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REVIEW

# Recent advances in synthetic carbohydrate-based human immunodeficiency virus vaccines

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**An effective vaccine for human immunodeficiency virus (HIV) is urgently needed to prevent HIV infection and progression to acquired immune deficiency syndrome (AIDS). As glycosylation of viral proteins becomes better understood, carbohydrate-based antiviral vaccines against special viruses have attracted much attention. Significant efforts in carbohydrate synthesis and immunogenicity research have resulted in the development of multiple carbohydrate-based HIV vaccines. This review summarizes recent advances in synthetic carbohydrate-based vaccines design strategies and the applications of these vaccines in the prevention of HIV.**

**KEYWORDS vaccine; human immunodeficiency virus (HIV); glycoprotein; N-glycosylation; neutralizing antibodies**

## INTRODUCTION

Human immunodeficiency virus (HIV) continues to be a major global public health issue, with more than 1.2 million acquired immune deficiency syndrome (AIDS)-related deaths occurring yearly (UNAIDS, 2015). In 2014, 2 million individuals were newly infected with HIV-1, and 36.9 million people were living with HIV-1 (UNAIDS, 2015). Combination antiretroviral therapy (cART) or highly active antiretroviral therapy (HAART) has been shown to reduce AIDS-related mortality and morbidity (de Goede et al., 2015). Although cART has the potential to dramatically prolong the life expectancy of HIV-infected individuals, it does not provide a cure; therefore, individuals receiving cART must continue therapy for their entire lives and must overcome issues associated with the side effects and high costs of therapy (Tongo and Burgers, 2014; de Goede et al., 2015). Moreover, cART may lead to the emergence of resistant mutants. Therefore, an effective HIV vaccine is still urgently needed to prevent HIV infection and progression to AIDS.

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Since the first use of a vaccine by Edward Jenner in 1796, the development of cell culture and recombinant DNA technologies has revolutionized vaccine design (Rodrigues et al., 2015). In the middle of the 20th century, attenuated and inactivated vaccines developed through animal cell culture became the most commonly used form of vaccination against viral infection. In the late 20th century, recombinant DNA technologies facilitated the development of subunit antiviral vaccines comprised of protein and/or DNA (Rodrigues et al., 2015). To date, vaccines have been successfully applied for eradication of several acute viral diseases, such as smallpox, poliomyelitis, and measles (Minor, 2015). However, the development of an HIV vaccine is more challenging for several reasons, including the absence of a single case of natural immunological protection against HIV infection and the marked genetic variability of HIV-1 (de Goede et al., 2015). Moreover, live attenuated forms of viruses and inactivated viruses, which are typically used in the development of many traditional vaccines, are not suitable for developing HIV vaccines for safety reasons (Tongo and Burgers, 2014).

Different HIV vaccine candidates have been tested in

human clinical trials, including virus-like particles, peptides, naked DNA, and viral vectors (Ensoli et al., 2014). Ideally, a highly effective HIV vaccine would elicit broadly neutralizing antibodies (bnAbs) to prevent infection and/or stimulate effective cytotoxic T lymphocyte (CTL) responses to slow disease progression (Mann and Ndung'u, 2015). To date, most vaccination trials have failed to provide suppression of HIV replication and prevention of AIDS progression (de Goede et al., 2015). Moreover, human vaccination studies have failed to induce bnAbs that target the envelope glycoprotein of HIV, suggesting that antibody-dependent cellular virus inhibition may play a key role (de Goede et al., 2015). A useful focus for HIV vaccine development is the reverse vaccinology approach, in which vaccines are designed based on epitopes recognized by biologically active monoclonal antibodies (mAbs) (Mayr and Zolla-Pazner, 2015).

To date, dozens of bnAbs have been isolated from patients exhibiting chronic HIV-1 infection (Figure 1) (McLellan et al., 2011; Pejchal et al., 2011; Walker et al., 2011; Julien et al., 2013; Kong et al., 2013; Blattner et al., 2014; Liu et al., 2015). HIV-1 envelope glycoprotein, which plays a key role in viral tropism and the membrane fusion process, is the only antigen accessible to bnAbs (Moulard and Decroly, 2000; Burton and Mascola, 2015). HIV-1 envelope glycoprotein, consisting of the exterior gp120 and the transmembrane gp41, can be presented to the human immune system in many forms: functional envelope trimers, nonfunctional and conformationally rearranged envelope protein, and envelope trimer after shedding of one gp120 and the gp41 stump (Burton and Mascola, 2015). Importantly, only functional envelope trimers can be available to elicit neutralizing antibody (nAb) response (Burton and Mascola, 2015). The five general targets of isolated bnAbs are 1) the gp120 variable loop1/2 (V1/V2) glycan (bnAbs: PG9, PG16), 2) the gp120 variable loop 3 (V3) glycan (bnAbs:

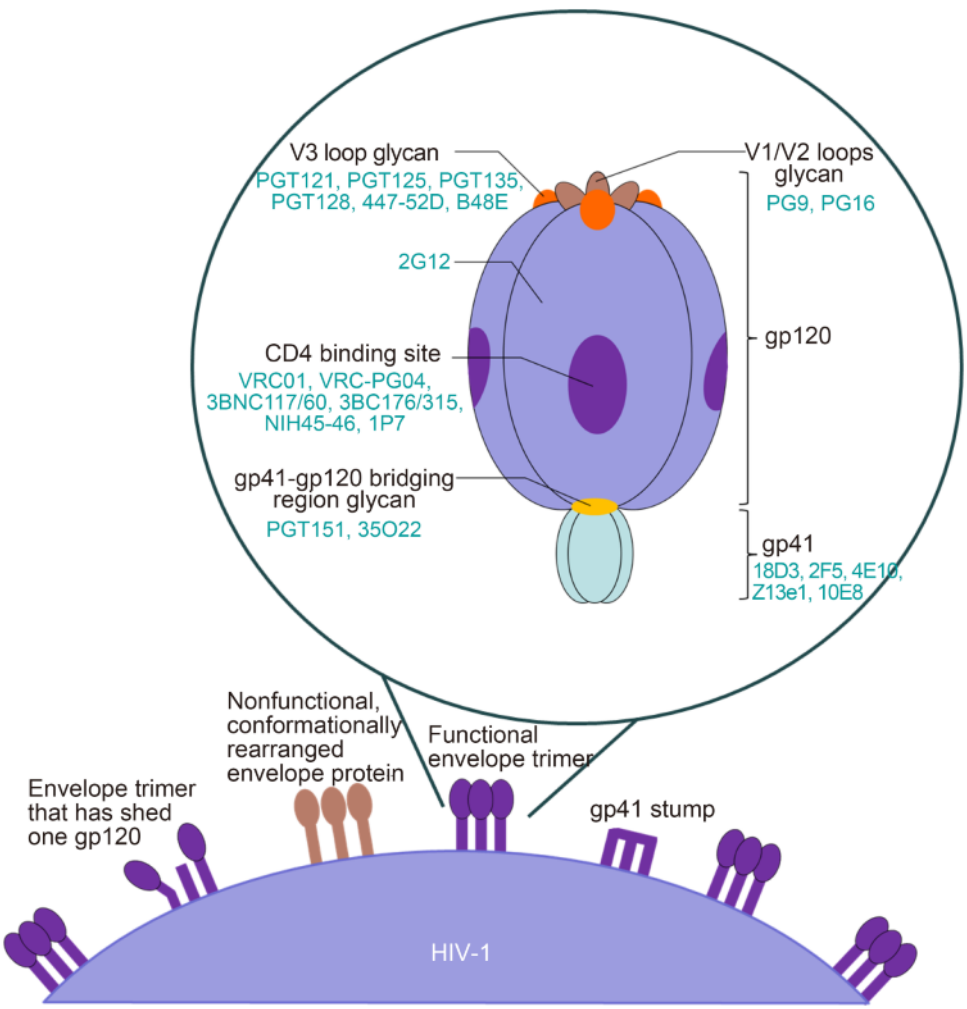


Figure 1. Schematic of the forms of HIV-1 envelope protein and the binding sites of broadly HIV-1-specific neutralizing antibodies. Below each bnAb site, the prototype antibodies that can bind at each site are listed.

PGT121, PGT125, PGT135, PGT128, 447-52D, B48E), 3) the CD4-binding site on gp120 (bnAbs: VRC01, VRC-PG04, 3BNC117/60, 3BC176/315, NIH45-46, 1P7), 4) the gp41 membrane proximal external region (bnAbs: 18D3, 2F5, 4E10, Z13e1, 10E8), and 5) the gp41-gp120 bridging region glycan (bnAbs: PGT151, 35O22) (Crispin and Doores, 2015; Fernandez-Tejada et al., 2015b; Haynes, 2015; Liu et al., 2015). Thus, most bnAbs are directed toward HIV-1 envelope glycans, which have previously been considered a "glycan shield" that masks vulnerable epitopes on the surface of HIV (Fernandez-Tejada et al., 2015b). Although envelope glycans are added by the host cell machinery, the clustering of glycans can be arranged in a "non-self" pattern (Crispin and Doores, 2015). As glycosylation of viral proteins becomes better understood, the development of carbohydrate-based antiviral vaccines against special viruses has been considered as a potential strategy (Swarts and Guo, 2009).

All cells are thought to be coated with different forms of carbohydrates, including glycoproteins and glycolipids in eukaryotic cells and capsular polysaccharides and lipopolysaccharides in bacterial cells (Haji-Ghassemi et al., 2015). The exposure of cell-surface carbohydrates and their unique structures on diverse pathogens make them attractive vaccine targets (Morelli et al., 2011). Polysaccharides and glycoconjugates isolated from pathogens have been referred to as valuable antigens for vaccine development and have been used clinically against a range of infectious diseases, including *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* (Astronomo and Burton, 2010; Fernandez-Tejada et al., 2015a). However, the isolation of carbohydrates from biological material is a tedious process often resulting in low yields of oligosaccharide mixtures and is limited to organisms that can be cultured (Hecht et al., 2009). Thus, many researchers have focused on chemical synthesis of structurally well-defined oligosaccharides, which have been widely used in preparing vaccines designed to target bacteria, virus, parasites, and cancer (Nikolaev and Sizova, 2011; Cai et al., 2012; Alama et al., 2013; Anish et al., 2014; Cavallari et al., 2014; Deng et al., 2014; Horiya et al., 2014b; Qin et al., 2014; Danishefsky et al., 2015; Thompson et al., 2015). One of the most successful examples of synthetic carbohydrate-based vaccines is the Cuban vaccine against Haemophilus influenza type b (Hib), which provides protection comparable to that of licensed vaccines comprised of natural capsular polysaccharides (Hsu et al., 2011).

In this review, we focus on recent carbohydrate-based vaccine design strategies and their applications in HIV vaccination.

## ACCELARATION OF CARBOHYDRATE SYNTHESIS

Regioselectivity and stereoselectivity are two major obstacles associated with approaching structurally well-defined oligosaccharides (Hsu et al., 2011). Despite ongoing challenges, tremendous progress in the preparation of chemically synthesized oligosaccharides has been achieved. Importantly, advances in glycochemistry, including protecting groups, linkers, solid supports, and glycosylation methods, have triggered the development of methods for automated carbohydrate synthesis (Seeberger, 2015). Over the past few decades, tremendous advances in automated oligosaccharide synthesis have facilitated the preparation of biologically important glycans (Seeberger and Werz, 2005; Hsu et al., 2011; Kröck et al., 2012; Eller et al., 2013; Seeberger, 2015). Plante and coworkers introduced an automated solid-phase oligosaccharide synthesizer, which was initially adapted from a peptide synthesizer by addition of a reaction vessel allowing for temperature adjustments (Plante et al., 2001). Over the past 13 years, several generations of home-built systems have been refined, resulting in a commercial system, the Glyconeer 2.1 (Figure 2) (Seeberger, 2015). This synthesizer was found to increase the efficiency of glycosylation reactions, as shown by the synthesis of chains as long as 30-mers (Calin et al., 2013), and could produce a variety of carbohydrates, including glycosaminoglycans (GAGs) (Eller et al., 2013; Kandasamy et al., 2014) and glycopeptides (Hurevich and Seeberger, 2014). Recently, preparation of bacterial (Kandasamy et al., 2013) and plant glycans (Schmidt et al., 2015) has also been achieved using an automated oligosaccharide synthesizer. Thus, most techniques now available for solution-phase glycan synthesis can be carried out with an automated solid-phase oligosaccharide synthesizer (Seeberger, 2015).

Figure 2. The first commercial "automated oligosaccharide synthesizer," Glyconeer 2.1. The first Glyconeer 2.1 in China was placed in Jiangnan University in 2015.

## ENHANCMENT OF CARBOHYDRATE IMMUNOGENICITY

The poor immunogenic response to carbohydrates is a major challenge encountered in the development of carbohydrate-based vaccines (Astronomo and Burton, 2010). Various methods have now been developed to overcome this serious problem. Major advances have been triggered by the discovery that protein-conjugated polysaccharides serve as T-cell dependent epitopes to acquire the requisite immunochemical ability (Roy and Chieh Shiao, 2011). These conjugates of B cell sugar epitopes and nonhomogeneous T-cell protein epitopes are considered semisynthetic carbohydrate vaccines (Peri, 2013). Recently, completely synthetic carbohydrate vaccines have been developed by replacing the nonhomogeneous protein with a homogeneous T-cell peptide epitope. Moreover, a single molecule assembled using different chemical units with various functions can be fully characterized by nuclear magnetic resonance (NMR) and mass and infrared (IR) spectroscopy (Peri, 2013).

Recently, the advantages of nanoparticle vaccines, including simultaneous antigen-loading, adjuvant codelivery, targeting properties, and increased circulation times, have triggered the development of glycosylated nanoparticle vaccines (Peri, 2013). Liposomes have been shown to be promising nanoparticles for multivalent display of synthetic carbohydrate epitopes, T-helper (Th) peptide epitopes, and adjuvants (Ingale et al., 2007; Said Hassane et al., 2009; Deng et al., 2014; Hu et al., 2015). Gold nanoparticles (GNPs) have also been intensively studied as carriers of carbohydrate vaccines (Safari et al., 2012).

## DESIGN AND APPLICATION OF CARBOHYDRATE-BASED HIV VACCINES

In addition to the "self" pattern feature of viral glycans, variable glycosylation sites due to the constant mutation of the viral genome is another obstacle in the application of antiviral vaccines (Morelli et al., 2011). Despite this challenge, tremendous advances in the characterization of viral glycosylation have promoted the development of carbohydrate-based HIV vaccines. The first broadly neutralizing antibody isolated from infected individuals is IgG 2G12, which targets high-mannose glycans of gp120 (Hsu et al., 2011). Thus, the highly conserved high-mannose-type N-glycan clusters (Figure 3) of gp120 have become attractive targets in the development of HIV-1 vaccines to induce a robust immune response (Horiya et al., 2014b).

### 2G12-targeted vaccine design

Biochemical studies have proposed that $Man_9GlcNAc_2$ and $Man_4$ are two favorable targets of 2G12 (Adams et al., 2004; Wang et al., 2004; Calarese et al., 2005); thus, various new 2G12-targeted immunogens have been designed and constructed through the regioselective coupling of synthetic oligomannose with multivalent scaffolds (Wang, 2006). Some of these glycoconjugates are further attached to rationally designed peptide (Li et al., 2005; Krauss et al., 2007; Wang et al., 2007; Joyce et al., 2008; Yang et al., 2010), carbohydrate (Wang et al.,

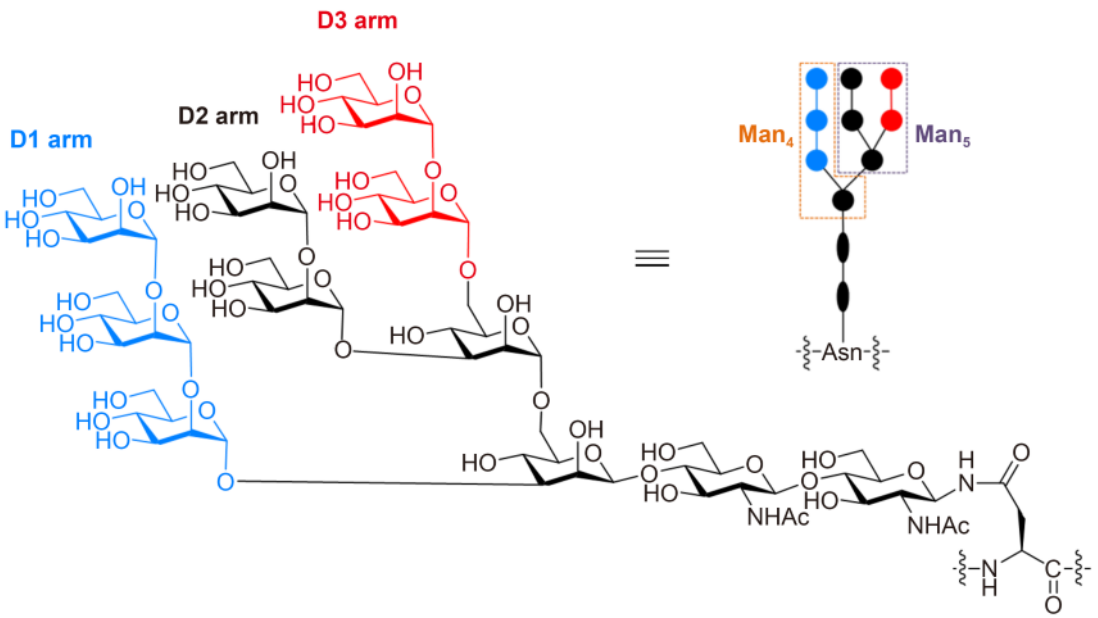


Figure 3. Chemical structure of high-mannose-type N-glycan $Man_9(GlcNAc)_2$ and its branches D1, D2, and D3.

2004; Ni et al., 2006), steroid (Li and Wang, 2004), peptide nucleic acid (Gorska et al., 2009; Ciobanu et al., 2011), dendrimer (Wang et al., 2008; Kabanova et al., 2010), and gold nanoparticle (Astronomo et al., 2008; Astronomo et al., 2010; Marradi et al., 2011) backbones and on biomacromolecules, such as Qβ phage particles (Astronomo et al., 2010) and bovine serum albumin (BSA) protein (Astronomo et al., 2008). Most glycoconjugates have been shown to have weaker (at least 50-fold) 2G12 affinity compared to gp120. High 2G12 affinity can be obtained through presentation of a higher number of oligomannoses compared with the natural 2G12-gp120 interaction involving three or four glycans (Horiya et al., 2014b). In some cases, robust mannose-binding antibody responses were detected; however, none of these antibodies can bind to gp120 or neutralize HIV (Ni et al., 2006; Astronomo et al., 2008; Joyce et al., 2008; Astronomo et al., 2010; Kabanova et al., 2010). One possible explanation is that none of the tested glycoconjugates exactly mimic the epitope of 2G12. Thus, a particular arrangement of oligomannoses is necessary to construct gp120-like immunogens (Horiya et al., 2014b).

### Directed evolution of 2G12 epitope mimics

Because there are just 3–4 oligomannoses in the 2G12 epitope of gp120 (Sanders et al., 2002; Scanlan et al., 2002; Calarese et al., 2003), it is necessary to synthesize constructs containing a similar number of oligomannoses. One new strategy is directed evolution of numerous random arrangements to highly antigenic glycoconjugates. Recently, both DNAs and peptides have been used as evolving scaffolds to develop oligomannose clusters (MacPherson et al., 2011; Temme et al., 2013; Horiya et al., 2014a; Temme et al., 2014). A library of ~$10^{13}$ multivalent $Man_9$ presentations is created by chemically attaching glycans to ~$10^{13}$ DNA scaffolds contained alkyne. These sequences are then subjected to 2G12 affinity tests and polymerase chain reaction (PCR) amplification. Using this method, highly antigenic structures were obtained through several rounds of glycosylation and selection. DNA libraries provided selection of 3–4 glycan-containing structures that had gp120-like affinity for 2G12. In further studies (Horiya et al., 2014a), a system for multivalent glycopeptide evolution yielded glycopeptides with comparable 2G12 affinity to natural gp120. Although no immunological studies of these evolved constructs have been reported, the directed evolution method may provide a helpful basis for developing highly antigenic glycoconjugates.

### PG9- and PG16-targeted vaccine design

PG9 and PG16, which show greater efficacy against HIV than 2G12 (Walker et al., 2009), are considered potential targets for anti-HIV vaccine design. Recent synthetic studies have demonstrated that glycoconjugates containing complex glycans at N173 or N156 exhibit tight binding with PG9 (Horiya et al., 2014b). In another study, dimers of synthetic glycopeptides bearing only high-mannose ($Man_5GlcNAc_2$) structures at both N156 and N160 positions were found to exhibit relatively tight binding with PG9. Notably, the dimeric $Man_5$ construct also recognized the germline precursor of PG9, thereby showing the potential to initiate a PG9-like response (Alama et al., 2013). Saturation transfer difference (STD)-NMR and crystallographic studies of PG16 have shown that the sialic acid residues of complex glycans are important contributors to the overall binding strength (Pancera et al., 2013).

### Non-self sugar mimic vaccine

D-Fructose has been reported to exhibit stronger 2G12-gp120 complex inhibition than mannose. Doores and coworkers (2010) created a library of non-self sugars by modifying different positions of the terminal sugar of $Man_4$ with different substitutions. They found that virus-like particles bearing these non-self sugars showed nanomolar affinities for 2G12. Although high titers of antibodies were elicited by non-self sugars containing constructs, no HIV-neutralizing antibody was observed (Doores et al., 2010).

## CONCLUSION

In recent years, the biological role of N-glycosylation of viral proteins has become better understood (Swarts and Guo, 2009). Although highly variable glycans are crucial for virus survival, some glycans with highly conserved structures are also essential for virulence, indicating that these highly conserved glycans may be utilized as targets for the development of vaccines and drugs (Swarts and Guo, 2009). To date, studies on synthetic carbohydrate-based antiviral vaccines have focused mainly on anti-HIV vaccines. The key challenges for achieving effective immunity using carbohydrate-based HIV vaccines are the design and construction of multivalent presentations of glycans for induction of several bnAbs and achieving a significant breadth of protection. Although much work is still needed to obtain effective carbohydrate-based HIV vaccines, the success of carbohydrate-based HIV vaccines can be forecasted on the basis of several facts, as follows: 1) the exposure of carbohydrates on the outer side of the HIV envelope glycoprotein enables these molecules to be accessible to the immune system; 2) a significant proportion of these glycans are highly conserved; 3) dozens of glycan-dependent HIV-1 bnAbs have been isolated; and 4) ad-

vances in carbohydrate synthesis are expected to facilitate the development of vaccines free of contaminants. The latest attempts at developing a carbohydrate-based HIV vaccine utilizing reverse vaccinology approaches may also provide important insights into the development of carbohydrate-based vaccines against other viruses.

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## COMPLIANCE WITH ETHICS GUIDELINES

The authors declare that they have no conflict of interests. This article does not contain any studies with human or animal subjects performed by any of the authors.

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