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Identification of individuals with broadly neutralizing serum activity against HIV-1

Dissertation

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Table of contents

1 Introduction

1.1 HIV

1.1.1 HIV/AIDS pandemic

In 1983, Human Immunodeficiency Virus (HIV) was identified as the virus that causes Acquired Immunodeficiency Syndrome (AIDS). Nearly four decades later, in 2021, 36.3 million people worldwide have died from AIDS-related illnesses so far with another 37.7 million people living with HIV in 2020 (UNAIDS, 2021), with the HIV pandemic affecting all continents of the world (Fig. 1). Since the advanced coverage of treatment with antiretroviral therapy (ART) in the 1990s, new infections are slowly decreasing, most probably due to the fact that ART limits transmission of HIV-1 (Cohen et al., 2011). But in 2020, still 1.5 million people were newly infected with HIV and 680000 AIDS-related deaths were reported worldwide (UNAIDS, 2021). In Germany in 2020, estimated 93000 adults and children lived with HIV (Fig. 2a), around 2600 new infections were recorded (Fig. 2b) and about 86% of the HIV-infected population received ART treatment that year (Fig. 2d) (UNAIDS, 2020). There were under 500 AIDS-related deaths of adults and children in Germany in 2020 (Fig. 2c) (UNAIDS, 2020).

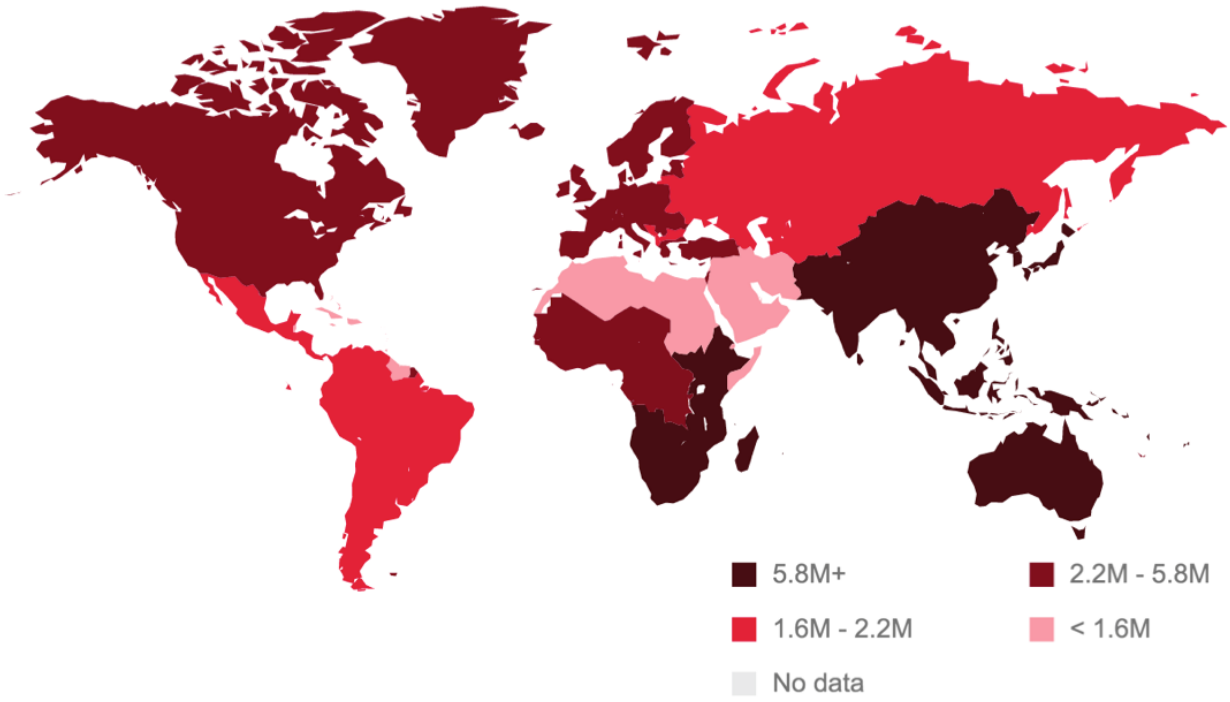

Figure 1 | World map of HIV infected populations. Numbers of people living with HIV worldwide in 2020 are shown related to regions as follows: Western and central Europe and North America; Latin America; Carribean; Africa - West and Central; Middle East and North Africa; Africa- Eastern and Southern; Eastern Europe and Central Asia; Asia and Pacific. Adapted from *www.unaids.org*.

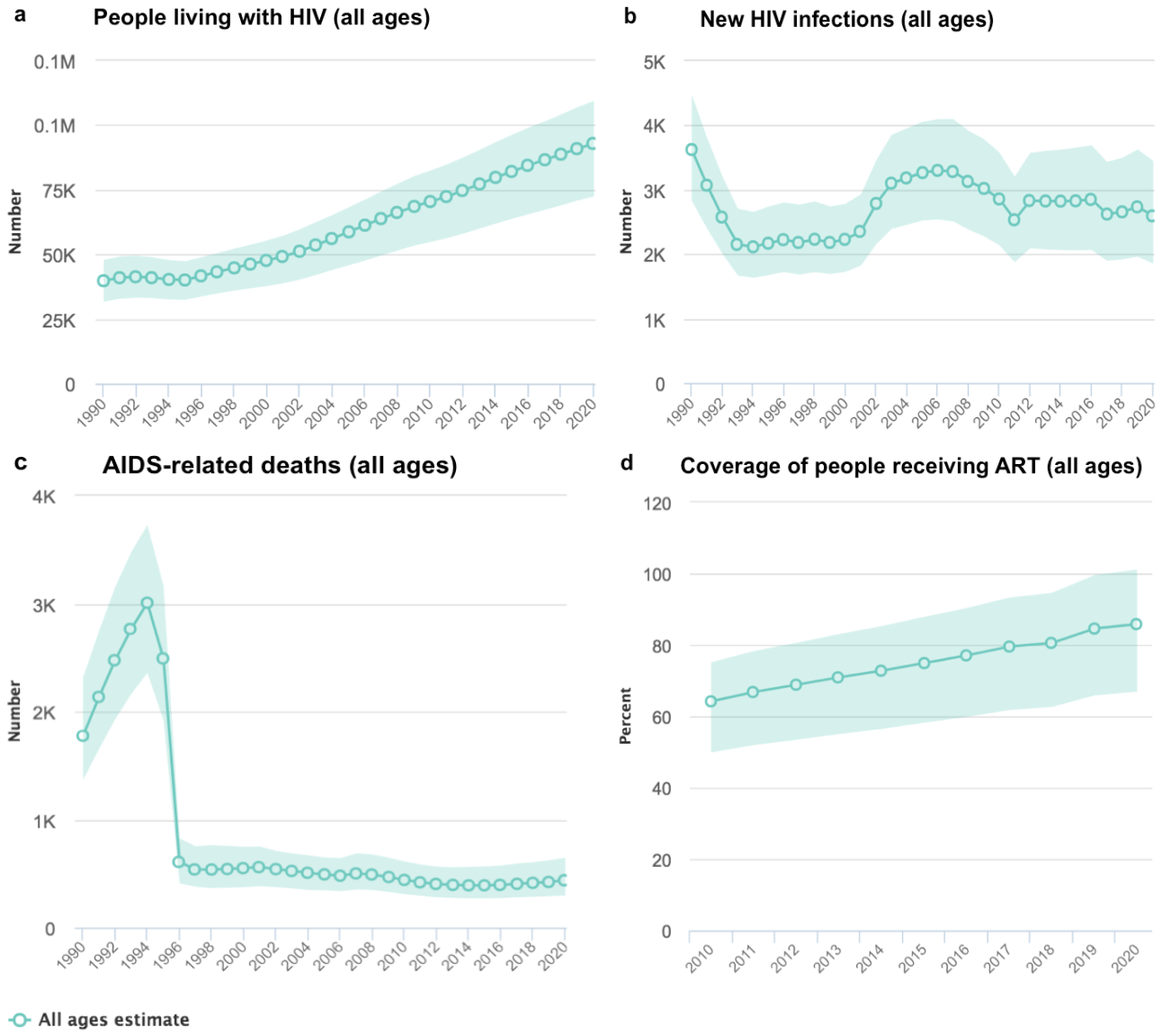

Figure 2 | Statistics on HIV, AIDS and ART treatment in Germany. a, This graph shows the rising number of people of all ages with a known HIV infection in Germany for each year from 1990 until 2020. **b,** New HIV infections in Germany are represented for each year between 1990 and 2020 with steady numbers between 2200 and 3300 new infections per year for the last two decades. **c,** The number of AIDS-related deaths in Germany is represented for each year since 1990 and besides a dramatic drop between the years 1994 and 1996, the death numbers stayed steady under 500 deaths per year in the recent years. **d,** More than 60% of HIV-infected individuals received ART treatment in Germany in 2010, with the percentage rising up to about 86% in 2020. Adapted from *www.unaids.org*.

These numbers prove that HIV still poses a challenge to mankind. Fig. 2c shows the strong decline of AIDS-related deaths between the years 1994 and 1996, a time when combination treatment in antiretroviral therapy rapidly increased (Kirk et al., 1998). Nevertheless, the burden of side effects of a life long antiretroviral therapy and the still high numbers of AIDS-related deaths worldwide justify the search for other preventive and

therapeutic options. A closer look on the structure and function of the virus is needed in order to understand the capability of the virus to escape neutralizing antibody responses of the immune system.

1.1.2 HIV structure and life cycle

HIV is a Lentivirus that is part of the familiy of Retroviridae (Luciw, 1996). Two non-covalently linked single-stranded, positive-sensed RNA molecules form the viral genome. The two RNA molecules together with nucleoprotein p7, reverse transcriptase, ribonuclease, integrase as well as further proteins with structural and regulatory functions are enveloped by a capsid consistent of copies of protein p24 (German Advisory Committee Blood, 2016). The matrix which is formed by copies of protein p17 encircles the viral capsid and connects it with the viral membrane (German Advisory Committee Blood, 2016). The structure of the viral envelope reflects its origin: the double phospholipid layer results from fusion with the human cell membrane in the context of infecting a human host cell. Besides non-specific human proteins, the envelope contains about 70 copies of a viral Envelope Protein (Env) (German Advisory Committee Blood, 2016). These structures are composed of an external part, gp120, and a transmembrane protein gp41 which both exist as trimers. gp120 and gp41 are bound loosely to each other and together they form the envelope spike (Weissenhorn et al., 1999).

The genome of HIV-1 is based on nine genes (Frankel and Young, 1998). Three of these, gag, pol and env, are found in most retroviruses that are able to replicate because they encode proteins that are essential for the structure and function of the virus (German Advisory Committee Blood, 2016). 'group antigen' (gag) contains the information for the capsid protein p24, the matrix protein p17 and the nucleoprotein p7. 'envelope' (env) encodes the glycoproteins gp120 and gp41 of the envelope protein. 'polymerase' (pol) carries the genetic information for the viral enzymes reverse transcriptase, integrase and protease (Frankel and Young, 1998). The six other genes tat, ref, nef, vif, vpr and vpu have regulatory functions which influence the life cycle of the virus (D'Aloja et al., 1998). Sequence analysis has revealed that gp120 consists of regions with highly variable sequences like the V1/V2 loop (Liu et al., 2008) and V3 loop (LaRosa et al., 1990, LaRosa et al., 1991). Another important structural detail on the gp120 protein is its massive glycosylation with a resulting carbohydrate shield that protects vulnerable epitopes of the virus and also plays a role in the interaction with the host cell (Stansell and Desrosiers, 2010).

The infection of host cells by HIV-1 happens in three steps: attachment, co-receptor binding and fusion (Barré-Sinoussi et al., 2013). Initially, HIV-1 gets entry into the human's body by having contact to an infected person's body liquids, mainly to blood, seminal or vaginal fluids. Target cells of HIV-1 have a CD4 molecule on its membrane: CD4+ T cells, monocytes, macrophages, dendritic cells and glia cells. The interaction between the viral envelope protein gp120 and CD4 is crucial for the first attachment of the virus to the cell. This binding induces conformational changes in gp120 and exposure of new epitopes that now bind to the chemokine co-receptors CCR5 or CXCR4 of the host cell (Feng et al., 1996). The completion of co-receptor binding induces the fusion process by changing the transmembranal protein gp41 from its native conformation in combination with gp120 (Weissenhorn et al., 1999) to a fusion active conformation (Eckert and Kim, 2001). A newly exposed hydrophobic N terminus of gp41 is now inserted into the target-cell membrane (Matthews et al., 2004) while the C terminus is still anchored in the viral membrane. In a process called "gp41 zipping", the C terminus folds back and forms a thermodynamically stable hairpin structure along the hydrophobic sites of the N terminus. This way, the viral and the host cell membrane are getting in closer proximity and finally the fusion of both membranes is taking place (Matthews et al., 2004). Viral RNA and viral proteins can now enter the host cell. The viral Reverse Transcriptase transforms the viral RNA to complementary DNA (cDNA) which can be transported into the nucleus of the host cell. Here, the cDNA is integrated into the host-cell-DNA with the help of viral Integrase enzyme (Matthews et al., 2004). The viral DNA stays either latent in the genome of the host cell (Siliciano and Greene, 2011) or the host cell's biosynthesis machinery is used to produce new viral proteins. Viral DNA is translated into viral mRNA which is spliced within the nucleus and then transported to the cytosol. Here, the mRNA is translated to a viral polypeptide which is cut into smaller proteins by viral protease. The resulting viral proteins and the viral RNA form a new viral core complex by assembling in proximity to the cell membrane from where they bud as a new virion ready to infect other host cells. gp120 and gp41 are integrated into the cellular membrane after translation and thus, become part of the new virion during the budding process (Sundquist and Krausslich, 2012).

1.1.3 HIV classification

HIV has a high genetic variability. HIV-1 and HIV-2 are identified as two types introduced to the human population from different animals. HIV-1 was transferred to humans by Chimpanzees and HIV-2 originates from Sooty Mangabeys. HIV-1 is further divided into groups M, N, O and P that stand for independent cross-species transmissions of simian

immunodeficiency viruses (SIVs) to humans (Sharp and Hahn, 2011). Group M of HIV-1 is the most virulent and predominant virus group of HIV and it includes those viruses that cause the HIV-1 pandemic. This group unites several subtypes (or clades) based on differences in their genetic sequence. Genetic variation between subtypes can be 25 to 35% whereas the variation within a subtype ranges from 15 to 20% (Hemelaar et al., 2006). Additionally, several recombinant forms of these subtypes have been identified. Recombination happens after coinfection of a person with two different subtypes of HIV-1: The newly budding virions include viral RNA from both subtypes and in the process of infecting the next host cell, reverse transcriptase writes one mosaic DNA from both subtype RNA strands that is now integrated into the host cell genome (Hemelaar et al., 2006). If identified in at least three people that show no direct epidemiologic linkage to each other, the recombinant forms are referred to as circulatory recombinant forms (CRF) and otherwise as unique recombinant forms (URF) (Hemelaar et al., 2006). Europe, North America and Australia are confronted with subtype B while Africa is dominated by HIV-1 subtypes A, C and D as well as HIV-2. HIV-1 subtype C is responsible for about half of all infections worldwide with a geographical dominance in Southern and East Africa, India and Nepal (McCutchan, 2006).

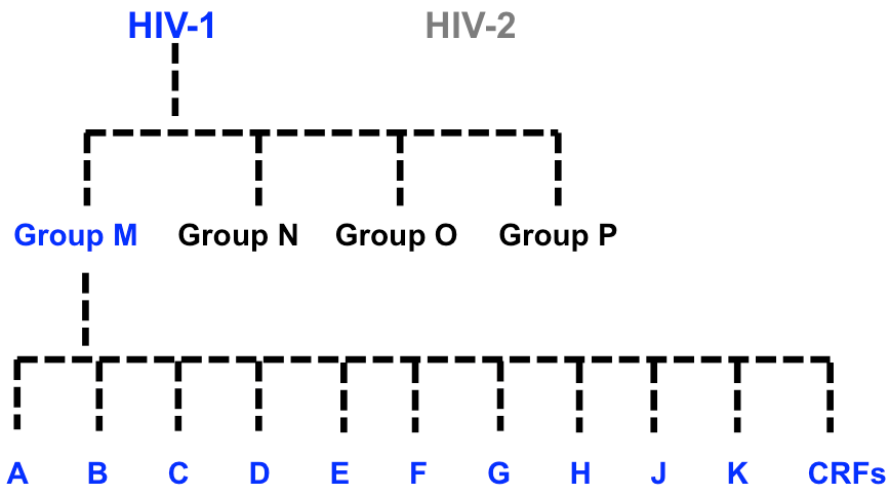

Figure 3 | HIV groups and subtypes. HIV-1 is divided into groups M, N, O and P which represent different transmissions of SIV to humans. Group M is the most virulent one and it includes viruses of several subtypes (A, B, C, D etc.), also called clades, based on differences in their genetic sequence.

1.1.4 Clinical course of HIV-1 infection

HIV-1 is mostly transmitted by sexual intercourse through the genital tract or rectal mucosa. Although diverse HIV-1 populations can exist in donors, disseminated infections in the recipient are usually established by only one or a few founder virus variants. This phenomenon is termed 'HIV-1 transmission bottleneck' and is not completely understood

yet (Kariuki et al., 2017). Infection usually occurs after multiple sexual exposures and the incidence is higher when the epithelial cell barrier is damaged by co-existing genital infections or physical trauma (McMichael et al., 2010). The transmission of HIV-1 is followed by the eclipse phase that endures about 10 days and ends with the moment of first detection of viral RNA in the plasma, known as T0 (Fig. 4). At the end of the eclipse phase, the virus reaches the draining lymph nodes and gets in contact with activated T cells amongst others. As a result, a systemic viral dissemination begins and plasma viral load increases exponentially. At day 21-28 days after infection, peak viremia is reached and at this time, individuals can become symptomatic (described later in the text) and a reservoir of latent virus begins to grow in cells that survive longer than infected T cells (McMichael et al., 2010). Subsequently, viral load decreases and reaches a stable level after 12 to 20 weeks after infection. This so called 'viral set point' reflects a counterbalance between viral replication and control by the host immune response (McMichael et al., 2010) and a higher value is associated with a faster progression to AIDS (Henrard et al., 1995, Lyles et al., 2000). Another study reported the HIV-1 viral set point to be reached at 21 to 119 days after infection (Huang et al., 2012b). These crucial first days and weeks after HIV-1 infection are categorized into six discrete stages based on the emergence of viral markers and antibodies (Fiebig et al., 2003). Concretely, viral RNA can be detected in stage 1, p24 antigen in stage 2 and anti-p24 IgM antibodies in stage 3. Stage 4 is marked by an indeterminate western blot, stage 5 by a positive western blot excluding p31 antigen and stage 6 generates a fully positive western blot (Fig. 4). The clinical term 'Acute HIV-1 infection' (AHI) describes the first period of exponential HIV-1 replication causing peak viremia and the collateral decline of CD4 T cells within 2 to 6 weeks after HIV-1 acquisition. HIV-1 infectiousness is very high during this period. There is no consensus in the literature about when AHI ends and when 'Early HIV-1 infection' (EHI) begins but a general conception is that AHI refers to the period from virus acquisition until completion of seroconversion, thus advancing to EHI at the end of Fiebig stage 5, approximately 100 days after infection (McMichael et al., 2010). EHI is therefore marked by a fully positive western blot and the achievement of a viral set point with a plateaued viral load (Kassutto and Rosenberg, 2004, McMichael et al., 2010). Another definition of EHI refers to an HIV-1 infection within the last 6 months including a documented negative HIV test within that time, independent from seroconversion (Rosenberg et al., 2015). 'Primary HIV infection' (PHI) encompasses both AHI and EHI and displays this dynamic relationship between virus and host immune system including seroconversion within the first months of HIV-1 infection (Kassutto and Rosenberg, 2004).

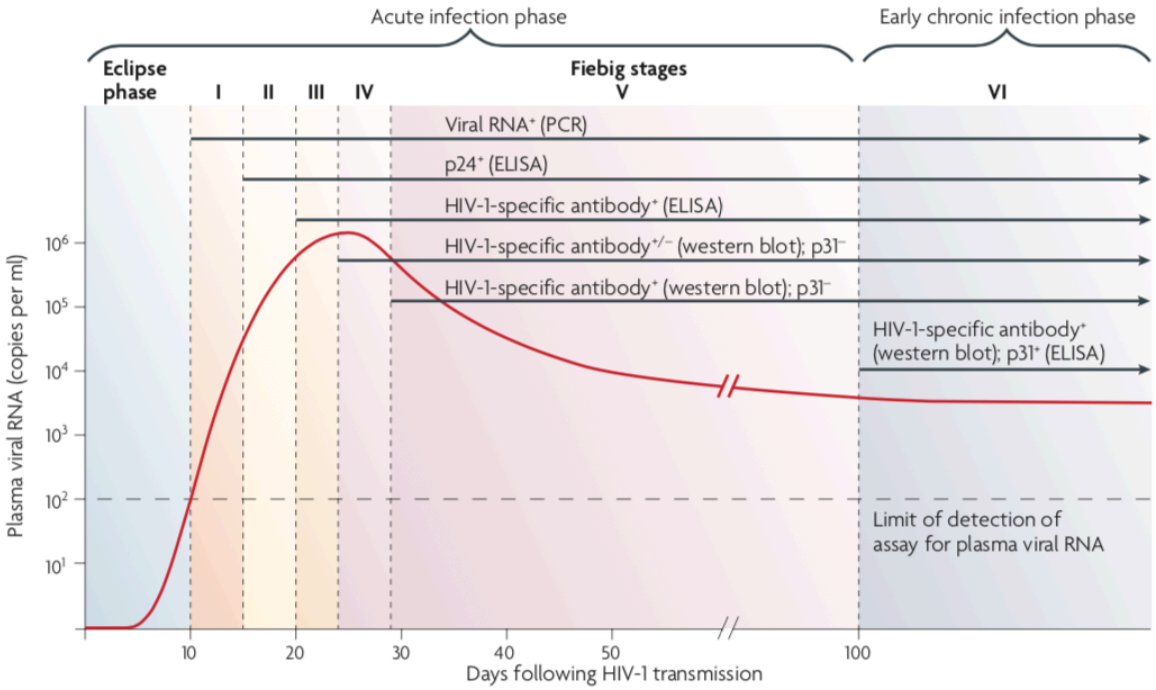

Figure 4 | Immunopathological classification of HIV-1 infection. After the eclipse phase, there are six stages of HIV-1 infection based on the occurrence of viral markers and anti-HIV-1 antibodies. Acute HIV-1 infection (AHI) comprises virus acquisition until Fiebig stage 5. Early HIV-1 infection begins with Fiebig stage 6 which marks the completion of seroconversion and also hitting the viral set point. Adapted from (McMichael et al., 2010, Fiebig et al., 2003).

The clinical presentation of affected individuals during AHI is unspecific and therefore prone to be misdiagnosed. 40% to 90% of affected individuals show temporary symptoms of an acute retroviral syndrome within days to weeks after HIV-1 acquisition. Most commonly, these are fever, fatigue, rash, headache, lymphadenopathy, pharyngitis, myalgia, arthralgia, aseptic meningitis, weight loss, depression, gastrointestinal distress, night sweats and oral or genital ulcers. The symptoms typically disappear in less than 14 days but they may also persist more than 10 weeks. A more prolonged and severe manifestation of the acute retroviral syndrome is associated with a faster progress of HIV-1 disease (Kahn and Walker, 1998).

During the course of HIV-1 infection, an individual usually undergoes the stages of acute and accordingly primary HIV infection, clinical latency and AIDS (Fig. 5) (Pantaleo and Fauci, 1996). After temporary illness during acute HIV infection in the first weeks postexposure, clinical latency endures, on average, about 8 years. In this time, the individuals generally show no symptoms but the virus keeps replicating which results in slowly decreasing CD4 cell numbers and increasing viral load. There is a progression to

symptomatic disease after 8 to 10 years during which the gap between CD4 cell and viral load numbers widens further. Finally, an AIDS defining illness can lead to death.

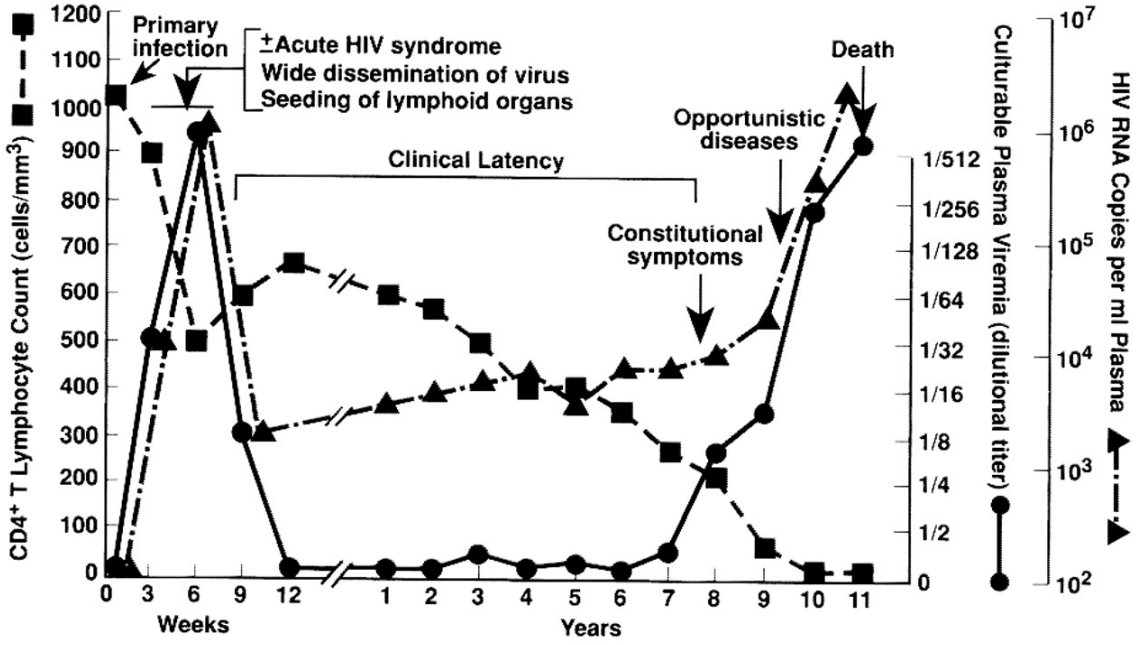

Figure 5 | Clinical course of HIV-1 infection. The acute HIV syndrome is marked by a fast drop of CD4 cell numbers and a fast increase of plasma viremia within weeks. During clinical latency, CD4 numbers show a steady decline while plasma viremia slowly increases over years. In the final clinical phase of HIV-1 infection, an AIDS-defining illness can lead to death. Adapted from (Fauci et al., 1996).

The World Health Organization (WHO) provides a definition for HIV-1 infection as well as a clinical staging and an immunological classification of HIV-1-related-disease. HIV-1 infection is defined by positive serological and/or virological testing each confirmed by second testing. Once the HIV-1 infection is diagnosed, HIV-1-infected individuals can be clinically staged in stages 1 until 4 that describe the severity of symptoms from asymptomatic, mild symptoms, advanced symptoms up to severe symptoms (Fig. 6). Each of these stages refers to a defined set of symptoms or illnesses (WHO, 2007). HIV-1-associated immunodeficiency is categorized based on the CD4 cell numbers in none or not significant, mild, advanced and severe (Fig. 7). Both clinical and immunological criteria are taken into account to specify HIV-1-related disease.

HIV-associated symptoms	WHO clinical stage
Asymptomatic	1
Mild symptoms	2
Advanced symptoms	3
Severe symptoms	4

Figure 6 | WHO clinical staging of established HIV-1 infection. Clinically, there are four stages of an established HIV-1 infection, each referring to a defined set of symptoms or illnesses. Adapted from (WHO, 2007).

HIV-associated immunodeficiency	Age-related CD4 values			
	<11 months (%CD4+)	12–35 months (%CD4+)	36–59 months (%CD4+)	>5 years (absolute number per mm^3 or %CD4+)
None or not significant	>35	>30	>25	> 500
Mild	30–35	25–30	20–25	350–499
Advanced	25–29	20–24	15–19	200–349
Severe	<25	<20	<15	<200 *or* <15%

Figure 7 | WHO immunological classification for established HIV-1 infection. The immunological classification for HIV-1 infection takes into account the absolute CD4+ cell numbers per mm^3 as well as percentage CD4+ cell counts and categorizes none or not significant, mild, advanced and severe immunodeficiency. Adapted from (WHO, 2007).

An advanced HIV-1 infection in adults is defined as a confirmed HIV-1 infection with a diagnosis of any stage 3 or 4 condition and/or a CD4 cell count below 350/mm^3. AIDS is defined as confirmed HIV-1 infection with a clinical diagnosis of any stage 4 condition and/or a severe status of immunodeficiency with CD4 cell numbers below 200/mm^3 (or below 15% of all lymphocytes) (WHO, 2007).

Ongoing viral replication and an advancing CD4 T cell depletion are the characteristics in most of the HIV-1-infected individuals. However, Long-Term Nonprogressors (LTNP) that make up 5 to 15% of untreated HIV-1-infected individuals stay clinically stable with high numbers of CD4 T cells for many years (Deeks and Walker, 2007). The definition of LTNP is based on duration of infection and CD4 cell numbers. In sight of differences between the precise numbers in the literature, the CD4 cell count of LTNP is most commonly defined as above 500 cells/ul (Okulicz, 2012). In further follow up, LTNP finally also show a decrease in CD4 cell counts and increase in viral load (Goudsmit et al., 2002). When viral load testing became available in the 1990s, a subgroup of LTNP, called Elite Controllers, emerged as a rare group of HIV-1-infected individuals (0,55%) whose viremia was not detectable with conventional PCR assays (<50 copies HIV-1-RNA/ml), also in the absence of antiretroviral therapy (Okulicz et al., 2009, Grabar et al., 2009).

1.2 Antibodies

1.2.1 Structure and function of antibodies

Antibodies are macromolecular proteins that play a key role in adaptive immunity. These 'Y'-shaped molecules consist for immunoglobulin G of two identical heavy chains and two identical light chains that are connected by disulfide bonds (Fig. 8). The antibody comprises one constant region in the base of a 'Y', namely Fc (Fragment, crystallizable) region, and two identical variable regions in both tips, called the Fab (Fragment, antigen-binding) regions. This allows antibodies to bind a vast variety of antigens with their variable Fab regions and at the same time to fulfill effector functions with the Fc region that interacts with a limited number of Fc receptors on other immune cells and the complement system. A closer look reveals that heavy and light chains comprise a specific number of immunoglobulin domains with a size of 12,5 kDa each. A light chain is made up of one variable (V_L) and one constant (C_L) immunoglobulin domain and a heavy chain includes one variable (V_H) and three constant (C_H1-3) immunoglobulin domains (Woof and Burton, 2004). Each Fab region contains one C_H1, V_H and a whole light chain with its antigen binding site being built by the heavy and light chains' variable domains V_H and V_L. Together, the two constant domains of each heavy chain (C_H2 and C_H3) form the Fc region (Woof and Burton, 2004). Moreover, a flexible polypeptide chain called the hinge region connects both Fabs with the Fc and serves as a joint that gives flexibility to the antibody (Fig. 8).

Different classes of antibodies, called isotypes, exist and differ in regard to structure, effector function and distribution in the human body. The five existing isotypes IgA, IgD, IgE, IgG, and IgM owe their names to the five different types of constant heavy chain domains (C_H) α, δ, ε, γ, and μ respectively, resulting from composition of class specific gene segments during genetic recombination (Maverakis et al., 2015). There are also two different types of light chains, lambda (ƛ) and kappa (***κ***), also derived from combination of gene segments from different loci (Das et al., 2008).

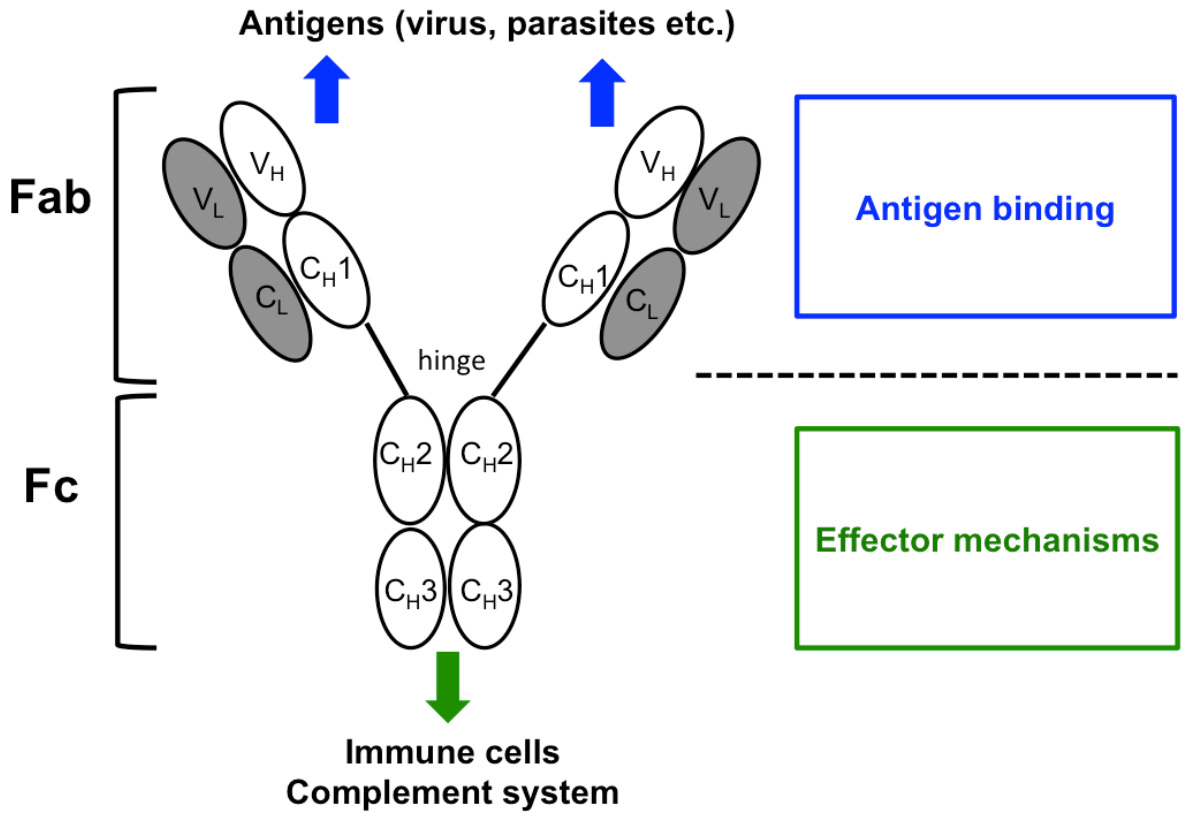

Figure 8 | Structure and function of an antibody. An antibody consists of two heavy and two light chains. A heavy chain has got three constant immunoglobulin (ig) domains and one variable immunoglobulin domain while a light chain consists of one constant and one variable immunoglobulin domain. As a result, each antibody is made up by one Fc and two Fab fragments. The Fc part can induce effector mechanisms in other immune cells or the complement system. Antigen binding is possible through each of the two Fab fragments. C_H=constant ig domain of heavy chain; V_H=variable ig domain of heavy chain; C_L= constant ig domain of light chain; V_L=variable ig domain of light chain.

Of all isotypes, IgG is the one that dominates the serum with 6.6 to 14 mg/ml in adult males and 5.8 to 16.3 mg/ml in adult females (Stoop et al., 1969) which is about 75 % of all immunoglobulines in the serum. With its long half-life of 3 weeks, IgGs grant long lasting immunity within the serum through its most important function, the neutralization of antigens. This immunity is also achieved in the extracellular fluid which is accessible to them because of their compact atomic structure: IgGs are by definition monomers and thus have an atomic mass of 150 kDa (Maverakis et al., 2015). In addition, IgGs are capable of entering the fetus's circulation through the placenta and thus granting passive immunity for the unborn child (Hay et al., 1971).

Antibodies usually occur as transmembranal proteins in B cells, known as B cell receptors (BCR). In the context of an immune response to an infection, B cells interact with T cells and transform to plasma cells that secrete the antibodies directly into the human body's fluids (Parker, 1993). There are several ways an antibody can protect the

body from pathogens: First, they can block the pathogen and thus disturb its interaction with the human host cell. Second, it can induce effector mechanisms against the captured antigen with its Fc region by activating phagocytes to do phagocytosis, mast cells to degranulate and release histamin and natural killer cells to release cytokines and cytotoxic molecules. Third, the complement system and its effector functions can be activated through binding of the complement component C1q to the Fc region of the pathogen-bound-antibody (Ravetch and Bolland, 2001).

1.2.2 Immunoglobulin diversity and specificity

The nearly infinite number of possible antigens that can challenge the immune system demands a highly diverse repertoire of antibodies. This diversity of antibodies is mainly driven by the recombination of variable gene segments and the acquisition of somatic mutations. To this end, the gene segments called V, D and J are recombined in a stepwise process referred to as somatic recombination or V(D)J recombination (Fig. 9). Here, single gene segments are arranged together in order to form the complete exon that translates into a variable domain. Concretely, a V and a J gene segment are joined together for the exon of a light chain variable domain and single V, D and J segments form the exon of a heavy chain variable domain. With each type of gene segment offering multiple variants for the combination with the counterparts, this process can generate a vast amount of different variable domains and therefore different antigen specificities of the resulting antibody (Market and Papavasiliou, 2003). In addition, random insertions and deletions of nucleotides during joining of V, D and J gene segments also increase the diversity of specificity as well as the combination of light and heavy chains. Following V(D)J recombination, the immunoglobulin is translated as membrane-bound antibody that becomes part of the B cell receptor. Through selection at multiple checkpoints, only those B cells that bind sufficiently to ligands but not to self-antigen survive. Thereafter, B cells enter the blood stream and travel to secondary lymphoid organs such as lymph nodes or spleen, where they bind to antigens and get activated by T helper cells. Once activated, B cells start to proliferate rapidly in germinal centers where their variable gene region undergoes somatic hypermutation with resulting changes in antibody affinity. Those B cells expressing a BCR with higher affinity are selected in several cycles until the specificity of the B cell response to an antigen is maximized. After this process of affinity maturation, B cells can undergo class switching in order to change the effector function of the immunoglobulin of the B cell. Then, B cells can further differentiate and leave the germinal

centers as antibody secreting plasma cells and as memory B cells (Mårtensson et al., 2010).

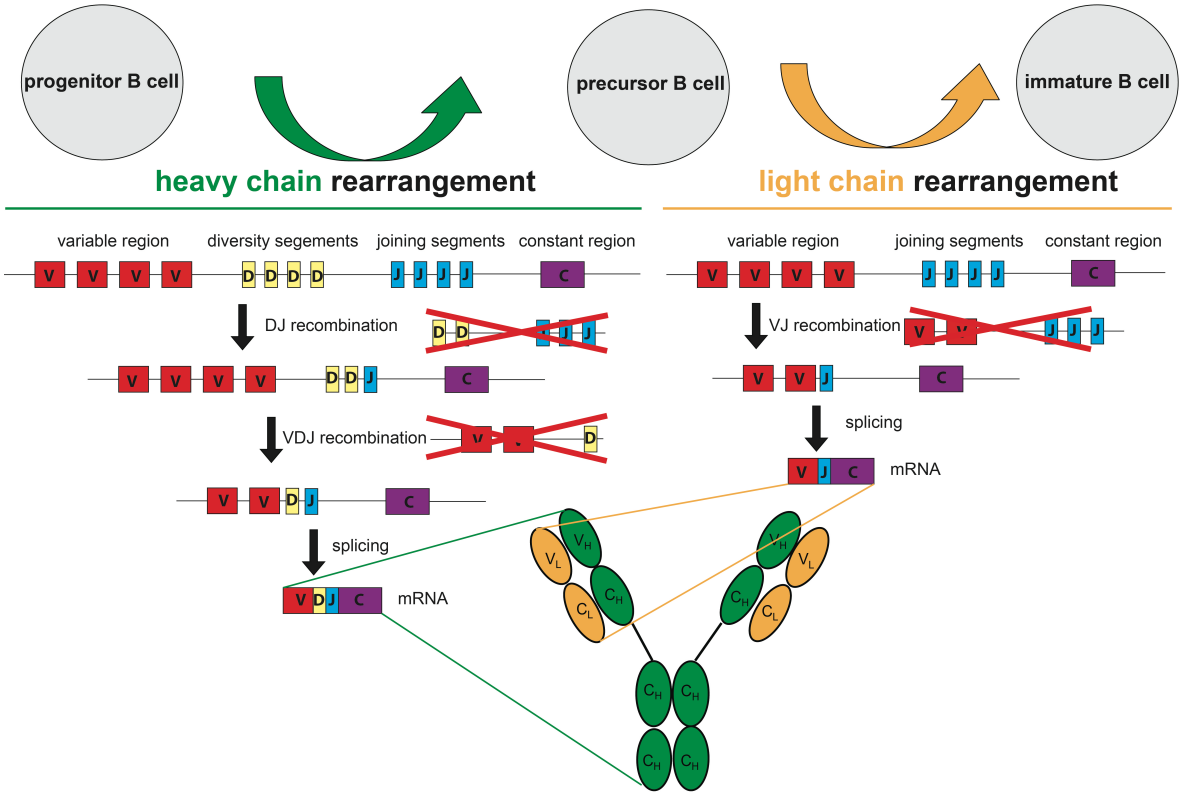

Figure 9 | VDJ recombination during B cell development. Heavy chains get rearranged by combination of variable (V), diversity (D) and joining segments while light chains are created by V and J segments. Both of these rearrangements result in a mRNA which is translated into a heavy and light chain. Each of these V, D and J segments exist in multiple variations on the genome which means that a highly diverse antibody repertoire is offered through combination of these segments. C_H=constant ig domain of heavy chain; V_H=variable ig domain of heavy chain; C_L= constant ig domain of light chain; V_L=variable ig domain of light chain.

1.3 Humoral immune response to HIV-1

The initial humoral immune response to an acute HIV-1 infection manifests itself with the occurrence of virion-antibody immune complexes around 8 days after first detection of plasma virus. Earliest IgG anti-gp41 and IgG anti-gp120 antibodies appear at a median time of 13 and 28 days after the onset of viremia, respectively (Tomaras et al., 2008). These antibodies are non-neutralizing and do not effect the kinetics of viral replication during acute infection (Tomaras et al., 2008). The first neutralizing antibodies (Nab) develop after a few months. They specifically bind to gp120 or gp41 on the autologous virus and provoke viral escape by exercising immune pressure on the virus. These neutralizing antibodies can neither control infection in the autologous immune system nor neutralize viruses of different strains on an in-vitro assay (Moore et al., 1994). The reasons

for the limitations of the humoral immune response to HIV-1 arise from antigenic qualities of Env: Gp120 and gp41 feature sequence-variable loops, extensive glycosylation and conformational masking of vulnerable epitopes that altogether restrict the accessibility of the immune system to conserved Env regions and thereby impede the induction of neutralizing antibody responses to these epitopes. Additionally, the virus can use these attributes to evade a neutralizing antibody response without losing fitness (Kwong and Mascola, 2012). Concretely, single amino acid substitutions, insertions and deletions within variable loops of Env entailing changes in the glycan shield enable the virus to change its surface (Sagar et al., 2006). The conformational masking of conserved epitopes during the process of viral entry also serves for viral escape from human neutralizing antibodies. The ongoing battle between virus and humoral immune response is portrayed in the viral evolution and the steadily renewed antibody response of the host immune system within one HIV-1 infected subject (Burton et al., 2005).

In contrast to this idea of a rather inefficient humoral immune response stands the fact that about 10-30% of HIV-1 infected patients generate cross-reactive neutralizing serum activity due to broadly neutralizing antibody responses (Sather et al., 2009, Simek et al., 2009). Doria-Rose et al. reported in 2008 (Doria-Rose et al., 2008) that at least 4 out of 5 different HIV-1 strains of an neutralization assay could be neutralized by sera of about 40% in Progressors and Slow Progressors and of 25% in Long-Term-Nonprogressors (LTNP). Longitudinal studies of chronically infected individuals showed that cross-reactive antibody responses were generated on average within the first 2.5 years while the earliest response was observed 1 year after infection (Mikell et al., 2011). Broadly neutralizing antibodies (bNAbs) are defined as antibodies that can block the function of various HIV-1 subtypes by binding to conserved epitopes that are found on these different virus strains despite of genetic variation (Scheid et al., 2009). The question is if cross-reactive serum activity is the result of individual bNAbs or rather a combined accomplishment of a polyclonal serum response. In some individuals, multiple antibodies of only modest breadth and potency are directed against different epitopes of Env and synergistically effect a broadly neutralizing serum activity (Scheid et al., 2009). This idea is contrasted by the fact that broad neutralizing activity in some sera is carried by certain highly effective antibodies (e.g. VRC01 showed 90% breadth of that seen in sera (Wu et al., 2010)). The finding of polyclonal antibody response underlined the so far difficulty to isolate bNAbs with unique capabilities out of sera with high neutralizing activities. As the first bNAbs have been purified, the question remained how the host immune system could create these efficiently neutralizing antibodies during natural HIV-1 infection. Analysis of B cell

transcripts and the antibodyomes of individuals helped to lay out B cell ontogeny of bNAbs. Understanding how bNAbs are generated during natural humoral immune response to HIV-1 might be useful to apply the same principles in the context of a vaccine (Kwong and Mascola, 2012).

1.4 Broadly neutralizing antibodies (bNAbs)

The very first broadly neutralizing antibodies from HIV-1 patients were isolated and characterized mostly during the 1990s. Antibodies b12, 2G12, 447-52D, 2F5a and 4E10 mainly targeted four epitopes on the viruses envelope protein (Fig. 10): the CD4 binding site (Roben et al., 1994), the carbohydrate shield (Calarese et al., 2003) and the V3 loop (Gorny et al., 1992), which are all parts of gp120, as well as the Membrane-Proximal External Region (MPER) of gp41(Muster et al., 1993, Stiegler et al., 2001). These antibodies have special features compared to non-neutralizing antibodies: an exceptionally long complementary-determining region 3 on the heavy chain that resembles a finger stretching out of the variable region which enables antibody b12 to bind to a less accessible epitope on the viral envelope protein (Roben et al., 1994, Sattentau et al., 1995, Fouts et al., 1997); a unique structure of the antigen binding site of 2G12 allows it to recognize whole clusters of oligomannoses as nonself in contrast to single glycans (Calarese et al., 2003) and the need of the proximity to the viral membrane for enhanced binding of the antibodies 2F5 and 4E10 (Muster et al., 1993, Stiegler et al., 2001, Ofek et al., 2004, Grundner et al., 2002).

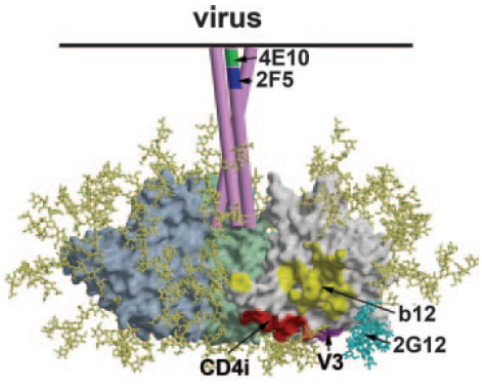

Figure 10 | Epitopes of first generation bNAbs on the HIV-1 trimer. The CD4 binding site and the V3 loop as well as the epitopes of the antibodies b12 and 2G12 are indicated on the gp120 protein. 4E10 and 2F5 target epitopes on the Membrane-Proximal External Region (MPER) of gp41. Published in (Burton et al., 2005).

The unusual recognition modes of b12 and 2G12 complicated vaccine design and the autoreactivity of 2F5 and 4E10 were problematic when facing tolerance mechanisms during B cell development (Kwong and Mascola, 2012). Furthermore, the antibodies b12, 2G12 and 2F5 only neutralized less than 50% of tested viral strains and hence were not that broad while 4E10 lacked a high potency (Kwong and Mascola, 2012). Thus, these antibodies were not ideal candidates for vaccine design. The discovery of the second generation of broadly neutralizing antibodies was triggered by two main advancements (Burton and Hangartner, 2016). First, large cohorts of HIV-1-infected individuals were screened for serum activity against HIV-1 by a high-throughput neutralization assay. 1% out of 1800 HIV-1-infected subjects were identified having exceptionally broad and potent serum activity and were termed as 'elite neutralizers', presenting a promising source of finding new bNAbs (Simek et al., 2009). Second, new developments in single cell antibody cloning led to the isolation of various new bNAbs. Until then, monoclonal antibodies from human blood were generated by rather inefficient techniques like random cloning of heavy and light chains from phage display libraries and hybridomas secreting antibodies. Now, two new approaches were used to generate monoclonal antibodies from human B cells. On the one hand, B cells could be caught directly by exposing the Env antigen as baits to the B cell receptor and sort these single-cells afterwards (Scheid et al., 2009). On the other hand, single B cells can be sorted first, cultured in each well so that their specificity transforms into secreted antibodies in the supernatant which then can be tested for neutralizing activity (Walker et al., 2009). Those B cells being caught by baits or having a supernatant positive for neutralizing activity, can be further analyzed in the next step by a method that builds the common denominator for both approaches: Heavy and light chain sequences of single cells are amplified by PCR and cloned into vectors that allow to express this specific antibody (Tiller et al., 2008). Hundreds of new broadly potent antibodies against HIV-1 have been characterized since 2009. These second generation antibodies showed significant higher breath and potencies than the first generation (Fig. 11).

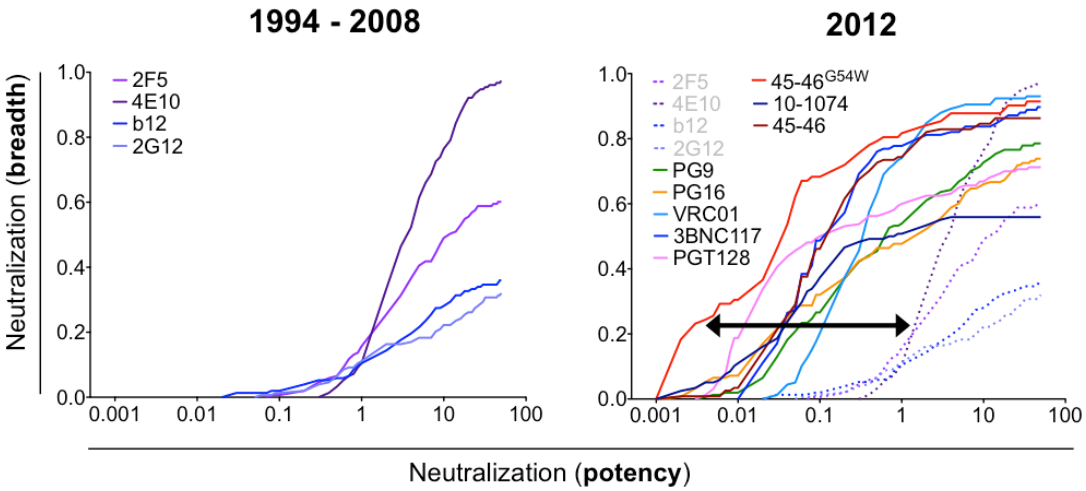

Figure 11 | Breadth and potencies of first and second generation bNAbs. The x- and y-axis present the potency and breadth of the bNAbs, respectively. While there were only a handful of known bNAbs between the years 1994 until 2008, the renaissance of bNAb discovery since 2009 brought many antibodies with a considerable increase in breadth and potency to the front. Adapted from Fig.1 in (Gruell and Klein, 2015).

With the discovery of the second generation of bNAbs, new epitopes have been described including a high mannose patch on the V1/V2 loop overlapping the 2G12 epitope, the apex of the glycan V3 loop as well as the interface of gp120 and gp41. Together with the CD4 binding site and the MPER on gp41, mainly five sites of vulnerability on the envelope protein are in focus (Fig. 12).

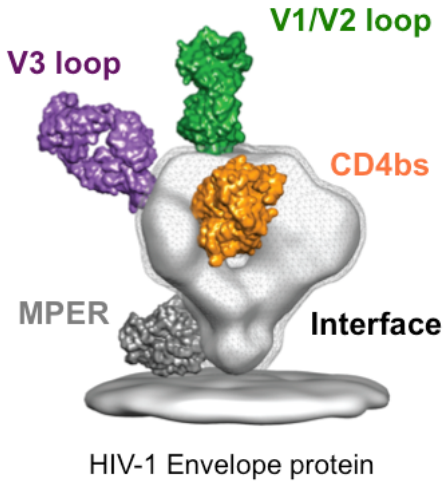

Figure 12 | Five main epitopes of bNAbs on the HIV-1 envelope protein. This graph shows the HIV-1 envelope protein highlighting the main epitopes CD4 binding site and the MPER on gp41 as well as the later described sites of vulnerability on the envelope protein including a high mannose patch on the V1/V2 loop overlapping the 2G12 epitope, the apex of the glycan V3 loop and the interface of gp120 and gp41. Adapted from (Klein et al., 2013b).

Screening supernatants of activated B cells proved better to catch broadly neutralizing antibodies against the V1/V2 loop, PG9 and PG16, that did not bind monomeric gp120 as

efficient as the trimeric envelope protein (Walker et al., 2009). The discovery of gp41-directed antibody 10E8 was also a result of screening B cell supernatants (Huang et al., 2012a). In contrast to that, differently designed gp120 baits led to the discovery of CD4 binding site directed antibodies VRC01 (Wu et al., 2010) as well as NIH45-46, 3BNC117 and 12A12 (Scheid et al., 2011).

Crystal structures of antibody and virus revealed different adaptions of bNAbs to the molecular evolution of HIV-1. Certain bNAbs bind to glycans linked to amino acids on the envelope protein that are conserved throughout the mutational escape of the virus. An N-linked glycan at position 160 is required for binding of antibodies PG9 and PG16 to the V1/V2 protein (Doores and Burton, 2010) while a glycan at N332 is needed for binding of PGT 128 and PGT 121 to the V3 protein (Walker et al., 2011). A glycan at N280 is crucial for antibodies binding to the CD4-binding site (Diskin et al., 2013). Another form of adaption is that most of these broadly neutralizing antibodies independently presented extraordinary properties compared to less or non-neutralizing antibodies, e.g. the unusual long CDRH3 in PG9 that is important for penetrating the glycan shield and binding parts of the Env protein or the ability to mimic CD4 binding in antibodies directed against the CD4 binding site (Kwong and Mascola, 2012, McLellan et al., 2011). Additionally, bNAbs show high numbers of somatic mutations which is believed to result from multiple rounds of affinity maturation in the germinal centers (Klein et al., 2013b). After reverting these mutations to sequences of germline ancestors, the neutralizing capacity of these antibodies decreased significantly. This essential contribution to broad neutralizing binding is also observed for somatic mutations in the framework of immunoglobulin variable regions (Klein et al., 2013a).

1.4.1 Broadly neutralizing antibodies in HIV-1 prevention

The discovery of the new generation bNAbs since 2009 has revived the idea of an HIV-1 vaccine. The characterization of recently discovered broadly neutralizing antibodies helped to describe sites of vulnerability on the HIV-1 Env protein which could be used in the design of a vaccine immunogen. So far, no vaccine could effectively elicit broadly neutralizing antibody responses in animal studies. The unusual features of bNAbs are considered as adaptions that result from a long-term exposure to antigen during active HIV-1 infection (Burton et al., 2005). Therefore, considerable effort has been put into the task to understand the maturation history of these broadly neutralizing antibodies. Next-generation sequencing of B cell transcripts helped to identify many related antibodies in donors of broadly neutralizing antibodies. The goal was to find early clonal variants from

the B cells that encode these bNAbs and to reconstruct the evolutionary pathway back to the unmutated germline ancestor. This may allow to induce bNAb precursors with a primary vaccination followed by several boosts that would represent the antigenic stimulation of a persistent HIV-1 infection that led to somatic maturation of bNAbs in individuals (Kwong and Mascola, 2012, Gruell and Klein, 2014). Besides serving as model for active vaccination, the focus shifted to the use of bNAbs in passive immunotherapy. Emil von Behring was awarded a Nobel prize in 1901 for showing that passively administered antibodies have the ability to prevent or combat infectious diseases (Graham and Ambrosino, 2015). This principle could be demonstrated when first generation bNAbs were shown to be effective against HIV-1 infection in macaques (Mascola et al., 1999, Hessell et al., 2010). Nevertheless, high doses of antibodies were needed for protection in macaques (Mascola, 2003) which represented rather poor in vivo activities of the first generation bNAbs. In contrast to that, in 50% of 60 macaques the second generation bNAbs showed a protective effect with small concentrations which were potentially achievable by vaccination (Shingai et al., 2014). Furthermore, the passive transfer by a single injection of second generation bNAbs has been shown to induce long-term protection in macaques for up to 23 weekly low dose viral challenges (Gautam et al., 2016). The long half life of broadly neutralizing antibodies and the possibility to stretch it by inserting mutations into the Fc part has elevated bNAbs to be considered as new alternative to standard HIV-1 drugs (Gautam et al., 2016). It also has been shown that a combination of bNAbs is needed for an adequate protection of macaques challenged with mixed viral variants of SHIV (Julg et al., 2017). Another study has shown that a trispecific anti-HIV-1 antibody is able to protect against a mixture of SHIV in macaques (Xu et al., 2017). These experiments with low dose viral challenges or mixed SHIV challenges to macaques were supposed to model human infection with HIV-1 where infection occurs after multiple exposures of low doses of virus (Gautam et al., 2016) and often multiple variants of the virus are included during sexual transmission (Julg et al., 2017). When administered to human beings, 3BNC117 and VRC01 fulfilled safety restrictions and showed good half-lives with 17 and 15 days, respectively (Caskey et al., 2015, Ledgerwood et al., 2015).

The Antibody-Mediated Prevention (AMP) trials evaluated if passive immunization with VRC01 could prevent an HIV-1 acquisition in healthy human beings who were at risk for HIV-1 infection (Corey et al., 2021). For this purpose, two placebo-controlled phase 2b studies were conducted in two cohorts, namely women living in sub-Saharan Africa as well as men and transgender persons who have sex with men. The results showed that VRC01

could only protect against viruses that were highly sensitive to the antibody, and did this with a protective efficacy of 75%. Thus, passive immunization of a single bNAb could not deliver overall protection. The emergence of resistant isolates emphasizes the upcoming challenge to test the preventive potential of bNAb combinations. Yet, these trials showed that serum neutralization titer against exposing viruses, measured with a standardized high-throughput assay, could predict the degree of protection. This could be useful for screening tests in future prevention trials (Corey et al., 2021).

1.4.2 Broadly neutralizing antibodies in therapy

Passive transfer of broadly neutralizing antibodies has been considered as a therapeutic modality for an established HIV-1 infection. Trkola et al. (Trkola et al., 2005) demonstrated that passive immunization with broad neutralizing antibodies led to a delay of viral rebound after interruption of antiretroviral therapy in humans. Also, it was observed that the virus can easily escape the immune pressure executed by those antibodies, mainly by 2G12. Furthermore, high quantities of 2G12 were needed to suppress viral replication in humans compared to in-vitro assays. This revealed the inefficiency of first generation bNAbs facing an established HIV-1 infection in the human immune system. The discovery of second generation bNAbs has put forth antibodies that are broad and potent enough to overcome the disadvantages of quantity and viral escape. Klein et al. (Klein et al., 2012) showed that combinations of these antibodies can suppress viral replication in a humanized mouse model for a prolonged period of time. Antibodies were transferred to mice either as monotherapy, a tri-mix (3BC176, PG16, 45-46G54W) or pentamix (tri-mix antibodies + PGT128, 10-1074). The monotherapy had a temporary effect on the infection course because of viral escape indicated by signature resistance mutations in the gp120 sequence. The viral loads in mice treated with the pentamix were all below or near the limit of detection for up to 60 days. After cessation of therapy, most mice showed viral rebound after an average of 60 days. Virus that rebounded showed no resistance to the pentamix indicating that this virus remained latent throughout therapy and thus kept unchallenged by the immune pressure of the bNAbs. While most of the mice treated with the tri-mix showed viral rebound during therapy (Klein et al., 2012), ART and tri-mix co-administered to mice led to complete viremic control as long as serum antibody level was detectable (Horwitz et al., 2013). The rise of signature mutations of the gp120 protein in the rebound virus against parts of the tri-mix but never against all three antibodies proved the efficiency of the bNAb therapy. Comparing another tri-mix group to ART as post-exposure prophylaxis in HIV-1 infected mice, Halper-Stromberg et al. (Halper-Stromberg et al., 2014) showed

that the bNAbs led to a longer lasting suppression of viremia than ART alone and also that Fc-mediated immune effects are playing a significant role in virus control. Another example for the significance of the Fc receptor is the fact that mutated Fc domains with enhanced binding capacity led to a faster and more sustained suppression of viremia in HIV-1 infected humanized mice (Bournazos et al., 2014).

In contrast to the humanized mouse model, macaques have a functional immune system which is able to generate innate and adapted immune responses. Some SHIV variants can escape immunotherapy with ART and bNAbs in macaques building a virus reservoir, keeping up a chronic SHIV infection for several months and leading to symptomatic immunodeficiency by irreversible CD4+ T cell depletion (Nishimura and Martin, 2017). Thus, testing bNAbs on this more complex animal model was the next step on the path to clinical trials with humans. In one of the first experiments, PGT121 therapy of chronically infected macaques led to suppression of viremia 35 to over 100 days with undetectable levels in 3 of 18 treated animals with no virus rebound (Barouch et al., 2013). Furthermore, cell-associated viral DNA in PBMC, lymph nodes and the GI-tract was 4 to 10-fold reduced. In another study, viral suppression lasted 4 to 7 days in a monotherapy with either 10-1074 or 3BNC117 in chronically SHIV-infected macaques and 18 to 36 days under a combination therapy with both antibodies (Shingai et al., 2013). Additional to chronic infection, bNAbs have been shown to be efficient also during early acute infection with SHIV in macaques: A single infusion of bNAbs on day 10 post infection followed by a 13 week regimen with ART starting 11 days after the bNAb injection can suppress viremia to the same extend as a continuous administration of only ART from day 10 post infection (Bolton et al., 2016). Additionally, early administration of bNAbs has been shown to not only block infection but also eradicate small foci of virus and thus prevent the dissemination of the virus into latent reservoirs (Hessell et al., 2016). Another study shows that long lasting elite controller status is achievable by single infusions of a bNAb combination on day 3, 10 and 17 post infection which is very likely attributable to the enhancement of a CD8+ T cell response (Nishimura et al., 2017).

Clinical trials conducted in the last several years in different populations and different clinical scenarios have shown that bNAbs are well tolerated by human beings. Their half-lives are approximately 2-3 weeks in uninfected adults and shorter in viremic HIV-1-infected individuals. This may result from a faster clearance in the presence of antigen (Caskey, 2020). In one of the first studies, Caskey et al. (Caskey et al., 2015) demonstrated that the CD4 binding site directed antibody 3BNC117 could suppress viremia in virus-infected individuals significantly for 4 to 28 days after a single infusion.

Furthermore, those individuals that received 3BNC117 showed enhanced neutralizing serum activity against a panel of tier-2 HIV-1 isolates which is a proof for enhanced host humoral immunity to HIV-1 mediated by immunotherapy (Schoofs et al., 2016). HIV-1-infected individuals with interrupted ART treatment are expected to show viral rebound after an average of 2.6 weeks. Scheid et al. (Scheid et al., 2016) demonstrated that two or four consecutive 3BNC117 infusions during analytical treatment interruption (ATI) prolonged this interval of viral rebound to an average of 6.7 and 9.9 weeks respectively. In 30% of these HIV-1-infected humans the virus rebounded only after antibody concentrations vanished and this occurred mostly with variants that showed no resistance to 3BNC117 suggesting that this bNAb executed strong immune pressure on the virus. 10-1074, a V3 glycan supersite directed monoclonal antibody, was well tolerated in a study on HIV-1 infected and uninfected humans (Caskey et al., 2017). It led to a virus reduction of 1.52 log10 copies/ml reaching the nadir at an average of 10.3 days after infusion. Resistant viral strains showed mostly mutations in N332S or S334N affecting the contact site of 10-1074 (Caskey et al., 2017).

However, the selection of HIV-1 resistant escape variants during bNAb monotherapy could be shown in those trials which tested 3BNC117, 10-1074 and VRC01 (Caskey et al., 2015, Caskey et al., 2017, Lynch et al., 2015). The combination of the two bNAbs, 3BNC117 and 10-1074, could suppress viremia on an average of 2.05 log10 copies/ml without development of *de novo* resistance in individuals infected with viruses sensitive to both antibodies. But complete suppression was not achieved in participants with high baseline viral loads (Bar-On et al., 2018). Full suppression could be maintained in ART-treated individuals with bNAb-sensitive viruses after cessation of ART treatment for periods for between 15 and more than 30 weeks, also without the selection of viruses resistant to both viruses (Mendoza et al., 2018). Ongoing trials test if repeated bNAb administrations or the use of bNAb variants with extended half-lives and increased potencies can extend the period of full viral suppression. Furthermore, trials with combinations of antibodies comprising specificities for 3 distinct epitopes on the envelope protein are underway. Engineered multispecific bNAbs covering up to 3 non-overlapping epitopes are also evaluated (Caskey, 2020). The results of future clinical studies will determine whether bNAbs should be delivered to HIV-1-infected individuals as addition to ART treatment, after cessation of ART treatment or as main therapy option replacing the ART drugs.

bNAbs are different from ART by their ability to activate the anti-HIV-1 host cellular immune response through Fc-mediated effector functions. This antibody-enhanced T cell

response could lead to a prolonged control of viremia and even clearance of latently infected cells. Clinical studies have to further examine the relationship between bNAbs and the cellular immune response as well as the latent reservoir in humans (Caskey, 2020).

2 Hypothesis and goal of this study

Nearly four decades after its discovery, HIV-1 still poses a threat to mankind. Efforts to induce neutralizing antibodies against the viral envelope protein (Env) through a vaccine have been hindered by the genetic diversity of HIV-1. However, recently discovered broadly neutralizing antibodies (bNAbs) targeting Env show great promise for prevention and therapy of HIV-1 infection. Combinations of bNAbs are efficient to suppress viral load and prevent viral escape in humanized mice (Klein et al., 2012) and nonhuman primates (Shingai et al., 2013). In clinical trials, the potential of bNAbs is restricted by the rebound of resistant viral strains. Therefore, a wide spectrum of bNAbs is required that target independent viral epitopes so that new bNAb combinations can contribute to a broader and more potent antiviral coverage (Caskey, 2020).

About 30% of HIV-1-infected individuals generate cross-reactive neutralizing serum activity due to broadly neutralizing antibody responses against Env (Doria-Rose et al., 2008), with 1 % of HIV-1-infected individuals being defined as 'elite neutralizers' because of exceptional breadth and potency. Recently discovered bNAbs have been purified by single B cell analysis from elite neutralizers. Therefore, screening individuals with broadly neutralizing serum activity against HIV-1 is the first step in the search for new bNAbs.

We want to to test if a combination of two different neutralization assays and a new ranking system can be useful to identify individuals with elite HIV-1 neutralizing activity. The first goal was to identify individuals with broadly neutralizing serum activity against HIV-1 and elite neutralizers out of two diverse cohorts with a neutralization screening method. The second goal was to identify potential factors that may correlate with the development of broadly neutralizing serum activity. This may help in future recruitments of HIV-1-infected individuals to more likely identify elite neutralizers. Finally, the third goal was to map anti-HIV-1 specificities found in sera of elite neutralizers. This might be critical to improve antibody isolation strategies using optimized antigens for identifying bNAbs (McCoy and Burton, 2017).

3 Material and Methods

3.1 Specimen

The Massachusetts General Hospital in Boston, MA, and the University Hospital of Cologne provided serum samples of HIV-1-infected individuals into this study. Before donation, all HIV-1-infected individuals signed an informed consent under approved protocols by their Institutional Review Boards (IRB) of the Massachusetts General Hospital (MGH cohort) and the University Hospital of Cologne (09-081). All experiments on the serum samples including an anonymized analysis of the individuals' clinical information were approved by the IRB of the The Rockefeller University (MNU-0628 and MNU-0625). All plasma samples were shipped in boxes with dry ice to Michel Nussenzweig's laboratory at The Rockefeller University. There, samples were heat-inactivated at 56°C for 1 hour to inactivate HIV-1 (Spire et al., 1985) and all samples were stored at -20°C. Blood donations from HIV-1-infected subjects took place in the time between 2010 and 2012.

3.2 Purification of IgG from plasma samples

Total IgG was purified from the individuals' plasma samples. 250 ul of heat-inactivated plasma was incubated with 200 ul of Protein G overnight. Protein G is a bacterial cell wall protein with Fc-binding capacity of IgG molecules from humans and other species (Björck and Kronvall, 1984). A human group G streptococcal strain has been the source for its isolation. After being detached from the bacterial wall through enzymatic digestion with papain, protein G has been purified (Björck and Kronvall, 1984). The IgG binding of Protein G derives from the COOH-terminal domains while the NH2-terminal domain can bind to serum albumin (Sjöbring et al., 1991). The latter domain is bound to 4% agarose in regard to immobilization of Protein G for laboratory applications (Protein G Sepharose 4 Fast Flow). The binding capacity of Protein G for human IgG is 17 mg/ml (Protein G Sepharose 4 Fast Flow), thus 200ul can bind 3.4 mg (17mg*200/1000) while 250 ul of plasma contain about 2.44 mg of human IgG (975 mg*250/1000/100; according to Stoop et al. (Stoop et al., 1969)). IgG was eluted from Protein G in *Bio-spin Chromatography Columns*, applying a pH change with Glycin (0.1M, pH=3). The flow through was collected in Tris (1M, pH=8) and the pH change was neutralized by a Tris-Glycin amount relation of 1:10. Before applying further experiments, the Tris-Glycin-buffer had to be exchanged with phosphate buffered saline (PBS). In Amicon Ultra 0.5 spin columns, samples were repeatedly concentrated and diluted by centrifugation and filling up with PBS. This way, only the buffer was exchanged while the membrane of the column kept the IgG back. After a 200-fold dilution in PBS was reached, samples were filled up with 250 ul PBS in

Eppendorf tubes and stored at 4º C. These samples were sent to *Monogram Biosciences, Inc.*

3.3 Neutralization assay

Neutralizing activity of total IgG purified from HIV-1-infected subjects' plasma was evaluated in a high-throughput capacity assay (Fig. 13). U87 cells expressing CD4 as well as CXCR4 and CCR5 got infected with HIV-1 pseudovirus. The amount of infective particles correlated with luciferase activity initiated in the target cell, resulting in a measureable luminescence. Before being added to the target cell, the virus particles were incubated with different dilutions of the tested samples. Reduction in luminescence compared to the virus control reflected the neutralization activity of the individuals' IgG (Montefiori, 2004). The pseudovirus was generated by transfection of HEK 293 cells with two plasmids: The gp160 encoding env genes of HIV-1-infected individuals were amplified and cloned into a pCXAS-expression vector with a cytomegalovirus immediate-early promoter that enhanced the expression of the envelope protein after transfection. A second plasmid carrying the HIV-1 genome and a firefly luciferase gene at the region of the silenced envelope gene was co-transfected. The pseudovirus was incubated with IgG samples for 1h at 37°C before the inoculation. After successful infection of the U87 cells, viral tat protein induced luciferase gene expression (Richman et al., 2003). Luciferase is an enzyme that catalyzes the reaction of luciferin with ATP and oxygen in the target cell with the outcome of light emission.

luciferin + ATP → luciferyl adenylate + PP_i

luciferyl adenylate + O_2 → oxyluciferin + AMP + light

Plasma samples were diluted consecutively threefold. Results are shown either as concentration in ug/ml or titer that reached a 50% inhibition of infection. Therefore, the titer correlated positively with the neutralizing activity while the IC_{50} correlated in revers.

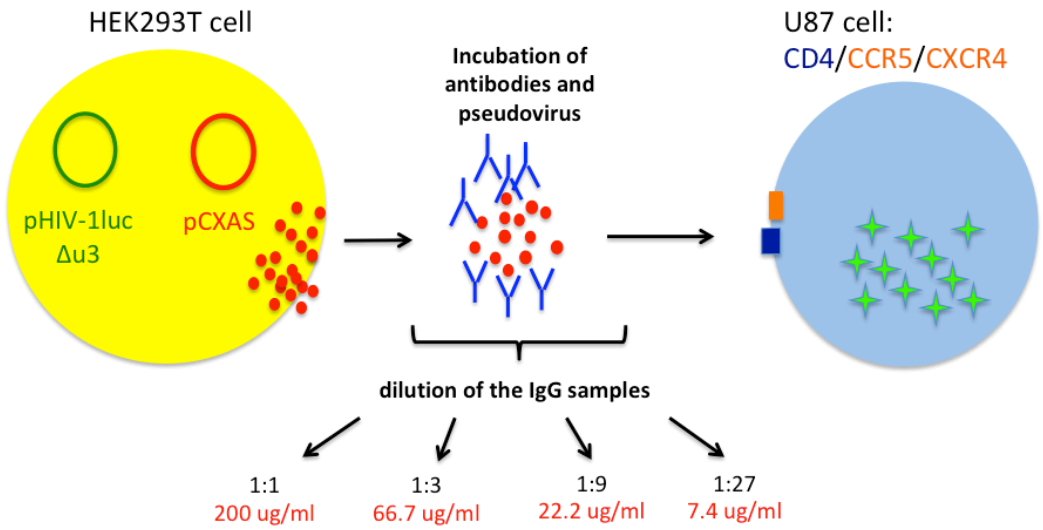

Figure 13 | *Monogram Biosciences* neutralization assay. Principles of the neutralization assay are shown. HIV-1 pseudoviruses are generated in HEK 293 cells that are transfected with two plasmids, one carrying the genome of HIV-1 and firefly luciferase and the other one carrying HIV-1-infected individuals' gp160 cloned in a pCXAS-expression vector. The resulting virus particles are incubated with different dilutions of the tested antibody samples. After that, U87 cells expressing CD4 as well as CXCR4 and CCR5 got incubated with the pseudovirus-antibody mix. Thus, infection of U87 cells with pseudovirus results in luciferase activity within the cells and therefore in a measurable luminescence. Compared to infection with pseudovirus only, the reduction in luminescence reflects the neutralization activity of the individuals' IgG.

Different strains representing different clades of HIV-1 were used as HIV-1 pseudovirus in this assay. A predictive panel of 5 strains was used for screening cohorts of HIV-1-infected individuals (Fig. 14 A). This panel and the service of pursuing a high output neutralization assay was provided by the company *Monogram Biosciences, Inc.* (Simek et al., 2009, Binley et al., 2004). For a second screening on the preselected specimen out of the first screening we used a broader panel for neutralization assays. The extended panel included 13 strains that were more resistant to neutralization than the *Monogram Biosciences* panel (Fig. 14 B and 15). It also covered a wider range of clades and CRFs. This helped to reflect breadth and potency of the analyzed samples in a more detailed way. Neutralization on this extended panel was performed by Dr. Michael Seaman at his laboratory at *The Center for Virology and Vaccine Research* at Beth Israel Deaconess Medical Center, Harvard Medical School in Boston, MA.

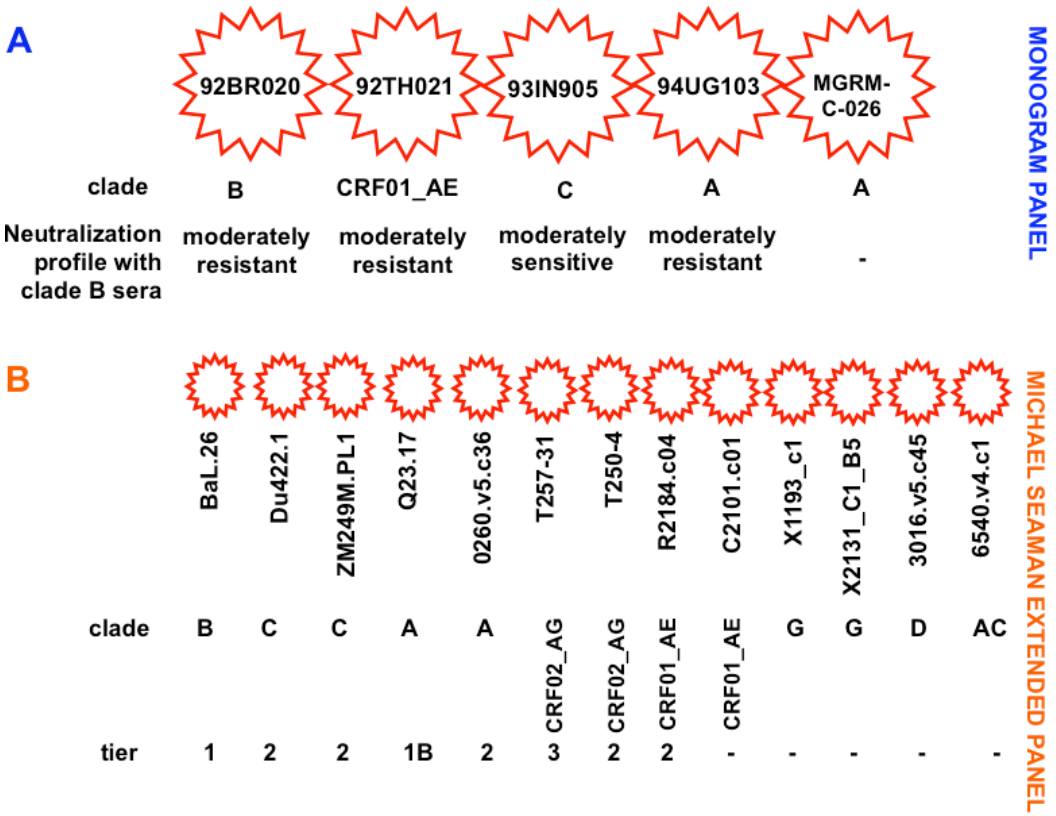

Figure 14 | *Monogram Biosciences* and extended panel. *Monogram Biosciences* panel (A) is shown with 5 strains of HIV-1 pseudoviruses as well as the extended panel by Dr. Michal Seaman (B) with 13 strains of pseudoviruses covering a broad range of clades and tiers.

HIV-1 isolate	Tier	Subtype	Country of origin	Stage of infection	Mode of transmission	Source	Gender	mo/yr isolated	weeks after est. infection date	plasma VL	CD4 count	CoR
BaL.26	1B	B	USA	Fiebig VI	Mother to child	Lung	n/a	n/a	n/a	n/a	n/a	n/a
Du422.1	2	C	South Africa	Fiebig V	M-F	ccPBMC	F	1998	8	17118	409	R5
ZM249M.PL1	2	C	Zambia	Fiebig II	F-M	plasma	M	2003	<1	1143760	NA	R5
Q23.17	1B	A1	Kenya	Fiebig VI	FSW	ccPBMC	n/a	n/a	n/a	n/a	n/a	n/a
0260.v5.c36	2	A	Tanzania	Fiebig V/VI	F-M	plasma	n/a	n/a	n/a	n/a	n/a	n/a
T257-31	3	CRF02_AG	Cameroon	Acute/early	n/a	ccPBMC	n/a	n/a	n/a	n/a	n/a	n/a
T250-4	2	CRF02_AG	Cameroon	Fiebig VI	n/a	ccPBMC	n/a	n/a	n/a	n/a	n/a	n/a
R2184.c04	n/a	CRF01_AE	Thailand	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
C2101.c01	n/a	CRF01_AE	Thailand	n/a	M-F	n/a	F	1999	n/a	n/a	n/a	n/a
X1193_c1	n/a	G	Spain	n/a	IDU	n/a	M	2002	n/a	n/a	568	R5
X2131_C1B5	n/a	G	Spain	n/a	F-M	n/a	M	2007	n/a	n/a	248	R5
3016.v5.c45	n/a	D	Tanzania	Fiebig I or II	heterosexual	plasma	n/a	2005	n/a	n/a	n/a	n/a
6540.v4.c1	n/a	AC	Tanzania	n/a	n/a	n/a	n/a	2004	n/a	n/a	n/a	n/a

Figure 15 | Demographic and biologic characteristics of HIV-1 pseudovirus strains of the extended panel.

Each pseudovirus has been cloned with an envelope protein that derives from infected HIV-1 individuals across the globe. Here, further properties of the HIV-1 pseudovirus strains are described with regard to country of origin, gender of the infected individuals, the mode of transmission, the stage of infection when the virus was isolated and further information in detail. References of the first description of these strains are: BaL.26 (Shu et al., 2007), Du422.1 (Li et al., 2006), ZM249M.PL1 (Li et al., 2006), Q23.17 (Poss and Overbaugh, 1999), 0260.v5.c36 (Seaman et al., 2010, Doria-Rose et al., 2012), T257-31 (Seaman et al., 2010, Kulkarni et al., 2009), T250-4 (Seaman et al., 2010, Kulkarni et al., 2009), R2184.c04 (Montefiori et al., 2012), C2101.c01 (Montefiori et al., 2012), X1193_c1 (Ana Revilla, 2011), X2131_C1B5 (Ana Revilla, 2011), 3016.v5.c45 (GenBank: HM215283.1), 6540.v4.c1 (GenBank: ADI62259.1). CoR, coreceptor tropism; M-F, male to female; F-M, female to male; FSW, female sex worker; IDU, intravenous drug use; ccPBMC, cocultured PBMC; n/a, not available.

3.4 Ranking system

In order to select the best samples out of the first screening, the results by *Monogram Biosciences* were ranked by a system that reflected the samples' breadth and potency. In our newly developed ranking system, 1 to 5 points were distributed for every neutralized strain, representing breadth. Potency was included by giving 1 to 2 points increasing in decimals for the average inhibitory concentrations of all positive neutralization reactions of a sample, decreasing from 200 ug/ml in steps of 20 ug/ml. Finally, both points were multiplied with each other resulting in a ranking score.

	average IC50 (ug/ml)	200	180-199	160-179	140-159	120-139	100-119	80-99	60-79	40-59	20-39	0-19
	potency points	**1**	**1,1**	**1,2**	**1,3**	**1,4**	**1,5**	**1,6**	**1,7**	**1,8**	**1,9**	**2**
neutralized strains	**1**	**1**	**1,1**	**1,2**	**1,3**	**1,4**	**1,5**	**1,6**	**1,7**	**1,8**	**1,9**	**2**
	2	**2**	**2,2**	**2,4**	**2,6**	**2,8**	**3**	**3,2**	**3,4**	**3,6**	**3,8**	**4**
	3	**3**	**3,3**	**3,6**	**3,9**	**4,2**	**4,5**	**4,8**	**5,1**	**5,4**	**5,7**	**6**
	4	**4**	**4,4**	**4,8**	**5,2**	**5,6**	**6**	**6,4**	**6,8**	**7,2**	**7,6**	**8**
	5	**5**	**5,5**	**6**	**6,5**	**7**	**7,5**	**8**	**8,5**	**9**	**9,5**	**10**

Figure 16 | Ranking system. This ranking system takes into account how many of the five virus strains and how potent they are neutralized by the tested antibody on the *Monogram Biosciences* panel. In order to get a ranking score (green digits), the amount of neutralized strains (red digits) is multiplied with the potency points (blue bold digits). Potency points are distributed for the IgG samples' average inhibitory concentrations of positive neutralization reactions in the assay, ranging from 0 to 200 ug/ml.

3.5 Statistical tools and software

Different statistical tests within the software program *'Prism 6'* have been used in order to compare different groups between the two cohorts. The Mann-Whitney-test was used for the comparison of CD4 lymphocytes and viral load counts between *LTNP-SELECTED* and *UNSELECTED* subjects as well as CD4 lymphocytes and viral load counts between treated and untreated individuals within the *UNSELECTED* group, as it is described in Fig. 21. The unpaired t test was used in Fig. 29 for the comparison of ranking point scores between all *LTNP-SELECTED* and *UNSELECTED* individuals as well as between the top individuals of these two cohorts. Furthermore, the data in Fig. 33, Fig. 34 and Fig. 36 was analyzed through linear regression. The neutralizing ability of treated versus untreated subjects of the *UNSELECTED* group in Fig. 37 was compared using an unpaired t test.

The graphs of this study were created with the use of *Microsoft Excel*, *Microsoft Powerpoint*, *Prism 6* and *Adobe Illustrator CS5.1*.

3.6 Mapping of specificity

In addition to the extended panel, the top individuals of both cohorts were tested against a panel using the HIV-1_{YU2} strain, a clade B virus, and four other mutated versions of this strain, namely YU2_N160K, YU2_N280Y, YU2_N332K and YU2_triple. The different mutations effect different binding sites on the virus and thus give hints about the samples' specificities by comparing neutralization of wildtype HIV-1_{YU2} with the mutated versions. N280Y is a mutation which alters the CD4 binding site of the virus. N332K changes the binding site on the V3 loop of the virus. The N160K mutation effects binding of antibodies like PG9 or PG16 that bind to variable loop V1/V2. The strain YU2_triple includes all three mutations.

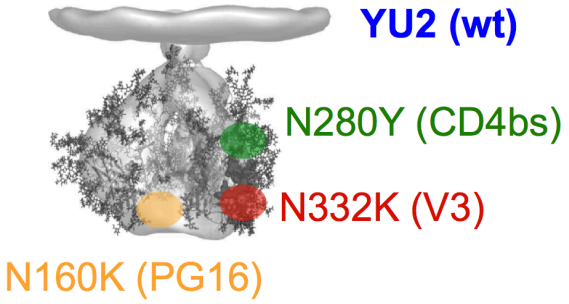

Figure 17 | YU2 and mutations. This graph depicts the trimer of a YU2 wildtype (wt) strain of a clade B HIV-1 virus. Three mutation positions are shown, namely N280Y on the CD4 binding site, N332K on the V3 loop and N160K on the binding site of the antibody PG16 (V1/V2 loop).

4 Results

4.1 Cohort description

294 plasma samples were collected from HIV-1-infected individuals from the *Massachusetts General Hospital* in Boston, MA, and 343 from HIV-1-infected individuals from the *University Hospital of Cologne* in Germany (Fig. 18). The individuals from Boston were all Long-Term-Nonprogressors (LTNP) whereas the cohort from Cologne was an unselected group of HIV-1-infected individuals. Therefore, these cohorts from Boston and Cologne are referred to as *LTNP-SELECTED* and *UNSELECTED* respectively.

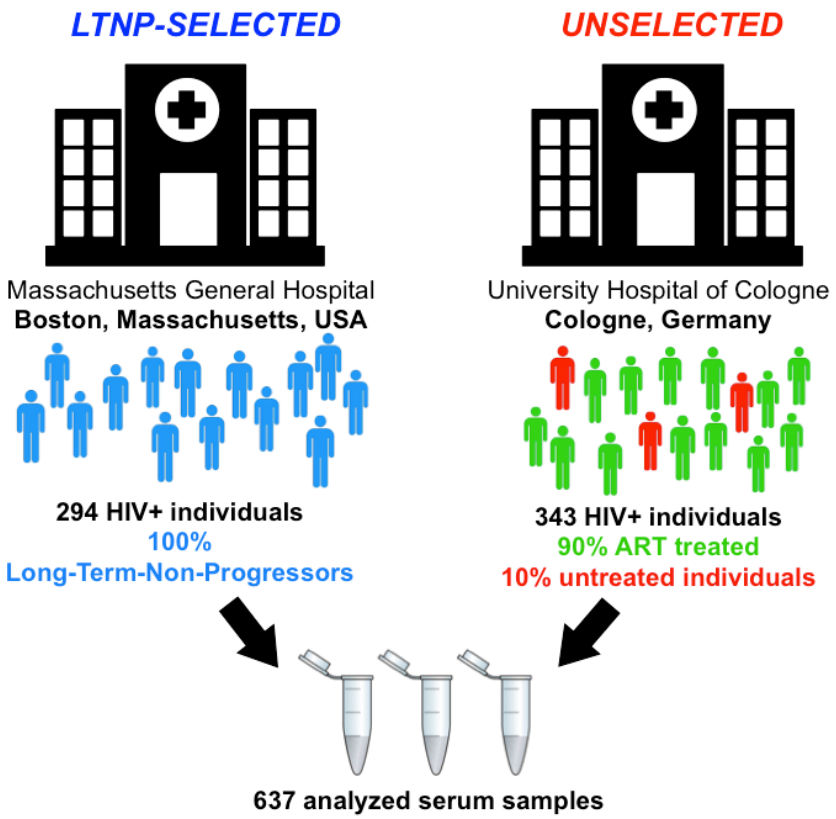

Figure 18 | Overview of cohorts. Two different cohorts are subject of this study: 294 HIV-1 positive individuals from Boston that are long-term-non-progressors (LTNP) and 343 unselected HIV-1 positive individuals from Cologne of whom 90% are treated with ART.

In both cohorts, most individuals are male with a fraction of 72% in the *LTNP-SELECTED* and 88% in the *UNSELECTED* group (Fig. 19). 90% of *UNSELECTED* individuals were treated with ART while *LTNP-SELECTED* individuals were mostly ART-naive (75%) and off therapy (23%) for over a year at the time of the neutralization analysis (Fig. 19). In terms of ethnicities, the *LTNP-SELECTED* group was more diverse than the *UNSELECTED* group, showing following distributions: 50% white, 33% Afro-American, 12% Hispanic, 0,35% Asian (*LTNP-SELECTED)* versus 89,8% white, 6,71% Afro-American, 1,75% Hispanic, 1,17% Asian (*UNSELECTED).* A closer look at the *UNSELECTED* individuals reveals that their age ranged from 25 to 55 years and that HIV-1 clade B was the dominating strain with a portion of 53 %. Homo-/bisexual contacts were the dominating cause for infection (63%), followed by heterosexual contacts (20%), endemic area (4%), intravenous drug use (2%) and blood transfusion (2%) (Fig. 20).

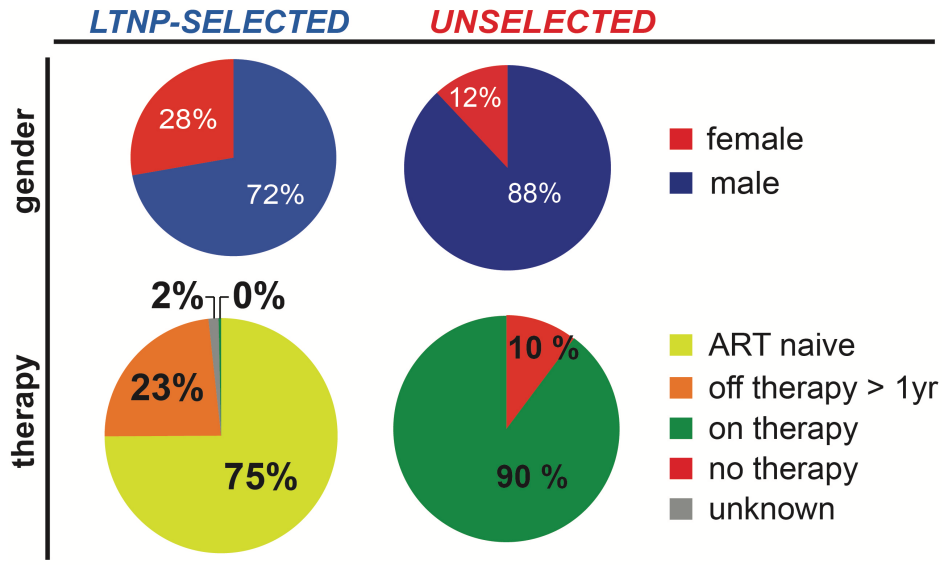

Figure 19 | Description of cohorts *LTNP-SELECTED* and *UNSELECTED*. This graph shows a comparison between the gender and therapy modes of both the *LTNP-SELECTED* and *UNSELECTED* cohorts. None of the *LTNP-SELECTED* individuals received ART at the moment of sample collection while 90% of *UNSELECTED* subjects were treated with ART. ART naive: individuals that have never received ART. Off therapy > 1year: individuals whose ART is terminated for more than a year. On Therapy: individuals that currently receive ART. No Therapy: individuals that don't receive ART.

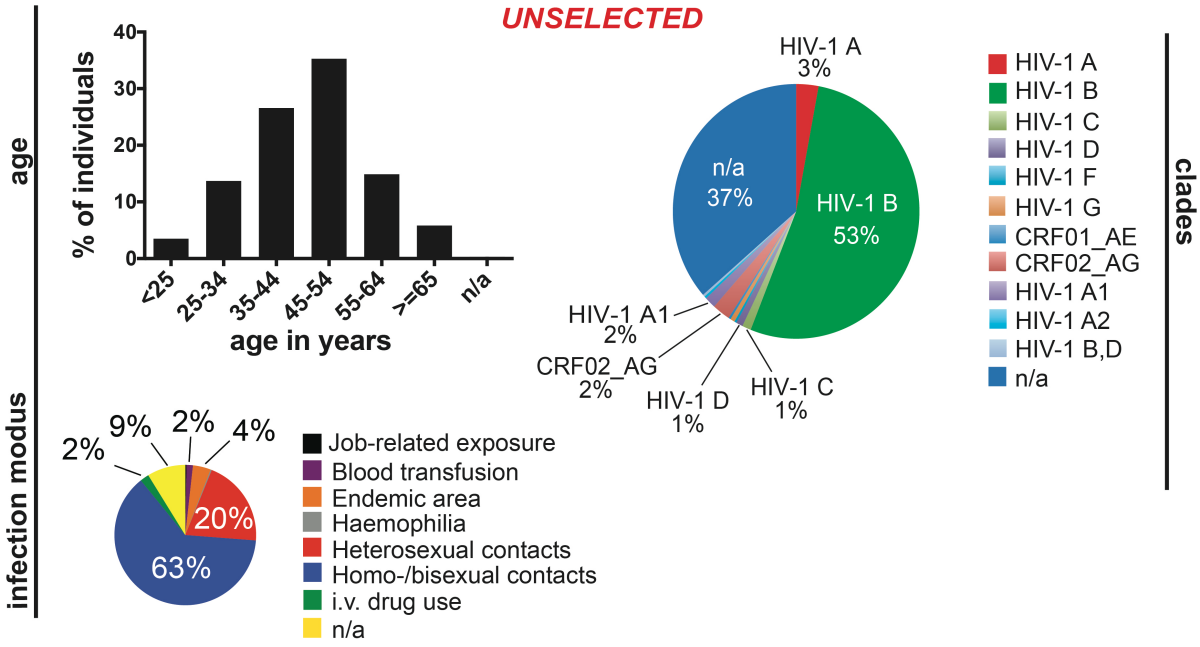

Figure 20 | Further description of the *UNSELECTED* cohort. Distributions of age, infection modes and clades of the *UNSELECTED* cohort are presented in this graph.

The *LTNP-SELECTED* individuals show on average significantly higher CD4 counts (mean 770 vs. 551 cells/ul) and lower viral load counts (mean 496 vs. 25758 copies/ml) than the *UNSELECTED* individuals (Fig. 21). Within the *UNSELECTED* cohort, treated

individuals show on average significant lower viral loads (mean 9158 vs. 169464 copies/ml) but similar CD4 counts compared to the untreated ones (mean 556 vs. 515 cells/ul) (Fig. 21). The majority of individuals in both cohorts have CD4 counts above 500 cells/ul (80% in *LTNP-SELECTED* and 54,84% in *UNSELECTED*) (Fig. 22). Most *UNSELECTED* individuals (76,33%) have viral load counts below 50 copies/ml and most *LTNP-SELECTED* subjects (54,89%) have viral loads between 50 and 2000 copies/ml (Fig. 22).

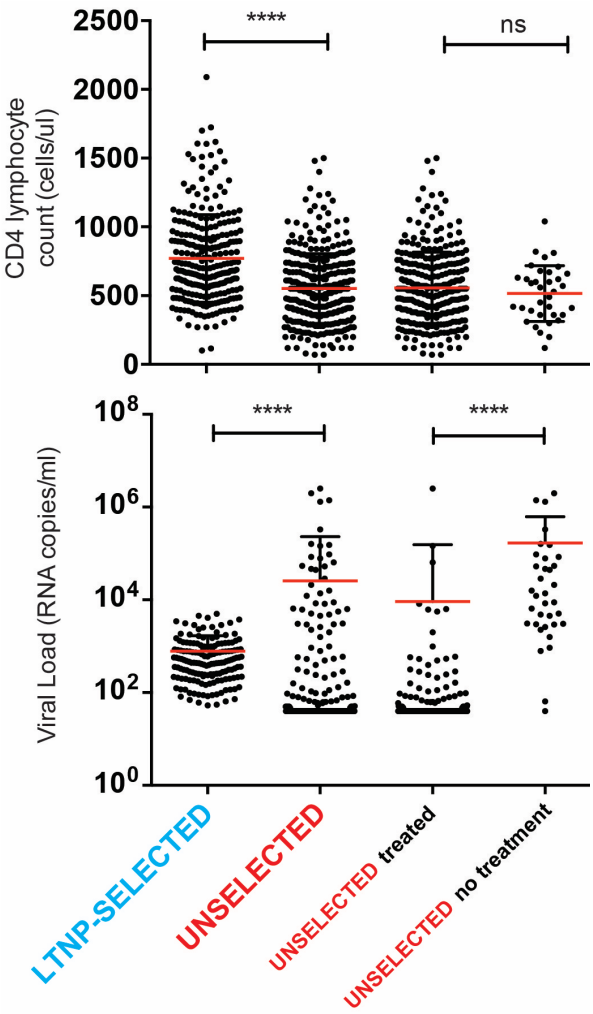

Figure 21 | Laboratory parameters of cohorts *LTNP-SELECTED* and *UNSELECTED*. Total CD4 lymphocyte counts (cells/ul) and total viral loads (RNA copies/ml) for the *LTNP-SELECTED* and *UNSELECTED* subjects are compared to each other. Furthermore, the CD4 counts and viral loads of the treated and untreated individuals of the *UNSELECTED* cohort are displayed.
ns = not significant = $p>0.05$; significance can be displayed in three steps: * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; **** = $p \leq 0.0001$.

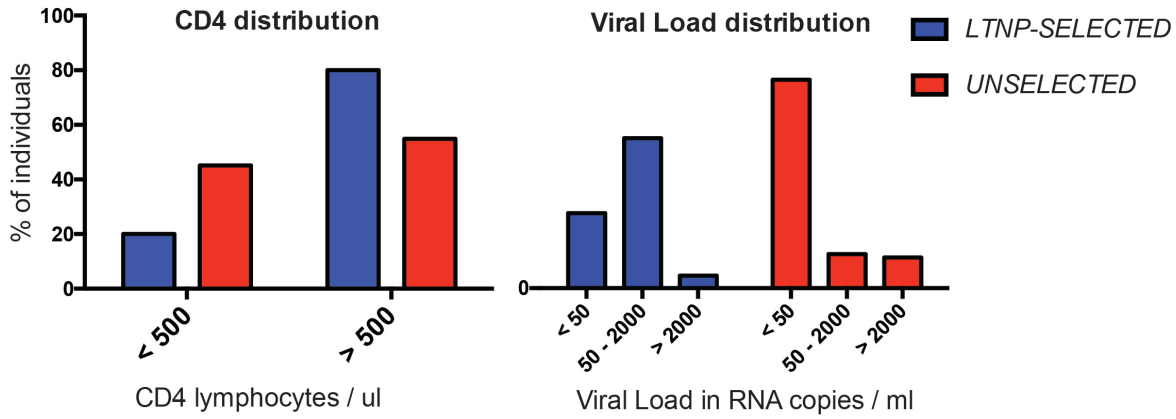

Figure 22 | CD4 and viral load distribution within both cohorts. CD4 and viral load counts are distributed differently in both groups of *LTNP-SELECTED* and *UNSELECTED* individuals. For better display, CD4 cell counts are grouped in <500 and >500 cells/ul and viral load counts are grouped in <50, 50-2000 and >2000 copies/ml.
CD4 distribution: n = 265 (*LTNP-SELECTED),* n = 341 (*UNSELECTED).* Viral load distribution: n = 266 (*LTNP-SELECTED), n=338 (UNSELECTED)*. Within the 266 *LTNP-SELECTED* individuals, 26 samples had viral loads specified as "below 75" and 9 samples specified as "below 400" so that these samples could not be assigned to one of the groupings in this graph.

4.2 Screening for HIV-1-infected individuals with broadly neutralizing serum activity

4.2.1 First screening on the *Monogram Biosciences* panel

The IgG samples of both cohorts were first analyzed on the *Monogram Biosciences* panel. Fig. 24 and Fig. 25 show the neutralization panel of the *LTNP-SELECTED* and *UNSELECTED* individuals. Every sample was tested against the five strains 92BR020, 92TH021, 93IN905, 94UG103, MGRM-C-026. They represent the HIV-1 subtypes B, CRF01_AE, C and A respectively. With this diversity of clades, this panel can serve as a first filter to catch individuals with broadly neutralizing serum activity against HIV-1. EB_151 was missing because we sent too low concentrations to *Monogram Biosciences* for being analyzed. *Monogram Biosciences* delivered the results of neutralization activity as inhibitory concentrations (IC_{50}) of 200, 66.7 and 22.2 ug/ml. These numbers arose from the threefold dilutions of the samples, starting with 200ug/ml and thus resulting in concentrations of 66.7 ug/ml, 22.2 ug/ml and 7.4 ug/ml. The neutralization activity of the samples was tested with each of these concentrations. The last concentration that showed a neutralization activity of at least 50% was marked as IC_{50} by *Monogram Biosciences*. Fig. 23 shows the example for sample EB_264 which has got a diverse range of inhibitory concentrations on this *Monogram Biosciences* panel.

ID	92BR020	92TH021	93IN 905	94UG103	MGRM-C-026
EB_264	200	N	22.2	200	66.7

Figure 23 | Example of EB_264 for different inhibitory concentrations. A wide range of inhibitory concentrations from 22.2 up to 200 ug/ml are displayed in this table for sample EB_264 from the Boston cohort, representing the varying ability of this patient's plasma to neutralize the five strains 92BR020, 92TH021, 93IN905, 94UG103 and MGRM-C-026. The color code underscores the neutralizing ability with yellow being a low neutralizing ability (inhibitory concentration of 200 ug/ml), orange being a moderate neutralizing ability (inhibitory concentration of 66.7 ug/ml) and red representing a high neutralizing ability (inhibitory concentration of 22.2 ug/ml) of this sample for a particular strain. The letter 'N' describes the fact that no neutralization of this strain by the sample was detected.

Every sample got a neutralization score from the ranking system. Based on these scores, the samples were ranked in an order starting with the highest score. This helped to choose the best neutralizing samples for the second neutralization panel. As visualized in Fig. 26 and Fig. 27, the ranking system captured the ones with the best breadth and potency in an order.

ID	92BR020	92TH021	93IN905	94UG103	MGRM-C-026
EB_101	N	N	N	N	N
EB_102	200	N	N	N	N
EB_103	N	N	N	N	N
EB_104	N	200	N	N	N
EB_105	200	N	N	N	N
EB_106	N	N	N	N	N
EB_107	N	N	N	N	N
EB_108	N	N	N	N	N
EB_109	N	N	N	N	N
EB_110	200	N	N	N	N
EB_111	200	N	200	N	N
EB_112	200	N	N	N	N
EB_113	N	N	N	N	N
EB_114	200	N	200	N	200
EB_115	N	N	N	N	N
EB_116	200	N	N	N	N
EB_117	N	N	N	N	N
EB_118	66.7	N	22.2	N	22.2
EB_119	N	N	200	N	N
EB_120	N	N	N	N	N
EB_121	N	N	N	N	N
EB_122	N	N	200	N	N
EB_123	N	N	N	N	200
EB_124	N	N	N	N	N
EB_125	N	N	N	N	N
EB_126	200	200	66.7	N	66.7
EB_127	N	N	N	200	N
EB_128	N	N	N	N	N
EB_129	N	N	N	N	N
EB_130	N	N	N	N	N
EB_131	N	N	N	N	N
EB_132	N	N	N	N	N
EB_133	200	N	200	N	200
EB_134	N	N	200	N	200
EB_135	N	N	N	N	N
EB_136	N	N	N	N	N
EB_137	N	N	N	N	N
EB_138	N	N	N	N	N
EB_139	N	N	N	N	N
EB_140	N	N	N	N	N
EB_141	N	N	N	N	N
EB_142	200	N	N	N	N
EB_143	N	N	N	N	N
EB_144	200	N	N	N	N
EB_145	N	N	N	N	N
EB_146	N	N	N	N	N
EB_147	N	N	N	N	200
EB_148	200	N	N	N	N
EB_149	N	N	N	N	N
EB_150	N	N	200	N	200
EB_152	N	N	N	N	N
EB_153	N	N	N	N	N
EB_154	200	N	N	N	N
EB_155	N	N	N	N	N
EB_156	N	N	N	N	N
EB_157	N	N	N	N	N
EB_158	N	N	N	N	N
EB_159	N	N	N	N	N
EB_160	N	N	N	N	N
EB_161	200	N	N	N	200
EB_162	N	N	N	N	N
EB_163	N	N	N	N	N
EB_164	N	N	N	N	N
EB_165	N	N	N	N	N
EB_166	N	N	N	N	N
EB_167	66.7	N	66.7	200	22.2
EB_168	N	N	N	N	N
EB_169	200	N	N	N	N
EB_170	200	N	N	N	N
EB_171	N	N	N	N	N
EB_172	N	N	N	N	N
EB_173	N	N	N	N	N
EB_174	N	N	N	N	N
EB_175	N	N	N	N	N

ID	92BR020	92TH021	93IN905	94UG103	MGRM-C-026
EB_176	N	N	N	N	N
EB_177	N	N	N	N	N
EB_178	N	N	N	N	N
EB_179	200	200	200	200	200
EB_180	N	N	N	N	N
EB_181	N	N	N	N	N
EB_182	N	N	N	N	N
EB_183	N	N	N	N	N
EB_184	200	N	N	N	200
EB_185	N	N	N	N	N
EB_186	200	N	N	N	N
EB_187	N	N	N	N	N
EB_188	66.7	N	200	N	N
EB_189	66.7	N	N	N	N
EB_190	N	N	N	N	N
EB_191	N	N	N	N	N
EB_192	200	N	N	N	N
EB_193	N	N	N	N	N
EB_194	N	N	N	N	N
EB_195	N	N	N	N	N
EB_196	N	N	N	N	N
EB_197	N	N	N	N	N
EB_198	N	N	N	N	N
EB_199	N	N	N	N	N
EB_200	N	N	N	N	N
EB_201	200	N	N	N	N
EB_202	200	N	66.7	200	200
EB_203	200	N	N	N	N
EB_204	N	N	N	N	N
EB_205	N	N	N	N	N
EB_206	N	N	N	N	N
EB_207	N	N	N	N	N
EB_208	N	N	N	N	N
EB_209	200	N	66.7	N	66.7
EB_210	N	N	N	N	N
EB_211	N	N	N	N	N
EB_212	N	N	N	N	N
EB_213	N	N	N	N	N
EB_214	200	N	N	N	N
EB_215	N	N	N	N	N
EB_216	200	N	200	N	N
EB_217	N	N	N	N	N
EB_218	N	N	22.2	N	N
EB_219	N	N	N	N	N
EB_220	N	N	N	N	N
EB_221	N	N	N	N	N
EB_222	N	N	N	N	N
EB_223	200	N	N	N	N
EB_224	200	N	N	200	N
EB_225	N	N	N	200	N
EB_226	N	N	N	N	N
EB_227	N	N	N	N	N
EB_228	N	N	N	N	N
EB_229	N	N	N	N	N
EB_230	N	N	200	N	N
EB_231	N	N	N	N	N
EB_232	200	N	200	N	N
EB_233	66.7	N	N	N	N
EB_234	N	N	N	N	N
EB_235	N	N	N	N	N
EB_236	N	N	N	N	N
EB_237	N	N	N	N	N
EB_238	N	N	N	N	N
EB_239	200	N	200	200	200
EB_240	200	N	N	N	200
EB_241	200	N	N	N	N
EB_242	N	N	N	N	N
EB_243	N	N	N	N	N
EB_244	N	N	N	N	N
EB_245	200	N	200	N	200
EB_246	N	N	N	N	N
EB_247	200	N	200	N	N
EB_248	N	N	N	N	N
EB_249	N	N	N	N	N

ID	92BR020	92TH021	93IN905	94UG103	MGRM-C-026
EB_250	N	N	N	N	200
EB_251	N	N	N	N	N
EB_252	N	N	N	N	N
EB_253	N	N	N	N	N
EB_254	N	N	N	200	N
EB_255	N	N	N	N	N
EB_256	N	N	N	N	N
EB_257	N	N	N	200	N
EB_258	66.7	N	200	N	N
EB_259	200	N	66.7	N	22.2
EB_260	N	N	N	N	N
EB_261	200	N	22.2	N	22.2
EB_262	N	N	N	N	N
EB_263	N	N	N	N	N
EB_264	200	N	22.2	200	66.7
EB_265	N	N	N	N	N
EB_266	N	N	200	N	N
EB_267	N	N	N	N	N
EB_268	N	N	N	N	N
EB_269	N	N	N	N	N
EB_270	200	N	200	N	66.7
EB_271	N	N	N	N	N
EB_272	200	N	N	N	200
EB_273	200	N	N	N	N
EB_274	N	N	N	N	N
EB_275	N	N	200	N	N
EB_276	N	N	N	N	N
EB_277	66.7	N	200	N	N
EB_278	N	N	N	N	N
EB_279	N	N	N	N	N
EB_280	N	N	N	N	N
EB_281	N	N	200	N	N
EB_282	N	N	N	N	N
EB_283	N	N	N	N	N
EB_284	N	N	N	N	N
EB_285	N	N	N	N	N
EB_286	N	N	N	N	N
EB_287	N	N	N	N	N
EB_288	N	N	N	N	N
EB_289	N	N	N	N	N
EB_290	N	N	N	N	N
EB_291	N	N	N	N	N
EB_292	N	200	200	N	N
EB_293	N	200	200	N	N
EB_294	N	N	N	N	N
EB_295	N	N	N	N	N
EB_296	N	N	N	N	N
EB_297	N	N	N	N	N
EB_298	N	N	N	N	N
EB_299	N	N	N	N	N
EB_300	66.7	N	200	N	N
EB_301	N	N	200	200	N
EB_302	N	N	N	N	N
EB_303	200	N	N	66.7	N
EB_304	N	N	N	N	N
EB_305	N	N	N	N	N
EB_306	N	N	N	N	N
EB_307	N	N	N	N	N
EB_308	200	N	N	N	N
EB_309	N	N	N	N	N
EB_310	N	N	N	N	N
EB_311	N	N	N	N	N
EB_312	N	N	N	N	N
EB_313	N	N	N	N	N
EB_314	N	N	N	N	N
EB_315	N	N	N	N	N
EB_316	N	N	N	N	N
EB_317	N	N	N	N	N
EB_318	N	N	N	N	N
EB_319	N	N	N	N	N
EB_320	N	N	N	N	N
EB_321	N	N	N	N	N
EB_322	N	N	N	N	N
EB_323	N	N	N	N	N

ID	92BR020	92TH021	93IN905	94UG103	MGRM-C-026
EB_324	N	N	N	N	N
EB_325	N	N	N	N	N
EB_326	N	N	N	N	N
EB_327	N	N	N	N	N
EB_328	N	N	N	N	N
EB_329	N	N	N	N	N
EB_330	N	N	N	N	N
EB_331	N	N	N	N	N
EB_332	200	N	200	N	200
EB_333	200	N	N	N	200
EB_334	N	N	N	N	N
EB_335	N	N	N	N	N
EB_336	N	N	N	N	N
EB_337	200	N	N	N	N
EB_338	N	N	N	N	N
EB_339	66.7	N	N	N	N
EB_340	N	N	N	N	N
EB_341	N	N	N	N	N
EB_342	N	N	N	N	N
EB_343	N	N	N	N	N
EB_344	N	N	N	N	N
EB_345	N	N	N	N	N
EB_346	N	N	N	N	N
EB_347	N	N	N	N	N
EB_348	N	N	N	N	N
EB_349	N	N	200	N	N
EB_350	N	N	N	N	N
EB_351	N	N	N	N	N
EB_352	N	N	N	N	N
EB_353	N	N	N	N	N
EB_354	66.7	N	22.2	200	22.2
EB_355	N	N	N	N	N
EB_356	N	N	N	N	N
EB_357	N	N	N	N	N
EB_358	200	N	N	N	200
EB_359	N	N	N	N	N
EB_360	200	N	200	66.7	66.7
EB_361	N	N	N	N	N
EB_362	N	N	N	N	N
EB_363	N	N	N	N	N
EB_364	200	N	N	N	N
EB_365	N	N	N	N	N
EB_366	N	N	N	N	N
EB_367	N	N	N	N	N
EB_368	N	N	N	N	N
EB_369	N	N	N	N	N
EB_370	N	N	N	N	N
EB_371	200	N	200	N	N
EB_372	N	N	N	N	N
EB_373	N	N	N	N	N
EB_374	N	N	N	N	N
EB_375	N	N	N	N	N
EB_376	N	N	N	N	N
EB_377	N	N	N	N	N
EB_378	N	N	200	N	200
EB_379	N	N	N	N	N
EB_380	N	N	N	N	200
EB_381	200	N	200	N	200
EB_382	N	N	N	N	N
EB_383	N	N	N	N	N
EB_384	N	N	N	N	N
EB_385	N	N	N	N	N
EB_386	N	N	N	N	N
EB_387	N	N	N	N	N
EB_388	N	N	N	N	N
EB_389	N	N	N	N	N
EB_390	N	N	200	N	200
EB_391	N	N	N	N	N
EB_392	N	N	N	N	N
EB_393	N	N	N	N	N
EB_394	200	N	N	N	N

Figure 24 | Monogram *LTNP-SELECTED* unranked. This graph shows the results by *Monogram Biosciences* with the neutralization activity of 394 *LTNP-SELECTED* samples against five strains of pseudovirus, namely 92BR020, 92TH021, 93IN905, 94UG103 and MGRM-C-026. The *LTNP-SELECTED* individuals are ordered by sample name and the inhibitory concentrations are laid out for each sample tested against each of the five strains of pseudovirus, including the same color code as described in Fig. 23 legend.

ID	92BR 020	92TH 021	93IN 905	94U G103	MGR M-C-026
CB_1	N	N	200	N	200
CB_2	N	N	N	N	N
CB_3	N	N	N	N	N
CB_4	N	N	N	N	N
CB_5	N	N	N	N	N
CB_6	N	N	N	N	200
CB_7	N	N	N	N	N
CB_8	N	N	N	N	N
CB_9	N	N	N	N	N
CB_10	N	N	N	N	N
CB_11	N	N	N	N	N
CB_12	N	N	N	N	N
CB_13	N	N	N	N	N
CB_14	N	N	N	N	N
CB_15	N	N	N	N	N
CB_16	N	N	N	N	N
CB_17	N	N	N	N	N
CB_18	N	N	200	N	N
CB_19	N	N	N	N	N
CB_20	N	N	N	N	N
CB_21	N	N	N	N	N
CB_22	N	N	N	N	N
CB_23	N	N	N	N	N
CB_24	N	N	N	N	N
CB_25	N	N	N	N	N
CB_26	N	N	N	N	N
CB_27	N	N	N	N	N
CB_28	N	N	N	N	N
CB_29	N	N	N	N	N
CB_30	N	N	N	N	N
CB_31	N	N	N	N	N
CB_32	N	N	N	N	N
CB_33	N	N	N	N	N
CB_34	N	N	N	N	N
CB_35	N	N	N	N	N
CB_36	N	N	N	N	N
CB_37	N	N	N	N	N
CB_38	N	N	N	N	N
CB_39	N	200	N	N	66,7
CB_40	200	N	67	N	N
CB_41	N	N	N	N	N
CB_42	N	N	N	N	N
CB_43	N	N	N	N	N
CB_44	N	N	N	N	N
CB_45	N	N	N	N	N
CB_46	N	N	N	N	N
CB_47	N	N	N	N	N
CB_48	N	N	N	N	N
CB_49	N	N	N	N	N
CB_50	N	67	N	N	N
CB_51	N	N	N	N	N
CB_52	N	N	N	N	N
CB_53	N	N	N	N	N
CB_54	N	N	N	N	N
CB_55	N	N	N	N	N
CB_56	N	N	N	N	N
CB_57	N	N	N	N	N
CB_58	N	N	N	N	N
CB_59	N	67	22	N	N
CB_60	N	N	N	N	200
CB_61	N	N	N	N	N
CB_62	N	N	N	N	N
CB_63	N	N	N	N	N
CB_64	N	N	N	N	N
CB_65	N	N	N	N	N
CB_66	N	N	N	N	200
CB_67	N	N	N	N	N
CB_68	N	N	N	N	N
CB_69	N	N	N	N	N
CB_70	N	N	N	N	N
CB_71	N	N	N	N	N
CB_72	N	N	N	N	N
CB_73	N	N	N	N	N
CB_74	N	N	N	N	N
CB_75	N	N	N	N	N
CB_76	N	N	N	N	N
CB_77	N	N	N	N	N
CB_78	N	N	N	N	N
CB_79	N	N	N	N	N
CB_80	N	N	N	N	N
CB_81	N	N	N	N	N
CB_82	N	N	N	N	N
CB_83	N	N	N	N	N
CB_84	N	N	N	N	N
CB_85	N	N	N	N	N
CB_86	66,7	N	67	200	22,2

ID	92BR 020	92TH 021	93IN 905	94U G103	MGR M-C-026
CB_87	N	N	N	N	N
CB_88	N	N	N	N	N
CB_89	N	N	N	N	N
CB_90	N	N	N	N	N
CB_91	N	N	N	N	N
CB_92	N	N	N	N	N
CB_93	N	N	N	N	N
CB_95	N	N	N	N	N
CB_96	N	N	N	N	N
CB_97	N	N	N	N	N
CB_98	N	N	N	N	N
CB_99	N	N	N	N	N
CB_100	N	N	N	N	N
CB_101	200	N	200	N	200
CB_102	N	N	N	N	N
CB_103	N	N	N	N	N
CB_104	N	N	N	N	N
CB_105	N	N	N	N	N
CB_106	N	N	N	N	N
CB_107	N	N	N	N	N
CB_108	N	N	N	N	N
CB_109	N	N	N	N	N
CB_110	N	N	N	N	N
CB_111	N	N	N	N	N
CB_112	N	N	N	N	N
CB_113	N	N	N	N	N
CB_114	N	N	N	N	N
CB_115	N	N	N	N	N
CB_116	N	N	200	N	N
CB_117	N	N	N	N	N
CB_118	67	N	200	N	N
CB_119	200	N	200	200	200
CB_120	N	N	N	N	N
CB_122	N	N	N	N	N
CB_123	N	N	N	N	N
CB_124	N	N	N	N	N
CB_125	N	N	N	N	N
CB_126	N	N	N	N	N
CB_127	N	N	N	N	N
CB_128	N	N	N	N	200
CB_129	N	N	200	N	N
CB_130	N	N	200	N	N
CB_131	N	N	N	N	N
CB_132	N	N	N	N	N
CB_133	N	N	N	N	N
CB_134	N	N	N	N	N
CB_135	N	N	200	N	N
CB_136	200	N	N	N	N
CB_137	N	N	N	N	N
CB_138	N	N	N	N	N
CB_139	N	N	N	N	N
CB_140	200	N	200	N	200
CB_141	200	N	200	N	N
CB_142	N	N	200	N	N
CB_143	N	N	N	N	N
CB_144	N	N	N	N	N
CB_145	N	N	N	N	N
CB_146	N	N	N	N	N
CB_147	N	N	200	N	200
CB_148	N	N	200	N	N
CB_149	N	N	N	N	N
CB_150	N	N	N	N	N
CB_151	N	N	N	N	N
CB_152	N	N	N	N	N
CB_153	N	N	N	N	N
CB_154	N	N	N	N	N
CB_155	N	N	N	N	N
CB_156	N	N	N	N	N
CB_157	N	N	N	N	N
CB_158	N	N	N	N	N
CB_159	N	N	N	N	N
CB_160	N	N	N	N	N
CB_161	N	N	N	N	N
CB_162	200	N	200	N	200
CB_163	N	N	N	N	N
CB_164	N	N	N	N	N
CB_165	200	N	67	67	N
CB_166	N	N	N	N	N
CB_167	N	N	N	N	N
CB_168	N	N	N	N	N
CB_169	N	N	N	N	N
CB_170	N	N	N	N	N
CB_171	N	N	N	N	N
CB_174	200	N	N	200	N
CB_175	N	N	N	N	N
CB_176	200	N	N	N	N

ID	92BR 020	92TH 021	93IN 905	94UG 103	MGR M-C-026
CB_177	N	N	N	N	N
CB_178	N	N	N	N	N
CB_179	N	N	N	N	N
CB_180	N	N	N	N	N
CB_181	N	N	N	N	N
CB_182	N	N	N	N	N
CB_183	N	N	N	N	N
CB_184	200	N	200	N	N
CB_185	N	N	N	N	N
CB_186	N	N	N	N	N
CB_187	N	N	N	N	N
CB_188	200	N	22,2	N	200
CB_189	N	N	N	N	N
CB_190	66,7	N	22,2	22,2	22,2
CB_191	N	N	N	N	N
CB_192	N	N	N	N	N
CB_193	N	N	N	N	N
CB_194	N	N	200	N	N
CB_195	N	N	N	N	N
CB_196	N	N	N	N	N
CB_197	N	N	N	N	N
CB_198	N	N	N	N	N
CB_199	N	N	N	N	N
CB_200	N	N	N	N	N
CB_201	N	N	N	N	N
CB_202	N	N	N	N	N
CB_203	N	N	N	N	N
CB_204	N	N	N	N	N
CB_205	N	N	N	N	N
CB_206	N	N	N	N	N
CB_207	200	N	22,2	N	22,2
CB_208	N	N	N	N	N
CB_209	66,7	N	N	66,7	N
CB_210	N	N	N	N	N
CB_211	N	N	N	N	N
CB_212	N	N	N	N	N
CB_213	N	N	N	N	200
CB_214	66,7	N	22,2	66,7	22,2
CB_215	N	N	200	N	200
CB_216	N	N	N	N	N
CB_217	N	N	N	N	N
CB_218	N	N	N	N	N
CB_219	N	N	N	N	N
CB_220	N	N	N	N	N
CB_221	N	N	N	N	N
CB_222	N	N	N	N	N
CB_223	N	N	N	N	N
CB_224	N	N	N	N	N
CB_225	N	N	N	N	N
CB_226	N	N	N	N	N
CB_227	N	N	N	N	N
CB_228	N	N	N	N	N
CB_229	N	N	N	N	N
CB_230	N	N	N	N	N
CB_231	N	N	N	N	N
CB_232	N	N	N	N	N
CB_233	N	N	N	N	N
CB_234	N	N	N	N	N
CB_235	N	N	N	N	N
CB_236	N	N	N	N	N
CB_237	N	N	N	N	N
CB_238	N	N	N	N	N
CB_239	N	N	N	N	N
CB_240	N	200	N	N	N
CB_241	N	N	N	N	N
CB_242	200	N	66,7	N	22,2
CB_243	N	N	N	N	N
CB_244	N	N	N	N	N
CB_246	N	N	N	N	N
CB_247	N	N	N	N	N
CB_248	N	N	N	N	200
CB_249	N	N	N	N	N
CB_250	N	N	N	N	N
CB_251	N	N	N	N	N
CB_252	N	N	N	N	N
CB_253	N	N	N	N	N
CB_254	N	N	200	N	N
CB_255	N	N	N	N	N
CB_256	N	N	N	N	N
CB_257	N	N	N	N	N
CB_258	N	N	N	N	N
CB_259	N	N	N	N	N
CB_260	N	N	N	N	N
CB_261	N	N	N	N	N
CB_262	N	N	N	N	N
CB_263	N	N	N	N	N

ID	92BR 020	92TH 021	93IN 905	94U G103	MGR M-C-026
CB_264	N	N	N	N	N
CB_265	N	N	N	N	N
CB_266	N	N	N	N	N
CB_267	N	N	N	N	N
CB_268	N	N	N	N	N
CB_269	N	N	N	N	N
CB_270	N	N	N	N	N
CB_271	N	N	N	N	N
CB_272	N	N	N	N	N
CB_273	N	N	N	N	N
CB_274	N	N	N	N	N
CB_275	N	N	N	N	N
CB_276	200	N	N	N	N
CB_277	N	N	N	N	N
CB_278	N	N	N	N	N
CB_279	N	N	N	N	N
CB_280	N	N	N	N	200
CB_281	N	N	N	N	N
CB_282	N	N	N	N	N
CB_283	N	N	N	N	N
CB_284	N	N	N	N	N
CB_285	N	N	N	N	N
CB_286	N	N	N	N	N
CB_287	200	N	67	N	67
CB_288	N	N	N	N	N
CB_289	N	N	N	N	N
CB_290	N	N	N	N	N
CB_291	N	N	N	N	N
CB_292	N	N	N	N	N
CB_293	N	N	N	N	N
CB_294	N	N	N	N	N
CB_295	N	N	N	N	N
CB_296	N	N	N	N	N
CB_297	200	N	N	N	N
CB_298	N	N	N	N	200
CB_299	N	N	N	N	N
CB_300	N	N	N	N	N
CB_301	N	N	N	N	N
CB_302	N	N	N	N	N
CB_303	N	N	N	N	N
CB_304	N	N	N	N	N
CB_305	N	N	N	N	N
CB_306	N	N	N	N	N
CB_307	N	N	N	N	N
CB_308	N	N	N	N	N
CB_309	66,7	200	22	67	22
CB_310	N	N	N	N	N
CB_311	N	N	N	N	N
CB_312	N	N	N	N	N
CB_313	N	N	N	N	N
CB_314	N	N	N	N	N
CB_315	N	N	N	N	N
CB_316	N	N	N	N	N
CB_317	N	N	N	N	N
CB_318	N	N	N	N	N
CB_319	N	N	N	N	N
CB_320	N	N	N	N	N
CB_321	N	N	N	N	N
CB_322	N	N	N	N	200
CB_323	N	N	N	N	N
CB_324	N	N	N	N	N
CB_325	N	N	N	N	N
CB_326	N	N	N	N	N
CB_327	N	N	N	N	N
CB_328	N	N	N	N	N
CB_329	N	N	N	N	N
CB_330	N	N	N	N	N
CB_331	N	67	67	N	N
CB_332	N	N	N	N	N
CB_333	N	N	N	N	N
CB_334	N	N	N	N	N
CB_335	N	N	N	N	N
CB_336	N	N	N	N	N
CB_337	N	N	N	N	N
CB_338	N	N	N	N	N
CB_339	N	N	200	N	N
CB_340	N	N	N	N	N
CB_341	N	N	N	N	N
CB_342	N	N	N	N	N
CB_343	N	N	N	N	N
CB_344	N	200	22	N	200
CB_345	N	N	N	N	N
CB_346	N	N	N	N	200
CB_347	N	N	N	N	N
CB_348	N	N	N	N	N

Figure 25 | Monogram *UNSELECTED* unranked. This graph shows the results by *Monogram Biosciences* with the neutralization activity of 348 UNSELECTED samples against five strains of pseudovirus, namely 92BR020, 92TH021, 93IN905, 94UG103 and MGRM-C-026. The *UNSELECTED* individuals are ordered by sample name and the inhibitory concentrations are laid out for each sample tested against each of the five strains of pseudovirus, including the same color code as described in Fig. 23 legend.

ID	rp	92BR020	92TH021	93IN905	94UG103	MGRM-C-026
EB_354	6,8	66.7	N	22.2	200	22.2
EB_167	6,4	66.7	N	66.7	200	22.2
EB_118	5,7	66.7	N	22.2	N	22.2
EB_126	5,6	200	200	66.7	N	66.7
EB_264	5,6	200	N	22.2	200	66.7
EB_360	5,6	200	N	200	66.7	66.7
EB_179	5	200	200	200	200	200
EB_202	4,8	200	N	66.7	200	200
EB_259	4,8	200	N	66.7	N	22.2
EB_261	4,8	200	N	22.2	N	22.2
EB_209	4,5	200	N	66.7	N	66.7
EB_239	4	200	N	200	200	200
EB_270	3,9	200	N	200	N	66.7
EB_114	3	200	N	200	N	200
EB_133	3	200	N	200	N	200
EB_245	3	200	N	200	N	200
EB_332	3	200	N	200	N	200
EB_381	3	200	N	200	N	200
EB_188	2,8	66.7	N	200	N	N
EB_258	2,8	66.7	N	200	N	N
EB_277	2,8	66.7	N	200	N	N
EB_300	2,8	66.7	N	200	N	N
EB_303	2,8	200	N	N	66.7	N
EB_111	2	200	N	200	N	N
EB_134	2	N	N	200	N	200
EB_150	2	N	N	200	N	200
EB_161	2	200	N	N	N	200
EB_184	2	200	N	N	N	200
EB_216	2	200	N	200	N	N
EB_224	2	200	N	N	200	N
EB_232	2	200	N	200	N	N
EB_240	2	200	N	N	N	200
EB_247	2	200	N	200	N	N
EB_272	2	200	N	N	N	200
EB_292	2	N	200	200	N	N
EB_293	2	N	200	200	N	N
EB_301	2	N	N	200	200	N
EB_333	2	200	N	N	N	200
EB_358	2	200	N	N	N	200
EB_371	2	200	N	200	N	N
EB_378	2	N	N	200	N	200
EB_390	2	N	N	200	N	200
EB_218	1,9	N	N	22.2	N	N
EB_189	1,7	66.7	N	N	N	N
EB_233	1,7	66.7	N	N	N	N
EB_339	1,7	66.7	N	N	N	N
EB_102	1	200	N	N	N	N
EB_104	1	N	200	N	N	N
EB_105	1	200	N	N	N	N
EB_110	1	200	N	N	N	N
EB_112	1	200	N	N	N	N
EB_116	1	200	N	N	N	N
EB_119	1	N	N	200	N	N
EB_122	1	N	N	200	N	N
EB_123	1	N	N	N	N	200
EB_127	1	N	N	N	200	N
EB_142	1	200	N	N	N	N
EB_144	1	200	N	N	N	N
EB_147	1	N	N	N	N	200
EB_148	1	200	N	N	N	N
EB_154	1	200	N	N	N	N
EB_169	1	200	N	N	N	N
EB_170	1	200	N	N	N	N
EB_186	1	200	N	N	N	N
EB_192	1	200	N	N	N	N
EB_201	1	200	N	N	N	N
EB_203	1	200	N	N	N	N
EB_214	1	200	N	N	N	N
EB_223	1	200	N	N	N	N
EB_225	1	N	N	N	200	N
EB_230	1	N	N	200	N	N
EB_241	1	200	N	N	N	N
EB_250	1	N	N	N	N	200
EB_254	1	N	N	N	200	N

ID	rp	92BR020	92TH021	93IN905	94UG103	MGRM-C-026
EB_257	1	N	N	N	200	N
EB_266	1	N	N	200	N	N
EB_273	1	200	N	N	N	N
EB_275	1	N	N	200	N	N
EB_281	1	N	N	200	N	N
EB_308	1	200	N	N	N	N
EB_337	1	200	N	N	N	N
EB_349	1	N	N	200	N	N
EB_364	1	200	N	N	N	N
EB_380	1	N	N	N	N	200
EB_394	1	200	N	N	N	N
EB_101	0	N	N	N	N	N
EB_103	0	N	N	N	N	N
EB_106	0	N	N	N	N	N
EB_107	0	N	N	N	N	N
EB_108	0	N	N	N	N	N
EB_109	0	N	N	N	N	N
EB_113	0	N	N	N	N	N
EB_115	0	N	N	N	N	N
EB_117	0	N	N	N	N	N
EB_120	0	N	N	N	N	N
EB_121	0	N	N	N	N	N
EB_124	0	N	N	N	N	N
EB_125	0	N	N	N	N	N
EB_128	0	N	N	N	N	N
EB_129	0	N	N	N	N	N
EB_130	0	N	N	N	N	N
EB_131	0	N	N	N	N	N
EB_132	0	N	N	N	N	N
EB_135	0	N	N	N	N	N
EB_136	0	N	N	N	N	N
EB_137	0	N	N	N	N	N
EB_138	0	N	N	N	N	N
EB_139	0	N	N	N	N	N
EB_140	0	N	N	N	N	N
EB_141	0	N	N	N	N	N
EB_143	0	N	N	N	N	N
EB_145	0	N	N	N	N	N
EB_146	0	N	N	N	N	N
EB_149	0	N	N	N	N	N
EB_152	0	N	N	N	N	N
EB_153	0	N	N	N	N	N
EB_155	0	N	N	N	N	N
EB_156	0	N	N	N	N	N
EB_157	0	N	N	N	N	N
EB_158	0	N	N	N	N	N
EB_159	0	N	N	N	N	N
EB_160	0	N	N	N	N	N
EB_162	0	N	N	N	N	N
EB_163	0	N	N	N	N	N
EB_164	0	N	N	N	N	N
EB_165	0	N	N	N	N	N
EB_166	0	N	N	N	N	N
EB_168	0	N	N	N	N	N
EB_171	0	N	N	N	N	N
EB_172	0	N	N	N	N	N
EB_173	0	N	N	N	N	N
EB_174	0	N	N	N	N	N
EB_175	0	N	N	N	N	N
EB_176	0	N	N	N	N	N
EB_177	0	N	N	N	N	N
EB_178	0	N	N	N	N	N
EB_180	0	N	N	N	N	N
EB_181	0	N	N	N	N	N
EB_182	0	N	N	N	N	N
EB_183	0	N	N	N	N	N
EB_185	0	N	N	N	N	N
EB_187	0	N	N	N	N	N
EB_190	0	N	N	N	N	N
EB_191	0	N	N	N	N	N
EB_193	0	N	N	N	N	N
EB_194	0	N	N	N	N	N
EB_195	0	N	N	N	N	N
EB_196	0	N	N	N	N	N

ID	rp	92BR020	92TH021	93IN905	94UG103	MGRM-C-026
EB_197	0	N	N	N	N	N
EB_198	0	N	N	N	N	N
EB_199	0	N	N	N	N	N
EB_200	0	N	N	N	N	N
EB_204	0	N	N	N	N	N
EB_205	0	N	N	N	N	N
EB_206	0	N	N	N	N	N
EB_207	0	N	N	N	N	N
EB_208	0	N	N	N	N	N
EB_210	0	N	N	N	N	N
EB_211	0	N	N	N	N	N
EB_212	0	N	N	N	N	N
EB_213	0	N	N	N	N	N
EB_215	0	N	N	N	N	N
EB_217	0	N	N	N	N	N
EB_219	0	N	N	N	N	N
EB_220	0	N	N	N	N	N
EB_221	0	N	N	N	N	N
EB_222	0	N	N	N	N	N
EB_226	0	N	N	N	N	N
EB_227	0	N	N	N	N	N
EB_228	0	N	N	N	N	N
EB_229	0	N	N	N	N	N
EB_231	0	N	N	N	N	N
EB_234	0	N	N	N	N	N
EB_235	0	N	N	N	N	N
EB_236	0	N	N	N	N	N
EB_237	0	N	N	N	N	N
EB_238	0	N	N	N	N	N
EB_242	0	N	N	N	N	N
EB_243	0	N	N	N	N	N
EB_244	0	N	N	N	N	N
EB_246	0	N	N	N	N	N
EB_248	0	N	N	N	N	N
EB_249	0	N	N	N	N	N
EB_251	0	N	N	N	N	N
EB_252	0	N	N	N	N	N
EB_253	0	N	N	N	N	N
EB_255	0	N	N	N	N	N
EB_256	0	N	N	N	N	N
EB_260	0	N	N	N	N	N
EB_262	0	N	N	N	N	N
EB_263	0	N	N	N	N	N
EB_265	0	N	N	N	N	N
EB_267	0	N	N	N	N	N
EB_268	0	N	N	N	N	N
EB_269	0	N	N	N	N	N
EB_271	0	N	N	N	N	N
EB_274	0	N	N	N	N	N
EB_276	0	N	N	N	N	N
EB_278	0	N	N	N	N	N
EB_279	0	N	N	N	N	N
EB_280	0	N	N	N	N	N
EB_282	0	N	N	N	N	N
EB_283	0	N	N	N	N	N
EB_284	0	N	N	N	N	N
EB_285	0	N	N	N	N	N
EB_286	0	N	N	N	N	N
EB_287	0	N	N	N	N	N
EB_288	0	N	N	N	N	N
EB_289	0	N	N	N	N	N
EB_290	0	N	N	N	N	N
EB_291	0	N	N	N	N	N
EB_294	0	N	N	N	N	N
EB_295	0	N	N	N	N	N
EB_296	0	N	N	N	N	N
EB_297	0	N	N	N	N	N
EB_298	0	N	N	N	N	N
EB_299	0	N	N	N	N	N
EB_302	0	N	N	N	N	N
EB_304	0	N	N	N	N	N
EB_305	0	N	N	N	N	N
EB_306	0	N	N	N	N	N
EB_307	0	N	N	N	N	N

ID	rp	92BR020	92TH021	93IN905	94UG103	MGRM-C-026
EB_309	0	N	N	N	N	N
EB_310	0	N	N	N	N	N
EB_311	0	N	N	N	N	N
EB_312	0	N	N	N	N	N
EB_313	0	N	N	N	N	N
EB_314	0	N	N	N	N	N
EB_315	0	N	N	N	N	N
EB_316	0	N	N	N	N	N
EB_317	0	N	N	N	N	N
EB_318	0	N	N	N	N	N
EB_319	0	N	N	N	N	N
EB_320	0	N	N	N	N	N
EB_321	0	N	N	N	N	N
EB_322	0	N	N	N	N	N
EB_323	0	N	N	N	N	N
EB_324	0	N	N	N	N	N
EB_325	0	N	N	N	N	N
EB_326	0	N	N	N	N	N
EB_327	0	N	N	N	N	N
EB_328	0	N	N	N	N	N
EB_329	0	N	N	N	N	N
EB_330	0	N	N	N	N	N
EB_331	0	N	N	N	N	N
EB_334	0	N	N	N	N	N
EB_335	0	N	N	N	N	N
EB_336	0	N	N	N	N	N
EB_338	0	N	N	N	N	N
EB_340	0	N	N	N	N	N
EB_341	0	N	N	N	N	N
EB_342	0	N	N	N	N	N
EB_343	0	N	N	N	N	N
EB_344	0	N	N	N	N	N
EB_345	0	N	N	N	N	N
EB_346	0	N	N	N	N	N
EB_347	0	N	N	N	N	N
EB_348	0	N	N	N	N	N
EB_350	0	N	N	N	N	N
EB_351	0	N	N	N	N	N
EB_352	0	N	N	N	N	N
EB_353	0	N	N	N	N	N
EB_355	0	N	N	N	N	N
EB_356	0	N	N	N	N	N
EB_357	0	N	N	N	N	N
EB_359	0	N	N	N	N	N
EB_361	0	N	N	N	N	N
EB_362	0	N	N	N	N	N
EB_363	0	N	N	N	N	N
EB_365	0	N	N	N	N	N
EB_366	0	N	N	N	N	N
EB_367	0	N	N	N	N	N
EB_368	0	N	N	N	N	N
EB_369	0	N	N	N	N	N
EB_370	0	N	N	N	N	N
EB_372	0	N	N	N	N	N
EB_373	0	N	N	N	N	N
EB_374	0	N	N	N	N	N
EB_375	0	N	N	N	N	N
EB_376	0	N	N	N	N	N
EB_377	0	N	N	N	N	N
EB_379	0	N	N	N	N	N
EB_382	0	N	N	N	N	N
EB_383	0	N	N	N	N	N
EB_384	0	N	N	N	N	N
EB_385	0	N	N	N	N	N
EB_386	0	N	N	N	N	N
EB_387	0	N	N	N	N	N
EB_388	0	N	N	N	N	N
EB_389	0	N	N	N	N	N
EB_391	0	N	N	N	N	N
EB_392	0	N	N	N	N	N
EB_393	0	N	N	N	N	N

Figure 26 | Monogram *LTNP-SELECTED* ranked. *Monogram Biosciences* results of neutralization activity of 394 *LTNP-SELECTED* samples against five strains of pseudovirus are shown in the same manner as in Fig. 24 but ordered by ranking points (rp). The color code is the same as described in Fig. 23 legend.

ID	rp	92BR 020	92TH 021	93IN 905	94UG 103	MGR M-C-026
CB_309	8,5	66,7	200	22,2	66,7	22,2
CB_190	7,6	66,7	N	22,2	22,2	22,2
CB_214	7,2	66,7	N	22,2	66,7	22,2
CB_86	6,4	66,7	N	66,7	200	22,2
CB_207	4,8	200	N	22,2	N	22,2
CB_242	4,8	200	N	66,7	N	22,2
CB_165	4,5	200	N	66,7	66,7	N
CB_287	4,5	200	N	66,7	N	66,7
CB_119	4	200	N	200	200	200
CB_188	3,9	200	N	22,2	N	200
CB_344	3,9	N	200	22,2	N	200
CB_59	3,6	N	66,7	22,2	N	N
CB_209	3,4	66,7	N	N	66,7	N
CB_331	3,4	N	66,7	66,7	N	N
CB_162	3	200	N	200	N	200
CB_101	3	200	N	200	N	200
CB_140	3	200	N	200	N	200
CB_39	2,8	N	200	N	N	66,7
CB_40	2,8	200	N	66,7	N	N
CB_118	2,8	66,7	N	200	N	N
CB_1	2	N	N	200	N	200
CB_141	2	200	N	200	N	N
CB_147	2	N	N	200	N	200
CB_174	2	200	N	N	200	N
CB_184	2	200	N	200	N	N
CB_215	2	N	N	200	N	200
CB_50	1,7	N	66,7	N	N	N
CB_6	1	N	N	N	N	200
CB_18	1	N	N	200	N	N
CB_60	1	N	N	N	N	200
CB_66	1	N	N	N	N	200
CB_116	1	N	N	200	N	N
CB_128	1	N	N	N	N	200
CB_129	1	N	N	200	N	N
CB_130	1	N	N	200	N	N
CB_135	1	N	N	200	N	N
CB_136	1	200	N	N	N	N
CB_142	1	N	N	200	N	N
CB_148	1	N	N	200	N	N
CB_176	1	200	N	N	N	N
CB_194	1	N	N	200	N	N
CB_213	1	N	N	N	N	200
CB_240	1	N	200	N	N	N
CB_248	1	N	N	N	N	200
CB_254	1	N	N	200	N	N
CB_276	1	200	N	N	N	N
CB_280	1	N	N	N	N	200
CB_297	1	200	N	N	N	N
CB_298	1	N	N	N	N	200
CB_322	1	N	N	N	N	200
CB_339	1	N	N	200	N	N
CB_346	1	N	N	N	N	200
CB_2	0	N	N	N	N	N
CB_3	0	N	N	N	N	N
CB_4	0	N	N	N	N	N
CB_5	0	N	N	N	N	N
CB_7	0	N	N	N	N	N
CB_8	0	N	N	N	N	N
CB_9	0	N	N	N	N	N
CB_10	0	N	N	N	N	N
CB_11	0	N	N	N	N	N
CB_12	0	N	N	N	N	N
CB_13	0	N	N	N	N	N
CB_14	0	N	N	N	N	N
CB_15	0	N	N	N	N	N
CB_16	0	N	N	N	N	N
CB_17	0	N	N	N	N	N
CB_19	0	N	N	N	N	N
CB_20	0	N	N	N	N	N
CB_21	0	N	N	N	N	N
CB_22	0	N	N	N	N	N
CB_23	0	N	N	N	N	N
CB_24	0	N	N	N	N	N
CB_25	0	N	N	N	N	N
CB_26	0	N	N	N	N	N
CB_27	0	N	N	N	N	N
CB_28	0	N	N	N	N	N
CB_29	0	N	N	N	N	N
CB_30	0	N	N	N	N	N
CB_31	0	N	N	N	N	N
CB_32	0	N	N	N	N	N
CB_33	0	N	N	N	N	N
CB_34	0	N	N	N	N	N
CB_35	0	N	N	N	N	N
CB_36	0	N	N	N	N	N
CB_37	0	N	N	N	N	N
CB_38	0	N	N	N	N	N
CB_41	0	N	N	N	N	N
CB_42	0	N	N	N	N	N
CB_43	0	N	N	N	N	N
CB_44	0	N	N	N	N	N
CB_45	0	N	N	N	N	N
CB_46	0	N	N	N	N	N
CB_47	0	N	N	N	N	N
CB_48	0	N	N	N	N	N
CB_49	0	N	N	N	N	N
CB_51	0	N	N	N	N	N
CB_52	0	N	N	N	N	N
CB_53	0	N	N	N	N	N
CB_54	0	N	N	N	N	N
CB_55	0	N	N	N	N	N
CB_56	0	N	N	N	N	N
CB_57	0	N	N	N	N	N
CB_58	0	N	N	N	N	N
CB_61	0	N	N	N	N	N
CB_62	0	N	N	N	N	N
CB_63	0	N	N	N	N	N
CB_64	0	N	N	N	N	N
CB_65	0	N	N	N	N	N
CB_67	0	N	N	N	N	N
CB_68	0	N	N	N	N	N
CB_69	0	N	N	N	N	N
CB_70	0	N	N	N	N	N
CB_71	0	N	N	N	N	N
CB_72	0	N	N	N	N	N
CB_73	0	N	N	N	N	N
CB_74	0	N	N	N	N	N
CB_75	0	N	N	N	N	N
CB_76	0	N	N	N	N	N
CB_77	0	N	N	N	N	N
CB_78	0	N	N	N	N	N
CB_79	0	N	N	N	N	N
CB_80	0	N	N	N	N	N
CB_81	0	N	N	N	N	N
CB_82	0	N	N	N	N	N
CB_83	0	N	N	N	N	N
CB_84	0	N	N	N	N	N
CB_85	0	N	N	N	N	N
CB_87	0	N	N	N	N	N
CB_88	0	N	N	N	N	N
CB_89	0	N	N	N	N	N
CB_90	0	N	N	N	N	N
CB_91	0	N	N	N	N	N
CB_92	0	N	N	N	N	N
CB_93	0	N	N	N	N	N
CB_95	0	N	N	N	N	N
CB_96	0	N	N	N	N	N
CB_97	0	N	N	N	N	N
CB_98	0	N	N	N	N	N
CB_99	0	N	N	N	N	N
CB_100	0	N	N	N	N	N
CB_102	0	N	N	N	N	N
CB_103	0	N	N	N	N	N
CB_104	0	N	N	N	N	N
CB_105	0	N	N	N	N	N
CB_106	0	N	N	N	N	N
CB_107	0	N	N	N	N	N
CB_108	0	N	N	N	N	N
CB_109	0	N	N	N	N	N
CB_110	0	N	N	N	N	N
CB_111	0	N	N	N	N	N
CB_112	0	N	N	N	N	N
CB_113	0	N	N	N	N	N
CB_114	0	N	N	N	N	N
CB_115	0	N	N	N	N	N
CB_117	0	N	N	N	N	N
CB_120	0	N	N	N	N	N
CB_122	0	N	N	N	N	N
CB_123	0	N	N	N	N	N
CB_124	0	N	N	N	N	N
CB_125	0	N	N	N	N	N
CB_126	0	N	N	N	N	N
CB_127	0	N	N	N	N	N
CB_131	0	N	N	N	N	N
CB_132	0	N	N	N	N	N
CB_133	0	N	N	N	N	N
CB_134	0	N	N	N	N	N
CB_137	0	N	N	N	N	N
CB_138	0	N	N	N	N	N
CB_139	0	N	N	N	N	N
CB_143	0	N	N	N	N	N
CB_144	0	N	N	N	N	N
CB_145	0	N	N	N	N	N
CB_146	0	N	N	N	N	N
CB_149	0	N	N	N	N	N
CB_150	0	N	N	N	N	N
CB_151	0	N	N	N	N	N
CB_152	0	N	N	N	N	N
CB_153	0	N	N	N	N	N
CB_154	0	N	N	N	N	N
CB_155	0	N	N	N	N	N
CB_156	0	N	N	N	N	N
CB_157	0	N	N	N	N	N
CB_158	0	N	N	N	N	N
CB_159	0	N	N	N	N	N
CB_160	0	N	N	N	N	N
CB_161	0	N	N	N	N	N
CB_163	0	N	N	N	N	N
CB_164	0	N	N	N	N	N
CB_166	0	N	N	N	N	N
CB_167	0	N	N	N	N	N
CB_168	0	N	N	N	N	N
CB_169	0	N	N	N	N	N
CB_170	0	N	N	N	N	N
CB_171	0	N	N	N	N	N
CB_175	0	N	N	N	N	N
CB_177	0	N	N	N	N	N
CB_178	0	N	N	N	N	N
CB_179	0	N	N	N	N	N
CB_180	0	N	N	N	N	N
CB_181	0	N	N	N	N	N
CB_182	0	N	N	N	N	N
CB_183	0	N	N	N	N	N
CB_185	0	N	N	N	N	N
CB_186	0	N	N	N	N	N
CB_187	0	N	N	N	N	N
CB_189	0	N	N	N	N	N
CB_191	0	N	N	N	N	N
CB_192	0	N	N	N	N	N
CB_193	0	N	N	N	N	N
CB_195	0	N	N	N	N	N
CB_196	0	N	N	N	N	N
CB_197	0	N	N	N	N	N
CB_198	0	N	N	N	N	N
CB_199	0	N	N	N	N	N
CB_200	0	N	N	N	N	N
CB_201	0	N	N	N	N	N
CB_202	0	N	N	N	N	N
CB_203	0	N	N	N	N	N
CB_204	0	N	N	N	N	N
CB_205	0	N	N	N	N	N
CB_206	0	N	N	N	N	N
CB_208	0	N	N	N	N	N
CB_210	0	N	N	N	N	N
CB_211	0	N	N	N	N	N
CB_212	0	N	N	N	N	N
CB_216	0	N	N	N	N	N
CB_217	0	N	N	N	N	N
CB_218	0	N	N	N	N	N
CB_219	0	N	N	N	N	N
CB_220	0	N	N	N	N	N
CB_221	0	N	N	N	N	N
CB_222	0	N	N	N	N	N
CB_223	0	N	N	N	N	N
CB_224	0	N	N	N	N	N
CB_225	0	N	N	N	N	N
CB_226	0	N	N	N	N	N
CB_227	0	N	N	N	N	N
CB_228	0	N	N	N	N	N
CB_229	0	N	N	N	N	N
CB_230	0	N	N	N	N	N
CB_231	0	N	N	N	N	N
CB_232	0	N	N	N	N	N
CB_233	0	N	N	N	N	N
CB_234	0	N	N	N	N	N
CB_235	0	N	N	N	N	N
CB_236	0	N	N	N	N	N
CB_237	0	N	N	N	N	N
CB_238	0	N	N	N	N	N
CB_239	0	N	N	N	N	N
CB_241	0	N	N	N	N	N
CB_243	0	N	N	N	N	N
CB_244	0	N	N	N	N	N
CB_246	0	N	N	N	N	N
CB_247	0	N	N	N	N	N
CB_249	0	N	N	N	N	N
CB_250	0	N	N	N	N	N
CB_251	0	N	N	N	N	N
CB_252	0	N	N	N	N	N
CB_253	0	N	N	N	N	N
CB_255	0	N	N	N	N	N
CB_256	0	N	N	N	N	N
CB_257	0	N	N	N	N	N
CB_258	0	N	N	N	N	N
CB_259	0	N	N	N	N	N
CB_260	0	N	N	N	N	N
CB_261	0	N	N	N	N	N
CB_262	0	N	N	N	N	N
CB_263	0	N	N	N	N	N
CB_264	0	N	N	N	N	N
CB_265	0	N	N	N	N	N
CB_266	0	N	N	N	N	N
CB_267	0	N	N	N	N	N
CB_268	0	N	N	N	N	N
CB_269	0	N	N	N	N	N
CB_270	0	N	N	N	N	N
CB_271	0	N	N	N	N	N
CB_272	0	N	N	N	N	N
CB_273	0	N	N	N	N	N
CB_274	0	N	N	N	N	N
CB_275	0	N	N	N	N	N
CB_277	0	N	N	N	N	N
CB_278	0	N	N	N	N	N
CB_279	0	N	N	N	N	N
CB_281	0	N	N	N	N	N
CB_282	0	N	N	N	N	N
CB_283	0	N	N	N	N	N
CB_284	0	N	N	N	N	N
CB_285	0	N	N	N	N	N
CB_286	0	N	N	N	N	N
CB_288	0	N	N	N	N	N
CB_289	0	N	N	N	N	N
CB_290	0	N	N	N	N	N
CB_291	0	N	N	N	N	N
CB_292	0	N	N	N	N	N
CB_293	0	N	N	N	N	N
CB_294	0	N	N	N	N	N
CB_295	0	N	N	N	N	N
CB_296	0	N	N	N	N	N
CB_299	0	N	N	N	N	N
CB_300	0	N	N	N	N	N
CB_301	0	N	N	N	N	N
CB_302	0	N	N	N	N	N
CB_303	0	N	N	N	N	N
CB_304	0	N	N	N	N	N
CB_305	0	N	N	N	N	N
CB_306	0	N	N	N	N	N
CB_307	0	N	N	N	N	N
CB_308	0	N	N	N	N	N
CB_310	0	N	N	N	N	N
CB_311	0	N	N	N	N	N
CB_312	0	N	N	N	N	N
CB_313	0	N	N	N	N	N
CB_314	0	N	N	N	N	N
CB_315	0	N	N	N	N	N
CB_316	0	N	N	N	N	N
CB_317	0	N	N	N	N	N
CB_318	0	N	N	N	N	N
CB_319	0	N	N	N	N	N
CB_320	0	N	N	N	N	N
CB_321	0	N	N	N	N	N
CB_323	0	N	N	N	N	N
CB_324	0	N	N	N	N	N
CB_325	0	N	N	N	N	N
CB_326	0	N	N	N	N	N
CB_327	0	N	N	N	N	N
CB_328	0	N	N	N	N	N
CB_329	0	N	N	N	N	N
CB_330	0	N	N	N	N	N
CB_332	0	N	N	N	N	N
CB_333	0	N	N	N	N	N
CB_334	0	N	N	N	N	N
CB_335	0	N	N	N	N	N
CB_336	0	N	N	N	N	N
CB_337	0	N	N	N	N	N
CB_338	0	N	N	N	N	N
CB_340	0	N	N	N	N	N
CB_341	0	N	N	N	N	N
CB_342	0	N	N	N	N	N
CB_343	0	N	N	N	N	N
CB_345	0	N	N	N	N	N
CB_347	0	N	N	N	N	N
CB_348	0	N	N	N	N	N

ID	rp	92BR 020	92TH0 21	93IN 905	94UG 103	MGR M-C-026

Figure 27 | Monogram *UNSELECTED* ranked. *Monogram Biosciences* results of neutralization activity of 348 UNSELECTED samples against five strains of pseudovirus are shown in the same manner as in Fig. 25 but ordered by ranking points (rp). The color code is the same as described in Fig. 23 legend.

The *Monogram Biosciences* panel shows different neutralizing patterns for these two different cohorts. The clade B virus 92BR020 is expected to be neutralized with the highest frequency within these two cohorts that are dominated by clade B infected individuals. But this is only true for the *LTNP-SELECTED* group where 21% of the individuals neutralize this strain while only 7% of the *UNSELECTED* individuals can neutralize it. The *UNSELECTED* individuals have their most frequent neutralization in the clade C virus 93IN905 with 9.6%. 7 individuals reached a high level of potency on this virus with an IC_{50} value of 22 ug/ml.

In both cohorts, 92TH021 and 94UG103 were less frequently neutralized than the other viral strains tested. These two viruses were hit by the *LTNP-SELECTED* group with lowest average potencies, displayed by an average IC_{50} of 200 and 181 ug/ml whereas in the *UNSELECTED* group, IC_{50} values of 143 and 111 ug/ml for these two strains represent two of the highest average potencies of this cohort. Nearly 71% from the *LTNP-SELECTED* and 85% of *UNSELECTED* individuals showed no neutralization at all.

The neutralization of certain viruses did not seem to be coupled to each other and thus happened independently. For example, samples could neutralize several of the viruses with a moderate neutralization activity like EB_239 or EB_179 while a sample like EB_218 only neutralized one strain pretty effectively. Notably, the *LTNP-SELECTED* samples neutralized the strains 92BR020 and 94UG103 with IC_{50} values never below 67 ug/ml and the strain 92TH021 never below 200 ug/ml. In the *UNSELECTED* group, 92TH021 also showed IC_{50} values of 67 ug/ml and 94UG103 was neutralized once with a peak IC_{50} of 22 ug/ml. For both cohorts, 93IN905 and MGRM-C-026 acculumated several IC50 values of 22 ug/ml throughout the neutralized viruses (in *LTNP-SELECTED* 7 and 7 times; in UNSELECTED 7 and 6 times, respectively).

Samples that stood out in the *LTNP-SELECTED* cohort were EB_354 and EB_167 because they neutralized 4 viruses with an average IC_{50} between 77 and 89 ug/ml. Subject CB_309 is the very best neutralizer of both cohorts with a breadth of 5 viruses and an average IC_{50} of 75.6 ug/ml. Other remarking samples of the *UNSELECTED* cohort were CB_190 and CB_214 which neutralized 4 strains with an average IC_{50} between 33 and 45 ug/ml. Thus, the top 3 *UNSELECTED* individuals (CB_309, CB_190, CB_214) perform better on the *Monogram Biosciences* panel than the top 3 *LTNP-SELECTED* individuals (EB_354, EB_167 and EB_118). This is also reflected in the ranking points. The highest score in the *LTNP-SELECTED* group was 6.8 whereas in the *UNSELECTED* cohort there are scores up to 8.5.

With this, 19 out of the *LTNP-SELECTED* individuals and 20 out of the *UNSELECTED* individuals were chosen for the second neutralization panel as seen in Fig. 28.

		Monogram Biosciences Screening Panel							*Monogram Biosciences* Screening Panel				
		92BR020	92TH021	93IN905	94UG103	MGRM-C-026			92BR020	92TH021	93IN905	94UG103	MGRM-C-026
ID	rp	B	CRF 01_AE	C	A	A	ID	rp	B	CRF 01_AE	C	A	A
EB_354	6,8	66,7	N	22,2	200	22,2	CB_309	8,5	66,7	200	22,2	66,7	22,2
EB_167	6,4	66,7	N	66,7	200	22,2	CB_190	7,6	66,7	N	22,2	22,2	22,2
EB_118	5,7	66,7	N	22,2	N	22,2	CB_214	7,2	66,7	N	22,2	66,7	22,2
EB_126	5,6	200	200	66,7	N	66,7	CB_86	6,4	66,7	N	66,7	200	22,2
EB_360	5,6	200	N	200	66,7	66,7	CB_207	4,8	200	N	22,2	N	22,2
EB_264	5,6	200	N	22,2	200	66,7	CB_242	4,8	200	N	66,7	N	22,2
EB_179	5	200	200	200	200	200	CB_165	4,5	200	N	66,7	66,7	N
EB_202	4,8	200	N	66,7	200	200	CB_287	4,5	200	N	66,7	N	66,7
EB_259	4,8	200	N	66,7	N	22,2	CB_119	4	200	N	200	200	200
EB_261	4,8	200	N	22,2	N	22,2	CB_188	3,9	200	N	22,2	N	200
EB_209	4,5	200	N	66,7	N	66,7	CB_344	3,9	N	200	22,2	N	200
EB_239	4	200	N	200	200	200	CB_59	3,6	N	66,7	22,2	N	N
EB_270	3,9	200	N	200	N	66,7	CB_209	3,4	66,7	N	N	66,7	N
EB_332	3	200	N	200	N	200	CB_331	3,4	N	66,7	66,7	N	N
EB_114	3	200	N	200	N	200	CB_162	3	200	N	200	N	200
EB_133	3	200	N	200	N	200	CB_101	3	200	N	200	N	200
EB_245	3	200	N	200	N	200	CB_140	3	200	N	200	N	200
EB_150	2	N	N	200	N	200	CB_39	2,8	N	200	N	N	66,7
EB_161	2	200	N	N	N	200	CB_40	2,8	200	N	66,7	N	N
							CB_118	2,8	66,7	N	200	N	N

Figure 28 | Top individuals from *LTNP-SELECTED* and *UNSELECTED*. Based on the highest score of ranking points in both cohorts, the best individuals of both cohorts are chosen for further analysis. The best 19 *LTNP-SELECTED* individuals and the best *UNSELECTED* 20 individuals can be compared in this graph with respect to their neutralization activities against the five *Monogram Biosciences* pseudovirus strains. The color code is the same as described in Fig. 23 legend.

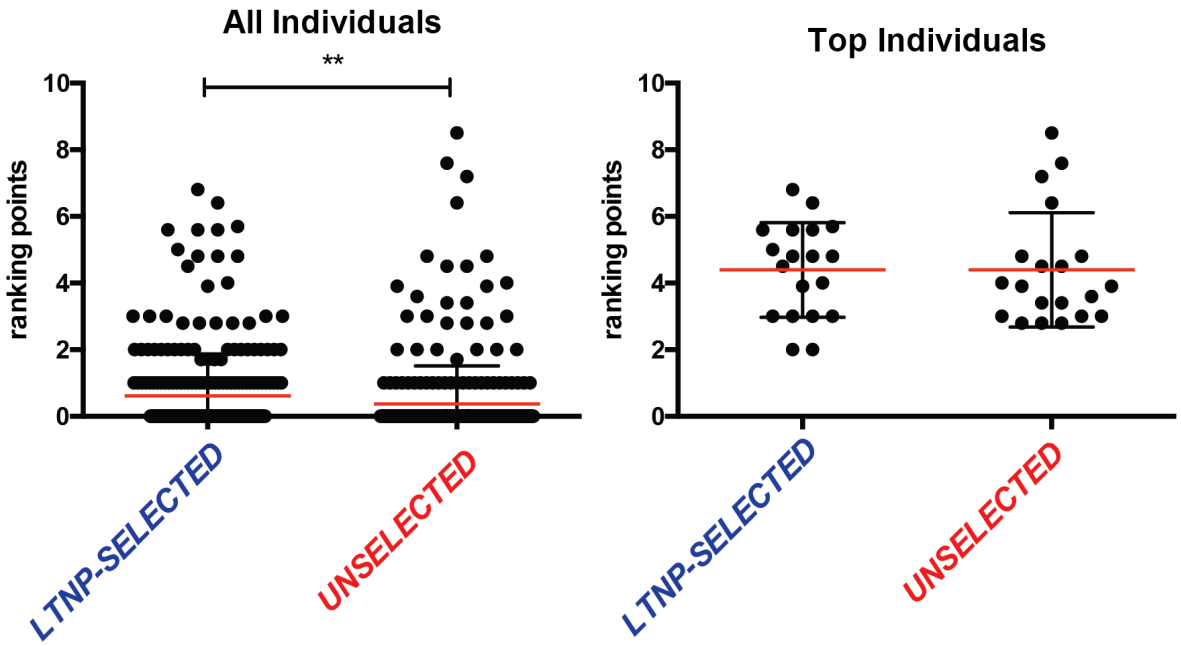

Figure 29 | Reproducibility of screening for HIV-1 infected individuals with sera of high neutralization activity. The ranking points of both *LTNP-SELECTED* and *UNSELECTED* cohorts are plotted and compared to each other (graph on the left). *LTNP-SELECTED* subjects achieve a significant higher average score than *UNSELECTED* subjects. In contrast, there is no significant difference between the average ranking points of the top individuals of both cohorts (graph on the right). Asterisk code as described in Fig. 21.

Fig. 29 shows that by comparing both cohorts there is a significant difference (p-value < 0.01) between the average neutralization scores each cohort achieves. *LTNP-SELECTED* individuals do slightly better with a mean ranking score of 0.6 versus the 0.37 of *UNSELECTED* individuals. But this is not the case for the chosen best individuals from both cohorts. Interestingly, the average ranking points of these both subsets of cohorts is the same at close under 5 points.

4.2.2 Second screening on the extended panel

In both cohorts, none of the samples neutralized all of the 13 strains on the extended panel. The strain Bal.26 was neutralized by all *LTNP-SELECTED* subjects with an average IC_{50} of 17.6 ug/ml. X2131_C1_B5 showed also a good rate of neutralization with 94.7% of the *LTNP-SELECTED* individuals but on a lower average potency, reflected by the average IC50 of 82.5 ug/ml. In contrast to that, the pseudoviruses C2101.c01 and 3016.v5.c45 were neutralized by only 10-16% of the *LTNP-SELECTED* subjects.

		Monogram Biosciences Screening Panel					Michael Seaman Extended Panel											
		92BR020	92TH021	93IN905	94UG103	MGRM-C-026	BaL.26	Du422.1	ZM249M.PL1	Q23.17	0260.v5.c36	T257-31	T250-4	R2184.c04	C2101.c01	X1193_c1	X2131_C1B5	3016.v5.c45	6540.v4.c1
ID	rp	B	CRF01_AE	C	A	A	B	C	C	A	A	CRF02_AG	CRF02_AG	CRF01_AE	CRF01_AE	G	G	D	AC
EB_354	6,8	66.7	N	22.2	200	22.2	12,8	65	56,6	8,6	60,8	160,1	6,95	39,3	>271	56,7	23,2	>271	>271
EB_167	6,4	66.7	N	66.7	200	22.2	25,7	76,9	463	29,7	351	>500	9,47	>500	>500	82,6	24,9	>500	218
EB_118	5,7	66.7	N	22.2	N	22.2	16,5	>500	290	21,2	237	>500	21,54	>500	>500	56,8	13,4	>500	>500
EB_126	5,6	200	200	66.7	N	66.7	7,84	93,5	139	33,6	500	>500	261,6	424	106	>500	26,3	>500	107
EB_360	5,6	200	N	200	66.7	66.7	27,2	477	268	67,2	>500	121,2	34,44	>500	>500	>500	>500	>500	84,1
EB_264	5,6	200	N	22.2	200	66.7	10,9	250	52,7	312	>500	>500	48,1	89,3	>500	70,3	53,6	>500	55,2
EB_179	5	200	200	200	200	200	24,5	>318	168	32,9	224	72,68	129,6	>318	82,5	198	90,4	>318	141
EB_202	4,8	200	N	66.7	200	200	9,17	105	167	14,2	189	98,5	9,16	>500	>500	>500	72,3	>500	9,76
EB_259	4,8	200	N	66.7	N	22.2	24	>500	>500	134	>500	>500	>500	>500	>500	62	78,2	>500	>500
EB_261	4,8	200	N	22.2	N	22.2	17,1	210	>500	173	>500	>500	>500	>500	>500	15,7	20,3	>500	>500
EB_209	4,5	200	N	66.7	N	66.7	18,4	272	>500	76,2	>500	>500	392,3	>500	>500	>500	39,7	>500	>500
EB_239	4	200	N	200	200	200	11,9	>500	434	>500	>500	>500	60,47	83,3	>500	>500	69,9	>500	>500
EB_270	3,9	200	N	200	N	66.7	20,1	>387	>387	>387	>387	>387	117,3	>387	>387	>387	85,6	>387	>387
EB_332	3	200	N	200	N	200	14,5	210	>375	73,9	287	>375	77,57	>375	>375	101	44,4	>375	327
EB_114	3	200	N	200	N	200	11,4	>500	>500	95	>500	>500	28,71	>500	>500	>500	244	>500	>500
EB_133	3	200	N	200	N	200	8,97	254	221	183	>500	>500	14,7	73,9	>500	>500	127	273	253
EB_245	3	200	N	200	N	200	12,8	>500	72,5	80,8	169	>500	>500	34,2	265	41,3	37,6	190	>500
EB_150	2	N	N	200	N	200	14,5	406	82,1	>500	>500	446,5	239,4	>500	>500	>500	236	>500	447
EB_161	2	200	N	N	N	200	45,5	>500	>500	>500	>500	>500	>500	>500	>500	>500	197	>500	>500

Figure 30 | Extended Panel *LTNP-SELECTED*. This graph shows the neutralization activities of the *LTNP-SELECTED* top 19 individuals against five strains of pseudovirus by *Monogram Biosciences* (as already shown in Fig. 28) as well as against 13 strains from Michael Seaman's extended panel, namely BaL.26, Du422.1, ZM249M.PL1, Q23.17, 0260.v5.c36, T257-31, T250-4, R2184.c04, C2101.c01, X1193_c1, X2131_C1B5, 3016.v5.c45 and 6540.v4.c1. The LTNP-SELECTED individuals are ordered by ranking points. The graph includes the same color code as described in Fig. 23 legend.

		Monogram Biosciences Screening Panel					Michael Seaman Extended Panel											
		92BR020	92TH021	93IN905	94UG103	MGRM-C-026	BaL.26	Du422.1	ZM249M.PL1	Q23.17	0260.v5.c36	T257-31	T250-4	R2184.c04	C2101.c01	X1193_c1	X2131_C1B5	3016.v5.c45	6540.v4.c1
ID	rp	B	CRF01_AE	C	A	A	B	C	C	A	A	CRF02_AG	CRF02_AG	CRF01_AE	CRF01_AE	G	G	D	AC
CB_309	8,5	66,7	200	22,2	66,7	22,2	7,02	49,6	76,5	16,4	462	129,5	115,9	>500	>500	304	44,9	120	69,4
CB_190	7,6	66,7	N	22,2	22,2	22,2	16	202	39,8	5,02	>500	>500	22,74	>500	31,7	>500	30,3	>500	243
CB_214	7,2	66,7	N	22,2	66,7	22,2	13,7	223	194	67,8	>500	>500	261,2	81,3	>500	>500	201	>500	>500
CB_86	6,4	66,7	N	66,7	200	22,2	31,4	234	>500	63,5	>500	>500	28,5	>500	>500	>500	119	>500	174
CB_207	4,8	200	N	22,2	N	22,2	7,14	73,7	85,4	30,5	375	>500	106,3	362	231	96	22,5	119	339
CB_242	4,8	200	N	66,7	N	22,2	15,4	255	>500	45,6	>500	268,5	263,5	>500	>500	>500	72,1	>500	>500
CB_165	4,5	200	N	66,7	66,7	N	23,3	>500	74,3	13,9	161	101,5	24,47	27,7	102	58,2	80,8	121	5,46
CB_287	4,5	200	N	66,7	N	66,7	207	315	>500	90,9	>500	153,7	52,6	>500	>500	>500	244	>500	>500
CB_119	4	200	N	200	200	200	22,8	60,3	145	113	>500	241,1	77,72	>500	>500	>500	>500	>500	101
CB_188	3,9	200	N	22,2	N	200	7,67	122	>500	>500	>500	>500	389,7	>500	>500	207	290	>500	>500
CB_344	3,9	N	200	22,2	N	200	90,6	480	>500	24,5	>500	59,8	12,46	32,6	39	37,9	13,9	>500	150
CB_59	3,6	N	66,7	22,2	N	N	138	>500	115	2,09	453	>500	7,25	452	>500	>500	45,5	>500	94,6
CB_209	3,4	66,7	N	N	66,7	N	11,8	>500	>500	>500	>500	>500	35,43	>500	>500	>500	>500	>500	93,5
CB_331	3,4	N	66,7	66,7	N	N	325	449	243	10,9	>500	102,5	11,97	>500	>500	287	62,2	>500	121
CB_162	3	200	N	200	N	200	12,3	>500	153	93,5	>500	131,2	252,8	>500	>500	>500	>500	>500	477
CB_101	3	200	N	200	N	200	259	400	>500	189	>500	>500	46,58	>500	>500	127	57,9	>500	>500
CB_140	3	200	N	200	N	200	20,4	>500	>500	>500	>500	>500	>500	>500	>500	160	63,3	>500	>500
CB_39	2,8	N	200	N	N	66,7	216	>500	>500	286	>500	>500	>500	>500	>500	>500	>500	>500	>500
CB_40	2,8	200	N	66,7	N	N	90	>500	383	132	>500	>500	352,2	>500	>500	>500	>500	>500	>500
CB_118	2,8	66,7	N	200	N	N	10,2	106	>500	12,8	47,1	>500	>500	>500	>500	13,6	38,2	181	>500

Figure 31 | Extended Panel *UNSELECTED*. This graph shows the neutralization activities of the *UNSELECTED* top 20 individuals against five strains of pseudovirus by *Monogram Biosciences* (as already shown in Fig. 28) as well as against 13 strains from Michael Seaman's extended panel, namely BaL.26, Du422.1, ZM249M.PL1, Q23.17, 0260.v5.c36, T257-31, T250-4, R2184.c04, C2101.c01, X1193_c1, X2131_C1B5, 3016.v5.c45 and 6540.v4.c1. The *UNSELECTED* subjects are ordered by ranking points. The graph includes the same color code as described in Fig. 23 legend.

On this extended panel, some HIV-1 subtypes were represented by two different pseudoviruses that differed in their resistance to neutralization, indicated by frequency and

potency of neutralization. A great difference in frequency of neutralization was found between Q23.17 and 0260.v5.c36 (clade A), T257-31 and T250-4 (CRF02_AG) as well as between X1193_c1 and X2131_C1_B5 (clade G). With the exception of X1193_c1 and X2131_C1_B5 (clade G), the virus with the higher neutralization frequency was also neutralized with better average potencies within these subtype pairs in the *LTNP-SELECTED* cohort. In the *UNSELECTED* cohort, this association of breadth and potency was found only in subtype pairs of clade A, CRF02_AG and G. R2184.c04 and C2101.c01 representing CRF01_AE were neutralized less frequently in both cohorts. Another strain that seemed to be resistant to neutralization was 3016.v5.c45, a clade D pseudovirus which was only neutralized by 10.5% in the *LTNP-SELECTED* cohort and 20 % in the *UNSELECTED* cohort. The virus 6540.v4.c1, a clade AC virus, can be seen in the middle range of both frequency and potency of neutralization in both cohorts. There were 8 *LTNP-SELECTED* and 5 *UNSELECTED* samples that showed a neutralization breadth of above 69% on the extended panel. With regards to potency, there were 7 *LTNP-SELECTED* and 6 *UNSELECTED* samples that showed an average IC_{50} of below 100 ug/ml. EB_354 and EB_202 united good neutralization breadth and high potency on this panel. Especially, EB_354 was performing well with neutralizing 77% of all viruses on the panel with an average IC_{50} of 49 ug/ml. EB_179, EB_126 and EB_245 also performed well on the extended panel. They neutralized the more resistant virus C2101.c01. Interestingly, all these good performers EB_354, EB_202, EB_179, EB_126 and EB_245 showed a different pattern of neutralization which seemed to be independent from each other. None of the more resistant strains that were neutralized with an overall frequency of less than 30% (T257-31, C2101.c01 and 3016.v5.c45) were neutralized with a notably high potency by these good neutralizers. Referring to this, the highest potency was seen for EB_179 neutralizing the strain T257-31 with an IC_{50} of 72.7 ug/ml. It is worth mentioning that EB_167 and EB_118 showed a similar neutralization pattern with same IC_{50} ranges in different virus subtypes. Some viruses on the extended panel showed a similar pattern of neutralization between both cohorts. Here again, BaL.26 stood out as the virus that is the easiest to neutralize, having the highest neutralization frequency with 100% and average IC50s in the lowest range on both panels. X2131_C1_B5, Q23.17 and T250-4 are among the most frequently and highly potently neutralized virus clades on both panels. The viruses that are least frequently neutralized were T257-31, C2101.c01 and 3016.v5.c45 in the *LTNP-SELECTED* cohort and 0260.v5.c36, R2184.c04, C2101.c01 and 3016.v5.c45 in the *UNSELECTED* cohort. In both cohorts, within most subtype pairs, the virus that was neutralized more frequently was also neutralized with a higher average potency than its

counterpart. The subtype pairs Q23.17 and 0260.v5.c36 (clade A) and T257-31 and T250-4 (clade AG) showed this tendency consistently in both cohorts. It is worthy to note that the *UNSELECTED* individuals covered almost both extremes concerning breadth on this extended panel: CB_165 and CB_207 got close to maximum breadth by neutralizing 12 strains which was about 92% while CB_39 only neutralized two clades with low potencies. Other candidates with great breadth on the extended panel were CB_309 and CB_344 with a virus coverage of 85% and 77% respectively. Looking at the top individuals of both cohorts in total, the very best individual was represented by the sample EB_354 based on the combination of great breadth of 76.9% and a very good average IC_{50} of 49 ug/ml. CB_165 from the *UNSELECTED* group had greater breadth with 92.3% but its average IC_{50} of 66.1 ug/ml was not as good as the one of EB_354. The samples CB_309, CB_190, CB_214, CB_207, CB_165, CB_344 as well as EB_354, EB_118, EB_264, EB_179, EB_202 and EB_245 were chosen as elite neutralizers (Fig. 32).

		Monogram Biosciences Screening Panel					Michael Seaman Extended Panel												
		92BR020	92TH021	93IN905	94UG103	MGRM-C-026	BaL.26	Du422.1	ZM249M.PL1	Q23.17	0260.v5.c36	T257-31	T250-4	R2184.c04	C2101.c01	X1193_c1	X2131_C1_B5	3016.v5.c45	6540.v4.c1
ID	rp	B	CRF 01_AE	C	A	A	B	C	C	A	A	CRF 02_AG	CRF 02_AG	CRF 01_AE	CRF 01_AE	G	G	D	AC
CB_309	8,5	66,7	200	22,2	66,7	22,2	7,02	49,61	76,52	16,36	461,5	129,5	115,9	>500	>500	304	44,9	120,3	69,42
CB_190	7,6	66,7	N	22,2	22,2	22,2	15,95	202,2	39,84	5,02	>500	>500	22,74	>500	31,73	>500	30,26	>500	242,5
CB_214	7,2	66,7	N	22,2	66,7	22,2	13,73	223,4	194,4	67,76	>500	>500	261,2	81,27	>500	>500	201,2	>500	>500
CB_207	4,8	200	N	22,2	N	22,2	7,14	73,67	85,42	30,49	374,9	>500	106,3	362,2	231,2	95,99	22,54	119	339,1
CB_165	4,5	200	N	66,7	66,7	N	23,32	>500	74,27	13,91	160,9	101,5	24,47	27,73	102	58,18	80,78	120,6	5,46
CB_344	3,9	N	200	22,2	N	200	90,64	479,7	>500	24,46	>500	59,8	12,46	32,55	38,97	37,87	13,87	>500	149,8
EB_354	6,8	66,7	N	22,2	200	22,2	12,76	64,99	56,6	8,6	60,81	160,1	6,95	39,25	>271	56,65	23,23	>271	>271
EB_118	5,7	66,7	N	22,2	N	22,2	16,5	>500	289,9	21,2	237,3	>500	21,54	>500	>500	56,83	13,37	>500	>500
EB_264	5,6	200	N	22,2	200	66,7	10,9	250,4	52,69	312	>500	>500	48,1	89,34	>500	70,3	53,55	>500	55,22
EB_179	5	200	200	200	200	200	24,52	>318	167,6	32,9	224,5	72,68	129,6	>318	82,52	198	90,38	>318	141
EB_202	4,8	200	N	66,7	200	200	9,17	104,7	166,9	14,19	189,1	98,5	9,16	>500	>500	>500	72,32	>500	9,76
EB_245	3	200	N	200	N	200	12,79	>500	72,54	80,8	169,2	>500	>500	34,21	264,9	41,27	37,59	190,1	>500

Figure 32 | Elite Neutralizers. The best performing samples from both cohorts were chosen as elite neutralizers and are displayed in this table. As in Fig. 30 and Fig. 31, both *Monogram Biosciences* panel and Seaman's extended panel are presented next to each other, this time with the elite neutralizers from both cohorts ordered by ranking points. This table includes the same color code as described in Fig. 23 legend.

4.3 Analysis of cohorts and panels

A good neutralization on the extended panel is crucial in the choice of the elite neutralizers. The fact that only the best individuals on the *Monogram Biosciences* panel were chosen for the extended panel raises the question if this choice of individuals was justified. A correlation between neutralizing performances of the top individuals on both panels would strengthen the idea that there were no elite neutralizers missed out by not testing the worse neutralizing *Monogram Biosciences* samples on the extended panel. As seen in Fig. 33, both cohorts present an association by trend between the score of a sample on the *Monogram Biosciences* panel and the breadth on the extended panel. The

breadth on the extended panel is presented as percentage of all the 13 strains that were hit with an IC_{50} up to 500 ug/ml. In both cohorts, the slope shows a deviation from zero with a p-value of 0.053 in the *LTNP-SELECTED* and 0.049 in the *UNSELECTED* group, thus presenting marginal significance in the *UNSELECTED* cohort while only a trend in the *LTNP-SELECTED* cohort. Similarly, the *Monogram Biosciences* panel does not seem to be predictive for determining the potency of a sample. In Fig. 33, a tendency of better ranked samples associated with lower IC_{50}s can be seen in both cohorts but also not proven with statistic significance.

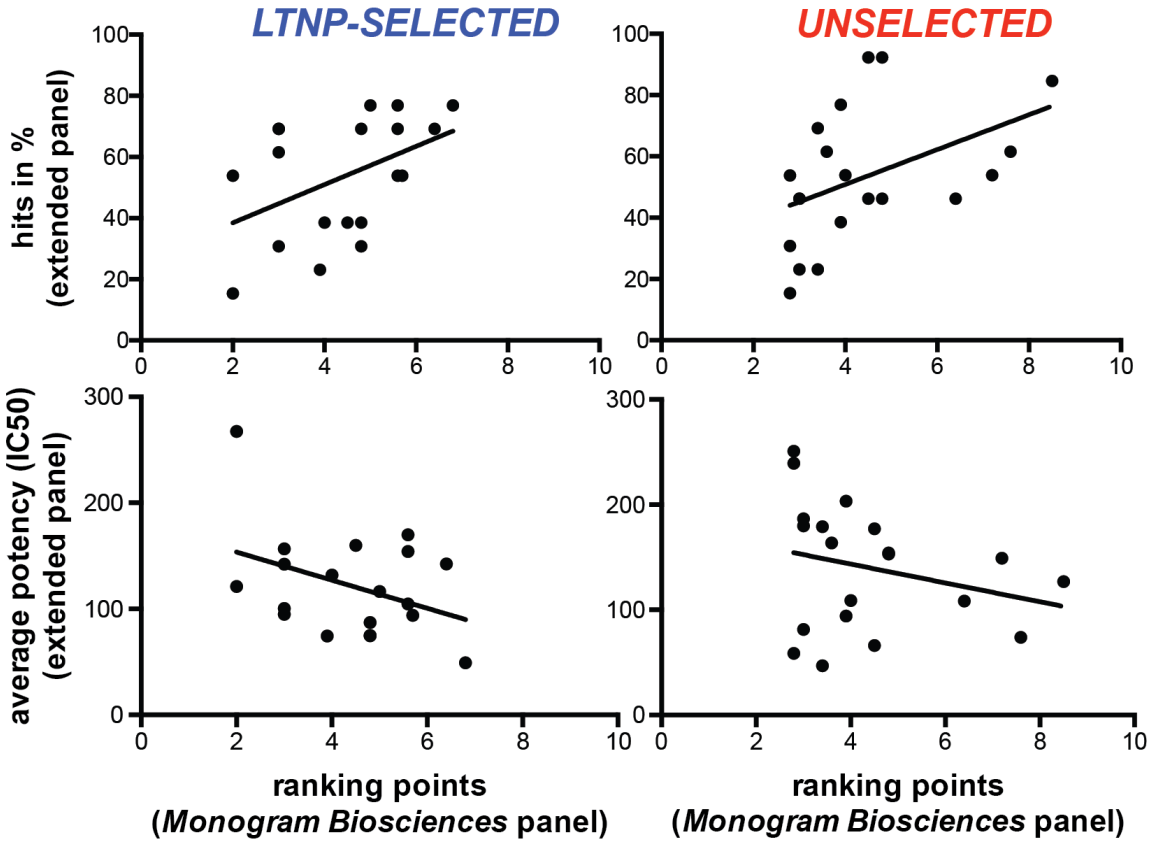

Figure 33 | Predictability of the *Monogram Biosciences* panel. These graphs depict the correlation between neutralization scores on the *Monogram Biosciences* panel and either breadth on the extended panel (upper two graphs) or average potency on the extended panel (lower two graphs). The *LTNP-SELECTED* cohort is presented on the two graphs on the left side and the *UNSELECTED* cohort is presented on the two graphs on the right side. The x-axis in all graphs present the ranking points from 0 to 10 on the *Monogram Biosciences* panel. On the upper two graphs, the y-axis show the percentage of all the 13 strains on the extended panel that were hit with an IC_{50} up to 500 ug/ml, representing breadth on this panel. The average potency achieved by both cohorts on the extended panel is displayed on the y-axis of the lower two graphs.

Since breadth and potency were the most important characteristics to describe the neutralization activity of the individuals' serum samples, it was interesting to see if there

was a correlation between these two variables. Fig. 34 shows a significant association of breadth and potency in the *LTNP-SELECTED* and *UNSELECTED* cohort on the *Monogram Biosciences* panel, with p-values of 0.0022 and <0.0001 respectively, thus having a stronger association in the *UNSELECTED* cohort. Looking at the extended panel, no significant association was seen between breadth and average potency.

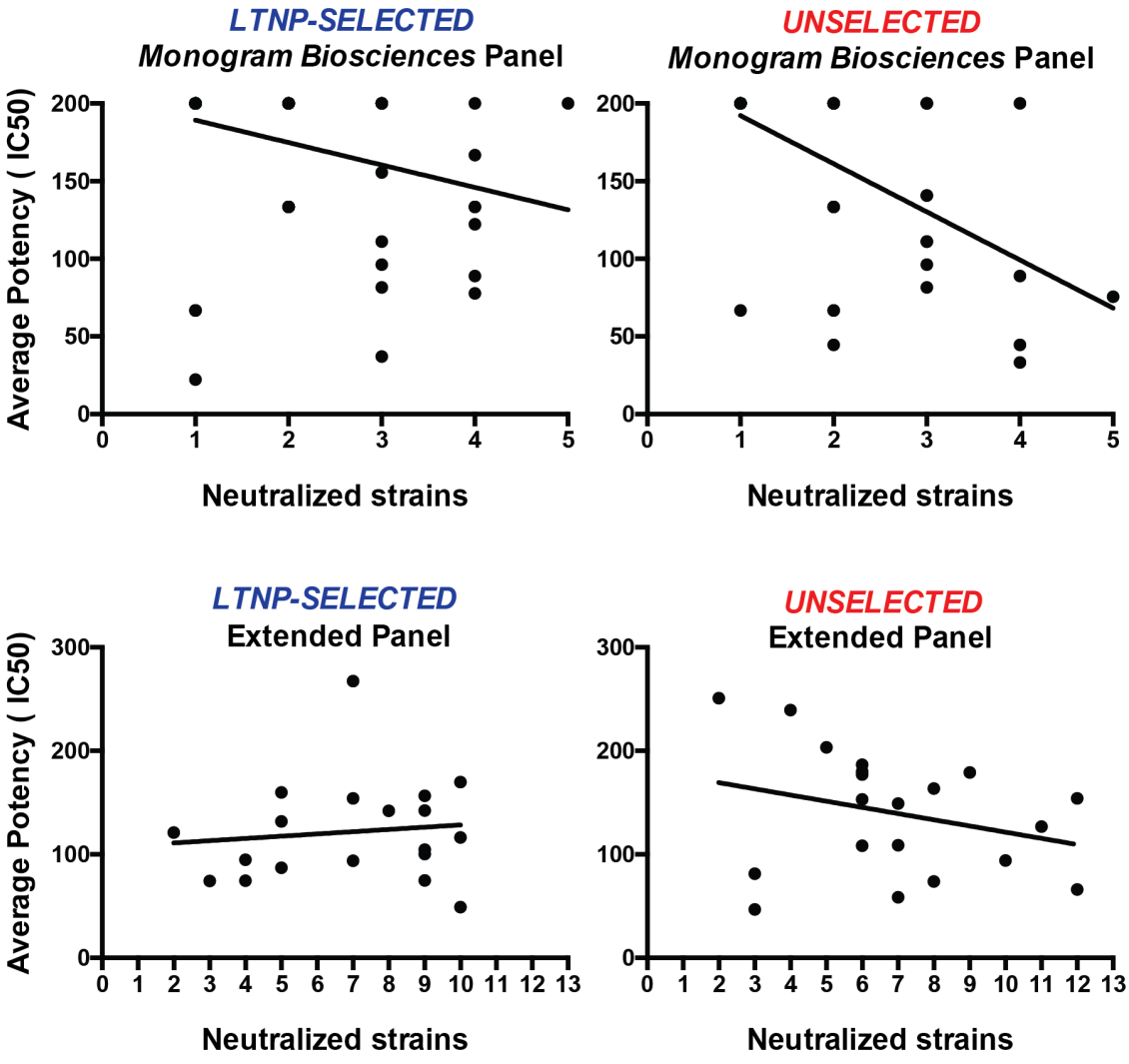

Figure 34 | Breath and potency association. These four graphs show the correlation between breadth and average potency on either the *Monogram Biosciences* panel (upper two graphs) or the extended panel (lower two graphs). Breadth is displayed on the x-axis by the number of strains (5 for the Monogram *Biosciences* panel, 13 for the extended panel) and average potency is depicted on the y-axis with a range of 0 to 200 ug/ml on the upper graphs and 0 to 300 ug/ml on the lower graphs. The *LTNP-SELECTED* cohort is presented on the two graphs on the left side and the *UNSELECTED* cohort is presented on the two graphs on the right side.

In order to compare the performances of both cohorts on the extended panel, the numbers of neutralized strains of each sample were calculated in percentage and the

average of these numbers has been compared (Fig. 35). The average IC_{50}s of all samples has been compared to each other too. With 53 % average neutralized strains in both cohorts and average IC_{50}s of 121ug/ml in the *LTNP-SELECTED* and 140ug/ml in the *UNSELECTED* group (Fig. 35), our top chosen individuals from both cohorts showed similar performances even on the extended panel. Fig. 29 already showed a similar level of performance of both cohorts on the *Monogram Biosciences* panel.

LTNP-SELECTED

	ranking points	Number of strains neutralized	Number of strains neutralized in %	Average IC50
EB_354	6,8	10	76,9	49
EB_167	6,4	9	69,2	142,3
EB_118	5,7	7	53,9	93,8
EB_126	5,6	10	76,9	169,8
EB_360	5,6	7	53,9	154,2
EB_264	5,6	9	69,2	104,7
EB_179	5	10	76,9	116,4
EB_202	4,8	9	69,2	74,9
EB_259	4,8	4	30,8	74,6
EB_261	4,8	5	38,5	87,2
EB_209	4,5	5	38,5	159,8
EB_239	4	5	38,5	131,9
EB_270	3,9	3	23,1	74,3
EB_332	3	8	61,5	142
EB_114	3	4	30,8	94,9
EB_133	3	9	69,2	156,5
EB_245	3	9	69,2	100,4
EB_150	2	7	53,9	267,4
EB_161	2	2	15,4	121
average values			53,4	121,8

UNSELECTED

	ranking points	Number of strains neutralized	Number of strains neutralized in %	Average IC50
CB_309	8,5	11	84,6	126,8
CB_190	7,6	8	61,5	73,8
CB_214	7,2	7	53,9	149
CB_86	6,4	6	46,2	108,4
CB_207	4,8	12	92,3	154
CB_242	4,8	6	46,2	153
CB_165	4,5	12	92,3	66,1
CB_287	4,5	6	46,2	177,1
CB_119	4	7	53,9	108,8
CB_188	3,9	5	38,5	203,3
CB_344	3,9	10	76,9	94
CB_59	3,6	8	61,5	163,5
CB_209	3,4	3	23,1	46,9
CB_331	3,4	9	69,2	179,1
CB_162	3	6	46,2	186,7
CB_101	3	6	46,2	179,9
CB_140	3	3	23,1	81,3
CB_39	2,8	2	15,4	250,8
CB_40	2,8	4	30,8	239,3
CB_118	2,8	7	53,8	58,5
			53,1	140

Figure 35 | Comparison of performance on the extended panel. Numbers of neutralized strains are listed in these two tables for the top individuals of *LTNP-SELECTED* and *UNSELECTED* cohorts. The numbers of neutralized strains on the extended panel which includes 13 strains in total are presented as digits as well as percentage. Of all the hits by one sample, the average IC50 has been calculated and presented here as well. The blue row on the low shows the average values of both neutralized strains in percent and the average IC50 for both cohorts.

Comparing the performance of the *LTNP-SELECTED* individuals on the *Monogram Biosciences* panel with time of infection, it could be seen that the individuals' neutralizing ability becomes better the longer the time of infection dates back. This was shown in Fig. 36 with a correlation between years of infection and ranking points on the *Monogram Biosciences* panel which has significance with a p-value < 0.0001.

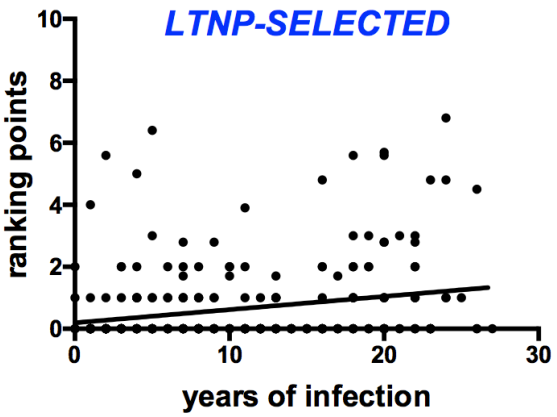

Figure 36 | Time of infection and neutralizing ability of the *LTNP-SELECTED* cohort. The x-axis represents the samples' passed years of infection while the y-axis shows the ranking points scored on the *Monogram Biosciences* panel from 0 to 10. This graph shows a significant correlation between neutralization activity (ranking points) and the passed years since infection (p-value < 0.0001).

Another observation of our data, as seen in Fig. 37, was that individuals without ART have on average a similar neutralizing activity like the treated *UNSELECTED* individuals.

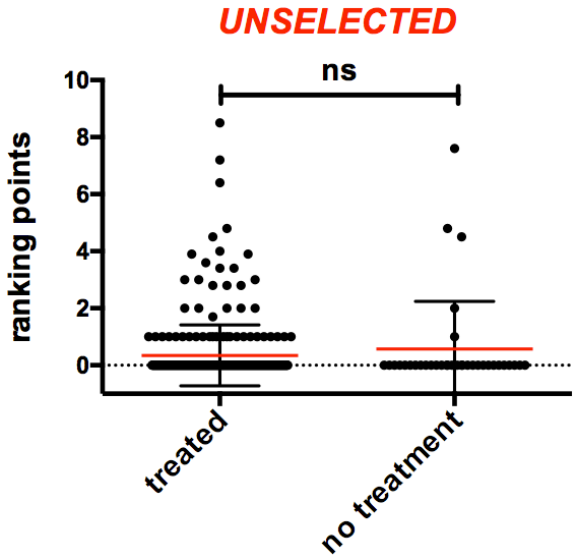

Figure 37 | Neutralizing ability of treated versus untreated *UNSELECTED* individuals. The ranking points of the treated and untreated subgroups within the *UNSELECTED* cohort are plotted separately. There is no significant difference between the means (red lines) of both subgroups (mean values: treated = 0,35; no treatment = 0,57). ns = not significant.

4.4 Mapping of specificity

Another goal of this study was to map the specificities of the individuals' sera from both cohorts. Top individuals from both cohorts have been tested on a panel that included the YU2 wildtype, a clade B virus, and four mutated versions of this strain, namely YU2_N160K, YU2_N280Y, YU2_N332K and YU2_triple. If neutralization was affected by a mutant version compared to the wildtype strain, this could give an idea about the binding sites and thus specificities of the individuals' IgG sample. Fig. 38 presents the neutralization results of both cohorts on the YU2 strain and its mutants.

LTNP-SELECTED						UNSELECTED					
	YU2 and mutants						YU2 and mutants				
	YU2.DG	YU2_ N160K	YU2_ N280Y	YU2_ N332K	YU2 triple		YU2.DG	YU2_ N160K	YU2_ N280Y	YU2_ N332K	YU2 triple
ID	B	B	B	B	B	ID	B	B	B	B	B
EB_354	53,16	43,24	42,84	82,71	27,14	CB_309	47,96	64,53	54,79	44,35	7,78
EB_167	92,21	98,15	83,02	54,71	9,35	CB_190	105,73	151,16	123,87	208,73	33,87
EB_118	359,08	478,53	307,51	>500	36,64	CB_214	139,76	245,52	259,39	258,4	79,88
EB_126	236,76	317,67	371,02	>141	19,73	CB_86	155,37	281,37	180,96	>500	199,47
EB_360	324,01	417,37	127,4	>500	187,32	CB_207	85,89	177,06	106,64	246,72	110,53
EB_264	54,48	67,68	69,45	106,32	26,12	CB_242	45,97	131,59	40,36	171,41	32,07
EB_179	138,11	182,64	>318	119,91	45,27	CB_165	44,4	77,92	138,33	94,6	76,91
EB_202	39,78	45,85	33,03	56,58	6,47	CB_287	>500	>500	227,49	>500	>500
EB_259	>500	>500	>500	>500	109,08	CB_119	95,36	107,82	83,73	82,72	10,63
EB_261	273,19	167,94	72,41	>500	143,9	CB_188	47,6	109,89	30,45	110,66	25,97
EB_209	172,61	168,33	173,07	494,7	36,98	CB_344	193,32	>500	66,71	466,95	117,82
EB_239	91,2	137,7	84,77	142,43	21,1	CB_59	>500	>500	>500	>500	>500
EB_270	>387	>387	>387	>387	64,39	CB_209	105,5	253,15	80,59	436,82	56,41
EB_332	76,9	110,77	89,72	147,78	24,02	CB_331	369,75	>500	221,41	>500	>500
EB_114	>500	>500	>500	>500	21,68	CB_162	107,62	154,14	121,83	132,65	40,45
EB_133	124,8	186,87	193,45	245,05	51,06	CB_101	137,95	189,8	106,42	>500	>500
EB_245	59,02	70,19	>500	82,44	298,5	CB_140	>500	481,56	282,85	>500	180,96
EB_150	242,41	>500	215,19	>500	59,11	CB_39	122,79	289,02	>500	284,83	386,53
EB_161	>500	>500	>500	>500	>500	CB_40	257,55	474,28	>500	444,08	487,79
						CB_118	41,33	62,64	29,41	216,74	162,18

Figure 38 | Neutralization of YU2 and mutants. Neutralization hits indicated as IC50 are presented for the top *LTNP-SELECTED* and *UNSELECTED* individuals with respect to the YU2 wildtype strain as well as the mutants YU2_N160K, YU2_N280Y, YU2_N332K and YU2_triple. The color code is adapted from the one described in Fig. 23 legend: Yellow = low neutralization activity (range 100-500); orange = moderate neutralization activity (range 22-99); red = high neutralization activity (below 22).

While the screening results in Fig. 38 are presented as IC_{50} values, the so-called neutralization 'hits', the neutralization curves in Fig. 39 and Fig. 40 are the extension of these IC_{50} values. They show the percentage of neutralization on the y-axis with each concentration of the individuals' sample after the step-fold dilutions on the x-axis. Thus, the IC_{50}s from each sample can be found on the x-axis where the curve hits the dashed line on the level of 50% neutralization. Each neutralization curve of the top individuals of both cohorts is presented and categorized by binding specificities. The loss of neutralization due to a mutation on the binding site of the virus could give an idea of the antibody specificity of the individuals' sample. Therefore, neutralization of YU2 wildtype is compared to each mutated virus by looking at the area under the neutralization curve (AUC). If the AUC of the neutralization curve of an YU2 mutant was below 90% than that of the wildtype, the area of that mutation has been counted as possible viral binding site of the individuals' antibodies.

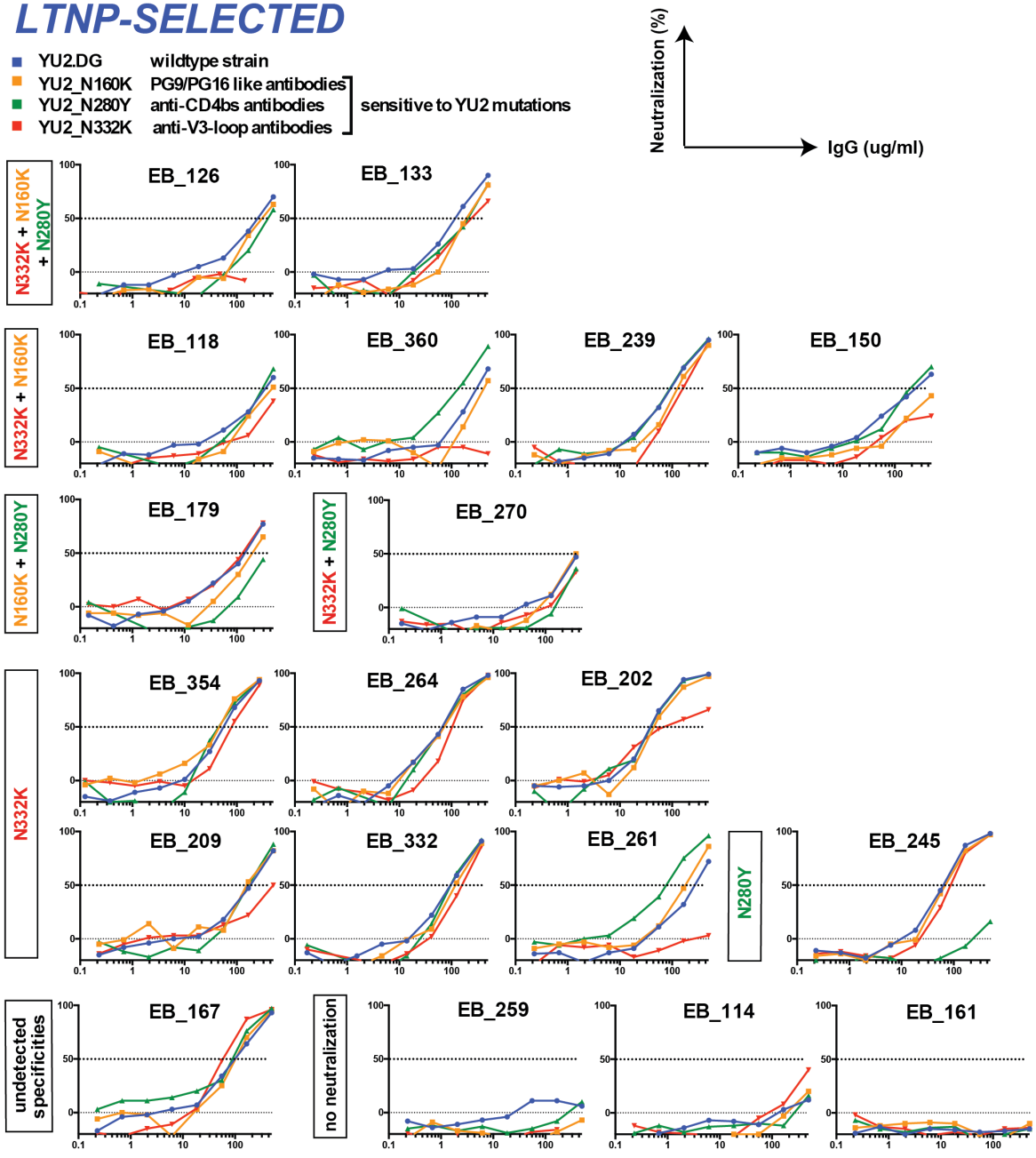

Figure 39 | Specificity mapping *LTNP-SELECTED* Top 19.

For each of the top *LTNP-SELECTED* individuals, a graph has been created in order to show the correlation between the IgG concentration and the neutralization of a YU2 strain and the YU2 mutants. The x-axis in all of the graphs shows the IgG concentration in ug/ml, the y-axis shows the achieved neutralization in percentage. The curves share the same color code in each of the graphs: blue=YU2 wildtype; orange= YU2_N160K; green=YU2_N280Y; red=YU2_N332K. The graphs have been categorized by binding specificities that were determined by looking at the reduction of the AUC of the mutated virus compared to the wildtype virus. If this reduction was at least 90% than that of the wildtype, the mutation of this neutralization curve was considered relevant for effacing viral binding of the individuals' antibodies.

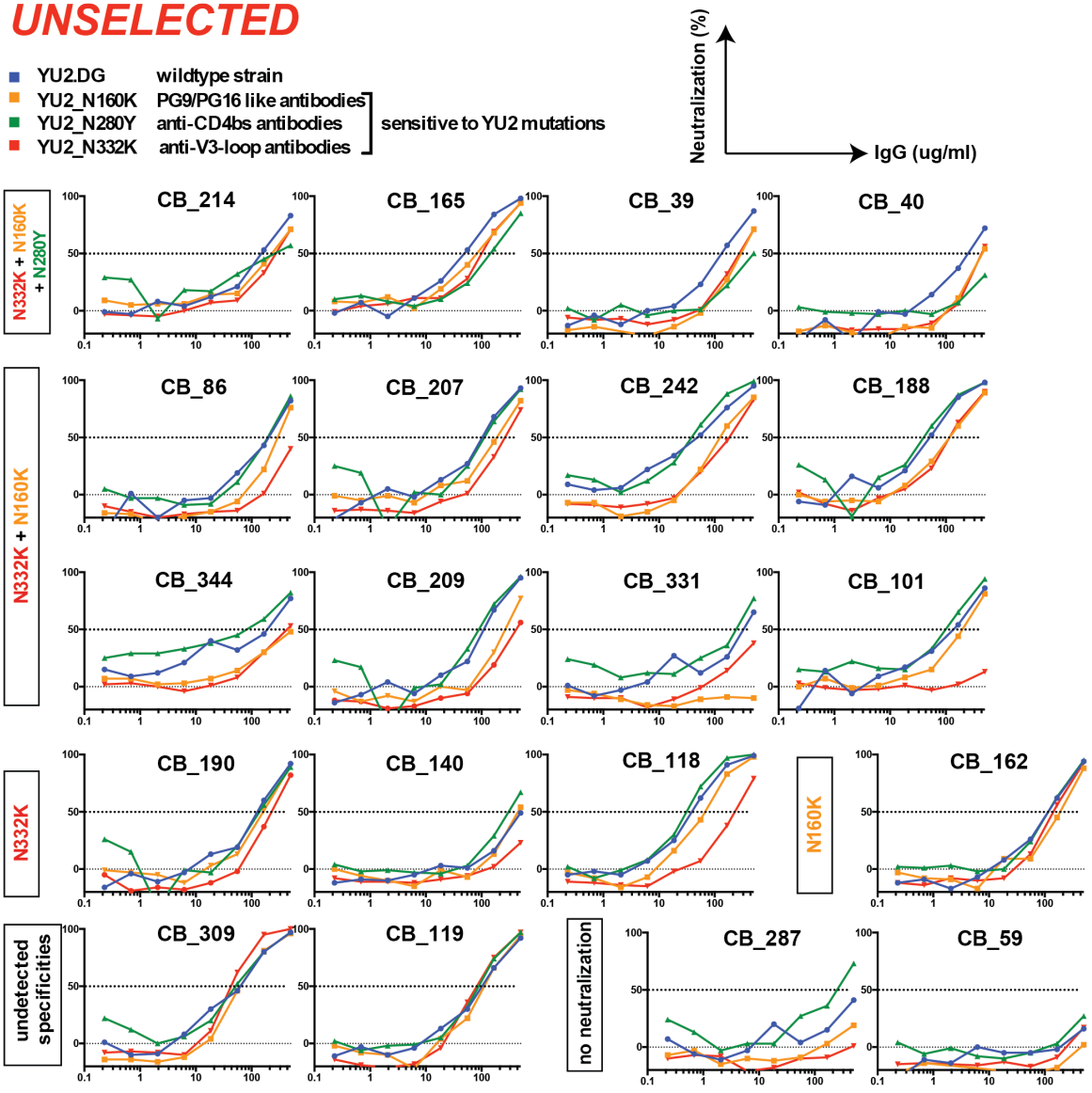

Figure 40 | Specificity mapping *UNSELECTED* Top 20. These graphs present the correlation between the IgG concentration and the neutralization of a YU2 strain and the YU2 mutants for the top *UNSELECTED* individuals. As described in Fig. 39 legend, the x-axis in all of the graphs shows the IgG concentration in ug/ml, the y-axis shows the achieved neutralization in percentage. The color code for the neutralization curves as well as the principle of categorizing the graphs by binding specificities are the same as in Fig. 39.

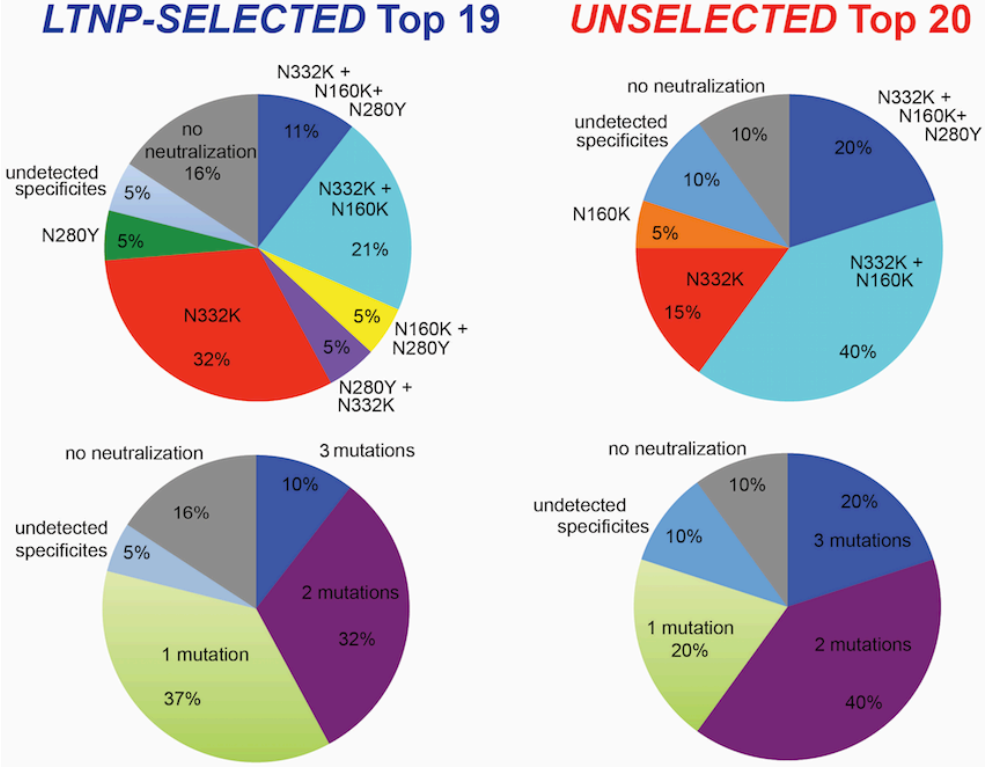

Figure 41 | Comparison of specificity mapping between two cohorts. The categories of binding specificity from Fig. 39 and 40 are presented in these graphs for the top *LTNP-SELECTED* and *UNSELECTED* individuals. The upper two pie charts show the actual combinations of specificities and their portion of the top subjects' samples of that cohort. These results were translated into the lower two pie charts which give an overview about how many mutations are involved in the characterized binding specificities of the individuals' samples.

Fig. 41 visualized the mapped specificities of both cohorts and compared them to each other. It revealed that N332K as only mutation was more prevalent in the *LTNP-SELECTED* than in *UNSELECTED* group whereas the combination of N332K+N160K was the dominating group with 40% in the *UNSELECTED* cohort. The *LTNP-SELECTED* cohort showed a higher heterogeneity of groups that were sensible to specific mutations or mutation combinations. It is worth to mention that the range of subjects that were sensitive to either one, two or three mutations, was covered in both cohorts. Individuals that showed no neutralization with YU2 and its mutants at all or that had specificities completely different than indicated by our chosen mutations constituted only small fractions in both cohorts with each about 20%. Thus, the chosen mutations covered up most of specificities in two different cohorts of HIV-1-infected individuals. Therefore it is a useful tool to categorize new cohorts so that a more specific search for antibodies in these individuals is possible.

5 Discussion

Out of two different cohorts of HIV-1-infected individuals, we identified the 1-2% top individuals that had broad and potent neutralizing activity in their sera. At the same time, we established an effective screening method including a new ranking system to select individuals with best broadly neutralizing serum activity. The cohort analysis helped to generate factors that should be considered in the recruitment of future HIV-1-infected subjects. The mapping of specificities of our top individuals with broad neutralizing activity can be helpful to select the right method for isolating novel bNAbs.

The description of the cohorts showed that both groups differed from each other not only regionally but also in the way they reacted to HIV-1 infection. *LTNP-SELECTED* individuals had lower viral loads and a higher CD4 count number although most of them were either ART naive or off therapy for over a year. In contrast, 90% of the *UNSELECTED* subjects were on therapy. Thus, the stable CD4 cell counts and low viral load of the *LTNP-SELECTED* subjects seemed to be the result of intrinsic mechanisms to control viral replication and cell damaging by HIV-1. It has been postulated that elite and viremic controllers seem to create a strong immune response against HIV-1 that dampens viral replication for a durable time rather than being less susceptive to HIV-1 infection due to their CD4 cells or being infected with replication-defective HIV-1 variants (Saag and Deeks, 2010). This strong immune response could be reflected in Fig. 29 where *LTNP-SELECTED* subjects in average scored higher than *UNSELECTED* subjects who are not viremic or elite controllers. In another study, long-term-non-progressors presented strong neutralizing serum activities and also robust CD8+ lymphocyte responses which both were seen as indicators for the continual exposure of the subjects' immune system to viral antigen (Cao et al., 1995). In this context, it seems logical that within the same cohort, a longer viral exposure leads to better neutralization ability as shown in Fig. 36 for the *LTNP-SELECTED* cohort. However, in the *UNSELECTED* cohort we did not detect a significant difference for the average neutralizing activity between ART treated and untreated individuals (Fig. 37). Hence, the theory of neutralizing activity increasing with viral exposure could not be supported within the cohort investigated. The heterogeneity of medication that *UNSELECTED* individuals have received could be a weak point of our study. Comparisons of treated and untreated individuals could be flawed and it was not clear whether the results in Fig. 37 would have been the same with a homologous treatment regiment of the existing cohort. Additionally, the study delivered the observation that the *LTNP-SELECTED* group included more Black individuals (33% vs. 7%) and more female individuals (28% vs. 12%) than the *UNSELECTED* group. This was consistent with

another study that described that HIV-1-infected individuals with elite control were more likely female and Black than those who were not LTNP (Crowell et al., 2015). Still, it remains to be determined how some HIV-1-infected individuals are able to control the infection over a durable period and others not. Beyond that, correlated factors of broad neutralizing activity were not congruently defined in other studies. Rusert et al. showed that Black individuals induced significantly more broad neutralizing antibody responses than white individuals (Rusert et al., 2016). In contrast, Doria-Rose et. al showed that breadth of sera with HIV-1- neutralizing activity had no correlation to gender, race/ethnicity, age or route of exposure (Doria-Rose et al., 2010).

We chose a small panel for first screening and an extended panel for second screening. The goal was to screen all individuals for neutralizing activity in their sera, rank them with the ranking score and finally screen them for elite neutralizing activity with a more resistant neutralization panel. The *Monogram Biosciences* panel with its five strains 92BR020, 92TH021, 93IN905, 94UG103 and MGRM-C-026 represented a mix of moderately resistant and sensitive strains which allowed to mask off neutralization activity and show the scope of breadth and potency between the samples. Only 29% of *LTNP-SELECTED* subjects and 15% of *UNSELECTED* subjects showed any kind of neutralization on the *Monogram Biosciences* panel. A possible disadvantage of the smaller *Monogram Biosciences* panel could be that some individuals with good neutralizing serum activity were not adequately represented in this first screening and thus completely missed out. But in both cohorts, the individuals with higher ranking points on the *Monogram Biosciences* panel showed a better breadth on the extended panel by trend (Fig. 32). This tendency could be a hint that we did not miss any good samples by the first screening. Following this trend, a sample that did not show any neutralization on the *Monogram Biosciences* panel is not expected to perform better on the extended panel. This emphasizes the sensitivity of the *Monogram Biosciences* panel. Looking at the top individuals out of both cohorts, 19 *LTNP-SELECTED* and 20 *UNSELECTED*, it became visible that the top *UNSELECTED* individuals spanned a higher range of ranking points than *LTNP-SELECTED* ones. Still, the median ranking score on the *Monogram Biosciences* panel of the top individuals of both cohorts was similar. The fact that top individuals out of two completely different cohorts showed the same results on this panel demonstrated that our screening process had the same effect in both cohorts. Thus, our logarithm seems to be universally applicable for screening of HIV-1-infected individuals with broadly neutralizing serum activity. The first group that identified elite neutralizers out of a large cohort representing different clades was Simek et. al. In multiple rounds of

screening process, they reduced the large pseudovirus panel to a five-virus-subset that explained approximately the same variation of neutralization scores in that cohort than with the bigger panel. This made the screening process more effective (Simek et al., 2009). We used exactly the same high-throughput screening panel resulting from this study. MGRM-C-026, also known as clade C virus IAVI_C22, was also included in the panel of Simek et al. Simek et al. suggested that in the future with new antibodies being identified, the panel should be constantly re-evaluated. The underlying principle was that these pseudoviruses should represent a balanced resistance profile against the common antibodies. Since the development of the screening panel, several new broadly neutralizing antibodies have been discovered. Thus, the resistance profile should be tested against these new bNAbs. Our second screening panel derived from a mix of virus envelopes that were categorized based on having high (tier 1A), above- average (tier 1B), moderate (tier 2) or low (tier 3) sensitivity to neutralization by antibodies (Seaman et al., 2010). The selection in our study represented all of these categories in a balanced manner.

We established a new ranking system for the neutralizing ability of serum samples. Different groups that have screened for broadly neutralizing serum activity against HIV-1 used other ranking scores. Weighted average of log-transformed titers across the pseudoviruses of the panel are one possible alternative (Simek et al., 2009). Another group scored neutralization activity of a sample by giving scoring points 0 to 3 for the percentage of neutralization of each virus of an 8-virus-panel and summing up all scores of that sample in order to rank them (Rusert et al., 2016). When discussing the legitimacy of the ranking system, one has to decide if breadth or potency is valued higher than one another. For example, EB_179 had 5 ranking points for neutralizing all five viruses of the *Monogram Biosciences* screening panel with a low potency (IC_{50} of 200ug/ml) while EB_261 only got 4.8 points for neutralizing three viruses, two of them with a high potency (IC_{50}s of 22.2ug/ml). In this case, breadth seemed to be more prioritized than potency. In contrast, EB_118 had 5.7 points for neutralizing only three viruses with high potency (IC_{50}s of 66.7 ug/ml, 22.2 ug/ml and 22.2 ug/ml) which meant that this high potency was prioritized over the breadth of EB_179. The missing assessment of the importance of breadth and potency and also the missing comparison of our ranking system with other alternatives might be limitations of our study. Fig. 29 showed that best individuals from both cohorts performed similar on *Monogram Biosciences* panel while the total sum of all individuals did not. This indicates that our ranking system selected the best individuals from two different cohorts in a reproducible way. Also, comparing both panels 'unranked'

and 'ranked', it could be seen that our ranking system did not miss out on any sample with neutralizing ability. With these tables, it became visually clear that the ranking system gave a comprehensible order to our individuals based on their sera's neutralizing ability. Since this was the purpose of our ranking system, it was sufficient for screening large cohorts of HIV-1-infected individuals and thus, choosing the best individuals from two different cohorts in an effective way.

Simek et al. identified 34% of 463 subjects having broad neutralizing activity and 1% having elite neutralizing ability (Simek et al., 2009). This was consistent with the findings of Doria-Rose et al. who claimed to have identified 30 % of individuals having broad neutralizing serum ability (Doria-Rose et al., 2008). Here, Doria-Rose et al. defined broad neutralizing serum activity as neutralizing four out of five pseudoviruses of their screening panel. Simek et al. defined broad neutralizing serum activity as an IC_{50} titer of at least 100 to at least one pseudovirus from four clade/CRF groups. Furthermore, elite activity was defined in Simek's study as the ability to neutralize more than one pseudovirus at an IC_{50} titer of at least 300 within a clade group and across at least four clade groups. An IC_{50} titer of 300 referred to a threefold dilution of the starting dilution of 1:100 which correlated to our IC_{50} concentration of 66.7 ug/ml after threefold dilution of the starting concentration of 200ug/ml. Transferring Simek's definitions to our results, individuals who showed neutralization with an IC_{50} of at least 200ug/ml to at least one pseudovirus out of the *Monogram Biosciences* panel were categorized as broadly neutralizing individuals. In this manner, 29.6 % of *LTNP-SELECTED* subjects and only 15% of *UNSELECTED* subjects showed broadly neutralizing activity. Following Simek's definition of elite neutralizers, 10 *LTNP-SELECTED* subjects (3.4%) and 8 *UNSELECTED* subjects (2.3%) should be identified as elite neutralizers. But in our study, only 1.8 % of 637 individuals were identified as elite neutralizers. In comparison of the results on *Monogram Biosciences* and extended panel, it could be seen that elite neutralizers did not follow the hierarchy of the ranking points. In both cohorts, the extended panel helped to identify elite neutralizers that performed less effective on the *Monogram Biosciences* panel than non-elite-neutralizers. EB_245 and CB_344 were the best examples for the importance of the second screening panel because otherwise these samples would not have been identified as elite neutralizers. New broad and potent antibodies against HIV-1 have been and are constantly identified due to technological advance in purifying these antibodies from individuals' sera and to a more efficient screening process (Simek et al., 2009). The selection of HIV-1-infected individuals and also our description of screening process might lead to purification of new broadly neutralizing antibodies that recently have been shown to

be effective in the fight against HIV-1. In this regard, the donor EB179, analyzed in a follow-up study, served as the source for the discovery of 179NC75, a glycan-dependent CD4-binding site neutralizing antibody (Freund et al., 2015). Furthermore, broad and potent neutralizing ability of donor EB354 was associated to 3 newly described antibodies that targeted non-overlapping sites on Env (Freund et al., 2017). In this latter study, an observation of serum samples from this donor over a period of 9 years showed that autologous virus strains remained largely sensitive to at least one of these temporarily coincident autologous bNAbs.

In recent clinical trials, it has been shown that current bNAbs may not be as broad or potent as predicted by *in vitro* assays. Therefore, *in vitro* screening methods have to be complemented by new assays to better predict *in vivo* bNAb sensitivity (Caskey, 2020).

A limitation of the first Simek study (Simek et al., 2009) that identified individuals with broadly neutralizing serum activities out of big cohorts has been the lack of knowledge about the specificities of these samples. After identification of broadly neutralizing serum activities, the next crucial step is to determine the specificities of these samples. This allows a more targeted search for broadly neutralizing antibodies since antibodies with different binding characteristics have been purified with the use of different baits. Thus, the mapping of specificities can be informative and helpful in the further search of antibodies. With only 3 mutations, it was possible to locate the specificities of 80% of the top individuals of both cohorts. The specificities of approximately 20% of the top individuals in each cohort could not be narrowed down to one of the present mutations either due to undetected specificities or failing neutralization of the YU2-virus. This is a significant number that should be reduced by using more and different mutated virus strains in the future. Specificities that could not be detected by our mutated viruses are 5% of *LTNP-SELECTED* subjects and 10% of *UNSELECTED* subjects compared to 12% of another study from Landais et al. (Landais et al., 2016). So far, purified bNAbs against HIV-1 helped to gain insight against the main epitopes on the virus targeted by the antibody. With CD4 binding-site (CD4bs), the V3-high mannose patch and the V2 apex, our YU2 mutants covered three important epitopes while two other main epitopes, the gp41 MPER and the gp120/gp41 interface, were not included in our specificity mapping. Landais et al. (Landais et al., 2016) showed that the predominant specificity of their identified broadly neutralizing sera was the V3 loop, also known as glycan supersite and represented in our study by the N332K mutation. The V2 apex was the target of 14% of the cohort's top neutralizing sera. Another significant portion was taken up by the gp120/gp41 interface with 12% while MPER got 2.5%. This could explain the big number of sera in our cohort

whose specificity remains unidentified because we did not use mutations representing the gp120/gp41 interface or the MPER epitope. In the Landais study (Landais et al., 2016), the CD4 binding site took a small portion with 5% while in our cohorts, the presence of the N280Y mutation representing the CD4 binding site varied from 5% to 20%, appearing mostly in combination with other mutations. In our cohort, it was also the V3 loop which was the predominating epitope either standing alone or in combination with other epitopes. Still, 12% of the top sera in the Landais study (Landais et al., 2016) remained unidentified despite mapping with mutations that covered the 5 most targeted epitopes on the HI-virus. With the discovery of more broadly neutralizing antibodies, more epitopes will be identified and thus mutations that are involved in these epitopes will be used in future screenings.

6 Summary

bNAbs present a promising new option in prevention and therapy of HIV-1 infection. Since the clinical use of bNAbs is limited by viral escape through mutations of the HIV-1 envelope glycoprotein (Env), more bNAbs need to be purified in order to unveil more viral vulnerabilities as well as various pathways of HIV-1 escape. Elite neutralizers have been the source of the second generation bNAbs, so it was the goal to identify these individuals within large cohorts of HIV-1-infected individuals.

In the first part of the study, we screened HIV-1-infected individuals out of two diverse cohorts for broadly neutralizing serum activity. The sera of 294 long-term-non-progressors (*LTNP-SELECTED cohort*) and 343 subjects mostly treated with ART (*UNSELECTED cohort*) were tested on the first neutralization assay by *Monogram Biosciences* (*MB*) with 5 different HIV–1 subtypes. With the help of a new scoring system, these sera were ranked based on their neutralizing activity. Subsequently, the top 19 *LTNP-SELECTED* individuals and the top 20 *UNSELECTED* individuals were chosen for a second screening on an extended neutralization panel with a broader set of HIV-1 isolates. Here, 12 individuals were identified as elite neutralizers making up 1.8% out of total 637 individuals. Our results show that the combination of two neutralization panels and the new ranking system can be used to identify individuals with elite HIV-1 neutralizing activity out of diverse cohorts.

In the second part of this study, the neutralization results of both cohorts were analyzed. A cohort's performance on the *MB* panel was associated by trend with greater breadth and higher potency on the extended panel. Another observation was that in both cohorts, breadth and potency of the samples correlated significantly on the *MB* panel but not on the extended panel. The top chosen *LTNP-SELECTED* and *UNSELECTED* individuals showed a similar level of neutralizing activity both on the *MB* and on the extended panel. Furthermore, the analysis of the *MB* panel revealed a significant correlation between neutralizing activity and the passed years since infection in the *LTNP-SELECTED* group. Also on the *MB* panel, no difference of HIV-1 serum neutralization was observed between treated and untreated *UNSELECTED* individuals.

In the third part, we used mutated variants of gp120 in order to narrow down the epitope specificities of the top 39 neutralizers. 80% of the samples had specificities linked to epitopes in the V1/V2 apex, the V3 loop or the CD4 binding site of the HIV-1 envelope. The specificities of 5-10 % of samples remained undetected.

7 Zusammenfassung

bNAbs stellen eine vielversprechende neue Option in der Prävention und Therapie einer HIV-1 Infektion dar. Die Selektion viraler Escape-Varianten durch Mutation des Env-Glykoproteinkomplex erschwert jedoch die klinische Anwendung von bNAbs. Daher sollten mehr bNAbs entdeckt werden, um weitere empfindliche Epitope sowie Escape-Mechanismen des HI-Virus zu beschreiben. Effektive bNAbs der zweiten Generation wurden aus Elite Neutralizern gewonnen, dementsprechend war das Ziel dieser Arbeit, Elite Neutralizer aus großen Kohorten von HIV-1-infizierten Individuen zu identifizieren.

Im ersten Teil der Dissertation untersuchten wurden die Seren von 294 Long-term-non-progressors (*LTNP-SELECTED Kohorte)* und 343 Individuen aus einer größtenteils mit ART behandelten Kohorte (*UNSELECTED)* auf dem ersten Neutralisationsassay von *Monogram Biosciences* (*MB*) mit 5 verschiedenen HIV-1 Subtypen getestet. Mithilfe eines neuen Punktesystems wurden diese Seren in einen Rang eingereiht, welcher die Neutralisationsaktivität berücksichtigte. So wurden die Top 19 *LTNP-SELECTED* und 20 *UNSELECTED* Individuen ausgewählt für das zweite Screening auf einem erweiterten Assay mit einem breiteren Set an HIV-1 Isolaten. Hieraus gingen 12 Individuen als Elite Neutralizer hervor und machten 1,8% der zu Beginn 637 eingeschlossenen Personen dieser Studie aus. Unser Ergebnis bestätigt, dass die Kombination aus beiden Neutralisationsassays und unserem Punktesystem geeignet ist, um Elite Neutralizer in unterschiedlichen Kohorten zu finden.

Im zweiten Teil dieser Studie wurden die Neutralisationsergebnisse beider Kohorten analysiert. Die Neutralisationsleistung auf dem *MB* Assay war tendenziell mit einer höheren Neutralisationsbreite und -potenz auf dem erweiterten Assay vergesellschaftet. Die Analyse der *LTNP-SELECTED* Kohorte auf dem *MB* Assay brachte eine signifikante Korrelation zwischen Neutralisationsaktivität und vergangene Jahre nach Infektion hervor. Zwischen den mit ART behandelten und nicht behandelten *UNSELECTED* Individuen gab es keinen Unterschied in der Neutralisationsaktivität auf dem *MB* Assay.

Im dritten Teil benutzten wir ein Assay mit mutierten Varianten von gp120, um die Bindungsspezifitäten der Top 39 Individuen aus beiden Kohorten einzugrenzen. 80% der Top Seren waren spezifisch für Epitope im V1/V2 Apex, im V3 Bogen oder an der CD4 Bindungsseite des HIV-1 Hüllenproteins. Die Spezifitäten von 5-10% der Seren konnten nicht detektiert werden.

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9 Contribution to this thesis

Blood samples of HIV-1-infected individuals from Boston were **recruited** by Bruce Walker, M.D. and Florencia Pereyra, M.D. at Massachusetts General Hospital in Boston, MA, USA. On the other hand, Professor Dr. Florian Klein, Professor Dr. Clara Lehmann, Professor Dr. Gerd Faetkenheuer from the University Hospital of Cologne recruited blood samples of HIV-1-infected individuals from Cologne, Germany.

The **purification of IgG from plasma samples** from both cohorts was accomplished by Reha-Baris Incesu under the supervision of Professor Dr. Florian Klein.

First screening for HIV-1 infected individuals with broadly neutralizing serum activity was done by the company *Monogram Biosciences Inc.* The neutralization panel and the service of pursuing a high output neutralization assay was provided by the company after Reha-Baris Incesu and Professor Dr. Florian Klein sent them the IgG samples existing in Eppendorf tubes in an ice package. **Second screening** was done by Dr. Michael Seaman in his lab at The Center for Virology and Vaccine Research at Beth Israel Deaconess Medical Center, Harvard Medical School in Boston, MA.

The **ranking system** was developed by Reha-Baris Incesu and Professor Dr. Florian Klein. **Analysis of cohorts and panels** and **mapping of specificity** was done by Reha-Baris Incesu under the supervision of Professor Dr. Florian Klein.

General counsel in writing the manuscript for this thesis was provided by Professor Dr. Florian Klein (Laboratory of Experimental Immunology, Institute of Virology, University Hospital of Cologne) and Professor Dr. Friedrich Koch-Nolte (Laboratory of Molecular Immunology, Institute of Immunology, University Medical Center Hamburg-Eppendorf).

Supervision in the lab was provided by Michel C. Nussenzweig, MD, PhD (Laboratory of Molecular Immunology, The Rockefeller University, New York, USA) and Professor Dr. Florian Klein.

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11 Curriculum vitae

Der Lebenslauf wurde aus datenschutzrechtlichen Gründen entfernt.

12 Publications and presentations

Christoph Würnschimmel, Randi Marisa Pose, Mike Wenzel, Zhe Tian, **Reha-Baris Incesu**, Pierre Karakiewicz, Markus Graefen, Derya Tilki. 2021. Validation of the STAR-CAP clinical prognostic system for predicting biochemical recurrence, metastasis, and cancer-specific mortality after radical prostatectomy in a European cohort. ***European Urology***, 80, 400-404.

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13 Eidesstattliche Erklärung

Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe.

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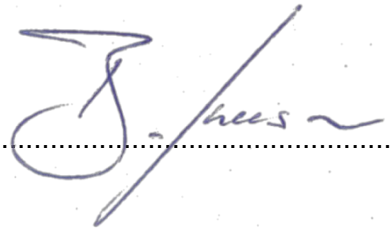
Ich erkläre mich einverstanden, dass meine Dissertation vom Dekanat der Medizinischen Fakultät mit einer gängigen Software zur Erkennung von Plagiaten überprüft werden kann.

Unterschrift: .....  .....