Molecular Science

AIMS Molecular Science, 5(1): 96–116.
DOI: 10.3934/molsci.2018.1.96
Received: 11 August 2017
Accepted: 14 March 2018
Published: 27 March 2018

http://www.aimspress.com/journal/Molecular

Review

Anti-HIV lectins and current delivery strategies

Fohona S. Coulibaly, Danielle N. Thomas and Bi-Botti C. Youan*

Laboratory of Future Nanomedicines and Theoretical Chronopharmaceutics, Division of Pharmaceutical Sciences, School of Pharmacy, University of Missouri Kansas City, USA

* **Correspondence:** Email: youanb@umkc.edu; Tel: +18162352410; Fax: +18162355779.

Abstract: Lectins, a class of carbohydrate binding agents (CBAs), have been widely studied for their potential antiviral activity. In general, lectins exert their anti-HIV microbicidal activity by binding to viral envelope glycoproteins which hinders a proper interaction between the virus and its host, thereby preventing viral entry and replication processes. Several natural lectins extracted from plant, fungi, algae, bacteria and animals, as well as boronic acid-based synthetic lectins, have been investigated against the Human Immunodeficiency Virus (HIV). This manuscript discusses the nature of HIV envelope glycoprotein glycans and their implication in lectin antiviral activity for HIV/AIDS prevention. In addition, anti-HIV lectins and their carbohydrate specificity is reported. Furthermore, current formulations of anti-HIV lectins are presented to illustrate how to overcome delivery challenges. Although antiviral lectins will continue to occupy a major stage in future microbicide research, further investigation in this field should focus on novel delivery strategies and the clinical translation of CBAs.

Keywords: anti-HIV lectins; synthetic lectins; HIV gp120; HIV gp41; glycans; drug delivery

Abbreviations: AIDS: Acquired Immune Deficiency Syndrome; AH: Actinohivin; BanLec: Banana lectin; BzB: Benzoboroxole; CBA: Carbohydrate Binding Agents; Con A: Concanavalin A; CVL: *Chaetopterus variopedatus* lectin; CV-N: Cyanovirin-N; DC-SIGN: Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin; FIPV: Feline Infectious Peritonitis Virus; Fuc: Fucose; Gal: Galactose; GlcNAc: N-acetylglucosamine; GRFT: Griffithsin; HexNAc: N-acetylhexoseamine (N-acetylglucoseamine, N-acetylgalactoseamine); HIV: Human Immunodeficiency Virus; HIV gp120: HIV envelope glycoprotein 120; HIV gp160: HIV glycoproteins precursor; HIV gp41: HIV envelope glycoprotein 41; Man: Mannose; MHC: Major Histocompatibility Complex; MHL: *Myrianthus holstii* lectin; MRC 1 & 2: Mannose Receptor C-Type 1 & 2; MVL: *Microcystis viridis* lectin; MVN: Microvirin; Neu5Ac: N-Acetylneuraminic acid (sialic acid); NPL: *Narcissus pseudonarcissus* lectin; OAA: *Oscillatoria agardhii* agglutinin; PBA: Phenylboronic acid;

SARS: Severe Acute Respiratory Syndrome; SIV: Simian immunodeficiency virus; SVL: *Serpula vermicularis* lectin; SVN: scytovirin

1. Introduction

Since its discovery in 1983 as the virus responsible for the Acquired Immune Deficiency Syndrome (AIDS), the Human Immunodeficiency Virus (HIV) has remained a scientific challenge; as a complete eradication strategy has yet to be successful [1]. Although early-generation microbicides, such as surfactant-containing spermicides, acid-buffering gels, and polyanionic gels have failed to demonstrate efficacy, advances in microbicide development using HIV-specific antiretroviral agents (ARV) have shown significant promise [2]. Part of the success demonstrated by ARV comes from their ability to target and block key stages in HIV replication including viral entry, reverse transcription, integration, and maturation. Despite recent progress in anti-HIV microbicide development and advances in access to antiretrovirals, an average of 1.9 million (1.9–2.2 million) new HIV infections still occur globally every year [3]. More alarmingly, the rate of new HIV-1 infections is believed to be outpacing the rate of new individuals receiving antiretroviral therapy by an average of 2.5:1 [4]. Furthermore, the widely promoted "ABC" approach (abstinence, being faithful, condom usage) aimed at fostering HIV prevention and reducing the rate of infection spread, has shown some limitations, especially in third world countries, which remain the most affected by the pandemic infection [5]. In fact, Cohen stated that the practice of abstinence "is only theoretical, since one can only be certain of one's own behavior, not the behavior of one's partner" [6]. Although HIV infection declines in Uganda and Thailand were attributed to reduction in partner number, its long-term application remains a challenge in rural areas, where polygamy is often deeply rooted in traditional and religious beliefs [7,8]. Condoms, which are known to be effective when used consistently and correctly, still face a strong rejection from certain users who often report reduced physical pleasure, embarrassment of purchasing condoms, and a general perception that condom use represents a sign of infidelity and/or HIV/STD-seropositive status [9]. Therefore, there is a critical need for the development of alternative, long-lasting, self-applied and effective microbicides. Such topically (vaginally or rectally) applicable microbicides are projected to ultimately protect women, since more than half of all new HIV infections worldwide occur in females [10].

Among the different classes of anti-HIV microbicides currently in use, agents targeting and preventing viral entry into target cells have shown remarkable promises, partly favored by fewer barriers that could potentially hinder their mechanism of action. HIV entry inhibitors are subdivided into three main groups: Attachment, co-receptor binding and fusion inhibitors [11]. Attachment inhibitors such as zintevir, BMS-378806, and BMS-488043 block a non-specific adsorption step between HIV virions and target cells' membrane, which is due to an interaction between the positively charged regions of the envelope glycoprotein gp120 and oppositely charged proteoglycans on cell surface. Co-receptor binding inhibitors are generally CCR5 antagonists such as aplaviroc, vicriviroc, and maravirok that bind to CCR5 and prevent further gp120-CCR5 interaction, which is critical for viral entry into host cells. Fusion inhibitors such as tifuvirtide, enfuvirtide, and sifuvirtide prevent the formation of the fusion pore by mimicking either heptad repeat 1 or 2 (HR1 or HR2) sequences in gp41. These sequences block the formation of a six-helix bundle structure necessary for HIV entry into host cells [11].

Lectins, which are carbohydrate binding proteins, have long being considered for their diagnostic and therapeutic potentials, as well as their pathogenic implication in many human diseases and conditions including various cancers [12], type 2 diabetes [13,14], cardiovascular disease [15], weight management [16,17], and HIV/AIDS [18]. The ability of lectins to recognize and bind several mannose oligosaccharides was long considered a viable example of anti-HIV therapeutic strategy. Primarily, anti-HIV lectins act as viral entry inhibitors by binding to oligosaccharide epitopes on HIV surface glycoproteins, which either hinder a proper interaction between HIV and receptors on target cell membranes or affect post-binding conformational alterations of key viral envelope and transmembrane glycoproteins (HIV gp120/HIV gp41). In this manuscript, we report the current trend in anti-HIV lectins research and emerging lectins formulations aiming at improving the delivery of these sugar-binding proteins.

2. HIV surface glycoproteins and glycans

HIV surface glycoproteins (HIV gp120 and HIV gp41) mediate host cell entry through interactions with CD4 receptor and CCR5/CXCR4 co-receptors on target cells. These glycoproteins are first expressed as HIV gp160 precursor before the proteolytic cleavage in the trans-Golgi by cellular furin or furin-like proteases that lead to the formation of envelope glycoprotein HIV gp120 and transmembrane glycoprotein HIV gp41. In mature HIV viruses, HIV gp120 and HIV gp41 remain linked by noncovalent interactions [19]. Most anti-HIV lectins target and bind specific glycan structures on HIV envelope glycoproteins. Understanding the glycosylation pattern of these glycoproteins is useful not only for anti-HIV vaccine design, but also for the selection of appropriate lectins for potential anti-HIV therapy. Glycans found on HIV surface glycoproteins may also help in understanding anti-HIV lectins overall mechanism of action. Moreover, the extent and variation in glycosylation pattern between HIV strands, as well as changes in the glycosylation pattern during HIV maturation may help explain resistance to certain anti-HIV vaccines and lectins, as well as the lack of broad activity usually observed with anti-HIV lectins [20,21].

2.1. HIV gp120 glycans and their function

HIV gp120 is the external envelope glycoprotein of HIV. It is a homotrimer with each subunit having a nominal molecular weight of 120 kDa. HIV gp120 plays an essential role in HIV entry into host cells. In fact, HIV entry into host cells is initiated by the binding of gp120 to CD4 receptor. This binding triggers a conformational change in HIV gp120, which, in turn, enhances its affinity to chemokine receptors CXCR4 or CCR5. This secondary binding induces another conformational change in the transmembrane glycoprotein HIV gp41 resulting in an intimate contact and fusion of both viral and host cell membranes. The membrane fusion process leads to the internalization of HIV viral capsid containing the viral mRNA and key viral proteins into host cells' cytoplasm. Ultimately, new HIV virus particles are produced which then propagate the infection [22,23]. Literature expounds on HIV gp120 biosynthesis, trafficking, and incorporation. Rather than the underlying biological mechanism involved in these processes, we will focus on the nature of glycosylation and its role in the membrane fusion.

Ratner et al. [24], Allan et al. [25] and Montagnier et al. [26] published some of the first studies that explored HIV gp120 glycosylation. Although these pioneering studies did not investigate HIV gp120 glycan structures in detail, they did report HIV gp120 glycosylation to account for approximately 27 to 50% of the overall glycoprotein molecular weight. Some of these early investigations also demonstrated the presence of 31 [25] or 32 [27] potential N-glycosylation sites on HIV gp120. Subsequent studies by Mizuochi et al. [28,29] further investigated HIV gp120 glycan structures. Mizuochi's findings showed in part that HIV gp120 is unique in its diversity of oligosaccharide structures. These studies also reported HIV gp120 glycans to be predominantly oligomannoses that are mostly comprised of five to nine mannose residues, and accounting for approximately 33% of the overall glycoprotein's carbohydrate structures [30]. Furthermore, this study projected the number of potential N-glycosylation sites on HIV gp120 to be 20. Besides the high-mannose type glycans, Mizuochi et al. also identified complex type glycan chains (34% of carbohydrate structures) mainly composed of four categories: mono-, bi-, tri- andtetra-antennary, with or without N-acetyllactosamine repeats, and with or without a core fucose residue [28,30–32]. A previous study by Geyer et al. [31] reported similar findings and showed that predominant oligomannose glycans in HIV gp120 are composed of seven to nine mannose residues, depending on whether the glycoprotein is excreted or expressed intracellularly. Geyer et al. also showed that HIV gp120 complex-type oligosaccharide are fucosylated with partial sialylated bi- and triantennary structures. Recent advances in glycoscience, genomic, proteomics, and mass spectrometry have led to more detailed and in-depth characterization studies of HIV gp120 glycosylation. In fact, recent mass spectrometry studies have confirmed HIV gp120 high mannose proportion for various viral clades and expression systems [32–36] and it is widely accepted that the number of HIV N-glycosylation sites range from 20–35 [19]. Following a matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) analysis, Bonomelli et al. showed that HIV gp120 oligosaccharides, derived from virus [clade A (92RW009), clade B (JRCSF), clade C (93IN905)] isolated from infected peripheral blood mononuclear cells (PBMCs), are almost entirely oligomannoses and varies from 62–79% for virion-associated versus 30% for recombinant monomeric HIV gp120 [33,37]. These studies also identified an "intrinsic" mannose patch in HIV gp120 composed essentially of $Man_{5-9}GlcNAc_2$ and conserved across primary isolates and geographically divergent HIV clades. Many other studies have confirmed the presence of the mannose patch on HIV gp120 and its relevance in the development of a successful anti-HIV vaccine [38–41]. HIV gp120 main glycan structures are summarized in Figure 1.

HIV gp120's heavy glycosylation is believed to play four key roles: Host immune evasion, pathogenesis, proper glycoproteins folding, and host cell surface recognition [42]. In fact, several studies have compared HIV gp120 extensive glycosylation to a protecting shield that prevents antibody access to underlying amino acid sequences and therefore limits their efficacy [40,43–46]. More specifically, Sanders et al. determined that the carbohydrate at asparagine 386 on HIV-1 gp120 enhances HIV immune evasion [47]. Furthermore, the general role of HIV gp120 glycosylation in HIV pathogenesis has widely been reported [48]. Besides its major implication in HIV gp120 proper folding and lysosomal degradation, Francois et al. [49] and Mathys et al. [50] showed that cleavage of glycan at asparagine 260 of HIV-1 gp120 results in loss of viral infectivity. Similarly, Huang et al. demonstrated that deletion of HIV gp120 glycans from asparagine proximal to the CD4-binding region (156, 262 and 410) impairs HIV viral infectivity [51]. The essential role of glycosylation in proper HIV gp120 folding was also elucidated by numerous reports [50,52]. For example, Li et al. showed that N-linked glycosylation is highly essential for a proper conformation of HIV gp120 capable of binding to CD4 receptor [53]. In a separate study Li et al. determined that deletion of the glycan at asparagine 448 can profoundly influence CD4 T-cell recognition of HIV-1 gp120 [54].

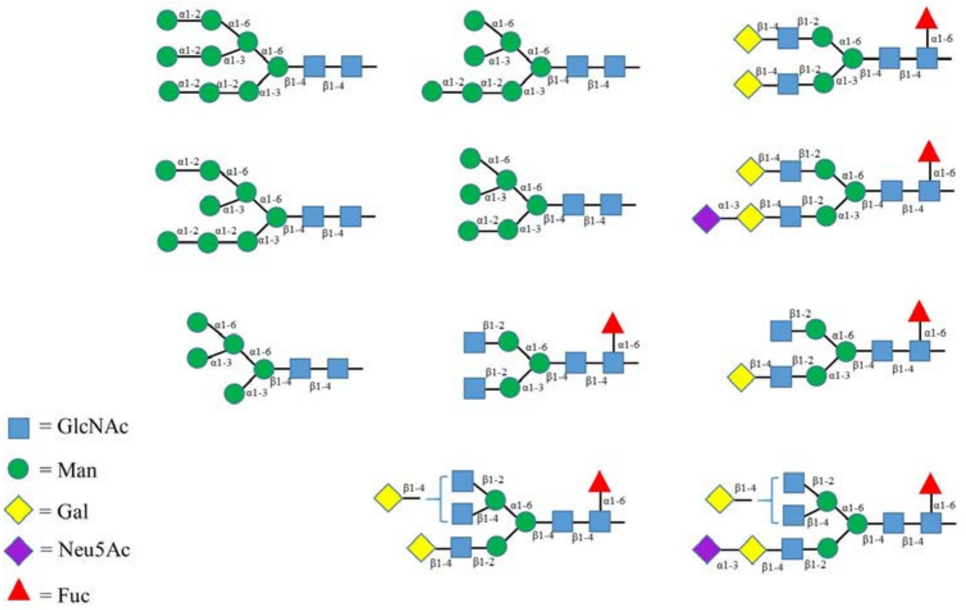

Figure 1. High mannose and complex glycan structures found in HIV gp120. Structures are adapted from the following references [28,30,31,55,56].

2.2. HIV gp41 glycans and their function

HIV gp41 is composed of 345 amino acids that are organized into three major domains: extracellular (ectodomain), transmembrane, and C-terminal cytoplasmic tail [19,57,58]. Unlike HIV gp120, the transmembrane glycoprotein contains fewer N-glycosylation sites. Nonetheless, there is an inconsistency pertaining to the number of N-glycosylation sites in HIV gp41, as various communications often report different numbers. This may be due to differences in expression systems, cell lines, and HIV strands. According to the current literature, the number of potential N-glycosylation sites in HIV gp41 vary between three to eight. In fact, Perrin et al. reported poor glycosylation of HIV gp41 ectodomain with only four or five potential glycosylation sites [59]. Following the screening of ten HIV-1 amino acid sequences, Johnson et al. determined that HIV gp41 typically contains three or four N-glycosylation sites, localized within a short stretch (20 to 30 amino acid residues) of the C-terminal half of the ectodomain [60]. The same number of HIV gp41 potential N-glycosylation sites was reported by Fenouillet et al. [61,62], Lee et al. [63], Ma et al. [64], and Wang et al. [65]. Furthermore, citing the work of Montefiori et al. [48] and Checkley et al. reported HIV gp41 N-glycosylation sites to vary between three to five [19]. The work of Balzarini et al. reported some of the highest number of HIV gp41 potential N-glycosylation sites which was thought to be seven with only four seemingly glycosylated [66]. Further studies by Mathys and Balzarini have reported N-glycosylation sites in HIV gp41 between four-eight with all four to five N-glycans on the ectodomain composed of complex-type glycans [67,68].

In contrast to HIV gp120, the transmembrane glycoproteins' glycans are known to be primarily composed of more complex carbohydrate types. In fact, following the analysis of HIV gp41 expressed from two different producer cells (Chinese hamster ovary cells and human embryonic kidney cells [293T]), Pritchard et al. determined that in combination with the presence of less oligomannose glycans (19–34%) compared to HIV gp120 (60–65%), HIV gp41 contains large populations of complex-type glycans on its ectodomain [56]. Regardless of the expression system, HIV gp41 oligomannose population was also found to be composed of $Man_{5–9}GlcNAc_2$. Like HIV gp120, complex glycans in HIV gp41 are composed of sialilated and asialilated bi-, tri-, and tetra-antennary structures usually containing N-lactosamine repeats with or without core fucose residue [34,55,56]. HIV gp41 main glycan structures are summarized in Figure 2.

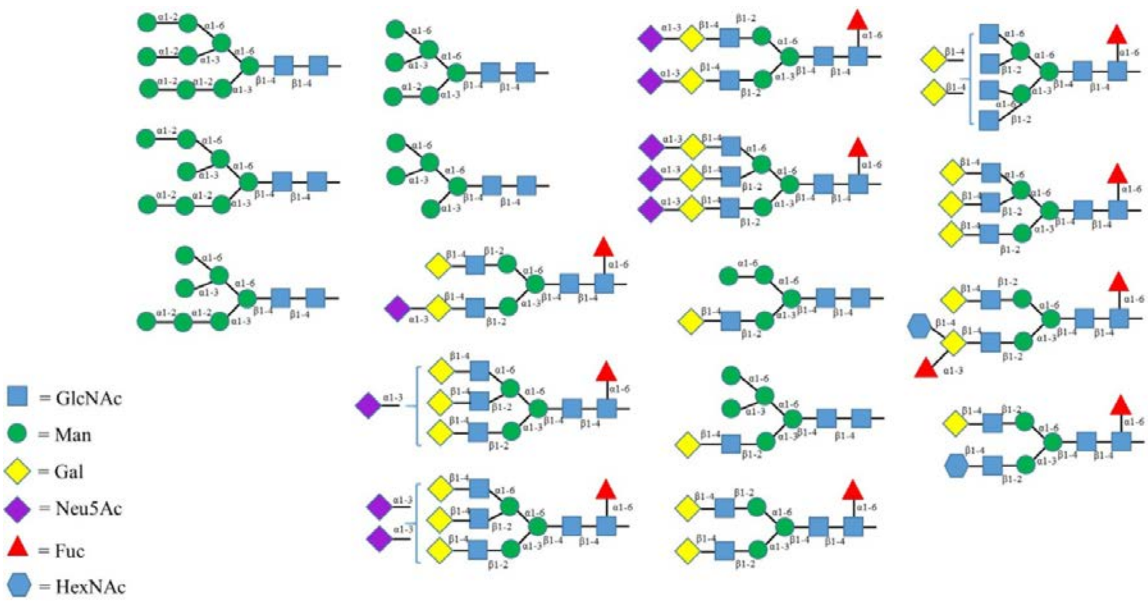

Figure 2. High mannose and complex glycan structures in HIV gp41. Structures are adapted from the following references [34,55,56].

HIV gp41 plays an equally critical role in HIV entry into target cells by mediating the membrane fusion process required for the internalization of the HIV viral capsid [23]. Glycans in HIV gp41 are reported to play key roles in viral entry, immune evasion, and infectivity. In fact, Fenouillet et al., reported a loss of HIV ability to enter target cells after complete removal of the glycan cluster from asparagines at positions 621, 630 and 642 [69] in HIV gp41. A follow up study by Perrin et al., determined the critical role of HIV gp41 glycosylation for an effective membrane fusion process. This study also reported that out of the four or five glycosylation sites in HIV gp41, only two sites are sufficient for efficient membrane fusion with a single site at asparagine 621 being the most critical of all positions [59]. Yuste et al. have also suggested that the function of HIV gp41 glycosylation from both HIV and SIV may be shielding underlying epitopes sequences, thereby allowing the virus to escape neutralizing antibodies [70]. Furthermore, Wang et al. reported that the glycan at asparagine 637 in HIV gp41 is composed of $Man_9GlcNAc_2$ and plays a critical role in immune evasion through the facilitation of the membrane fusion process [65]. A later study by Mathys and Balzarini lead to a rather different conclusion regarding the importance of this glycan [68]. By following the generation of several HIV-1 mutants lacking HIV gp41 *N*-glycans and assessing their influence on viral infectivity, this study determined that besides the glycan at asparagine 616 that when deleted, leads to a complete loss of HIV-1 infectivity, deletion of glycans on asparagines 611 and 637, displayed marginal effect on overall viral infectivity. In addition, this study concluded that glycans on asparagines 625 and 674 do not play any significant role in HIV infectivity, since their deletion did not influence viral infectivity.

3. Anti-HIV lectins

3.1. Natural lectins

Owing to HIV-gp120 high mannose content, various mannose binding natural lectins have been investigated as HIV entry inhibitors. Primarily, these lectins specifically bind mannose oligosaccharides on HIV-gp120, thus hindering a proper interaction between the envelope glycoprotein and its host cell receptor (CD4). This may ultimately prevent the membrane fusion step and the production of new HIV virions. Actinohivin (AH), a prokaryotic lectin derived from the gram-positive bacteria actinomycete *Longispora albida* (K97-0003T) successfully prevented HIV-1 entry into $CD4^+$ T lymphocytes ($IC_{50} \approx$ 2–110 nM) [71]. It was determined that AH binds α(1-2)-mannose oligosaccharides present in HIV gp120 and HIV gp41. Furthermore, AH did not induce any mitogenic activity or cytokine/chemokine production in PBMC cultures, suggesting that this lectin could be a safe and effective microbicide candidate [72]. Recently, Zhang et al. reported that Man_1 and Man_2 residues, found on HIV gp120 high-mannose type glycans structures, occupy two of the three carbohydrate binding sites of AH, while Man_3 residues interact with conserved hydrophobic amino acid residues (Tyr and Leu) of AH [73]. Cyanovirin-N (CV-N) is a cyanobacterial lectin with broad-spectrum antiviral activity. The potential use of CV-N as anti-HIV microbicide has widely been reported ($IC_{50} \approx$ 3.9–31 nM) [74–78]. CV-N inhibits HIV replication in part by binding to HIV-gp120 high mannose glycans, thus preventing the envelope glycoprotein from binding to its cell surface receptor (CD4), thereby blocking the glycoprotein-mediated membrane fusion process required for HIV-1 entry [79]. Hu et al. have determined that CV-N binding interaction is mediated through three to five high-mannose residues from 289 to 448 in the C2–C4 region of HIV gp120 and deglycosylation of these residues resulted in a resistance to CV-N [80,81]. It was also shown that CV-N inhibits HIV replication by interacting with the chemokine receptors CXCR4 and CCR5 [82]. Recently, a CV-N oligomer ($CV\text{-}N_2$) was designed and demonstrated an increased HIV-1 neutralization activity by up to 18-fold compared to the wild-type CV-N ($IC_{50} \approx$ 0.1–41 nM) [81]. *Oscillatoria agardhii* agglutinin (OAA) is a newly discovered cyanobacterial lectin that was shown to prevent HIV transmission, replication, and syncytium formation between HIV-1-infected and uninfected T cells ($IC_{50} \approx$ 24–30 nM) [83]. OAA is known for having two sugar binding sites that recognize Manα(1–2)Man, Manα(1–6)Man and the branched core unit of Man_9 (3α,6α-mannopentaose) [83–86]. Similar to OAA, scytovirin (SVN) is a cyanobacterium lectin isolated from *Scytonema varium* [87]. SVN was also shown to possess potent anti-HIV activity through its binding interaction with HIV gp160, HIV gp120, and HIV gp41 and binds Manα(1–2)Manα(1–6)Manα(1–6)Man tetrasaccharide in high mannose type oligosaccharides ($IC_{50} \approx$ 24.1 nM) [88–90]. In addition, MVL, a lectin isolated from the cyanobacterium *Microcystis viridis* also showed strong anti-HIV activity at nanomolar concentrations by binding to Manα(1–6)Manβ(1–4)GlcNAcβ(1–4)GlcNAc oligosaccharides on the surface of HIV gp120 ($IC_{50} \approx$ 30 nM) [91,92]. Another cyanobacterial lectin, microvirin (MVN), isolated from *Microcystis aeruginosa* has shown anti-HIV activity comparable to CV-N with a much better cytotoxicity profile ($IC_{50} \approx$ 2–12 nM) [93]. It was further shown that MVN binds Manα(1–2)Man residues on HIV gp120 [93,94]. Plant lectins such as *Narcissus pseudonarcissus* lectin (NPL) ($EC_{50} \approx$ 0.17–2.76 μg/mL) [95,96] and *Myrianthus holstii* lectin (MHL) ($EC_{50} \approx$ 150 nM) [97] have also shown potential HIV inhibition *in vitro*. Concanavalin A (Con A), one of the most studied plant lectins, is a mannose binding lectin extracted from jack bean. Con A binds sugars, glycoproteins, and glycolipids, containing internal and nonreducing terminal α-D-mannosyl and α-D-glucosyl groups (K_D ≈ 0.05 μM to 1.5 μM) [98,99]. Several studies have demonstrated the ability of Con A to bind HIV gp120 and inhibit the fusion process during HIV infection (EC_{50} ≈ 98 nM) [100–103]. Furthermore, BanLec is a lectin isolated from the banana fruit (*Musa acuminata*), which has shown potent anti-HIV activity (IC_{50} ≈ 2.5–694 nM) [104]. Like Con A, BanLec inhibits HIV by binding to high mannose carbohydrate structures found in HIV gp120, thus blocking the virus entry into host cells. In fact, in a comparative study, BanLec showed similar inhibitory activity like T-20 and maraviroc, two FDA approved HIV entry inhibitor microbicides [4].

Griffithsin (GRFT), a lectin isolated from the red algae *Griffithsia* inhibited cell-to-cell fusion between HIV infected and uninfected cells (IC_{50} ≈ 4 nM) [105]. GRFT also inhibited HIV-1 transmission by binding to glucose, mannose, and *N*-acetylglucosamine residues in HIV glycoproteins (HIV gp120, HIV gp41 and HIV gp160) [106]. Emau et al. [107] have also established that GRFT strongly blocked CXCR4- and CCR5-tropic viruses at concentrations less than 1 nM, with low cytotoxicity, rapid onset of antiviral activity and long-term stability in cervical/vaginal lavage. GRFT tandemers, recently reported by Moulaei et al., have shown anti-HIV activities five to ten-fold higher than native GRFT (IC_{50} ≈ 0.02–0.274 nM) [108]. Table 1 summarizes natural anti-HIV lectins and major properties discussed above.

Table 1. Example of natural anti-HIV lectins.

Lectin	Glycan preference	Target	IC_{50}/EC_{50}	Origin	References
AH	Manα(1–2)Man, Manα(1–3)Man, Manα(1–6)Man, GlcNAc	gp120 and gp41	IC_{50} ≈ 2–110 nM	*Actinomycete Longispora albida*	[71,72]
CV-N	Manα(1–2)Man in Man_8 or Man_9	gp120, CXCR4 and CCR5	IC_{50} ≈ 0.1–41 nM	*Nostoc ellipsosporum*	[80,81]
OAA	Manα(1–2)Man, Manα(1–6)Man, 3α,6α-mannopentaose	gp120	IC_{50} ≈ 24–30 nM	*Oscillatoria agardhii*	[83–86]
SVN	Man	gp120	IC_{50} ≈ 24.1 nM	*Scytonema varium*	[88,89]
MVL	Manα(1–6)Manβ(1–4)GlcNAcβ(1–4)GlcNAc	gp120	IC_{50} ≈ 30 nM	*Microcystis viridis*	[91]
MVN	Manα(1–2)Man	gp120	IC_{50} ≈ 2–12 nM	*Microcystis aeruginosa*	[93,94]
NPL	Manα(1–3)Man; Manα(1–2)Man	gp120	EC_{50} ≈ 0.17–2.76 μg/mL	*Narcissus Pseudonarcissus*	[95,96]
MHL	GlcNAc	gp120	EC_{50} ≈ 150 nM	*Myrianthus Holstii*	[97]
Con A	α-D-Man and α-D-Glc	gp120	EC_{50} ≈ 98 nM	Jack bean	[98]
BanLec	Man	gp120	IC_{50} ≈ 2.5–694 nM	*Musa acuminata*	[4]
GRFT	Glc, Man and GlcNac	gp120, gp41, gp160, CXCR4 and CCR5	IC_{50} ≈ 4 nM	*Griffithsia*	[106]
CVL	β-Gal	gp41, gp120	EC_{50} ≈ 73 nM	*Chaetopterus variopedatus*	[110]
Mermaid	Manα(1–3)Manα(1–6)Man	gp120	IC_{50} ≈ 3.1 μg/mL	*Laxus oneistus*	[111,112]
SVL	GlcNAc	gp41, gp120	IC_{50} ≈ 0.15–0.23 μg/mL	*Serpula vermicularis*	[113]

Chaetopterus variopedatus lectin (CVL) is a β-galactose-specific lectin extracted from the marine worm *Annelida*. CVL was shown to inhibit both HIV attachment to host cells and the fusion process between HIV and target cells (EC_{50} ≈ 73 nM) [109]; suggesting that CVL might be exerting its action through interaction with complex glycan type found in HIV gp120 and HIV gp41 [110]. In addition, Mermaid, a calcium (Ca^{2+}) dependent lectin isolated from the marine nematode (*Laxus oneistus*) was shown to have structural similarities and similar glycan specificity with the Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN). Mermaid, which binds mannose oligosaccharides on HIV gp120 prevented HIV-1 binding to DC-SIGN on dendritic cells, which ultimately blocked HIV transmission (IC_{50} ≈ 3.1 μg/mL) [111,112]. Another marine lectin *Serpula vermicularis* lectin (SVL) isolated from the sea worm *Serpula vermicularis* was also shown to bind GlcNAc and inhibited the production of viral p24 antigen and cytopathic effect induced by HIV-1 (EC_{50} ≈ 0.15–0.23 μg/mL) [113,114].

3.2. Synthetic lectins

Carbohydrate binding agents (CBAs) can bind to carbohydrate residues on the surface of viral envelopes, as for HIV gp120. This binding could lead to an inhibition of viral entry. Moreover, mutations of the envelope glycoproteins can improve drug pressure and lead to viral immune response neutralization. Because manufacturing natural plant-based lectins can be expensive, synthetic lectins have been considered as potential inhibitory alternative [115]. Synthetic lectins are cheaper to mass-produce as compared to their plant-based counterparts. As a response to the high cost and potential mitogenecity of natural lectins, Mahalingan et al. developed a benzoboroxole (BzB) polymeric synthetic lectin. Like natural plant-based lectins, BzB targets and binds carbohydrates on HIV viral envelope. At pH 7, BzB demonstrated increased binding efficiency to reducing sugars, such as fructose and weak binding affinity to non-reducing sugars, such as galactopyranose, a terminal sugar found on the surface of HIV gp120 complex glycans. This study further showed that BzB polymers of high molecular weight increased antiviral activity, proving that polyvalent interactions between inhibitor and glycosylated sites on HIV viral envelope improved with increased molecular weight. For example, increasing the mole percent of BzB functionalization showed an increase in EC_{50} [EC_{50} = 15 uM (25 mol%); EC_{50} = 15 nM (75 mol%)]. Moreover, substituting the polymer backbone with 10% sulfonic acid, resulted in an increased synergistic anti-HIV activity, as well as a 50-fold increase in aqueous solubility of the polymer. Furthermore, BzB-sulfonic acid showed an improved selectivity to HIV gp120, and the presence of fructose from seminal fluid did not decrease its antiviral activity [116]. Similarly, synthetic lectins containing phenylboronic acid (PBAs) could potentially exhibit carbohydrate recognition like that of CBA. For instance, Trippier et al. synthesized mannose selective PBA-based synthetic lectins that were tested for binding affinity against HIV gp120 [117]. Because the mono-PBA synthetic lectins tested did not bind HIV gp120 and were not good antiviral candidates, bis-PBA synthetic lectins were further investigated [118]. Although the bis-PBA did not demonstrate pronounced antiviral activity, these compounds were however found to be relatively noncytotoxic. It was also suggested that the lack of HIV gp120 binding could be due to the lack of multivalency and the small size of the PBA compounds.

4. Current anti-HIV lectins delivery strategies

4.1. Challenges in anti-HIV lectins drug development

The clinical translation of anti-HIV natural lectins faces numerous challenges including stability, solubility, resistance, toxicity, and manufacturing. These factors have seriously limited the progress in the field and often overshadow the potential benefits that anti-HIV lectins may have. For example, BanLec has been shown to partially dissociate into its monomeric forms in acidic conditions (pH 2) while maintaining a dimeric structure at neutral pH. The monomeric form of BanLec also offered more resistance towards chemical denaturation than the native dimeric form [119]. In addition, AH was shown to display low solubility in neutral buffer solutions with an enhanced solubility in acidic conditions [120]. This lack of solubility in neutral pH conditions could dramatically limit AH use as a topical microbicide for the prevention of HIV sexual transmission. It is established that vaginal pH increases from acidic (pH ≈ 4.5) to neutral (pH ≈ 7.5) during intercourse [121]. Furthermore, lectin resistant HIV strands have been reported [20]. The mutation of certain glycan structures in HIV gp120 was shown to be responsible for CV-N and Con A resistant HIV strands [100]. Although the development of HIV resistance to lectin may ultimately undermine the potency of these proteins, this is however viewed as an indirect route for exposing underlying amino acid sequences that could potentially be targeted by antibodies [80]. Anti-HIV lectins have also been associated with strong toxicity. In particular, Con A was shown to be mitogenic toward T-cells and induced cytotoxicity at high concentrations [122,123]. Similarly, CV-N and BanLec induced pronounced mitogenic activities on PBMCs and T-cells respectively [124,125]. Nonetheless, by replacing histidine 84 with a threonine in BanLec, Swanson et al. have demonstrated the possibility of bioengineering anti-HIV lectins to suppress their mitogenicity while maintaining their antiviral activity [104]. The high cost of natural anti-HIV lectins mass production and purification presents another particularly difficult challenge [126]. Although recombinant technology was proposed to overcome this limitation, improving fermentation yield, controlling mutation, and addressing potential immune system insults need to be studied [127]. Besides their ability to address some of these limitations and inhibit HIV transmission with relatively good safety profiles, synthetic lectins usually lack carbohydrate specificity and often require extensive optimizations to improve their binding affinity for HIV surface glycoproteins [117,118,126].

4.2. Anti-HIV lectin formulations

A potential barrier to the development of antibody-based vaccines against HIV is the oligosaccharide layer that provide a protective covering to the underlying antigens on the viral envelope surface [128]. Carbohydrate-lectin complexes are a promising therapeutic strategy because various proteins interact with oligosaccharides on the surface of many human cells. Glycoproteins and glycolipids can also interact with lectins and enhance mucosal absorption of drugs and vaccines [129]. Taking advantage of the so-called “lectin direct targeting”, potential efficacious HIV vaccine nanoformulations have targeted endogenous lectins for antigen delivery to immune cells [130]. Dendritic cell lectins are often targeted in this strategy. Those anti-HIV vaccines strategy activate various receptors on antigen presenting cells or C-type lectins to illicit immune responses.

The mannose receptor, a C-type lectin found on the vaginal epithelium, is known to bind HIV gp120 [131,132]. Binding of the mannose receptor to HIV gp120 allows the virus to cross the vaginal epithelium [133]. Humans have two types of mannose receptors, type 1 (MRC1) and type 2

(MRC2) and both can stimulate active and adaptive immunity. Because mannose receptors are highly expressed on dendritic cells as well as macrophages, these receptors are important for antigen recognition. Mannose receptors on dendritic cells take up antigen which stimulates robust T-cell activation via both major histocompatibility complexes (MHC) I and II molecular uptake mechanisms. This T-cell activation plays a critical role in the successful anti-HIV vaccine development [131]. When HIV-1 DNA was encapsulated in mannan coated-cationic liposomes targeting MRC, the nanoformulation successfully activated immunological responses, such as cytotoxic T cells, IgA, and other hypersensitivity responses [131]. These cationic nanoparticles showed 50% higher uptake than non-coated mannan nanoparticles in the macrophage cell line J774E [133]. Similarly, Espuelas et al. showed that a liposome nanoformulation containing mono-, di-, and tetra antennary mannosyl lipid derivatives could potentially achieve identical mannose receptor targeting on dendritic cells for a potential mannose-targeted vaccination strategy [134]. Furthermore, this study proved that liposome formulations containing higher mannose density result in more efficient interactions with mannose receptors. DC-SIGN is a Ca^{2+} binding adhesion lectin present on the surface of immature dendritic cells that play an important role in modulating host response to infection and inflammatory stimuli [135]. Because of its implications for antigen targeting and stimulation of T-cell responses, DC-SIGN has been considered a potential receptor for HIV vaccine targeting. In fact, DC-SIGN recognizes various high mannose oligosaccharides on HIV gp120 [136]. *In vitro* studies using DC-SIGN-targeted PLGA nanoparticles have shown that these nanoformulations deliver antigens to human dendritic cells [137]. DC-SIGN also increased antigen presentation, which translated into an improved activation of CD4 + and CD8 + T-cells.

The development of additional HIV nanovaccine immunogens utilized envelope glycoprotein mimetics. Ingale et al. investigated liposomes-grafted high-density enveloped HIV glycoprotein trimers that were recognized by anti-HIV-1 antibodies and activated B-cells [138]. These liposome constructs may lead to a promising HIV neutralization vaccine. Moreover, He et al. designed nanoparticles containing native like trimeric structures of V1V2 and gp120. These nanoformulations presented a variety of gp140 trimers that displayed 20 spikes like that of other virus like particles. This study showed high B cell stimulation, which may lead to further investigation in the development of a multivalent HIV vaccine [139].

Other lectin-based anti-HIV strategies have focused on “lectin indirect targeting” instead. In this case, lectins (natural or synthetic) are included in formulations to target HIV envelope glycoproteins. This “virion capture” approach may lead to a successful HIV prevention by hindering a proper interaction between HIV virus and its targets. Virion and HIV gp120 antigen capture could potentially lay the foundation for a mucosal anti-HIV vaccine. Akashi et al. proposed a Con A immobilized polystyrene nanospheres capable of capturing HIV virions through binding interactions with HIV gp120 high mannose glycans [140]. Hayakawa et al. further investigated a similar strategy using nanoparticles prepared via co-polymerization of polystyrene and poly methacrylate[141]. Recently, Coulibaly et al. developed a mannose specific lectin-based HIV-1 gp120 responsive microbicide formulation capable of the control release of the nucleotide reverse transcriptase inhibitor Tenofovir (TFV) [142]. In this study, Con A’s ability to bind glycogen (a glucose-based polysaccharide) was used to engineer a self-assembled layer-by-layer drug delivery system. Drug release was achieved through a controlled and reverse disassembly of Con A/glycogen layers in seminal and vaginal fluid simulants at HIV-1 gp120 concentrations $\geq$ 25 μg/mL. Con A/glycogen layers disassembly was also shown to be primarily due to the lectin’s higher binding affinity for mannose glycans in HIV-1 gp120. Moreover, the amount of TFV released was shown to potentially inhibit HIV sexual transmission. This system also appeared to be safer on vaginal (VK2), murine macrophage (RAW 264.7) and *Lactobacillus crispatus* cell lines. Although this system could be a safe and effective template for HIV vaginal microbicide drug delivery, future studies still need to prove its anti-HIV activity and *in vivo* safety. Current anti-HIV lectins' formulations, discussed above, have been summarized in Table 2.

Table 2. Summary of anti-HIV lectin formulations.

Formulation	Lectin	Target	References
Mannan coated-cationic liposomes	Mannose receptors, C-type lectins	MRC (Dendritic cells)	[131,133]
Mannosylated liposome	Mannose receptors, C-type lectins	MRC (Dendritic cells)	[134]
PLGA nanoparticles	DC-SIGN	Dendritic cells	[137]
High density enveloped HIV glycoprotein liposomes	N/A	BRC (B cells receptor)	[138]
Con A immobilized polystyrene nanospheres	Con A	HIV gp120	[140]
Con A immobilized polystyrene/methacrylate nanospheres	Con A	HIV gp120	[141]
Layer-by-layer engineered Con A/glycogen microparticles	Con A	HIV gp120, methyl α-D-mannopyranoside	[142]

5. Conclusion

In general, the field of lectinology has greatly contributed in the structural elucidation, the mechanistic understanding and the advancement of lectin based alternative antiviral therapy for various enveloped viruses including HIV, Zika, Ebola, Marburg, Herpes, Hepatitis-C, influenza, Severe Acute Respiratory Syndrome (SARS), Feline Infectious Peritonitis Virus (FIPV), and many more [143–150]. Despite test tube promises shown by lectins (natural and synthetic) against these pathogens, lectin-based antiviral clinical translation still faces great challenges including, resistance, cytotoxicity, immunogenicity, antigen specificity, and limited stability [151]. Nonetheless, current research on selected lectin candidates, such as BanLec and Griffithsin, may potentially lead to the first clinically available lectin-based antiviral therapy in the future [4,152]. Although anti-HIV lectins research is projected to grow, future investigations in the field would likely have to address novel delivery strategies to significantly improve CBA clinical translation.

Acknowledgments

This work is supported by Grant Number R01AI087304 from the National Institute of Allergy and Infectious Diseases. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Allergy and Infectious Diseases or the National Institutes of Health.

Conflict of interest

All authors declare no conflicts of interest in this manuscript.

References

1. Hodder SL, Justman J, Haley DF, et al. (2010) Challenges of a hidden epidemic: HIV prevention among women in the United States. *J Acquir Immune Defic Syndr* 55 Suppl 2: S69–S73.
2. Shattock RJ, Rosenberg Z (2012) Microbicides: Topical prevention against HIV. *Cold Spring Harb Perspect Med* 2: a007385.
3. UNAIDS. Available from: http://www.unaids.org/en/resources/documents/2016/Global-AIDS-update-2016.
4. Swanson MD, Winter HC, Goldstein IJ, et al. (2010) A lectin isolated from bananas is a potent inhibitor of HIV replication. *J Biol Chem* 285: 8646–8655.
5. Murphy EM, Greene ME, Mihailovic A, et al. (2006) Was the "ABC" approach (abstinence, being faithful, using condoms) responsible for Uganda's decline in HIV? *PLoS Med* 3: 1–5.
6. Cohen SA. Available from: https://www.guttmacher.org/gpr/2004/11/promoting-b-abc-its-value-and-limitations-fostering-reproductive-health.
7. Sovran S (2013) Understanding culture and HIV/AIDS in sub-Saharan Africa. *Sahara J* 10: 32–41.
8. Reniers G, Watkins S (2010) Polygyny and the spread of HIV in sub-Saharan Africa: A case of benign concurrency. *Aids* 24: 299–307.
9. Helweglarsen M, Collins BE (1994) The UCLA Multidimensional Condom Attitudes Scale: Documenting the complex determinants of condom use in college students. *Health Psychol* 13: 224–237.
10. UNAIDS. Available from: http://www.unaids.org/globalreport/Global_report.htm.
11. Qian K, Morris-Natschke SL, Lee KH (2009) HIV entry inhibitors and their potential in HIV therapy. *Med Res Rev* 29: 369–393.
12. Fohona S, Coulibaly BBC (2017) Current status of lectin-based cancer diagnosis and therapy. *AIMS Mol Sci* 4: 1–27.
13. Hansen TK, Gall MA, Tarnow L, et al. (2007) Mannose-binding lectin and mortality in type 2 diabetes. *Arch Intern Med* 166: 2007–2013.
14. Guan LZ, Tong Q, Xu J (2015) Elevated serum levels of mannose-binding lectin and diabetic nephropathy in type 2 diabetes. *PLoS One* 10: 1–10.
15. Losin IE, Shakhnovich RM, Zykov KA, et al. (2014) Cardiovascular diseases and mannose-binding lectin. *Kardiologiia* 54: 64–70.
16. Kelsall A, Fitzgerald AJ, Howard CV, et al. (2002) Dietary lectins can stimulate pancreatic growth in the rat. *Int J Exp Pathol* 83: 203–208.
17. Hoyem PH, Bruun JM, Pedersen SB, et al. (2012) The effect of weight loss on serum mannose-binding lectin levels. *Clin Dev Immunol* 2012: 1–5.
18. Akkouh O, Ng TB, Singh SS, et al. (2015) Lectins with anti-HIV activity: A review. *Molecules* 20: 648–668.
19. Checkley MA, Luttge BG, Freed EO (2011) HIV-1 envelope glycoprotein biosynthesis, trafficking, and incorporation. *J Mol Biol* 410: 582–608.

20. Balzarini J, Van LK, Hatse S, et al. (2004) Profile of resistance of human immunodeficiency virus to mannose-specific plant lectins. *J Virol* 78: 10617–10627.
21. Huskens D, Van LK, Vermeire K, et al. (2007) Resistance of HIV-1 to the broadly HIV-1-neutralizing, anti-carbohydrate antibody 2G12. *Virology* 360: 294–304.
22. Blumenthal R, Durell S, Viard M (2012) HIV entry and envelope glycoprotein-mediated fusion. *J Biol Chem* 287: 40841–40849.
23. Wilen CB, Tilton JC, Doms RW (2012) HIV: Cell binding and entry. *Cold Spring Harb Perspect Med* 2: 1–13
24. Ratner L, Haseltine W, Patarca R, et al. (1985) Complete nucleotide sequence of the AIDS virus, HTLV-III. *Nature* 313: 277–284.
25. Allan JS, Coligan JE, Barin F, et al. (1985) Major glycoprotein antigens that induce antibodies in AIDS patients are encoded by HTLV-III. *Science* 228: 1091–1094.
26. Montagnier L, Clavel F, Krust B, et al. (1985) Identification and antigenicity of the major envelope glycoprotein of lymphadenopathy-associated virus. *Virology* 144: 283–289.
27. Wainhobson S, Sonigo P, Danos O, Cole S, et al. (1985) Nucleotide sequence of the AIDS virus, LAV. *Cell* 40: 9–17.
28. Mizuochi T, Spellman MW, Larkin M, et al. (1988) Carbohydrate structures of the human-immunodeficiency-virus (HIV) recombinant envelope glycoprotein gp120 produced in Chinese-hamster ovary cells. *Biochem J* 254: 599–603.
29. Mizuochi T, Spellman MW, Larkin M, et al. (1988) Structural characterization by chromatographic profiling of the oligosaccharides of human immunodeficiency virus (HIV) recombinant envelope glycoprotein gp120 produced in Chinese hamster ovary cells. *Biomed Chromatogr* 2: 260–270.
30. Mizuochi T, Matthews TJ, Kato M, et al. (1990) Diversity of oligosaccharide structures on the envelope glycoprotein gp120 of human immunodeficiency virus 1 from the lymphoblastoid cell line H9. Presence of complex-type oligosaccharides with bisecting N-acetylglucosamine residues. *J Biol Chem* 265: 8519–8524.
31. Geyer H, Holschbach C, Hunsmann G, et al. (1988) Carbohydrates of human immunodeficiency virus. Structures of oligosaccharides linked to the envelope glycoprotein 120. *J Biol Chem* 263: 11760–11767.
32. Go EP, Hewawasam G, Liao HX, et al. (2011) Characterization of glycosylation profiles of HIV-1 transmitted/founder envelopes by mass spectrometry. *J Virol* 85: 8270–8284.
33. Bonomelli C, Doores KJ, Dunlop DC, et al. (2011) The glycan shield of HIV is predominantly oligomannose independently of production system or viral clade. *PLoS One* 6: 1–7.
34. Go EP, Herschhorn A, Gu C, et al. (2015) Comparative Analysis of the Glycosylation Profiles of Membrane-Anchored HIV-1 Envelope Glycoprotein Trimers and Soluble gp140. *J Virol* 89: 8245–8257.
35. Raska M, Takahashi K, Czernekova L, et al. (2010) Glycosylation patterns of HIV-1 gp120 depend on the type of expressing cells and affect antibody recognition. *J Biol Chem* 285: 20860–20869.
36. Zhu X, Borchers C, Bienstock RJ, et al. (2000) Mass spectrometric characterization of the glycosylation pattern of HIV-gp120 expressed in CHO cells. *Biochemistry* 39: 11194–11204.
37. Doores KJ, Bonomelli C, Harvey DJ, et al. (2010) Envelope glycans of immunodeficiency virions are almost entirely oligomannose antigens. *Proc Natl Acad Sci U S A* 107: 13800–13805.
38. Behrens AJ, Harvey DJ, Milne E, et al. (2017) Molecular Architecture of the Cleavage-Dependent Mannose Patch on a Soluble HIV-1 Envelope Glycoprotein Trimer. *J Virol* 91: 1–16.

39. Sok D, Doores KJ, Briney B, et al. (2014) Promiscuous glycan site recognition by antibodies to the high-mannose patch of gp120 broadens neutralization of HIV. *Sci Transl Med* 6: 1–15.
40. Pritchard LK, Spencer DI, Royle L, et al. (2015) Glycan clustering stabilizes the mannose patch of HIV-1 and preserves vulnerability to broadly neutralizing antibodies. *Nat Commun* 6: 1–11.
41. Coss KP, Vasiljevic S, Pritchard LK, et al. (2016) HIV-1 Glycan Density Drives the Persistence of the Mannose Patch within an Infected Individual. *J Virol* 90: 11132–11144.
42. Raska M, Novak J (2010) Involvement of envelope-glycoprotein glycans in HIV-1 biology and infection. *Arch Immunol Ther Exp* 58: 191–208.
43. Wang SK, Liang PH, Astronomo RD, et al. (2008) Targeting the carbohydrates on HIV-1: Interaction of oligomannose dendrons with human monoclonal antibody 2G12 and DC-SIGN. *Proc Natl Acad Sci U S A* 105: 3690–3695.
44. Balzarini J (2005) Targeting the glycans of gp120: A novel approach aimed at the Achilles heel of HIV. *Lancet Infect Dis* 5: 726–731.
45. Koch M, Pancera M, Kwong PD, et al. (2003) Structure-based, targeted deglycosylation of HIV-1 gp120 and effects on neutralization sensitivity and antibody recognition. *Virology* 313: 387–400.
46. Rathore U, Saha P, Kesavardhana S, et al. (2017) Glycosylation of the core of the HIV-1 envelope subunit protein gp120 is not required for native trimer formation or viral infectivity. *J Biol Chem* 24: 10197–10219.
47. Sanders RW, Anken EV, Nabatov AA, et al. (2008) The carbohydrate at asparagine 386 on HIV-1 gp120 is not essential for protein folding and function but is involved in immune evasion. *Retrovirology* 5: 1–15.
48. Montefiori DC, Jr RW, Mitchell WM (1988) Role of protein N-glycosylation in pathogenesis of human immunodeficiency virus type 1. *Proc Natl Acad Sci U S A* 85: 9248–9252.
49. Francois KO, Balzarini J (2011) The highly conserved glycan at asparagine 260 of HIV-1 gp120 is indispensable for viral entry. *J Biol Chem* 286: 42900–42910.
50. Mathys L, Francois KO, Quandte M, et al. (2014) Deletion of the highly conserved N-glycan at Asn260 of HIV-1 gp120 affects folding and lysosomal degradation of gp120, and results in loss of viral infectivity. *PLoS One* 9: 1–11.
51. Huang X, Jin W, Hu K, et al. (2012) Highly conserved HIV-1 gp120 glycans proximal to CD4-binding region affect viral infectivity and neutralizing antibody induction. *Virology* 423: 97–106.
52. Binley JM, Ban YE, Crooks ET, et al. (2010) Role of complex carbohydrates in human immunodeficiency virus type 1 infection and resistance to antibody neutralization. *J Virol* 84: 5637–5655.
53. Li Y, Luo L, Rasool N, et al. (1993) Glycosylation is necessary for the correct folding of human immunodeficiency virus gp120 in CD4 binding. *J Virol* 67: 584–588.
54. Li H, Jr CP, Tuen M, Visciano ML, et al. (2008) Identification of an N-linked glycosylation in the C4 region of HIV-1 envelope gp120 that is critical for recognition of neighboring CD4 T cell epitopes. *J Immunol* 180: 4011–4021.
55. Behrens AJ, Vasiljevic S, Pritchard LK, et al. (2016) Composition and Antigenic Effects of Individual Glycan Sites of a Trimeric HIV-1 Envelope Glycoprotein. *Cell Rep* 14: 2695–2706.
56. Pritchard LK, Vasiljevic S, Ozorowski G, et al. (2015) Structural Constraints Determine the Glycosylation of HIV-1 Envelope Trimers. *Cell Rep* 11: 1604–1613.
57. Steckbeck JD, Craigo JK, Barnes CO, et al. (2011) Highly conserved structural properties of the C-terminal tail of HIV-1 gp41 protein despite substantial sequence variation among diverse clades: Implications for functions in viral replication. *J Biol Chem* 286: 27156–27166.

58. Dimonte S, Mercurio F, Svicher V, et al. (2011) Selected amino acid mutations in HIV-1 B subtype gp41 are associated with specific gp120v(3) signatures in the regulation of co-receptor usage. *Retrovirology* 8: 1–11.
59. Perrin C, Fenouillet E, Jones IM (1998) Role of gp41 glycosylation sites in the biological activity of human immunodeficiency virus type 1 envelope glycoprotein. *Virology* 242: 338–345.
60. Johnson WE, Sauvron JM, Desrosiers RC (2001) Conserved, N-linked carbohydrates of human immunodeficiency virus type 1 gp41 are largely dispensable for viral replication. *J Virol* 75: 11426–11436.
61. Fenouillet E (1993) La N-glycosylation du VIH: Du modèle expérimental à l'application thérapeutique. *J Libbery Eurotext Montrouge* 9: 901–906.
62. Fenouillet E, Jones IM (1995) The glycosylation of human immunodeficiency virus type 1 transmembrane glycoprotein (gp41) is important for the efficient intracellular transport of the envelope precursor gp160. *J Gen Virol* 76: 1509–1514.
63. Lee WR, Yu XF, Syu WJ, et al. (1992) Mutational analysis of conserved N-linked glycosylation sites of human immunodeficiency virus type 1 gp41. *J Virol* 66: 1799–1803.
64. Ma BJ, Alam SM, Go EP, et al. (2011) Envelope deglycosylation enhances antigenicity of HIV-1 gp41 epitopes for both broad neutralizing antibodies and their unmutated ancestor antibodies. *PLoS Pathog* 7: 1–16.
65. Wang LX, Song H, Liu S, et al. (2005) Chemoenzymatic synthesis of HIV-1 gp41 glycopeptides: Effects of glycosylation on the anti-HIV activity and alpha-helix bundle-forming ability of peptide C34. *Chembiochem* 6: 1068–1074.
66. Balzarini J, Van LK, Hatse S, et al. (2005) Carbohydrate-binding agents cause deletions of highly conserved glycosylation sites in HIV GP120: A new therapeutic concept to hit the achilles heel of HIV. *J Biol Chem* 280: 41005–41014.
67. Van AE, Sanders RW, Liscaljet IM, et al. (2008) Only five of 10 strictly conserved disulfide bonds are essential for folding and eight for function of the HIV-1 envelope glycoprotein. *Mol Biol Cell* 19: 4298–4309.
68. Mathys L, Balzarini J (2014) The role of N-glycans of HIV-1 gp41 in virus infectivity and susceptibility to the suppressive effects of carbohydrate-binding agents. *Retrovirology* 11: 1–18.
69. Fenouillet E, Jones I, Powell B, et al. (1993) Functional role of the glycan cluster of the human immunodeficiency virus type 1 transmembrane glycoprotein (gp41) ectodomain. *J Virol* 67: 150–160.
70. Yuste E, Bixby J, Lifson J, et al. (2008) Glycosylation of gp41 of simian immunodeficiency virus shields epitopes that can be targets for neutralizing antibodies. *J Virol* 82: 12472–12486.
71. Tanaka H, Chiba H, Inokoshi J, et al. (2009) Mechanism by which the lectin actinohivin blocks HIV infection of target cells. *Proc Natl Acad Sci U S A* 106: 15633–15638.
72. Hoorelbeke B, Huskens D, Ferir G, et al. (2010) Actinohivin, a broadly neutralizing prokaryotic lectin, inhibits HIV-1 infection by specifically targeting high-mannose-type glycans on the gp120 envelope. *Antimicrob Agents Chemother* 54: 3287–32301.
73. Zhang F, Hoque MM, Jiang J, et al. (2014) The characteristic structure of anti-HIV actinohivin in complex with three HMTG D1 chains of HIV-gp120. *Chembiochem* 15: 2766–2773.
74. Bewley CA, Gustafson KR, Boyd MR, et al. (1998) Solution structure of cyanovirin-N, a potent HIV-inactivating protein. *Nat Struct Biol* 5: 571–578.

75. Barrientos LG, Louis JM, Ratner DM, et al. (2003) Solution structure of a circular-permuted variant of the potent HIV-inactivating protein cyanovirin-N: Structural basis for protein stability and oligosaccharide interaction. *J Mol Biol* 325: 211–223.
76. Esser MT, Mori T, Mondor I, et al. (1999) Cyanovirin-N binds to gp120 to interfere with CD4-dependent human immunodeficiency virus type 1 virion binding, fusion, and infectivity but does not affect the CD4 binding site on gp120 or soluble CD4-induced conformational changes in gp120. *J Virol* 73: 4360–4371.
77. Alexandre KB, Gray ES, Mufhandu H, et al. (2012) The lectins griffithsin, cyanovirin-N and scytovirin inhibit HIV-1 binding to the DC-SIGN receptor and transfer to CD4(+) cells. *Virology* 423: 175–186.
78. Buffa V, Stieh D, Mamhood N, et al. (2009) Cyanovirin-N potently inhibits human immunodeficiency virus type 1 infection in cellular and cervical explant models. *J Gen Virol* 90: 234–243.
79. Boyd MR, Gustafson KR, Mcmahon JB, et al. (1997) Discovery of cyanovirin-N, a novel human immunodeficiency virus-inactivating protein that binds viral surface envelope glycoprotein gp120: Potential applications to microbicide development. *Antimicrob Agents Chemother* 41: 1521–1530.
80. Hu Q, Mahmood N, Shattock RJ (2007) High-mannose-specific deglycosylation of HIV-1 gp120 induced by resistance to cyanovirin-N and the impact on antibody neutralization. *Virology* 368: 145–154.
81. Keeffe JR, Gnanapragasam PN, Gillespie SK, et al. (2011) Designed oligomers of cyanovirin-N show enhanced HIV neutralization. *Proc Natl Acad Sci U S A* 108: 14079–14084.
82. Dey B, Lerner DL, Lusso P, et al. (2000) Multiple antiviral activities of cyanovirin-N: Blocking of human immunodeficiency virus type 1 gp120 interaction with CD4 and coreceptor and inhibition of diverse enveloped viruses. *J Virol* 74: 4562–4569.
83. Férir G, Huskens D, Noppen S, et al. (2014) Broad anti-HIV activity of the Oscillatoria agardhii agglutinin homologue lectin family. *J Antimicrob Chemother* 69: 2746–2758.
84. Koharudin LM, Gronenborn AM (2011) Structural basis of the anti-HIV activity of the cyanobacterial Oscillatoria Agardhii agglutinin. *Structure* 19: 1170–1181.
85. Koharudin LM, Furey W, Gronenborn AM (2011) Novel fold and carbohydrate specificity of the potent anti-HIV cyanobacterial lectin from Oscillatoria agardhii. *J Biol Chem* 286: 1588–1597.
86. Carneiro MG, Koharudin LM, Ban D, et al. (2015) Sampling of Glycan-Bound Conformers by the Anti-HIV Lectin Oscillatoria agardhii agglutinin in the Absence of Sugar. *Angew Chem* 54: 6462–6465.
87. Bokesch HR, O’Keefe BR, Mckee TC, et al. (2003) A potent novel anti-HIV protein from the cultured cyanobacterium Scytonema varium. *Biochemistry* 42: 2578–2584.
88. Adams EW, Ratner DM, Bokesch HR, et al. (2004) Oligosaccharide and glycoprotein microarrays as tools in HIV glycobiology; glycan-dependent gp120/protein interactions. *Chem Biol* 11: 875–881.
89. Mcfeeters RL, Xiong C, O’Keefe BR, et al. (2007) The novel fold of scytovirin reveals a new twist for antiviral entry inhibitors. *J Mol Biol* 369: 451–461.
90. Alexandre KB, Gray ES, Lambson BE, et al. (2010) Mannose-rich glycosylation patterns on HIV-1 subtype C gp120 and sensitivity to the lectins, Griffithsin, Cyanovirin-N and Scytovirin. *Virology* 402: 187–196.

91. Williams DC, Lee JY, Cai M, et al. (2005) Crystal structures of the HIV-1 inhibitory cyanobacterial protein MVL free and bound to Man3GlcNAc2: Structural basis for specificity and high-affinity binding to the core pentasaccharide from n-linked oligomannoside. *J Biol Chem* 280: 29269–29276.
92. Ziółkowska NE, Wlodawer A (2006) Structural studies of algal lectins with anti-HIV activity. *Acta Biochim Pol* 53: 617–626.
93. Shahzadulhussan S, Gustchina E, Ghirlando R, et al. (2011) Solution structure of the monovalent lectin microvirin in complex with Man(alpha)(1–2)Man provides a basis for anti-HIV activity with low toxicity. *J Biol Chem* 286: 20788–20796.
94. Huskens D, Ferir G, Vermeire K, et al. (2010) Microvirin, a novel alpha(1,2)-mannose-specific lectin isolated from Microcystis aeruginosa, has anti-HIV-1 activity comparable with that of cyanovirin-N but a much higher safety profile. *J Biol Chem* 285: 24845–24854.
95. López S, Armandugon M, Bastida J, et al. (2003) Anti-human immunodeficiency virus type 1 (HIV-1) activity of lectins from Narcissus species. *Planta Med* 69: 109–112.
96. Müller WEG, Forrest JMS, Chang SH, et al. (1991) Narcissus and Gerardia lectins: Tools for the development of a vaccine against AIDS and a new ELISA to quantify HIV-gp 120. *Lectins Cancer* 1991: 27–40.
97. Charan RD, Munro MH, O'Keefe BR, et al. (2000) Isolation and characterization of Myrianthus holstii lectin, a potent HIV-1 inhibitory protein from the plant Myrianthus holstii(1). *J Nat Prod* 63: 1170–1174.
98. Coulibaly FS, Youan BB (2014) Concanavalin A-polysaccharides binding affinity analysis using a quartz crystal microbalance. *Biosens Bioelectron* 59: 404–411.
99. Bhattacharyya L, Brewer CF (1989) Interactions of concanavalin A with asparagine-linked glycopeptides. Structure/activity relationships of the binding and precipitation of oligomannose and bisected hybrid-type glycopeptides with concanavalin A. *Eur J Biochem* 178: 721–726.
100. Witvrouw M, Fikkert V, Hantson A, et al. (2005) Resistance of human immunodeficiency virus type 1 to the high-mannose binding agents cyanovirin N and concanavalin A. *J Virol* 79: 7777–7784.
101. Hansen JE, Nielsen CM, Nielsen C, et al. (1989) Correlation between carbohydrate structures on the envelope glycoprotein gp120 of HIV-1 and HIV-2 and syncytium inhibition with lectins. *Aids* 3: 635–641.
102. Matsui T, Kobayashi S, Yoshida O, et al. (1990) Effects of succinylated concanavalin A on infectivity and syncytial formation of human immunodeficiency virus. *Med Microbiol Immunol* 179: 225–235.
103. Pashov A, Macleod S, Saha R, et al. (2005) Concanavalin A binding to HIV envelope protein is less sensitive to mutations in glycosylation sites than monoclonal antibody 2G12. *Glycobiology* 15: 994–1001.
104. Swanson MD, Boudreaux DM, Salmon L, et al. (2015) Engineering a therapeutic lectin by uncoupling mitogenicity from antiviral activity. *Cell* 163: 746–758.
105. Alexandre KB, Gray ES, Pantophlet R, et al. (2011) Binding of the mannose-specific lectin, griffithsin, to HIV-1 gp120 exposes the CD4-binding site. *J Virol* 85: 9039–9050.
106. Mori T, O'Keefe BR, Bringans S, et al. (2005) Isolation and characterization of griffithsin, a novel HIV-inactivating protein, from the red alga Griffithsia sp. *J Biol Chem* 280: 9345–9353.
107. Emau P, Tian B, O'Keefe BR, et al. (2007) Griffithsin, a potent HIV entry inhibitor, is an excellent candidate for anti-HIV microbicide. *J Med Primatol* 36: 244–253.

108. Moulaei T, Alexandre KB, Shenoy SR, et al. (2015) Griffithsin tandemers: Flexible and potent lectin inhibitors of the human immunodeficiency virus. *Retrovirology* 12: 1–14.
109. Zhou X, Liu J, Yang B, et al. (2013) Marine natural products with anti-HIV activities in the last decade. *Curr Med Chem* 20: 953–973.
110. Wang JH, Kong J, Li W, et al. (2006) A beta-galactose-specific lectin isolated from the marine worm Chaetopterus variopedatus possesses anti-HIV-1 activity. *Comp Biochem Physiol C Toxicol Pharmacol* 142: 111–117.
111. Bulgheresi S, Schabussova I, Chen T, et al. (2006) A new C-type lectin similar to the human immunoreceptor DC-SIGN mediates symbiont acquisition by a marine nematode. *Appl Environ Microbiol* 72: 2950–2956.
112. Nabatov AA, Jong MAWPD, Witte LD, et al. (2008) C-type lectin Mermaid inhibits dendritic cell mediated HIV-1 transmission to CD4+ T cells. *Virology* 378: 323–328.
113. Molchanova V, Chikalovets I, Chernikov O, et al. (2007) A new lectin from the sea worm Serpula vermicularis: Isolation, characterization and anti-HIV activity. *Comp Biochem Physiol C Toxicol Pharmacol* 145: 184–193.
114. Vo TS, Kim SK (2010) Potential anti-HIV agents from marine resources: An overview. *Mar Drugs* 8: 2871–2892.
115. Mahalingam A, Geonnotti AR, Balzarini J, et al. (2011) Activity and safety of synthetic lectins based on benzoboroxole-functionalized polymers for inhibition of HIV entry. *Mol Pharmaceutics* 8: 2465–2475.
116. Berube M, Dowlut M, Hall DG (2008) Benzoboroxoles as efficient glycopyranoside-binding agents in physiological conditions: Structure and selectivity of complex formation. *J Org Chem* 73: 6471–6479.
117. Trippier PC, Mcguigan C, Balzarini J (2010) Phenylboronic-acid-based carbohydrate binders as antiviral therapeutics: Monophenylboronic acids. *Antivir Chem Chemother* 20: 249–257.
118. Trippier PC, Balzarini J, Mcguigan C (2011) Phenylboronic-acid-based carbohydrate binders as antiviral therapeutics: Bisphenylboronic acids. *Antivir Chem Chemother* 21: 129–142.
119. Khan JM, Qadeer A, Ahmad E, et al. (2013) Monomeric banana lectin at acidic pH overrules conformational stability of its native dimeric form. *PLoS One* 8: 1–12.
120. Suzuki K, Ohbayashi N, Jiang J, et al. (2012) Crystallographic study of the interaction of the anti-HIV lectin actinohivin with the alpha(1-2)mannobiose moiety of gp120 HMTG. *Acta Crystallogr Sect F Struct Biol Cryst Commun* 68: 1060–1063.
121. Tevibénissan C, Bélec L, Lévy M, et al. (1997) *In vivo* semen-associated pH neutralization of cervicovaginal secretions. *Clin Diagn Lab Immunol* 4: 367–374.
122. Ballerstadt R, Evans C, Mcnichols R, et al. (2006) Concanavalin A for *in vivo* glucose sensing: A biotoxicity review. *Biosens Bioelectron* 22: 275–284.
123. Krauss S, Buttgereit F, Brand MD (1999) Effects of the mitogen concanavalin A on pathways of thymocyte energy metabolism. *Biochim Biophys Acta* 1412: 129–138.
124. Balzarini J, Laethem KV, Peumans WJ, et al. (2006) Mutational pathways, resistance profile, and side effects of cyanovirin relative to human immunodeficiency virus type 1 strains with N-glycan deletions in their gp120 envelopes. *J Virol* 80: 8411–8421.
125. Gavrovicjankulovic M, Poulsen K, Brckalo T, et al. (2008) A novel recombinantly produced banana lectin isoform is a valuable tool for glycoproteomics and a potent modulator of the proliferation response in CD3+, CD4+, and CD8+ populations of human PBMCs. *Int J Biochem Cell Biol* 40: 929–941.

126. Mahalingam A, Geonnotti AR, Balzarini J, et al. (2011) Activity and safety of synthetic lectins based on benzoboroxole-functionalized polymers for inhibition of HIV entry. *Mol Pharm* 8: 2465–2475.
127. Lam SK, Ng TB (2011) Lectins: Production and practical applications. *Appl Microbiol Biotechnol* 89: 45–55.
128. Scanlan CN, Offer J, Zitzmann N, et al. (2007) Exploiting the defensive sugars of HIV-1 for drug and vaccine design. *Nature* 446: 1038–1045.
129. Gupta A, Gupta RK, Gupta GS (2009) Targeting cells for drug and gene delivery: Emerging applications of mannans and mannan binding lectins. *J Sci Ind Res* 68: 465–483.
130. Ghazarian H, Idoni B, Oppenheimer SB (2011) A glycobiology review: Carbohydrates, lectins and implications in cancer therapeutics. *Acta Histochem* 113: 236–247.
131. Toda S, Ishii N, Okada E, et al. (1997) HIV-1-specific cell-mediated immune responses induced by DNA vaccination were enhanced by mannan-coated liposomes and inhibited by anti-interferon-gamma antibody. *Immunology* 92: 111–117.
132. Zelensky AN, Gready JE (2005) The C-type lectin-like domain superfamily. *FEBS J* 272: 6179–6217.
133. Cui Z, Hsu CH, Mumper RJ (2003) Physical characterization and macrophage cell uptake of mannan-coated nanoparticles. *Drug Dev Ind Pharm* 29: 689–700.
134. Espuelas S, Thumann C, Heurtault B, et al. (2008) Influence of ligand valency on the targeting of immature human dendritic cells by mannosylated liposomes. *Bioconjugate Chem* 19: 2385–2393.
135. Zhang Q, Su L, Collins J, et al. (2014) Dendritic cell lectin-targeting sentinel-like unimolecular glycoconjugates to release an anti-HIV drug. *J Am Chem Soc* 136: 4325–4332.
136. Hong PWP, Flummerfelt KB, Parseval AD, et al. (2002) Human immunodeficiency virus envelope (gp120) binding to DC-SIGN and primary dendritic cells is carbohydrate dependent but does not involve 2G12 or cyanovirin binding sites: Implications for structural analyses of gp120-DC-SIGN binding. *J Virol* 76: 12855–12865.
137. Cruz LJ, Tacken PJ, Fokkink R, et al. (2010) Targeted PLGA nano- but not microparticles specifically deliver antigen to human dendritic cells via DC-SIGN *in vitro*. *J Controlled Release* 144: 118–126.
138. Ingale J, Stano A, Guenaga J, et al. (2016) High-Density Array of Well-Ordered HIV-1 Spikes on Synthetic Liposomal Nanoparticles Efficiently Activate B Cells. *Cell Rep* 15: 1986–1999.
139. He L, Val ND, Morris CD, et al. (2016) Presenting native-like trimeric HIV-1 antigens with self-assembling nanoparticles. *Nat Commun* 7: 1–15.
140. Akashi M, Niikawa T, Serizawa T, et al. (1998) Capture of HIV-1 gp120 and virions by lectin-immobilized polystyrene nanospheres. *Bioconjugate Chem* 9: 50–53.
141. Hayakawa T, Kawamura M, Okamoto M, et al. (1998) Concanavalin A-immobilized polystyrene nanospheres capture HIV-1 virions and gp120: Potential approach towards prevention of viral transmission. *J Med Virol* 56: 327–331.
142. Coulibaly FS, Ezoulin MJM, Purohit SS, et al. (2017) Layer-by-layer engineered microbicide drug delivery system targeting HIV-1 gp120: Physicochemical and biological properties. *Mol Pharm* 14: 3512–3527.
143. Takahashi K, Moyo P, Chigweshe L, et al. (2013) Efficacy of recombinant chimeric lectins, consisting of mannose binding lectin and L-ficolin, against influenza A viral infection in mouse model study. *Virus Res* 178: 495–501.

144. Sato Y, Morimoto K, Kubo T, et al. (2015) Entry Inhibition of Influenza Viruses with High Mannose Binding Lectin ESA-2 from the Red Alga Eucheuma serra through the Recognition of Viral Hemagglutinin. *Mar Drugs* 13: 3454–3465.
145. Kachko A, Loesgen S, Shahzad-Ul-Hussan S, et al. (2013) Inhibition of hepatitis C virus by the cyanobacterial protein Microcystis viridis lectin: Mechanistic differences between the high-mannose specific lectins MVL, CV-N, and GNA. *Mol Pharmaceutics* 10: 4590–4602.
146. Gadjeva M, Paludan SR, Thiel S, et al. (2004) Mannan-binding lectin modulates the response to HSV-2 infection. *Clin Exp Immunol* 138: 304–311.
147. Eisen S, Dzwonek A, Klein NJ (2008) Mannose-binding lectin in HIV infection. *Future Virol* 3: 225–233.
148. Ji X, Olinger GG, Aris S, et al. (2005) Mannose-binding lectin binds to Ebola and Marburg envelope glycoproteins, resulting in blocking of virus interaction with DC-SIGN and complement-mediated virus neutralization. *J Gen Virol* 86: 2535–2542.
149. Keyaerts E, Vijgen L, Pannecouque C, et al. (2007) Plant lectins are potent inhibitors of coronaviruses by interfering with two targets in the viral replication cycle. *Antiviral Res* 75: 179–187.
150. Hamel R, Dejarnac O, Wichit S, et al. (2015) Biology of Zika Virus Infection in Human Skin Cells. *J Virol* 89: 8880–8896.
151. Clement F, Venkatesh YP (2010) Dietary garlic (Allium sativum) lectins, ASA I and ASA II, are highly stable and immunogenic. *Int Immunopharmacol* 10: 1161–1169.
152. Lusvarghi S, Bewley CA (2016) Griffithsin: An Antiviral Lectin with Outstanding Therapeutic Potential. *Viruses* 8: 1–18.