~7.4) and release these antigens for lysosomal degradation during the endosomal FcRn-mediated recycling system in the endothelial cells lining the blood vessels. Here, the pH is lower than in circulation (pH ~6), which allows the antibody to be recycled into the bloodstream ready to bind more antigens (100). However, these approaches are highly experimental at this point, and their potential translational value in relation to recombinant antivenom development is still unknown.

Other areas of science and technology are also starting to affect the development of recombinant antivenoms. In particular, the use of bioinformatics and approaches involving machine learning and artificial intelligence are expected to increase and enable both entirely in silico design of toxin-neutralizing antibodies and/or antibody-like proteins and optimization of such molecules. There is no doubt that the increased use of computational tools can help researchers extract valuable information for early discovery (79). However, it remains to be seen what the impact of these tools will be on actual product development and whether their contribution will significantly speed up the development process toward clinical use.

Finally, we speculate that the emerging field of snakebite envenoming diagnostics could affect how recombinant antivenom products are designed (i.e., what snake species to be covered) (101–103), as improved diagnostics may help provide better epidemiological data on snakebite cases. Snakebite diagnostics may also affect how to approach the discovery of monoclonal antibodies and antibody-like proteins, as it may suddenly become more feasible to deploy species- or genus-specific antivenom products in the clinical setting.

4. TOXIN-TARGETING SMALL-MOLECULE INHIBITORS

Synthetic low-molecular-mass compounds as potential snakebite treatments have received renewed attention in recent years and represent an alternative or complementary therapeutic approach to conventional antivenoms. Similar to recombinant antibodies, synthetic small-molecule-based toxin inhibitors have been under study for several decades (104–106), with natural compounds contained in plant-derived traditional medicines having been tested as snakebite treatments for several centuries prior to that (9). Nonetheless, there are currently no clinically approved toxin-inhibiting snakebite drugs on the market, and only two are in clinical development (107, 108): the metal ion-chelating antimetalloproteinase agent DMPS (2,3-dimercapto-1-propanesulfonic acid) (unithiol) and the PLA_2 inhibitor varespladib, which have recently completed Phase I and Phase II clinical trials, respectively (109–111).

Small-molecule drugs are particularly interesting for snakebite because of their potential to generically inhibit the active sites of enzymatic toxin families, thereby offering the potential for desirable, cross-toxin isoform inhibition (112). This is particularly advantageous given the global context of venom toxin variation limiting the geographical efficacy of conventional antivenoms (14). The small size of such molecules may also enable access to active-site pockets in a manner unachievable using larger biologics. This characteristic may also facilitate more rapid tissue penetration, which could be beneficial in the context of local envenoming (113–115), particularly since conventional antivenoms appear largely ineffective at preventing local tissue damage (116–118). However, with reduced size comes pharmacokinetic challenges, especially relating to short half-lives compared with those of most types of antibodies. Route of administration is a key consideration in this context, with small molecules most rapidly cleared when delivered intravenously. Consequently, much recent research has focused on exploiting the potential for certain small molecules to be delivered orally. For example, both DMPS and the prodrug of varespladib, methyl-varespladib, are orally bioavailable and have been developed in the broader context of

field- or community-level delivery soon after a snakebite, potentially preceding onward transport to a clinical environment for secondary delivery of antivenom (106, 119). Similarly, such molecules have also been discussed in the context of transdermal delivery, with the goal of rapid toxin inhibition at the bite site to prevent severe local envenoming (113, 115, 120). Ideally, such molecules should be selected with a clearly defined target candidate profile, such that discovery and development can be directed toward desirable drug metabolism and pharmacokinetic properties amenable to the anticipated delivery route. Even with optimization, small-molecule therapeutics will require careful consideration of the potential need for repeat dosing. Consequently, generating a rationally designed dosing regimen informed via robust pharmacokinetic data (111) is likely to be critical to the clinical success of such molecules, particularly given that their pharmacokinetic profiles will be distinct from current standard of care (i.e., antivenom).

While plant-based sources of toxin-inhibiting molecules have been used for centuries as traditional medicines for treating snakebite envenoming, there remains an absence of robust clinical data supporting the efficacy of these medicines, and only a few studies have described the isolation and structure-function characterization of toxin-inhibiting compounds from this source (9, 121–123). Much contemporary research has focused on the discovery of synthetic small-molecule inhibitors of venom toxins. These have been inspired by earlier studies showing that specific toxin activities, such as metalloproteinase activity, could be abrogated via the use of repurposed drugs like the matrix metalloproteinase inhibitors (MMPIs) batimastat or marimastat or chelating agents like ethylenediaminetetraacetic acid (EDTA) (104, 105, 124). These studies provided proof of principles that key venom pathologies, such as hemorrhage or coagulopathy, could be reduced in severity or prevented via the delivery of specific drugs. At the same time, the advent of transcriptomic and proteomic technologies led researchers to identify the full diversity and relative abundance of different toxin families and their isoforms found in the venoms of different snake species (125, 126). Collectively, these studies laid the groundwork for current snakebite drug discovery efforts (33, 75), which have focused predominantly around the identification of inhibitors against snake venom metalloproteinase (SVMP) and PLA_2 toxin families (9, 62, 107). Despite their considerable structural and functional diversity, broad-spectrum inhibitors for both toxin families have been identified. Marimastat and other MMPIs (e.g., prinomastat) show considerable therapeutic promise against SVMPs due to their similarity to human matrix metalloproteinases (114, 127, 128), and varespladib has proved to be a class leader against PLA_2 toxins due to its seemingly broad-spectrum inhibition of PLA_2 activity across humans and snakes (106, 129, 130). Metal ion chelators also remain an interesting class of therapeutics for inhibiting the zinc-dependent SVMPs, with the lead candidate DMPS (unithiol) outperforming EDTA and other chelators (119), resulting in downstream clinical development for snakebite indication (110, 111).

The different lead candidates outlined above have all stemmed from a repurposing approach, whereby the drugs were originally developed for other disease indications, namely MMPIs for cancer, DMPS for heavy metal poisoning, and varespladib for cardiovascular diseases. Such repurposing has enabled the rapid translation of varespladib and DMPS into snakebite clinical trials due to the extensive prior safety data and regulatory dossier information available, which allowed for a reduced timeline to enter clinical development. Varespladib previously entered a (later halted) Phase III clinical trial for the treatment of acute coronary syndrome (131) and recently completed Phase II for snakebite envenoming in a randomized placebo-controlled study conducted in the United States and India (109). DMPS is already a registered drug used to treat heavy metal poisoning in Europe but was recently used in a Phase I dose escalation study in Kenya to explore the safety of increasing oral doses with a view to treating cases of snakebite envenoming in Phase II (110, 111). Promisingly, both drugs are available in formulations amenable to both oral and parenteral administration.

Venom variation and the different toxin targets for the abovementioned drugs (i.e., PLA_2 and SVMPs) have also led to several recent studies exploring the potential utility of small-molecule drug combinations. Such studies have shown that combination therapies can protect against both local and systemic toxic effects in rodent models and can either increase the inhibitory potency of each drug or broaden the indication by expanding the number of snake species that can be treated by the combination therapy (113, 128). Combination therapies therefore hold considerable long-term promise, though their clinical development will be greatly facilitated by clinical trials first demonstrating efficacy for the individual components.

Despite the achieved progress, the chemical space explored for the identification of synthetic toxin inhibitors remains narrow. Given the high attrition rate in drug development (132), it remains important to identify backup molecules or new chemistries amenable to optimization for snakebite indications. To this end, validated high-throughput screening protocols for assaying both SVMP and PLA_2 enzymatic activities have been recently described (133, 134), and these could readily be applied for discovery campaigns involving large drug libraries (or recombinant antibodies). Such activities could also be facilitated by academic–industry collaborations, such as those mediated by public–private partnership organizations (such as WIPO Re:Search) acting as facilitators for academics to access large commercial drug libraries or screening resources (135). Alternative approaches to larger-scale screening, such as virtual drug screening, remain underutilized for snakebite, and recent advances in bioinformatics tools for protein modeling could help to circumvent wet lab–related resource roadblocks (136).

Even though most recent research has focused on the identification of SVMP or PLA_2 inhibitors, other enzymatic snake toxin families have been targeted via the use of synthetic molecules. These include the coagulopathic snake venom serine proteases with the anticoagulant drug nafamostat mesylate (128), the venom spreading factor hyaluronidase with sodium cromoglycate and sodium aurothiomalate (137), and the identification of propionic acid derivatives via virtual screening as potential inhibitors of venom L–amino acid oxidases (138). However, certain pathogenic toxin families, particularly those that are nonenzymatic, show a high level of unrelatedness to human homologs, making a repurposing approach challenging. Perhaps more importantly, such toxins are often isoform rich and of low mass, thereby offering limited biophysical possibilities for intermolecular interactions with drugs, and thus may be more challenging to inhibit with small-molecule therapeutics. One such example is the three finger toxins, several of which interact with high affinity with nicotinic acetylcholine receptors (i.e., α-neurotoxins). Only a few potential drugs have been proposed for inhibiting these pathogenically important toxins, but none showed breadth of inhibition or comparable potency with recently discovered monoclonal antibodies (47). Other therapeutic modalities, such as recombinant antibodies (48, 64, 66, 67, 71, 87), but also perhaps previously described receptor-mimicking molecules (70), synthetic nanoparticles (139) and/or aptamers (140–142), may be more amenable to preventing or displacing such high-affinity toxin–receptor interactions.

Although much of the recent literature has focused on identifying and testing toxin-specific small-molecule drugs, several groups have also identified promising molecules that act via an indirect (i.e., not directly on toxins) mechanism of action. These molecules include the acetylcholinesterase inhibitor neostigmine, which acts to reduce neuromuscular blockade caused by venom toxins at the neuromuscular junction by retaining acetylcholine. Intranasal delivery of neostigmine showed promising preclinical efficacy against *Naja naja* cobra envenoming (143), and although clinical evidence has since been variable, the WHO guidelines for managing snakebite in Southeast Asia advocate trialing intramuscular use of this adjunct drug in cases of neurotoxic cobra envenoming (144). More recently, the melatonin G protein–coupled receptor (MT1) agonists ramelteon and agomelatine have shown efficacy in the preclinical setting by promoting

the recovery of function of the neuromuscular junction following degeneration of motor axon terminals caused by presynaptic PLA_2 toxins (145). Their mechanism of action is thought to be via stimulation of the MT1 receptor present on the surface of perisynaptic Schwann cells, which in turn activates the CXCR4 receptor on the stump of the damaged axon by the chemokine CXCL12α, resulting in the promotion of regrowth (145). These drugs could potentially be particularly beneficial for promoting the recovery of respiratory function following neurotoxic envenomings by snakes like kraits (*Bungarus* spp.) and are already licensed for other indications, providing a potentially rapid pathway into clinical trials.

Despite the promise of small-molecule drugs, considerable challenges remain, not least how these new treatment modalities, particularly oral therapeutics, can be effectively integrated into the patient treatment pathway and the health systems of low- and middle-income countries. Community sensitization and education will be critical to ensure that appropriate health-seeking behavior enables the effective use of inhibitory drugs and to also ensure that onward clinical presentation continues for evaluation of the potential secondary need for intravenous antivenom. Before this can happen, robust clinical trials must be performed. These trials will require careful design and selection of outcome measures due to variability in snakebite pathologies (57) and robust evaluations of tolerance due to the risk of off-target effects, i.e., where inhibitory molecules might interact with human proteins related to the toxin targets (e.g., human PLA_2 and matrix metalloproteinases) (146, 147). Finally, ethical considerations will likely dictate that snakebite drugs are clinically trialed in conjunction with current standard of care, but efficacy signals are likely to be challenging to achieve if oral drugs are delivered concurrently with intravenous antivenom.

5. CHALLENGES AHEAD AND FUTURE DIRECTIONS

Fulfilling the goals of the WHO strategy to prevent and control snakebite envenoming demands renewed multisectorial efforts to improve the treatment of this neglected tropical disease. This involves strengthening animal-derived antivenom production and developing novel therapies based on recombinant antibodies and toxin inhibitors. In the case of antivenoms derived from animal plasma, key challenges are designing novel immunization strategies, including new adjuvants; improving the safety of antivenoms; assessing preclinical efficacy; developing alternative quality control tests within the 3Rs paradigm; improving the stability of products; clinical testing of efficacy and safety; strengthening the universe of manufacturers and regulatory agencies; and improving availability, accessibility, and affordability. These tasks demand renewed interdisciplinary and intersectorial partnerships.

The development of novel therapies based on recombinant antibodies and toxin inhibitors involves a different set of challenges. One issue has to do with intellectual property (IP) rights, since these are key to incentivize developers to bring new molecules into the clinic, but may limit accessibility. In some cases, it might be beneficial if IP is held by institutions based in countries with high incidence of snakebites, as recently proposed (148), or if IP regulations are flexibilized as to allow the local production of these therapeutics. However, market factors will make this challenging for many translational projects, and diverse options on how to manage IP in this field must be considered. Irrespective of IP rights, it should nevertheless be of paramount importance that access to new snakebite therapeutics at affordable prices is ensured for low- and middle-income countries.

The testing of new therapies must consider the design and implementation of clinical trials, including the selection of appropriate sites for these trials, the clinical outcome measures, and whether these therapies will be tested alone or in combination with current standard of care (i.e., antivenoms). Moreover, the regulatory framework for new therapies should be carefully

considered, and protocols for the approval of these products need to be agreed upon. Furthermore, regulators in developing countries, where these trials are likely to be developed, need to be strengthened to support product authorization, particularly in the context of recombinant biologics.

The various stages in the development pipeline for novel therapeutics should be carefully considered, including the identification of stakeholders to be involved in each step. Research and development groups with expertise in generating recombinant antibodies and synthetic inhibitors, together with new stakeholders in the downstream stages of development and manufacture, should be incorporated into this field. In this landscape, new partnerships are needed, and lessons can be learned from the field of drug development for other neglected tropical diseases. Researchers should take a comprehensive approach to the development of new types of therapies so that they both choose molecular scaffolds that are actually developable and manufacturable and consider the economical and deployment aspects in the early discovery and development process (112, 149).

Likewise, models of manufacture and delivery of these new therapies should ensure high production scales and sustainability. A key aspect in the emergence of these new therapies is thus the need to ensure availability, affordability, and accessibility, particularly in countries with high incidence of snakebites (150). To this end, it might be relevant to strengthen the manufacturing capacity in low- and middle-income countries to create better conditions and incentives for bringing antivenoms to the clinic in the regions with the largest need. In addition, the introduction of these new therapies should involve training programs for health staff dealing with snakebite envenoming (55).

There are plenty of challenges and opportunities to improve the therapy of envenomings (**Table 1**), and the field is ripe for these developments. To achieve such goals, current and new stakeholders from diverse areas of expertise, from basic science to manufacture and delivery, should

Table 1 Overview of the challenges and opportunities for current and new snakebite envenoming therapies

	Plasma-derived antivenoms	Recombinant antivenoms	Small-molecule inhibitors
Challenges	Improving efficacy Optimal animal care Improving quality and purity Improving affordability and accessibility Clinical assessment	Establishing cost-competitive manufacturing Regulatory approval of such products not tried before Clinical assessment	Obtaining specificity toward certain toxins (e.g., neurotoxins) Dose optimization Clinical assessment Integration of new treatment modality into health system
Opportunities	Use of recombinant/purified toxins New types of adjuvants Novel immunization strategies (e.g., DNA/mRNA immunization) Strengthening current manufacturing infrastructure Expanded shelf life New preclinical assays for quality control (3Rs principles)	Earlier administration due to improved safety Use of new low-cost biomanufacturing systems Fortification of existing antivenoms Control over toxin specificity (i.e., species coverage can be designed) Independence of snakes and production animals Routine product batch quality control without animal use Combination therapies	Alternative routes of administration Long shelf life In-field use Adjunct therapy with antivenom Combination therapies Independence of snakes and production animals Routine product batch quality control without animal use

Abbreviation: 3Rs, replacement, reduction, and refinement.

be involved. Access to current antivenoms and new therapeutics for people with the highest risk of snakebites should be prioritized and ensured. In the long term, this effort will contribute to fulfilling the goal of reducing the global suffering inflicted by snakebite envenoming.

DISCLOSURE STATEMENT

J.M.G. works at Instituto Clodomiro Picado (Universidad de Costa Rica), which manufactures and distributes antivenoms for the treatment of snakebite envenoming. The other authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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