Chapter 11
General discussion
Towards an HIV-1 vaccine that induces broadly neutralizing antibodies; lessons from the past and directions for the future

The last century vaccinology has been one of the greatest success stories in public health care. Since the discovery by Edward Jenner in 1796 that protection against smallpox infection can be achieved by inoculation of the cowpox virus, millions of lives have been saved by vaccination. The eradication in 1980 of smallpox, but also the near-eradication of polio in the 1950s, both exemplify the great benefits of vaccine induced protection. However, there are still many pandemic infectious diseases for which protective vaccines remain elusive. One notorious example is HIV-1, which infects more than 2 million new people every year. The continuously expanding HIV-1 pandemic, necessitates the discovery of a vaccine and the acceleration of vaccine trials. In a two-pronged approach, on one side basic science to understand the mechanisms of immune responses in natural infection and the subsequent usage in vaccine design are supported, while empirical approaches testing available vaccines to obtain information on vaccine-induced immune responses in humans are also pursued [1]. Lessons from both approaches are probably needed to eventually end the worldwide HIV-1 pandemic.

Efficacy trials with HIV-1 vaccines that originally appeared promising in preclinical studies and safety trials, resulted in big disappointments [2]. The major mechanism of vaccine-induced protection is the generation of neutralizing antibodies (NAbs) that bind to the surface of a pathogen, and thereby block new infections [3]. Recently, antiviral activity of non-NAbs, through their Fc-receptor, has also been appreciated as a potential mechanism to protect against infection [4, 5]. The Vaxgen trials using a gp120 subunit vaccine immunogen and aimed at inducing NAb against the envelope glycoprotein (Env), showed no protective efficacy [6, 7]. These results turned the focus of vaccine design in the direction of vaccines that could induce CD8+ T-cell responses. The intention of these vaccines was not only to protect against infection, but also to lower the level of plasma virus at set-point, thereby lowering the damaging effects of the virus, in case these vaccines could not prevent the establishment of HIV-1 infection. Two large trials, the STEP and Phambili trials, showed a lack of efficacy and even showed evidence of increased susceptibility to HIV-1 acquisition [8-10]. Vaccines aimed at inducing NAb and CD8+ T-cell responses were then combined and renewed interest in Abs was raised after the results of the RV144 trial were published, in which a canarypox viral vector expressing HIV-1 proteins was combined with the Vaxgen gp120. Modest efficacy (31%) was reported and, surprisingly, the correlates of protection were not NAb (NAbs against primary viruses were not induced) or CD8+ T-cells, but non-NAbs against variable regions 1 and 2 of Env [11-13]. Although a causal relation has yet to be established, these results formed the rationale to also go for the induction of non-NAbs with, for example, antibody-cell-mediated cytotoxicity (ADCC) functions [14].
Due to the successes with antiretroviral therapy (ART) and prevention programs worldwide, we can even ask ourselves the question why we still pursue the goal of a protective HIV-1 vaccine even after more than 30 years of failures and very limited successes? However, in the low-developed countries, especially in the vulnerable populations, some of the successes seem to be counterbalanced by a multitude of reasons [15]. Therefore, it should be clear that the search for an HIV-1 vaccine that can protect against HIV-1 infection and prevent its establishment, should continue. One of the approaches is the development of a vaccine that elicits broadly neutralizing antibodies (bNAbs) that can protect against a broad range of genetic variants from the different subtypes that are circulating worldwide. Two observations support the idea that this could be achieved by an Env-based vaccine immunogen. First, in many HIV-1 infected individuals broadly neutralizing activity (bNAc) with the capability to neutralize different viral variants from different subtypes is detected in serum [16-22]. Second, isolated monoclonal bNAbs from these individuals can protect against HIV/SHIV infection in non-human primates [23-28]. In addition, some of the more potent bNAbs can reduce plasma viral load and in some cases induce periods of viral load control in infected macaques [29, 30]. Recently it has been shown that a bNAb could reduce viraemia in HIV-1 infected individuals [31]. Passively administered bNAbs are able to neutralize infecting or circulating viruses, as these Abs did not co-evolve with the viral variants, as observed in natural infection, hence the viruses are still sensitive. Additionally, immune escape is minimal, as numerous viral mutations are needed.

Here we review the role of Env in the development of NAb responses in HIV-1 infected participants from the Amsterdam Cohort Studies on HIV-1 infection and AIDS (ACS), and how this can be used in the development of an effective Env-based vaccine immunogen. The results described in this thesis will be discussed in relation to findings of the wider field.

The humoral immune response in HIV-1 infected individuals

Ab responses against HIV-1 have been studied extensively since the discovery of HIV-1, especially because their specificities provide information about sites of vulnerability on Env. The first B-cell responses can be detected within one week after detectable viraemia, however these usually target epitopes which are not exposed on the functional viral Env, hence non-neutralizing, and are not able to control initial viral levels [32]. Non-NAs are probably induced by non-native forms of Envs (including cleaved gp160 and dissociated gp120 and gp41 subunits) that are abundantly present and are highly immunogenic [33-35]. Furthermore, in the gastrointestinal tract, which is a prominent replication area early during infection, clonal expansion has been observed of memory B-cells that are primed by gut commensal bacteria and have cross-reactive non-neutralizing activity against gp41 [36, 37].
NAb responses directed against the transmitted/founder (T/F) virus can be observed about 12-20 weeks post-infection. It is still unknown why their development is slow, however the immunodominance of non-NAbs (see above) and the impaired CD4⁺ T-cell help (as a consequence of massive depletion) are possible explanations [5]. These early autologous NAb responses are strain-specific and mainly directed against the variable regions of Env [38-43]. Viral escape from the autologous NAb responses, mediated by mutations of only a few amino acids, can be observed in almost all infected individuals, eventually resulting in resistance of circulating viral variants to the contemporaneous antibodies. Indeed, we (Chapter 4) and others have shown that the arms race between virus and humoral immune system results in the continuous evolution of Env to escape from the co-evolving immune responses [39, 40, 44-47]. As a consequence, NAbs elicited by the virus during natural infection do not clear the infection and do not protect against disease progression [16, 21, 22, 48], an observation that we have also made in the Injecting Drug User (IDU) cohort of the ACS (Chapter 2). It has been suggested that one or two Ab specificities define the autologous NAb response in the first year of infection and viral escape stimulates the induction of more specificities later during infection [49]. In 10-30% of infected individuals the arms race eventually results in development of bNAc [16-22]. Although, the presence of bNAb does not benefit the individuals in whom this response developed, because of the rapid viral escape, their induction by a vaccine should prevent infection [23-31].

It is unclear why some individuals develop bNAb and others do not, but typically a high viral load and low CD4⁺ T-cell counts at set-point, and the duration of infection are associated with their development [16, 20, 21, 48, 50, 51]. In Chapter 2 we observed that in IDU infected ACS participants, the percentage of individuals with bNAc was lower compared to HIV-1 infected men who have sex with men (MSM), 19% vs. 27% respectively. However, both percentages are within the range of what is observed in other cohorts (mentioned above) with individuals who became infected via homo- or heterosexual transmission, suggesting that the route of transmission is not a possible predictor for bNAb development. In the MSM and IDU cohorts from the ACS, high viral load and low CD4⁺ T-cell count at set-point were strongly associated with potency and breadth of bNAbs, which is in line with other studies. The lower percentage of bNAc in the IDU compared to the MSM cohort was mainly attributable to the presence of women, who are known to have higher CD4⁺ T-cell counts at set-point [52]. A multivariate analyses showed that both gender and higher CD4⁺ T-cell counts were independent predictors for bNAC outcome, indicating that the emergence of bNAC may be dependent on multiple factors. Around 1% of the HIV-1 infected individuals, termed “elite neutralizers”, develop unusually potent bNAb responses [19, 20]. In the ACS, four individuals qualified as elite neutralizers, one in the MSM cohort and three in the IDU cohort (Chapter 2) [53]. Interestingly, all these elite neutralizers were males, again emphasizing the association of low CD4⁺ T-cell counts with bNAc.
The presence of bNAbs in a considerable proportion of HIV-1 infected individuals indicates that the induction of bNAbs is possible in humans, and of course these individuals are of great interest for vaccine design. However, the development of bNAbs is associated with duration of infection, and normally takes 2-4 years, which is problematic in a vaccine setting (Chapter 4) [16, 54]. In Chapter 3 we show that the development of bNAC in the ACS elite neutralizers is more accelerated, and takes around one year, although bNAC continued to gain potency and breadth up to more than three years post-SC. It is unknown if the elite bNAC responses were dependent on host specific factors in these particular individuals, but it shows us that under some circumstances bNAbs can develop in a relatively short timeframe.

Factors that are associated with the development of broadly neutralizing antibodies

To induce bNAbs by means of vaccination, we need to understand the host factors and viral factors that contributed to the development of these antibodies in HIV-1 infected individuals. Host factors associated with bNAbs development are the presence of specific HLA genes, low numbers of CD4+ T-cells (even before infection), and high numbers of circulating T-follicular helper (Tfh) cells in the periphery [16, 22, 55-57]. The levels of circulating Tfh cells in the periphery are associated with the levels of Tfh cells in germinal centers (GCs), where they are important in providing help to B-cells to produce high affinity antibodies. The association of high Tfh cell numbers and low total CD4+ T-cell numbers with bNAb development seems paradoxical and the underlying mechanism is not fully understood. However, it has been proposed that the preservation of Tfh cells early in infection is a consequence of immune activation, for example by IL-6 secretion, and that this early preservation is important for bNAb development [56, 58]. The functional state of the B- and T-cell (especially memory Tfh cells) compartments probably also play an important role, although conflicting results are reported [57, 59, 60].

Other factors involved in bNAb development are associated with the virus itself, and provide opportunities for exploitation in immunogen design. A high viral load at set-point and duration of infection have been associated with the development of bNAbs [16, 18, 20, 48], implying that the induction of bNAbs is dependent on high antigen levels and prolonged antigenic stimulation. However, many infected individuals with a high viral load or long duration of infection fail to induce bNAbs, and bNAbs have also been described in individuals with low viral loads, suggesting that antigenic diversity, rather than antigen quantity, is important [16]. Indeed, a study in a cohort of subtype A infected individuals showed that early viral diversity was associated with the development of bNAbs [48]. In addition, in a subpopulation of the ACS MSM cohort, we found that the overall mean diversity within the first years of infection was correlated with the development of bNAC,
although the enhanced diversity itself might be indicative for strong immune pressure (Chapter 4). Conversely, when we compared 38 individuals of the MSM and IDU ACS who did or did not develop bNAb, we found no association between viral diversity and bNAb (Chapter 2). These conflicting results suggest that the role of viral diversity in the induction of bNAb is not yet fully understood.

The influence of viral diversity on the development of bNAb may become clear in HIV-1 infected individuals who get infected with a second HIV-1 virus, a so called “superinfection”. The superinfecting virus, containing distinct Env antigens, might broaden the NAb response. However, in Chapter 5 we show that there is no significant difference in bNAb induction between individuals with single or multiple infections. Even in an individual with two superinfections (i.e. three different infections), we did not observe an increase in the breadth and potency of the bNAb response. These results are in concordance with a study in two subtype B superinfected MSM from the San Diego Primary Infection Cohort [61], but are at odds with findings in a cohort of African women, where superinfection did broaden and strengthen the bNAb response [62, 63]. One explanation could be that the Env on the superinfecting virus is antigenically too distinct from the Env on the initial virus, facilitating escape from the strain-specific NAb response present in the recipient, but also lacking the ability to boost and broaden these strain-specific NAb responses.

As mentioned earlier, the duration of infection is also an important predictor for the development of bNAb. This can be explained by prolonged antigen stimulation as a consequence of the ongoing arms race between the virus and the immune system. In a cohort of chronically infected individuals with unknown sero-conversion dates, the percentage of individuals who developed bNAb reached levels of around 50% [64]. Thus, the percentage of individuals eventually developing bNAb could be even higher than the 10-30% observed in other cohorts, where generally individuals were sampled at 2-4 years post-SC (Chapter 2).

Other important factors that could play a role in the development of bNAb are the Env characteristics on the virus. Earlier studies suggested that bNAb development was associated with the length of the variable regions [65-67]. However, all these studies used viral sequences from the same time point during infection at which bNAb activity was measured. Therefore, it cannot be excluded that the associated Env characteristics were a consequence rather than the cause of the measured bNAb. In Chapter 6 we show that in 31 subtype B infected individuals from the ACS, a short V1 region, and low probability of glycosylation was associated with the development of bNAb at 2-4 years post-SC. In contrast to the previous mentioned studies, we used early viral sequences, from within the first year post-SC, i.e. before the presence of bNAb. These results support the hypothesis of early Envs with a more open structure, allowing access to bNAb epitopes and the subsequent induction of bNAb. Thus, there seems to be a role for early viral characteristics to drive the development of bNAb. Overall, it is well possible that in the
individuals who developed bNAbs multiple of the above mentioned factors contributed to bNAb development. In light of vaccine design this would be an extra hurdle that should be overcome, and its feasibility is unknown.

Viral escape and maturation of the immune response

The notion that bNAb responses normally require years to develop, emphasizes the need for in-depth longitudinal studies to elucidate the precise interaction between the evolving virus and the humoral immune response. Env evolution through substitutions, insertions/deletions and changes in PNGS, is a consequence of the pressure from autologous NAb responses, yet also drives the maturation of these responses and eventually, in some infected individuals, the development of bNAc (Chapter 4 and 6) [39, 40, 42, 47, 50, 68-70]. The unmutated common ancestors (UCAs) of bNAbs only sporadically bind different Env's, suggesting that multiple rounds of somatic hypermutations are needed for an Ab lineage to acquire breadth. Longitudinal studies have elucidated three evolutionary pathways that can lead to bNAb development.

One pathway constitutes the induction of strain-specific autologous antibodies that drive viral escape, thereby creating a new epitope which can be targeted by antibodies from an independent lineage that have the capacity to become bNAbs [49, 69]. In two individuals that were studied in detail the early strain-specific NAb response was directed against the C3. The virus escaped from this response by introducing a glycan at position 332, and as this glycan is present on almost three quarter of the globally circulating viruses, it became the stimulus to develop N332-directed bNAbs [49]. The N332 glycan, which is targeted by multiple bNAbs, is one of the more common NAb specificities in HIV-1 infected individuals, constituting a “supersite of vulnerability” on Env [71, 72]. In Chapter 7 we describe a bNAb response directed against the N332 glycan in one elite neutralizer from the ACS, although we could not determine if this glycan was already present on early viral variants or was introduced to mediate escape from an earlier NAb response. However, viral escape from these N332-directed (b)NAbs was observed, and was mediated by a large increase in V1 length, rather than direct mutation of the glycan as observed in other individuals [49, 73]. Elongation of variable regions as a protection mechanism has widely been described, but remarkably in this individual the elongation of the V1 corresponded with the introduction of additional cysteine residues, which is a rare phenomenon. In a different study the deletion of a glycan at position 167, due to escape from strain-specific NAbs, created an epitope that became the later target for the bNAbs response [69].

While researchers have studied the presence of a PNGS at position 332, the probability that this PNGS is actually occupied by a glycan has been ignored. In Chapter 6 we found that a low probability of glycan occupancy at position 332 on early viral variants was associated with bNAc development, suggesting that the absence of the glycan might reveal (a) vulnerable bNAb epitope(s). Paradoxically, when present, the N332 glycan can
become the target of bNAb responses, as observed in multiple studies [49, 69, 73]. However, the absence of an N332 glycan early during infection and its subsequent introduction, does not always result in the development of N332-directed bNAbs, suggesting that observations involving this glycan and bNAb development are context-dependent [74]. Interestingly, in Chapter 8 we observed that the second position of the Asn-X-Ser PNGS at position 332, could influence the presence of a glycan at this position and thereby providing a previously unappreciated source of glycan heterogeneity.

In the same study of the individual who developed bNAbs against the N167 glycan a second mechanism of Ab escape was observed [69]. The deletion of this glycan not only exposed the V2 epitope, but also exposed the CD4bs epitope, which later in infection became the target of a second bNAb wave. These results suggest that viral escape can lead to the exposure of an unrelated previously occluded bNAb epitope, and that consecutive bNab specificities can be generated within one individual.

The third bNAb maturation pathway describes the direct maturation of the bNAb response from autologous NAbs, probably by incomplete viral escape from bNAb precursors [50, 68, 70]. Here, strain-specific autologous antibodies acquire breadth through the toleration of escape mutations.

**Broadly neutralizing antibodies and the constraints in their induction**

An important question relating to HIV-1 vaccine design is why it takes so long for NAb lineages to develop into bNAbs in HIV-1 infected individuals? During an immune reaction, B-cells get activated by an antigen and move towards the B-cell follicles in lymph nodes. Here, they undergo cycles of proliferation and Ab affinity maturation in the dark zone, followed by selection of potent binders in the light zone [75]. This process eventually results in the production of neutralizing antibodies within a couple of weeks, however during an HIV-1 infection mainly non-neutralizing antibodies are produced. The mechanisms by which the activation, proliferation, affinity maturation and selection of B-cells and eventually bNAb development in an HIV-1 infection takes place, and why this takes so long is now starting to become unraveled by the longitudinal characterization of bNAb lineages.

The last few years, single B-cell sorting and Ab cloning techniques generated hundreds of HIV-1 specific antibodies [76]. This has not only provided information on the vulnerable epitopes on Env, but also on the specificities, abnormalities and developmental pathway of these antibodies. Five main bNAb-epitope specificities have been defined, namely the CD4 binding site (CD4-bs), the membrane-proximal external region (MPER), the highmannose patch, the V2 trimer apex, and the interface between gp120 and gp41. Most of the isolated bNAbs targeting one of these epitopes have one or more unusual features
(reviewed in [77]). The complementarity determining region (CDR) loops in many of the bNAb s are extremely long, sometimes twice as long compared to normal human antibodies. For example, PG9 and PG16 need the long CDR loops to penetrate through the glycan shield and engage with the underlying protein domains in the V1 and V2 regions of Env [78]. A feature observed in almost all bNAb s is the high degree of somatic hypermutation. Human antibodies normally carry 15-20 somatic mutations, whereas bNAb s can carry 40-100 of these mutations [77, 79]. For many of these bNAb s most of the mutations are a necessity, as reverting the antibodies to the germline sequence results in complete loss of neutralizing activity [80, 81]. The high degree of somatic hypermutations can explain why it takes a long time before these antibodies generate breadth. Furthermore, almost half of the bNAb s exhibit poly- and/or autoreactivity [82]. All the above mentioned traits are normally under negative B cell selection pressure and negatively affect the generation of such antibodies, therefore also complicating their induction. In Chapter 9 we describe the isolation of a potent bNAb from an ACS elite neutralizer. This bNAb, termed ACS202, neutralizes 45% of a large panel of representative viruses, including viruses from all clades. Furthermore, it binds to a novel epitope at the gp120-gp41 interface that includes the glycan at position 88 and neighbouring residues 83, 87 and 229 in gp120, as well as the residues 516-520 in the fusion peptide in gp41. Considering these unusual traits of bNAb s and their development as a result of iterative cycles of Ab affinity maturation and Env escape, it has been suggested that sequential immunizations to mimic such co-evolutionary pathways are probably necessary to generate bNAb s [49, 50, 68-70].

The use of native-like trimers to induce NAb s

The recent development of soluble recombinant (SOSIP) Env trimers that resemble the native trimer and display multiple epitopes for bNAb s, while occluding epitopes for non-NAb s, present a major step forward towards the induction of bNAb s [83-86]. Monomeric gp120 or uncleaved gp140 immunogens have failed to generate NAb s against neutralization-resistant (Tier-2) primary viruses, simply because they did not resemble the native spike [87-90]. In contrast, native-like SOSIP trimers based on the BG505 and B41 isolates induced strong and consistent NAb responses against the autologous Tier-2 viruses [88]. As most of the commonly T/F viruses have a Tier-2 phenotype, broadening these responses to target heterologous tier-2 NAb s should be the next step. In Chapter 9 and 10, we used the design of the BG505.664 SOSIP protein as the blueprint to construct four new recombinant native-like Env trimers, termed AMC009, AMC011, AMC016 and AMC018 SOSIP, that are based on env genes from recently transmitted viruses of ACS participants. Initially, we used the original SOSIP.664 modifications [91-93], but further improvements were made by adding novel stabilizing mutations when they became available [86][Torrents de la Peña et al. manuscript in preparation]. A number of
rabbits immunized with single immunogens elicited NAb responses against the autologous Tier-2 viruses, as well as sporadic low titer neutralization against heterologous Tier-2 viruses. These heterologous responses were most frequent in the animals immunized with AMC011 SOSIP. Interestingly, sera from the AMC011 SOSIP immunized animals competed with ACS202, the bNAb isolated from the same participant from whom AMC011 SOSIP was derived, suggesting that the response targeted overlapping gp120-gp41 interface epitopes. In addition, a trivalent cocktail consisting of AMC008 [86], AMC009 and AMC011, induced consistent low titer heterologous responses against several Tier-2 viruses. These responses were significantly stronger than those observed in the animals receiving monovalent immunogens. Overall, these findings show that patient-derived SOSIP-based immunogens form a platform to develop immunogens that are capable of eliciting protective NAb responses. Whether these SOSIP immunogens are also able to induce NAbs against Tier-2 viruses in non-human primates and/or humans, remains to be determined.

Important hurdles to overcome: Where do we go from here

Recapitulating the bNAb development in natural infection probably requires guiding the B-cell affinity maturation along specific pathways. This targeted approach is called “B-cell lineage immunogen design” [94]. The first step would be to prime the naïve B-cell precursor (UCA) of the bNAb. This is not straightforward because most HIV-1 isolates and their derivative recombinant Env proteins do not bind to UCAs [81, 95], although recent studies showed that various SOSIP trimers can bind to the UCAs of V1V2-directed bNAb [96, 97]. In addition, two longitudinal studies showed that the earliest isolated viruses were able to bind the UCA of the bNAb lineage, while the later viruses lost that ability [50, 68].

The responses induced by these priming immunogens should then be boosted with the appropriate boosting reagents. One strategy is to isolate bNAbS from different stages in the maturation pathway from infected individuals, then design Env proteins or retrieve Env proteins from the same individuals that optimally bind these intermediate antibodies to simulate the natural maturation pathway [98]. In light of these ideas, follow-up studies are being conducted with the ACS participants from which we generated SOSIP trimers from early after infection (Chapters 9 and 10). Viral sequences from multiple time points during their infection are isolated and will be used to generate SOSIP immunogens for longitudinal immunization regimens. In addition, single cell technology will be used to isolate monoclonal antibodies from the same ACS participants, again from different time points during infection, to follow the evolution of the antibody specificities in parallel with the viral evolution. These antibodies can subsequently be used for the optimization of the already existing immunogens to enhance their affinity.
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Other important considerations are the way the immunogens will be delivered, and the use of adjuvants to enhance vaccine efficacy, both, however, beyond the scope of this thesis. Vaccine delivery systems can consist of immunogens attached to liposomes, virus-like particles or nanoparticles, and are normally used to mimic the various key features of the pathogens, such as size and shape, thereby increasing its immunogenicity. Indeed, BG505 SOSIP trimers attached on ferritin-nanoparticles, induced higher NAb responses against most tier 1 viruses, as well as the autologous tier 2 virus than when the same trimers were delivered as soluble proteins [99]. Furthermore, adjuvants are normally used to create an antigen depot for prolonged exposure to the immune system and activation of antigen presenting cells [100-103].

Accelerating HIV-1 vaccine efficacy trials in humans with new and promising HIV-1 vaccine candidates is another important step that should be more actively pursued [104]. The correlates of protection identified by the RV144 trial are encouraging, however it is not clear if this can be generalized to other studies. In addition, the multiple challenge studies with non-human primates have been very informative, however these do not represent validated animal models that are predictive for human efficacy trials. Therefore, it is vital for the HIV-1 vaccine field that carefully designed immunogens that could induce broadly neutralizing antibodies or non-neutralizing antibodies with antiviral activity, get tested in clinical efficacy trials. Regardless of the trial outcomes, the results will have a major impact on the vaccine field, and will probably guide new immunogen designs and strategies. Clinical efficacy studies are large and complex, and as a consequence also very expensive and challenging. However, the acquired information with these clinical trials will provide substantially more understanding for immune correlates of protection.

Concluding remarks

The ultimate goal of HIV vaccine research is the development of Env based antigen which is capable of eliciting bNAb with the ability to protect against HIV-1 acquisition. Although many challenges lay ahead, recent advances provide confidence that this will eventually be possible. The rational design of immunogens that mimic the native Env spike, the isolation of many bNAb and the characterization of their epitopes, and the understanding of bNAb development in HIV-1 infected individuals are all important recent achievements that will facilitate new vaccine design efforts. In particular, the interplay between the virus and the immune system in HIV-1 infected individuals who developed bNAb responses provides important information on which strategies have a chance at succeeding. However, the success of these advances can only be revealed during trials in humans.
Reference List

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