transmission to CD4 T cells [15,30,34,37,38]. DC-SIGN interaction with HIV-1 is thus implicated in the initial stages of virus acquisition and spread from the mucosal site of virus entry [39]. However, the relative importance of these CLR expressing cells particularly DC-SIGN in HIV-1 transmission remains controversial. In some studies, antibody blockage of DC-SIGN on DCs was found to reduce virus capture only up to 50% [40,41], but other studies have demonstrated 70%-75% inhibition of virus capture by immature monocyte derived dendritic cells (MDDCs) and almost complete blockage of transmission to CD4 T cells [31]. The siRNA-based knock down of DC-SIGN in immature dendritic cells also been able to decrease the HIV-1 transfer to CD4 T cells by 75% [42].

Figure 1. Schematic representation of HIV-1 Env glycan composition governing the balance between virus capture and transmission vs. degradation by antigen presenting cells (APCs). The innate immune cells (macrophages and dendritic cells) recognize HIV-1 via CLRs expressed by these cells lining the mucosal surface of the host. DC-SIGN expressed by mucosal innate immune cells has a very high affinity to bind to oligomannose glycans. It captures the virus, and the downstream processing and fate of virus within these APCs whether directed to viral degradation and presentation pathway towards CD4 cells or transmission and dissemination within the host is determined by the glycan composition of the virus envelope glycoprotein. (A)HIV-1 with Env expressing homogenous oligomannose type of glycans are taken by the APCs via DC-SIGN and endocytosed. Viruses expressing abundantly $Man_{5\text{-}9}GlcNAc_2$ oligomannose type of glycans bind with high affinity to DC-SIGN rendering their release to other cells unlikely. These viruses are shuttled to lysosomal degradative pathway, processed and presented through MHC II to TCR. (B)Viruses with heterogenous glycans of different levels of complexities with complex, hybrid and oligomannose glycans representing the heterogeneity bind to DC-SIGN relatively with lower affinity and avidity. These weakly bound viruses are released from the DC-SIGN and transferred directly through trans-infection pathways. Within APCs the weakly bound viruses are preserved within the endosomal compartments to prevent degradation and protect the insidious reservoir of viral particles. The infectious viral particles within the APCs along with MVBs are transferred to target CD4 cells or the virus is recycled with or without DC-SIGN to the cell surface and transferred to CD4 cells through free virus particles or trans-infection.

The glycan composition expressed on HIV Env is a key factor determining DC-SIGN-mediated virus transmission and fate of virus being taken up by the cells expressing these CLRs. Previously, it has been shown that HIV-1 enriched with high-mannose glycans was captured more efficiently by DC-SIGN and shuttled towards a degradation pathway, which augmented the MHCII-antigen presentation but impeded trans-infection [30]. The process of decorating surface of Env with glycans adds a layer of diversity to the already extremely high level of Env variation found among circulating HIV-1 isolates. At the genetic level, N-linked glycosylation is dictated by the N-X-S/T motif, where X is any amino acid except P. The Env proteins have varying number of these potential N-glycosylation sites (PNGSs), ranging from 23 to 34 per gp160 protomer; the majority of these are in the gp120 surface subunit, whereas only four to eight are in the external domain of the gp41 subunit [4]. Most PNGSs are not conserved. In fact, only six to eight PNGSs found in Env of clades A, B, C, G, and CRF_01.AE are >90% conserved, and for several PNGSs, conservation is <20% **(Figure 2)** [4,43]. The PNGSs on HIV-1 Env are not fully occupied [44,45]. The level of PNGS occupancy is dictated by site accessibility for a series of enzymes participating in the glycan maturation process [46]. The glycosylation pathway is initiated by the addition of $Glc_3Man_9GlcNAc_2$en bloc onto a nascent protein in the ER. As the protein is transported across the ER and the Golgi apparatus, the high-mannose structure is trimmed and subsequently elaborated with hybrid- and complex-type glycans [19]. In contrast to cellular glycoproteins, which are usually adorned with mature complex-type glycans, virus Env carries all three glycan types, including early and intermediate high-mannose, intermediate hybrid, and mature complex glycans [47-49]. In fact, various glycan types and glycoforms are found in proportions that vary depending on Env strain and host cell type [4,23,44,45,50]. On soluble and membrane-anchored Env mimics, the high-mannose-type glycans range from 60–70%, with the most prominent being the least processed Man9GlcNAc2 glycans at 20–40% [19,47,48]. Analysis of soluble, uncleaved, prefusion-optimized BG505 Env gp140 trimers produced in 293F cells, similarly has shown that 56% are high-mannose type composed of Man5 (6%), Man6 (3%), Man7 (6%), Man8 (15%), and Man9 (26%) [51] hen the same proteins were produced in ExpiCHO cells, the total oligomannose content increased to 64%, with observable changes in glycoform proportions. Site-specific analysis further revealed that each PNGS on gp120 incorporated multiple glycoforms of only the oligomannose type or a mix of oligomannose and complex type, whereas the gp41 PNGSs had mainly complex type glycoforms [46,51].

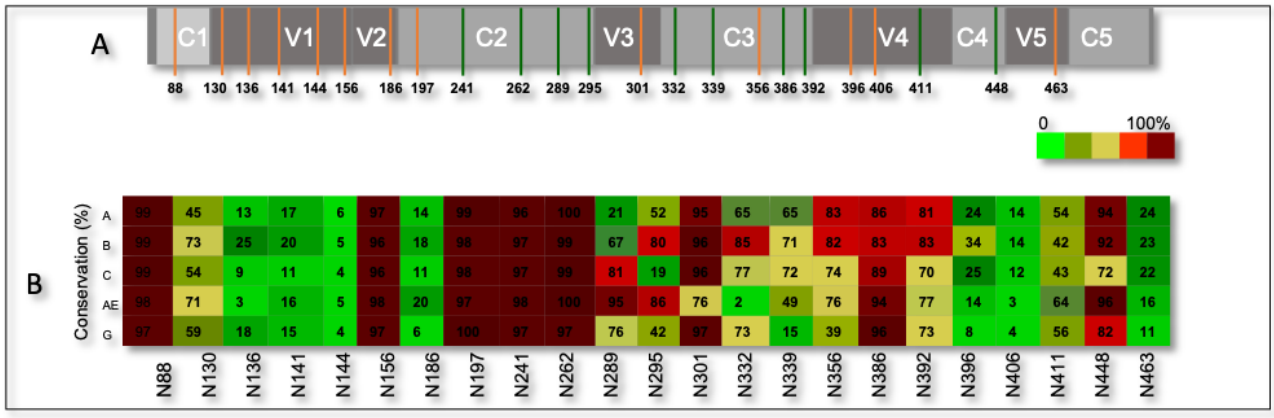

Figure 2. Schematic representation of glycan distribution, occupancy and conservation on HIV-1 gp120. (A) The distribution of potential N-linked glycosylation sites (PNGSs) encoded by N-X-S/T (X#P) of $gp120_{Bal}$. The various PNGSs are represented as putative complex type (orange) or putative oligomannose-type (green) based on previous findings [54-56]. (B) Percent conservation and occupancy of the various PNGSs across different clades calculated from Env sequences available from LANL database. (Adapted from Pritchard et al. 2015) [43].

The abundance of high-mannose glycans on HIV-1 Env and predominant binding of high-mannose glycans by DC-SIGN has been implicated in viral capture upon exposure and viral dissemination within the host. The interaction between HIV Env and DC-SIGN is influenced to a great extent by the glycan composition of virus [30,33,52]. Our recent findings also provide supporting evidence that virions carrying gp120 with higher numbers of oligomannose-type glycans are more efficiently endocytosed through DC-SIGN and more proficiently processed for antigen presentation than HIV-1 containing gp120 with heterogeneous glycans [34]. The transmission of oligomannose-enriched HIV-1 was relatively inefficient. Thus, the expression of oligomannose by HIV-1 enhances capture of DC-SIGN and transmission, but presence of heterogenous-glycansnegatively affect transmission by enhancing viral degradation **(Figure 1)**. The mechanisms by which hybrid and complex glycans help HIV-1 evade degradation are not well understood yet. DC-

SIGN has been shown to shuttle HIV-1 bearing high-mannose glycans to the degradative pathway, similar to other pathogens with such glycans [30]; this was indicated by an increased association with vesicles containing early and late endosomal markers and by more efficient antigen processing and presentation to MHCII-restricted CD4 T cells [30,34,53]. This study demonstrates that HIV-1 evades this degradative pathway by signals that prevent virus transport to endolysosomal compartments and preserve infectious virions on dendrites to promote their transfer to T cells [34].

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