Broadly Neutralizing Antibodies between HIV Vaccination and Treatment the Beginning of the End?

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Abstract

For many years, HIV has been a major concern for both health professionals and researchers. Some of the HIV infected individuals develop broadly neutralizing antibodies (bNAb). Different studies have shown that these bNAb can be used in HIV prevention and treatment. BNAbs mostly act on four main sites (sites of vulnerability) on the viral spike of the conserved epitope known as CD4-Binding Site, First and Second Variable Domains (V1/V2) of the Env trimer, Third Variable Domain (V3) Oligomannose Glycan Patch Involving Asn332 Glycan and Membrane Proximal External Region (MPER). Reverse vaccinology is considered a different concept of HIV prevention through figuring out the structure of bNAb. Although several trials were done in the field of HIV vaccination, none of them showed great success. However, there are other approaches paving the way for delivering an effective vaccine such as delivery of genes encoding neutralizing antibody which is known as antibody gene transfer. Another promising method is to target specific germ line precursor B cells to produce bNAbs against HIV by certain immunogens and there are a lot of trials showed great success. Concerning HIV treatment, research is also now directed to using these broadly neutralizing antibodies. Depending on the idea that antagonizing the CD4 cell and its co receptor can abolish the infection, many clinical trials have been done to find out a promising HIV antibody therapy. There are antibodies against CD4 cells such as Ibalizumab, and others against the coreceptor CCR5 such as Pro140 which are used in HIV therapy trials. The story does not end there, there are other concerns, due to HIV latency inside several body cells, targeting these HIV reservoir cells is indispensable. This can be achieved through antibody based molecules called CD3-targeted-bispecific-DARTs. Also an important aspect to be considered is to prevent the HIV cell to cell infection. Within this short review, we will discuss different approaches targeting HIV therapy and vaccination.

Keywords: HIV Vaccination; HIV therapy; Neutralizing antibodies; Vaccines

Introduction

HIV continues to be a major global public health issue. In 2016, an estimated 36.7 million people were living with HIV – with a global HIV prevalence of 0.8% among adults. Around 30% of these people do not know that they are infected with the virus [1].

Since the start of the epidemic, an estimated 78 million people have become infected with HIV and 35 million people have died of AIDS-related illnesses. In 2016 alone, 1 million people died of AIDS-related illnesses [1].

Most of these infected individuals develop a humoral immune response weeks to months after infection, but it is strain specific and therefore instead of conferring immunity, it induces viral mutation [2,3]. Ten to thirty percent of these individuals will develop broadly neutralizing antibodies (bNAb). Such antibodies can act antivirally against a wide spectrum of viruses by targeting relatively conserved regions on the HIV envelope trimmer spike. However, a minority (~1%) will produce antibodies with extensive breadth and potency (these are termed “elite neutralizers”) [2,4]. This process can take between 2 to 4 years. Yet, these are insufficient to control viral replication [5], because the native HIV1 Env have several characteristics that could limit their ability to elicit bNAb such as; protection of the conserved structures by variable loops [6-8], remarkable genetic diversity [9], a glycan shield [10], steric occlusion [11] and conformational masking [12].

However, human antibodies that effectively neutralize HIV1 target the HIV1 viral spike, a trimeric heterodimer composed of gp120 Env, which is involved in recognizing the CD4 receptor, and of gp41 transmembrane glycoprotein, which in turn is responsible for fusing the viral and target cell membranes [13,14].

These neutralizing Ab co-evolve together with the viruses. Eventually, mature bNAb has unusual characteristics, particularly high somatic hyper mutations, extremely long heavy-chain complementarity determining region 3 (HCDR3) loops, and polyreactivity [3]. Moreover, isolation of the corresponding monoclonal antibodies has been facilitated by identification of favorable donors with potent bNAb containing sera and by development of improved methods for human antibody generation. Molecular studies of recombinant Envtrimers, alone and in interaction with bNAbs, are providing new insights that are fueling the development and testing of promising immunogens aimed at the elicitation of bNAb [15].

Such bNAb will play a significant role in guiding the new structure-based immunogenic designs and also contribute to both HIV-1 prevention and treatment fields.

Most broadly neutralizing antibodies can be placed into four categories on the basis of the location on the viral spike of the conserved epitopes that they recognize Figure 1 [16].

Sites of Vulnerability

CD4-Binding site

The CD4-binding site is one of the conformational epitopes responsible for CD4 receptor binding. It is recessed and surrounded by glycans and variable regions which make it a conserved and protected...
VRC01-class bNAbs were found in the sera of many donors, it was to be one of the most bNAbs. Envtrimer is considered a great target for vaccine design [18].

First and second variable domains (v1/v2) of the envtrimer

This epitope includes the trimer apex of the Env quaternary structure and is highly variable. To escape neutralization, the bNAbs have developed a special and long CDRH3 "hammer-head" structure that allows antibodies to bind to the underlying protein surface (residues 165–171, as well as an Asn160 glycan). PGDM1400, is known to be one of the most efficient bNAbs which recognize V1/V2 domain, bearing a broad (83%) and potent cross-clade neutralizing activity with a median IC50 of 0.003 µg/mL [20].

Third variable domain (v3)/oligomannose glycan patch involving asn332 glycan

The V3-glycan epitopes are considered the "supersite of vulnerability" and depend on the N332 glycan [21], with several bNAbs identified to date. The first glycan-dependent bNAb to be identified was 2G12. A glycan cluster is a target of humoral immune recognition, and a glycan cluster-shielded Envtrimer is one of the classical viral evasion mechanisms [22]. Targeting these epitopes is an attractive site for vaccine development.

Membrane proximal external region (MPER)

MPER is a highly conserved region of gp41 and plays an important role in the viral fusion process [23]. It is a vulnerable site, and thus a valuable tool for HIV-1 vaccine design for the induction of neutralizing antibodies. However, the structural flexibility of the MPER is responsible for its poor immunogenicity in neutralization studies [24]. Several reports suggest that the MPER is relatively inaccessible to antibodies in the pre-fusion conformation and is exposed only transiently after CD4 binding [25-28].

A promising start

One of the most important early vaccine trials was the RV144 trial with a 31% (P=0.04) reduction in infection that correlated with the presence of antibodies that bind to the V1/V2 region of the envelope spike [29,30]. However, the antibody response induced by the vaccine did not neutralize primary HIV-1 isolates, and it has been proposed that the effects were mediated instead by antibody-dependent cellular cytotoxicity (ADCC) [31].

bNAbs as vaccines

Antibodies play a crucial role as passive as well as active immunization against HIV. The combination of poor pharmacokinetic properties and limited overall breadth and potency made passive immunization with first generation bNAbs unfavourable [32-34]. Despite several achievements, there was little enthusiasm for the possibility of an antibody-based vaccine, because all of the initially characterized antibodies were unusual. There were several problems faced, including; having three combining sites, instead of the usual two (2G12 targeting V3) [35,36], being self-reactive (2F5 and 4E10 targeting MPER) [37,38] and finally, being a phage-derived antibody generated by random pairing of heavy and light chains that may have never existed in nature (b12 targeting CD4bs) [39]. Most important, however, the amount of these antibodies required for complete protection in macaques was thought to be too high to be achieved by vaccination [40]. Later on second-generation bNAbs with greater neutralization breadth and potency were developed against HIV-1. They proved to be far more effective than first-generation bNAbs in Human clinical trials as displayed in Table 1.

<table>
<thead>
<tr>
<th>Antibody binding site</th>
<th>Antibody</th>
<th>Example of clinical trials</th>
<th>Location Population</th>
<th>Phase</th>
<th>Recruitment status Primary purpose</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 binding site</td>
<td>VRC01</td>
<td>NCT02716675, United States, Brazil, Peru, Switzerland Estimated enrollment: 2700 participants</td>
<td>Phase 2</td>
<td>Still recruiting Prevention</td>
<td>[79]</td>
<td></td>
</tr>
<tr>
<td>3BNC117</td>
<td>NCT02018510, United states, Germany Actual enrollment: 49 participants</td>
<td>Phase 1</td>
<td>Completed Prevention</td>
<td>[80]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VRC07-523IS</td>
<td>NCT03015181, United States Actual enrollment: 26 participants</td>
<td>Phase 1</td>
<td>Active Non-recruiting Prevention</td>
<td>[81]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N6</td>
<td>Planned for clinical trial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[82]</td>
</tr>
<tr>
<td>gp41 (membrane proximal external region)</td>
<td>10e8</td>
<td>Planned for clinical trial</td>
<td></td>
<td></td>
<td></td>
<td>[83]</td>
</tr>
</tbody>
</table>
Table 1: Shows recent clinical trials using different antibodies categorized by their binding sites to achieve protection against HIV infection.

Reverse vaccinology (RV)

Reverse vaccinology refers to a reversed approach whereby starting from the known structure of bnMAbs and using them as a template, the epitope recognized by the Ab is in vitro reconstructed [41,42]. However, because HIV particles contain only a small number of proteins useful for vaccination purposes, which require a particular tertiary or quaternary conformation to be effective vaccine immunogens, this approach seems to have failed to deliver an effective, preventive HIV-1 vaccine after prolonged intensive research [43].

Germline targeting approach

Germline targeting is a vaccine priming strategy through which the affinity maturation of specific germline-precursor B cells is initiated by preferentially activating bnAb precursors [44,45]. Activating naïve B cells that express specific germline BCRs is the main purpose of germline targeting immunogens. This is achieved through: activating bnAb-precursor B cells, selecting productive (bnAb-like) somatic mutations, and finally producing memory B cells that can be boosted subsequently to select additional productive mutations [46-48].

Unfortunately, there are some limitations to the germ-line targeting approach. The limited number of available “germline-targeting” immunogens is still an obstacle and the search for additional “germline-targeting” immunogens is very active through different strategies by several groups. Also in humans, there will be competition between the B cells that express the desired BCR and a majority of B cells that bind outside the desired epitope. Moreover, it has been found in several studies that repeated immunizations with the “germline targeting” immunogen will not lead to extensive somatic hypermutation in HC and LCs and will not lead to the development of bNAbs due to viral diversity and lacking detectable affinity for supersite-bnAb germline precursor [48,49]. Additional Env variants will be required for boosting [46,50].

Furthermore, although the immunogens employed during the boost will amplify the desired B-cell clone stimulated during the prime, they will also stimulate undesirable B-cell lineages denovo [51].

Antibody gene transfer

Genetic delivery of antibody molecules: Passive transfer of monoclonal antibodies can successfully prevent HIV1 infection in humans as shown in clinical trials. However, considerable challenges regarding the cost and scaling up of the antibody product are causes of concern. So, using vector -based delivery of genes encoding antibody

<table>
<thead>
<tr>
<th>Gp120-41 interface</th>
<th>BANC195</th>
<th>Not yet planned for clinical trial</th>
<th>[84]</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1/V2-glycan</td>
<td>CAP256-VRC26</td>
<td>Planned for clinical trial</td>
<td>[85]</td>
</tr>
<tr>
<td>PG9</td>
<td>UK</td>
<td>Actual enrollment: 21 participants</td>
<td>Phase 1</td>
</tr>
<tr>
<td>V3-glycan</td>
<td>PGT121</td>
<td>United States Estimated enrollment: 63 participants</td>
<td>Phase 1</td>
</tr>
<tr>
<td>10-1074</td>
<td>NCT02960581</td>
<td>United States Estimated enrollment: 63 participants</td>
<td>Phase 1</td>
</tr>
<tr>
<td>Combinations</td>
<td>3BNC117+10-1074</td>
<td>United States Actual enrollment: 24 participants</td>
<td>Phase 1</td>
</tr>
<tr>
<td>PGDM1400 and CAP256-VRC26</td>
<td>Planned for clinical trial</td>
<td>[90]</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1: Different CD4 binding sites vulnerable for various types of CD4 binding site antibodies [91].

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molecules as a means to produce sufficient levels of antibodies over a sustained period of time, is a suggested alternative approach [52].

**Vectored ImmunoProphylaxis (VIP):** It is a gene therapy approach for generating vaccine-like protection by delivering genes (through a single injection of adeno associated virus based vectors (AAVs)) encoding nAbs into nonhematopoietic tissues, such as muscles as an alternative to traditional vaccination methods, as depicted in Figure 2 [53,54]. The advantage of this approach lies in the direct provision of nAbs through transgene expression in host cells, bypassing the reliance on the natural immune system for mounting desired humoral immune responses. VIP extends the application of monoclonal antibodies (mAbs) from passive immunization to a new form of gene therapy that is based on transfer of antibody genes and their subsequent expression in host tissues [55,56]. In fact, two recent studies in animal models have demonstrated that VIP against HIV is feasible, paving the way for more clinical testing in humans [55,56].

Some concerns in VIP approach are: constitutive expression could induce immune responses against the VIP antibody and/or the F2A linker, in turn, leading to reduced antibody production and other unknown effects. So, as a safety precaution, it is desirable to develop a regulated expression system, such as the dimerizer-regulated gene expression system [57-59] to switch antibody expression on and off.

**Treatment modalities:** Based on the fact that ART is known for its ability to interfere with the virus life cycle to prevent replication, it is postulated that using FcFc receptor-mediated effector mechanism, bNAbs can neutralize the free virus and kill the infected cells. Despite obstacles in effective HIV vaccine design, several bNAbs are considered alternative approaches for HIV-1 therapy during chronic infection.

Viral load is a major obstacle as it rebounds quickly in the infected individuals treated by either high or low dose of neutralizing therapeutic antibody. That is because of the outgrowth of pre-existing or de novo viral escape variants. So, elimination of the virus and virus-infected cells in vivo is very challenging once these resistant mutants rapidly emerge [61].

The relationship between broadly neutralizing immunity and disease progression is never correlated with the rapid virus escape, and decrease in the humoral responses along the course of the disease [61].

**Targets for therapeutic bNAbs**

**Antibodies against CD4:** HIV mediates its entrance mainly through a CD4 receptor together with its co-receptors CCR5 and CXCR4 [62]. An allogenic stem cell transplantation with 32bp homozygous deletion in the CCR5 allele to an HIV patient with acute myeloid leukemia led to undetectable HIV viral load even with discontinuation of the antiretroviral therapy [63]. Based on the same principle, it is proposed that by targeting the CD4 receptor or one of its co-receptors, HIV infection could be controlled. Some monoclonal antibodies were developed against human CD4 cellular receptor such as Ibalizumab (iMab), 6H10 and 13B8-2 [64-66]. Others were developed against CCR5 co-receptor such as the humanized mAb PRO140 [64]. The role of these antibodies in HIV therapy is still limited. Nevertheless, the second generation iMab which is a mAb that has broad and potent activity against HIV-1 by binding mainly to domain 2 (D2) of CD4 on host target cells and inhibiting a mechanical extension which occurs in beta strand between D1 and D2. This process is dependent on both time and the infectivity of the virus [64,68]. This leads to inhibition of post-CD4 binding events required to infect cells [69]. Another example of second generation mAb is PRO140 which is a monoclonal antibody directed against the CCR5 CoR (CCR5 antagonist), and it potently inhibits CCR5-mediated HIV-1 entry without blocking the natural activity of CCR5 in vitro [70].

Accordingly, these antibodies may provide new alternatives in both HIV treatment and prevention [64,65]. Triple- and quadruple-monomonal antibody (Mab) combinations have been used to substantially improve the neutralization breadth of the treatment, as compared with the delivery of single Mab [71].

Engineered, bispecific antibodies are potentially promising candidates for HIV-1 prevention and therapy.

In 2013, Pace et al. [72], created bispecific antibodies that combine Ibalizumab (iMab) with anti-gp120 bNAbs. Two of these bispecific bNAbs are PG9-iMab and PG16-iMab.

**Further concerns facing antibody therapy**

Reservoir targeting: Even with potent ARVs and undetectable HIV-1 levels in the blood, HIV-1 persists in reservoirs within multiple organ systems, including the reticuloendothelial system and is never fully eradicated [73]. Accordingly, once infection is established, the high replication and mutation rates present major obstacles to the immune system as it attempts to clear the infection [74]. Thus in order to target these reservoir cells CD3-targeted bi-specific DARTs were developed. The dual-affinity re-targeting (DART) scaffold is a bi-specific, antibody based modality offering better stability and manufacturability. It has the ability to bind viral envelope with CD3-binding specificity to recruit and activate cytotoxic T cells. Accordingly, CD3-targeted bi-specific DARTs are antibody-based
molecules that elicit a cell-mediated killing mechanism which is different from the Fc effector-competent bNAbs. HIVxsCD3 DART molecules specifically redirect cytotoxic T cells to Env-expressing target cells and induce their lysis [75-77]. They showed enhanced potency against various HIV reservoir cells [78].

Cell to cell transmission: HIV-1 is consistently less susceptible to neutralization by multiple classes of bNAbs. The susceptibility is dependent upon both the viral strain and epitope targeted by the antibody. An important fact to be considered is that cell to cell infection cannot be prevented by some bNAbs even at high concentrations, and this is a major concern for antibody-based therapeutics and protection strategies (Table 2) [75].

<table>
<thead>
<tr>
<th>Number of Population</th>
<th>Location</th>
<th>Examples of clinical trials</th>
<th>Binding site</th>
<th>Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 participants</td>
<td>Thailand</td>
<td>NCT02664415</td>
<td>CD4 binding site</td>
<td>VRCO1</td>
</tr>
<tr>
<td>5 participants</td>
<td>USA</td>
<td>NCT01056393</td>
<td>Conformational epitope on CD4 cell</td>
<td>Ibalizumab</td>
</tr>
<tr>
<td>12 participants</td>
<td>USA</td>
<td>NCT0219966</td>
<td></td>
<td>Monoclonal Antibodycocktail (C2F5, C2G12, C4E10)</td>
</tr>
<tr>
<td>10 participants</td>
<td>USA</td>
<td>PM2C2045579</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 participants</td>
<td>Switzerland</td>
<td>15880120</td>
<td>Gp41</td>
<td>2G12, 2F5, and 4E10</td>
</tr>
<tr>
<td>300 participants</td>
<td>USA</td>
<td>NCT02859961</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28 participants</td>
<td>USA</td>
<td>NCT02355184</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 participants</td>
<td>USA</td>
<td>NCT02483078</td>
<td>Host CCR5 receptor</td>
<td>PRO 140</td>
</tr>
<tr>
<td></td>
<td>USA</td>
<td>NCT02446847</td>
<td>Gp41</td>
<td>3BNC117</td>
</tr>
</tbody>
</table>

Table 2: Clinical trials to treat HIV infection using bNAbs targeting different epitopes in HIV virion and CD4 cells.

Conclusions

Despite all efforts to find a highly effective vaccine against/treatment for HIV, the situation is still far from being resolved. With the unleashing of viral immunopathogenesis and discovery of bNAbs, the hope for a better outcome was heightened. Many ongoing and planned trials aiming at curbing the AIDS pandemic are undertaken. Virus reservoirs and cell to cell transmission are still formidable barriers to complete eradication. Germline targeting approaches, reverse vaccinology and genetic delivery of antibody molecules through antibody gene transfer or vectored immunophrophylaxis are among the novel vaccination modalities attempted but with moderate success. Any success in such attempts will have major impact on the public health concern.

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