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HIV Vaccine Development: The Importance of Gp120 Variable loop 3 for the survival of the Human Immunodeficiency Virus and the Potency of the Neutralizing Antibody to bind to the Variable Loop 3

Riham Hussien

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Riham Hussien

Master's in human biology

South Dakota State University

Dr.Greg Heiberger

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Table of Content

Abstract

The tendency for the Human Immunodeficiency Virus (HIV) to proliferate and mutate it's trimeric envelop (Env), is one of the reasons why it's a challenge for an antibody-based vaccine to be developed. Glycoprotein 160 (gp160,) which consists of glycoprotein 120 (gp120) and glycoprotein 41 (gp41), is the main focus for a research vaccine design. Gp120 consists of an outer membrane, inner membrane and bridging sheet. The protein is trimeric, and non-covalently binds to gp41. The outer domain is where the neutralizing antibody bind, such as 2G12, immunoglobulin G1b12, and anti-V3 antibodies. V3 stands for the Variable loop 3, gp120 has 5 variable loops that contribute to the binding of the envelope to the CD4 binding sites. These loops are important in immunogenicity and the binding of monoclonal neutralizing antibodies (mNAb). The V3 loop has a conserved structural element despite its variation sequence, and due to this conserved element, the anti-V3 antibodies can recognize and neutralize the virus. The anti-V3 antibodies can tolerate the sequence changes in the V3 loop in certain strains of the virus and at a certain stage of infection in patients, but this is still controversial. This paper will look at the challenges in developing body neutralizing antibodies, and the potency for anti-V3 mature antibody to neutralize and decrease the viral load during infection. It will also cover recent and future vaccine trials using variable loops to directly neutralize viral fusion.

Introduction

Human immunodeficiency virus (HIV) was discovered in 1983 by researchers at the Pasteur institution in France. Since the epidemic began approximately 75 million people have been infected worldwide, 32 million people have died of the infection, and 37.9 million people

are currently living with HIV (CDC, 2019). Since then, researchers have been able to categorize the virus genomic structure as part of the retrovirus family and identify certain characteristics of the virus. HIV is a single-stranded positive RNA, Diploid, containing highly mutagenic glycoproteins, reverse transcriptase (RT) which has a no proof-reading function, integrase (IN), and protease. Only one major vaccine trial that was somewhat successful, with efficacy at preventing infection in approximately 30% of vaccinated individuals. Despite the low efficacy, this trial was a major development in the scientific community, many scientific journals were published based on the outcomes of RV144 vaccine success.

The error-prone reverse transcriptase is one of the major contributing factors to the persistence of the infection, which makes the development of vaccine for the virus exceptionally hard. HIV's viral RNA integrates into the host cell chromosome and enters the germline through vertical transmission. The RT can induce a high mutation rate in the variable domains of gp120, which is the binding site for the CD4 T cells and other antigen-presenting cells.

Gp120 trimeric structure contains multiple variable loops that are involved in the fusion of its envelope with the host's cell membrane. These variable loops are important for body neutralizing antibodies that target gp120. Certain variable loops, if deleted will cause the virus to be susceptible to neuralization but still fuses with the cell membrane, while other variable loop (aka variable loop 3) if neutralized the virus will not fuses with the host's cell transmembrane

Variable loop neutralizing antibodies' potency and efficacy in decreasing viral load depends on the breadth of the antibody. Breadth is important for neutralizing antibodies to recognize its target epitope and neutralize it, the higher the breadth of the antibody the more efficient it is at neutralizing. Researchers were able to increase the potency and the breadth of these antibodies causing them to become polyreactive, where the antibody had low affinity and

became reactive to a variety of unrelated antigens. This polyreactivity, what makes it difficult to engineer a neutralizing antibody that can recognize and specifically bind it's target antigen.

Variable loop 3 structure is variable but has a conserved region if neutralize the viral fusion will be inhibited. The challenges that come with engineering a neutralizing antibody for variable loop 3 is the V1/V2 masks V3 loop, preventing it from being recognized and neutralized. N-glycosylation of V3 can also resist neutralizing antibodies. We will cover recent research regarding the potency and breadth of different neutralizing antibodies, as well as recent vaccine trials and past trials that show promising results for future research in vaccine design.

The Variable loops

Multiple regions within the gp120 glycoprotein are involved in the receptor bindings of the virus, these include the Five variable loops. Variable loop 1, is involved in the syncytium formation of the virus forming a multi-nucleated enlarged cell. The loop is a site for potent neutralizing monoclonal antibodies, there is still much to learn about the loop. The virus is susceptible to neutralizing antibodies upon the deletion of its variable loop 2, but the deletion of V1V2 does not inhibit the virus ability to replicate, the envelope still functions (Saunders,2005)

Variable loop 3, the amino acids of this loop are highly variable, Clade C virus contains a conserved sequence on its V3 crown. The loop is imbedded deep, with limited breadth, despite its strong immunogenicity and its ability to induce monoclonal neutralizing antibody it's very difficult to induce these antibodies due to its limited accessibility. Variable loop 4 (V4) and Variable loop 5 (V5), are poorly defined. V4/V5 are both involved in neutralization escape, they do go through variations during early infection, but they are not a good target for an antibody-based vaccine.

Gp120 fusion and Variable Loop 3 structure/interaction with Cell's Co-receptors

The role of the V3 loop is very essential for coreceptor binding (CCR5 or CXR4), these coreceptors are important for the fusion and the entry of the virus, they interact with the loop through hydrogen binding and occur on the N-terminus of the coreceptor and the extracellular loop, as shown in figure 1. The binding of the coreceptor on the N-terminal usually occurs around the core and the base of the V3 loop. The loop consists of approximately 39 amino acids, it acts as a molecular hook for the coreceptor and it can alternate certain subunits within the viral spike (Huang,2005). The loop can sub-divide into three regions; the Conserved base, where the core's integral portion; flexible stem, extend from the core, Beta-hairpin tip. The way the loop differentiates between the coreceptor is through their charge. The loop will bind to CXCR4 if their 11th or the 25th position is positively charged (Huang,2005). Once the gp120 binds to CD4 a bridging sheet is formed. The variable loop is close enough to the

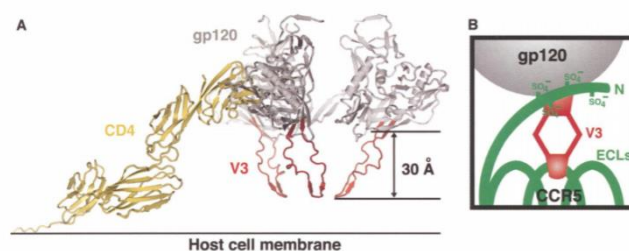Figure 1. Schematic model of the gp120 as it binds to the cell's coreceptors and CD4 binding sites. A) once gp120 bind it brings the V3 loop tip closer to the cell membrane. B) closer representation of the V3 loop as it binds to N terminus of the CCR5 coreceptor (Huang, 2005).

coreceptor for tethering and exposure of the hydrophobic gp41 region for fusion peptide located on the host's membrane.
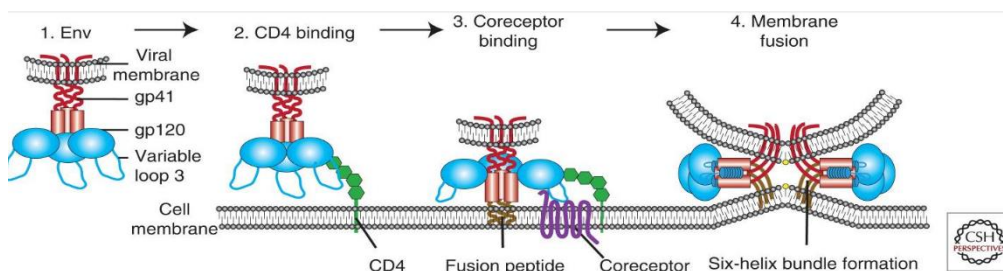
Figure 2. Overview of HIV fusion. CD4bs of gp120 initiate it's binding to the CD4 transmembrane protein. Next the V3 binds to the coreceptor and bringing gp41 hydrophobic region closer to the fusion peptide. Six helix bundle is formed creating a fusion pore (Huang,2005).

A six helical bundle is formed when the amino-terminal region and carboxyl-terminal region are brought closer together forming the bundle and a fusion pore, as shown in figure 2. Where V3 binds to the coreceoper and bringing the fusoin protein closer to gp41. Once the membrane fusion pore is formed and the virus is fused, it will release its viral content into the host's cytosol (Huang,2005).

The Development of Broadly neutralizing antibodies

Neutralizing antibodies(bNAbs) is the major focus for HIV vaccine development. Individuals react differently to the HIV infection. Some might develop extremely broad and potent neutralizing antibodies while others might not. These neutralizing antibodies have a high level of somatic hypermutation, and their long/short antigen-binding loops can be challenging in the development of the vaccine (Landais, 2018). These antibodies can target multiple sites on the HIV-1 envelope, the Variable loops, membrane-proximal external region (MPER) and the CD4 binding sites. Different regions on the envelope can react and bind differently to these antibodies,

some might require a long CDRH3 to penetrate the glycan at the apex of the trimer, while others might require the short loop to avoid steric hindrance (Landais,2018).

The reason why antibodies can recognize multiple variable epitopes is due to their somatic hypermutation, but many of these mutations are neutral with no breadth (breadth, is important for antibodies to recognize their target epitope). Scientists were able to engineer a body neutralizing antibody to achieve enhanced breadth and potency, but this usually resulted in an enhanced polyreactive (Landais,2018) which posed a challenge to vaccine development.

The development of these antibodies depends on a feedback loop, where the increase in the diversity of the envelope leads to an increase in the diversity of the antibody's response, this will result in an exposed bNAb with multiple related antigens over multiple escape pathways. This process can aid in vaccine design through the diversification of the envelope that leads to identifying the breadth of the antibodies. The overall purpose of a body neutralizing antibodies is to neutralize viral effectivity from fusion with the host's transmembrane (Landais,2018).

Challenges of V3 neutralizing antibodies

V1/V2 masking V3 loop

Variable loops of the gp120 play an important role in antibody recognition and epitope masking. V1/V2 mask the V3 loop, it has been proven that the deletion of the V1/V2 loop would enhance the neutralization of the virus by the nAbs. The study was done at the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda (Liu,2011). Where they investigated
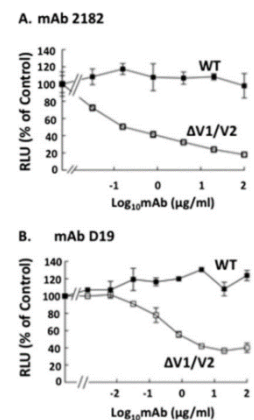

Figure 3. This is a graph that measure viral activity when variable loop 1 and 2 are mutated and neutralized by mature antibody D19 and 2182 (Liu, 2011).

the interaction between two monoclonal antibodies that are subject to mask trimer envelope of gp120. They also looked at whether the masking was on the same protomer or adjacent protomer. They used a firefly luciferase gene, that is used to encodes oxidizing enzymes and a fluorescent measurement. This genome is highly used in HIV neutralization and epitope masking.

The neutralizing antibodies were obtained from individuals who were infected with clade A of the virus, mAb 2182 which binds to V3 loop peptide. 2182 competes with 447-52D other neutralizing antibodies that bind to the tip of the V3 loop. They deleted the V1V2 region and as shown in Figure 3 the deletion resulted in a drastic decrease in the survival of the virus in both mAb2182 neutralizing antibody and the D19 another neutralizing antibody that is generated with immunization with clade B (Liu,2011).

What's different in the clade B is that the inhibition was plateaued at approximately between 63% and 80% this indicates that the V1/V2 loop might not be the only one that is masking the epitope region (Liu,2011). GPGR which is a tetra G protein-peptide varies from virus to virus is found at the V3 tip, it has also been proven that mutation in that region will abolish the mAB D19 antibody sensitivity but also preserver the overall fusion of the virus to the cell.

*N-glycosylation of gp120 and V3 loop causes the resistance of the neutralizing antibodies (*McCaffrey, 2004)

Glycosylation limits the binding of antibodies to their viral epitopes. The genetic drift of the virus cause changes in glycosylation pattern. HIV uses glycan as a shield to protect itself from being recognized by neutralizing antibodies, while still maintaining the ability to recognize and interact with the CD4 binding site. Individuals glycans play different roles in protecting the virus from neutralization. The elimination of the N-linked glycosylation on the amino-terminal and carboxyl of the V3 loop (most HIV strains V3 loops contain single glycosylation site), can silent coreceptor usage and the susceptibility for antibodies to mediate neutralization (McCaffrey, 2004).
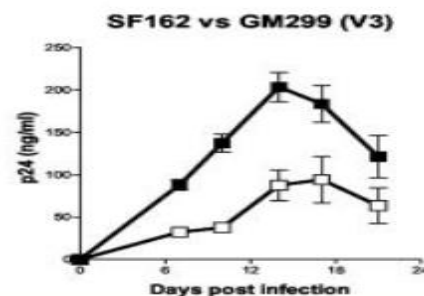

Figure 4. Replication Kinetics in human PBMC. Open boxes mutant viruses, while solid boxes are the parental SF162 (McCaffrey, 2004).

To examine if the elimination of glycosylation from the viral envelope does affect the phenotype. SF162 and HIV type-1 strain is being used to determine the viral load in human peripheral blood mononuclear cells (PBMC) after the elimination of the N-glycosylation in variable loops (McCaffrey, 2004). One thing to keep in mind is certain positions of glycan can impact replication while other residues if deleted the virus can still replicate. Shown in figure 4, GM 299 is a V3 mutated form of the virus, compared to the parental SF162 strain.

The viral load of the mutant V3 significantly decreased compared to the parent SF162. This shows the importance of V3 in viral infectivity(McCaffrey, 2004).
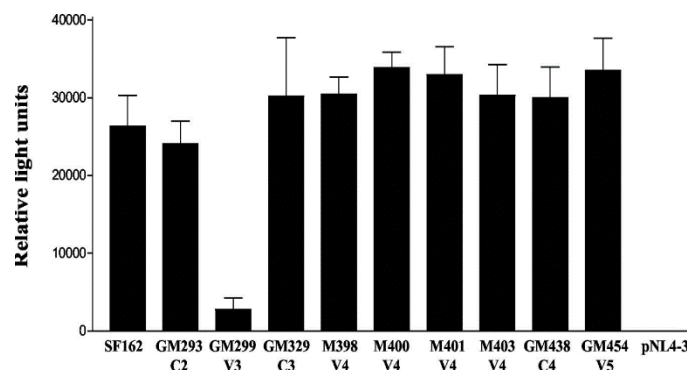
Figure 5. bar graph measure the relative light unites of efficiency of different mutant viruses entering host' cells when different viral

SF162 is unable to enter the cell without V3 binding to Coreceptor, to determine the inability for the virus to enter the host's cell is due to the inhibition of the V3 loop glycosylation and not to the fewer envelope molecules in the GM299 virions (McCaffrey, 2004). An engineered virus that express either parental or the mutant SF162 envelope protein infected U87 human astroglioma cell line, this cell line expresses multiple coreceptors. The Virus is luciferase reporter capable of a single round of replication to either express the parental strain or the mutated strain. As shown in the bar graph below in Figure 5, except for the V3 mutant almost every other mutant the virus was still able to replicate and infect the U87 cell (McCaffrey, 2004).

While the V3 mutant GM299 the virus did not enter the target cell and the relative target units were significantly low compared to the parental SF162 control. This again, evidence that V3 loop glycan has an important role in ensuring the viral entry into the host's cell.

To determine if glycosylation can protect the viral envelope from neutralizing antibodies, a neutralization assay was performed where infectious doses were mixed with diluted Mature Neutralizing antibodies (MAbs) and PBMC was added. They were mixed in a different mutated forms of the virus at mutliple

Table 1. IC90 and IC80 concentrations of Mab that inhibited infection of the virus. The bold indicated that it increased the susceptibility to neutralizing compared to control SF162 (McCaffrey, 2004).

Neutralization of SF162 glycosylation mutants by antibodies

MAb or Fab	Epitope specificity	IC90, IC80 [a]					
		SF162	GM293 (C2)	GM299 (V3)	GM329 (C3)	GM438 (C4)	GM454 (V5)
IgG-CD4	CD4 binding site	5.2, 4.1	0.3, 0.2	0.2, 0.1	1.0, 0.5	1.6, 1.1	1.7, 1.2
IgG1 b12	CD4 binding site	6.1, 4.8	0.3, 0.2	0.9, 0.2	3.3, 2.1	2.0, 1.5	1.7, 1.1
391-95D	V3 loop	—, —	0.3, 0.2	<0.08, <0.08	0.6, 0.3	5.6, 2.7	6.0, 3.0
447D	V3 loop	—, —	3.3, 1.6	0.3, 0.3	1.6, 1.0	—, —	—, —
G3.4	V2 loop	—, —	—, —	—, —	—, —	—, —	—, —
48d	CD4i	—, —	—, —	—, —	—, —	—, —	—, —
17b	CD4i	—, —	—, —	—, —	—, —	—, —	—, —
Fab X5	CD4i	—, —	4.2, 3.1	4.5, 3.3	—, 4.0	—, —	—, —
Fab M18	Conserved, unknown	—, —	—, 6.4	3.7, 2.5	—, —	—, —	—, —
50-69	gp41	—, —	—, —	—, —	—, —	—, —	—, —
2F5	gp41	—, —	7.7, 5.3	—, —	—, —	—, 5.0	—, 6.7

variable loops. As shown in table 1,

Variable loop 3 had signficant value as indicated in bold, where the susceptiblity to neutralize

compare to the parent strain SF162 is greater. It had p value of <.08 indicates a signficant increase in neutralization, due to the V3 mutated form.

Recent Neutralizing Antibodies targeting V3 Loop

10A3/10A37

The Apex crown of the V3 loop is a great HIV vaccine target design. The study which was published by the Department of Biochemistry and Molecular Pharmacology, NYU and Biomedical Sciences at Iowa State University (Ruimin Pan,2017) presented a crystal structure of two neutralizing V3 mature Rabbit antibodies 10A3 and 10A37. These neutralizing antibodies were capable to neutralize the V3 crown region of the HIV-1 strain, they acquired different neutralizing capabilities. Antibody 10A3 binds to the V3 crown and stem region, in a similar fashion to human neutralizing antibodies. What differentiates the 10A3 and the 10A37, is the epitope region where 10A37 binds, it exists as a helical turn as shown in figure 7. This turn seems to increase the breadth and the potency of 10A37, which seems to be a potential vaccine study. The Rabbit neutralizing antibodies they presented were very similar to the human neutralizing antibodies, this makes the rabbit be an excellent animal model for future vaccine candidates.

The binding mode of these antibodies are very similar to human's V3 specific mature antibodies derived from IGHV3 germline as shown in figure 6. Where the darker the color, the higher the antibody antigens contact area.

Figure 6. Comparison of the V3 epitopes of rabbit and human MAbs. Plot showing the distribution and number of contacts formed with the V3 loop by different MAbs (Ruimin Pan,2017).
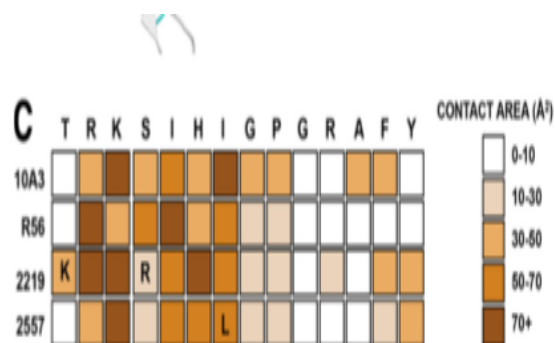
Despite the low amino acids sequence similarity to human antibody germ line, rabbit model can still serve as a useful animal model for future vaccine development.
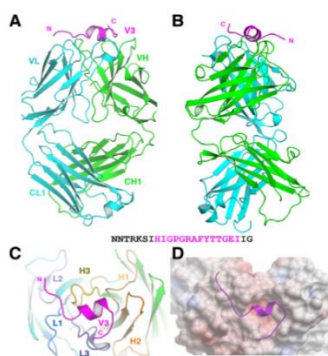
Figure 7. Structure of Fab 10A37 in complex with V3. (A and B) Ribbon representations of the Fab 10A37 in complex with V3(C) The antigen binding site of 10A37 with the CDR loops. (D) Electrostatic potential surface of the antigen binding site of 10A37. (Ruimin Pan,2017)

The breadth and Potency of the Anti-V3 Loop neutralizing antibodies (Hioe,2010)

The conserved element of V3 can bind the host's co-receptor and fuses, despite its variability. Seven different pseudoviruses of HIV-1 strain were tested against anti-V3

neutralizing antibody 2191 on different target cells lines. This demonstrated the ability that an anti-V3-mAb was capable of neutralizing around 42% of these pseudoviruses (Hioe,2010). To determine the potency of the antibodies, an inhibitory concentration (lC50) and the measurement of the titration curve with its slope and area under the curve were calculated. Using two different target cell lines the U87 and the TZM.bl cell line that expressed CD4, CCR5, and CXCR4 on its surface (Hioe,2010). They then were treated with 50ug/ml of diluted mAbs. The luciferase activity was measured after 72 hours of incubation. To measure the titration curve a polynomial regression model was used to best fit the curve. The IC50 value represents the mature antibodies concentration which corresponds with 50% of the neutralization in the titration curve. The area under the curve represents the average neutralization within the concentration range (Hioe,2010).

Now, comparing the Area under the curve and the IC50 values to identify the neutralization activity of the anti-V3 mAbs, as shown in Figure 8 the neutralization curve of the seven pseudoviruses, neutralized by the mAb2191 using U87 as target cell were measured. Significant values were denoted with * after the 50 percent neutralization dash line. The confidence level of neutralization was at a p-value of <.001(calculated by comparing the area under the curve value with negative control and the titration curve slope). The curve was representative against a negative control pseudovirus an MLV and a positive control SF162, as shown in the curve B-011, B-034, B029, A-015, and B-016 were positive,
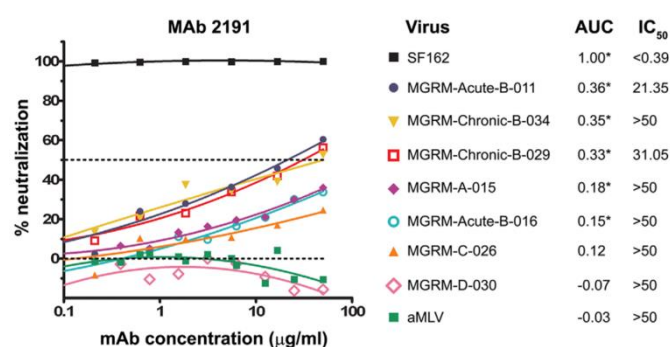

Figure 8. Neutralization curves of the anti V3 mature antibody 2191 against 9 of the pseudoviruses, as well as their IC50 and their Area under the curve, are shown to the right The confidence level of p<.001 which is considered significant and is denoted with * (Hioe,2010).

while the neutralization curves against C-026, D-030, and aMLV are negative and not significant (Hioe,2010).

These polyclonal antibodies cross-react and neutralize the conserved element of the V3 loop. The structural element of the loop has three regions; base attached to the gp120, flexible stem, and a crown where the anti-V3 mAbs recognizes the epitopes. The crown has a conserved features GPG motif, which is located at the tip of the crown in a b-hairpin structure, the mature antibody 447 interacts with this motif in a hydrophobic interaction. The V3 N-terminal will flank the GPG turn rendering the effect of the 447 antibodies. The polar interaction with the side chains in the GPGR motif restricts the activity of the mAb447 (Hioe,2010).

The variability in V3 is restricted at the hydrophilic region of the V3 loop b-hairpin. Regardless of the variable region of the loop, the constant conserved region is what the co-receptor manly needs to participate in binding. The breadth and potency of the anti-V3 mAbs are not that different from other neutralizing antibodies like b12 and 2G12 which has an IC50 value of 35% and 42% against the U87 assay (Hioe,2010). The polyclonal activity of the neutralizing activity against the V3 loop or the binding site for CD 4 has greater breadth than any singly mature antibody (Hioe,2010).

The neutralizing ability of seven anti-V3 mature antibodies and the 860 control against the represented pseudoviruses carrying different Env,
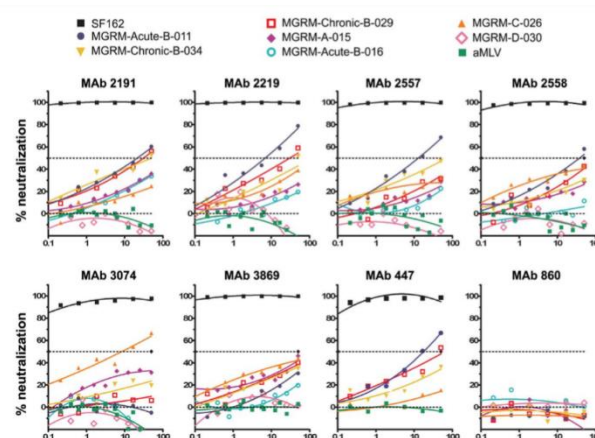

Figure 9. Neutralization curves of different anti-V3 mature antibodies against different HIV pseudo viruses, where U87 is the target cell line. The Seven antibodies were, 2191, 2219, 2557, 2558, 3074, 3869, and 447. Mab 860 is the positive control while the aMLV Env is a negative control (Hioe, 2010).

were observed in figure 9. 100% of antibodies neutralized the positive control SF162 and in all of the trials the antibodies were dose-dependent. During the IC 50 value calculation, it was shown that 32% of the viruses were neutralized by more than one anti -V3 mature antibodies at 50 mg/ml (Hioe,2010).

Also shown, each of these antibodies displayed different patterns of neutralization mAbs 2191 was found to has the highest proportions against pseudovirus and it was observed in both target cell U87 and TZM.bl. approximately 42 % of viruses were significantly neutralized by mAbs 2191 (Hioe,2010). As for the other anti-V3 mAbs, they were also effective in neutralizing 28-36% of the pseudoviruses and they were statistically significant. This show that anti-V3 neutralizing antibodies are capable to mediate neutralization against HIV-1 (Hioe,2010).

Vaccine Trials

RV144 Trial Phase III

The United State HIV research military research unit head the investigation of the RV144 Thai trials (Rerks-Ngarm, 2009), and on the 24th of September 2009 the results were published. This research yielded a P value of 0.04 and vaccine efficacy (VE) of 30%. This value represents how efficient the virus was at preventing the likely of the vaccinated individual to be infected with HIV. The trial had 56,000 people volunteers, out of the 56,000 only 16,000 were vaccinated. Half were placebo and the other half were vaccinated. Primary goal was to prevent further spread of HIV infection, and to decrease the viral load of infected individuals.

These trials took place in North America, Bangkok, and Thailand where they evaluated the efficacy of the gp120 envelope protein-based vaccine by using prim-boost of ALVAC and

AIDSVAX. ALVAC-HIV (vCP1521) is genetically engineered recombinant canarypox version

of HIV genes (env, gag, and pol) linked to transmembrane 3 anchoring portion of gp41.

AIDSVAX, is a genetically engineered gp120 boost. Figure 10 shows that the results from the

trials in the America and Bangkok was ineffective but the trails in Thailand had an estimated VE

of 31% and P value of 0.04 (Peter ,2011). "These results show that development of a safe and

effective preventive HIV vaccine is possible," said Colonel Nelson Michael, Director, Division

of Retrovirology, Walter Reed Army Institute of Research and 99Director, U.S. Military HIV

Research Program (MHRP).

Table 1. Summary of Trial Results for Evaluating Human Immunodeficiency Virus Vaccine Efficacy

Efficacy trial	HIV risk group	Population	Nv (nv)	Np (np)	Estimated VE, %	(95% CI)	2-Sided P value
RV144[1]	General Population	ITT	8202 (56)	8200 (76)	26	(−4 to 48)	.08
Thailand	Mostly at	MITT	8197 (51)	8198 (74)	31	(1−51)	.04
	Heterosexual risk	PP	6176 (36)	6366 (50)	26	(−13 to 52)	.16
	61% Men						
Vax004[7]	MSM	MITT	3598 (241)	1805 (127)	6	(−17 to 24)	.59
North America	and Women; 94% Men	PP	3330 (191)	1679 (98)	4	(−23 to 24)	.77
Vax003[8]	Injection Drug	MITT	1267 (106)	1260 (105)	0	(−31 to 24)	.99
Bangkok	Users; 93% Men	PP	1193 (86)	1167 (79)	−8	(−46 to −21)	.64
Step[12]	MSM;	MITT	914 (49)	922 (33)	−50	(−141 to 5)	.07
Americas	100% Men	PP	835 (41)	840 (23)	−60	(−160 to 1)	.05

Figure 10. The vaccine trials took place at Thailand, North America, Bangkok, and Americas. The HIV

risk group were the general population who were mostly at heterosexual risk 61%. Men having sex with

other Men (MSM)and women ;94%. Men injection drug users were 93%. The Thailand trial had 2-sided

P value of 0.04 compared to the rest of the countries where their P value was between 0.05 (Americas)

and 0.99 Bangkok (Peter ,2011).

The vaccine was administered at 0 week, 4 weeks, 12 weeks, and 24weeks. A booster vaccine was given at each of the four visits, the individuals were monitored under Division of Acquired Immunodeficiency Syndrome of the National Institute of Allergy and Infectious Diseases (DAIDS). Subjects reported local and systemic vaccine reaction. Serious adverse reactions were reported and graded on a scale used for rating adverse events associated with vaccines.

Figure 11. Of the ntention-to- treat analysis group (A) all vaccinated individuals, VE of 26.4%. Per-protocol (B)particpanets who were eligbale to containue further tirals, VEof 26.2%. Modified intention-to-treat (C) excluded those who were tested positive after screeening but before vaccination, VE of 31% (Rerks-Ngarm, 2009).
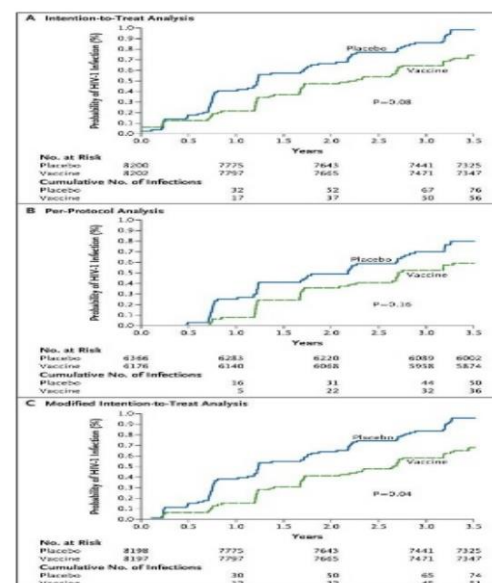
The results of Figure11 had the intention-to- treat analysis group, they involved all those who were picked for vaccination and they had a vaccine efficacy of 26.4%. In the per-protocol analysis involved particpanets who were eligbale to containue further tirals, they had a vaccine efficay of 26.2%, as for the modified intention-to-treat analysis involved every particpant excluding those who were tested positive after screeening but before vaccination, they had vaccine efficacy of 31%. Third grpah of figure 11 (Rerks-Ngarm, 2009) was the most relevant of this study, that group was 31% more likely to be protected against the virus than the intention to treat analysis group and the per protocol analysis group. This trial identfied the correlation between IgG antibody binding to the variable loops of HIV Env decreased risk of transmission, and plasma IgA to Env decreased vaccine efficacy.

Another study was done in 2012 and developed by the Pox-Protein Public Private Partnership. The vaccine efficacy results will be out in 2020. HVTN702 2b/3 phase trial was a build on the RV144 trial. The trial test the subtype C prime-boost vaccine efficacy in South Africa. They have a threshold requirement for advancement; at what magnitude did gp120 Env binds to the antibodies, prevalence of specific V1V2-directed binding antibodies, and Env-specific CD4 T-cell binding (Russell,2016).
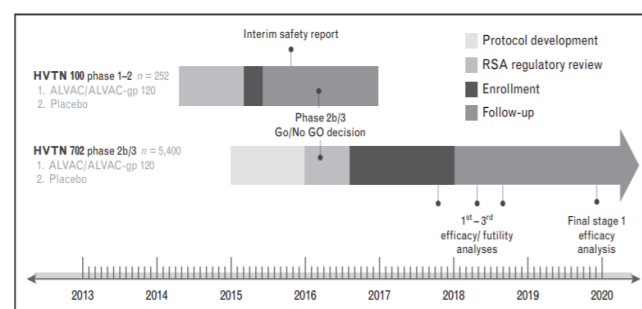

Figure 12. The timeline for HVTN HIV vaccine trial. Protocol review, regulatory review, enrollment and follow up which end in 2020.

Future course of the current partially effective vaccine in hand (Phillips,2014)

A calculated study was done in Southern Africa (Phillips,2014) to measure the effect of antiretroviral therapy and the partially effective vaccine in hand (developed in the RV 144 trial) was measured over the next 45 years. The vaccine with a 30% efficacy rate at preventing HIV infection projected 67% lower HIV incidence in 2060. The vaccine will also prevent an 8% reduction in death rate in the next 45 years, this was calculated using multivariable uncertainty analysis.

The study was an individual-based model to investigate the impact of prophylactic vaccines in developing country settings. This projected 67% decline in future infection can protect the population in a herd immunity form. The study also predicted if a vaccine had a 50%

but no effect on the viral load, will not have much impact on prevention compared to no vaccine (Phillips,2014).

The number of HIV cases been lower in the past decade due to Antiviral medication and educational awareness in the different regions around the world. Therefore despite the low efficacy the RV144 prevented infection, it can prevent 8% of future death tolls.

Conclusion

Although researchers have not yet developed a vaccine for HIV, the achievement that been accomplished in learning about the virus is remarkable. Variable loops of gp120 are essential for coreceptor binding (CCR5 or CXR4), these loops interact with the host cell through hydrogen bonding on the N-terminus of the coreceptor. The loops will sub-divide and bind to the coreceptor, where a bridging sheet will form causing the tethering and exposure of the hydrophobic gp41 region. what researchers were able to discover, is the inhibition of the variable loops through neutralizing antibodies will neutralize the virus and decrease in viral load.

The development of these neutralizing antibodies can be a challenge, they have a high level of somatic hypermutation and different breadth levels. Engineering these antibodies to increase their breadth level can lead to polyreactivity where the antibody becomes reactive with multiple antigens at once. Variable loop 3 is a good target to completely neutralize the virus despite its variability, its crown region contains a constant and conserved region that if neutralize viral fusion will be inhibited. N-glycosylation and the V1/V2 can prevent the recognition of the V3 loop by anti-V3 mAbs.

Variable loop 3 is masked by variable loop 1 and 2, as shown in the previous study that was done by the national institute of allergy and infectious diseases. They obtained mature

antibody 2182 from an infected individual with clade A, which binds to V3 loop peptide. The V1V2 loop was deleted, resulted in a drastic decrease in the survival of the virus in both the mAb 2182 neutralizing antibody and the D19 antibody (another neutralizing antibody).

N-glycosylation of gp120 and V3 loop causes the resistance of neutralizing antibodies. The mutated form of N-glycosylation did indeed decrease significantly compared to the non-mutated form. The mutation of the glycosylation resulted in a more efficient way to neutralize the V3 loop as shown in Table 1 where the IC90 and the IC80 showed significant p value of <.08. GM299 is the V3 glycosylation mutant form, which is important for the survival of the virus and its neutralization, and it's mutation increase it's neutralization by V3 nAb.

In the past decades, multiple neutralizing antibodies were discovered that neutralizes the V3 loop. A study was done at NYU and ISU found two mature Rabbit antibodies 10A3 and 10A37 that neutralized aV3 loop, specifically the crown of the loop region 308HIGPGRAFYTTGEI323, these antibodies had different properties from one another, 10A37 can neutralize better than 10A3 due to its structural properties. They analyzed the Fab/peptide structure of these anti V3 neutralizing antibodies and defined their properties at the atomic level.

They are very similar in structure except for the 10A37 target the C- terminal end of the V3 crown, and is slightly complex due to its hairpin structure, this structure seems to play a role in the potency of the antibody. The main take away of the study was to compare these neutralizing antibody capabilities to neutralize the V3 loop compare to human neutralizing antibodies. The Rabbit antibodies were very similar to human antibodies which make them a good animal model for further vaccine design.

To further investigate the importance of the Anti-V3 mature antibodies and their capabilities in neutralizing the fusion of the viral envelope. A study was conducted to test nine more neutralizing antibodies against the V3 loop, some of those antibodies had different neutralizing capabilities. mAb 2191 had the highest potency than the other neutralizing antibodies. The IC50 and the area under the curve were statistically significant with a p value of <.08 The neutralization efficiency of the strain increased due to the mutation in the V3 loop.

The final take away of this paper is to realize the importance of mature neutralizing antibodies and their role in neutralizing the viral envelop from being fused with host' cells. Multiple studies were able to provide evidence of these antibodies to neutralize HIV strains. There are still challenges that face research in designing antibodies that can match the ever-changing structural envelope of HIV. Anti-V3 mature antibodies that can neutralize variable loop 3, targeting V3 is a promising vaccine research design.

These mAbs showed great potency and breadth in inhibiting the fusion of the viral envelope with the host's cell. Despite the immunogenicity of the V3, there come multiple challenges that prevent it from being recognized by Anti-V3 mAbs, including V1/V2 masking and N-glycosylation.

RV144 was a vaccine trial that was directed towards the V1/V2 region of gp120, where it showed some protective measures against the virus. It had 30% preventable efficacy against the transmission of HIV-1 strain. Variable loops regions are important to constitute in the process of viral integration into the host's cell. Despite the capabilities of anti V1/V2 neutralizing antibodies to prevent infection in 30% of the population. For future vaccine research design, focusing on variable loop 3 antibodies and improving their potency and their breadth to

neutralize viral fusion, without compromising them to become polyreactive might show better vaccine efficacy.

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References

1- HIV/AIDS. (2019, August 19). Retrieved from https://www.who.int/gho/hiv/en/.

2- Peter B. Gilbert,1-2 James 0. Berger.. Victor G. DeGruttola8. Statistical Interpretation of the RV144 HIV Vaccine Efficacy Trial in Thailand: A Case Study for Statistical Issues in Efficacy. (2011, April 1). Retrieved from: https://www.nejm.org/doi/suppl/10.1056/NEJMoa0908492/suppl_file/nejm_rerks-ngarm_2209sa1.pdf

3- Rerks-Ngarm, S., Pitisuttithum, P., Nitayaphan, S., Kaewkungwal, J., Chiu, J., Paris, R., … Kim, J. H. (2009). Vaccination with ALVAC and AIDSVAX to Prevent HIV-1 Infection in Thailand. *New England Journal of Medicine*, *361*(23), 2209–2220. doi: 10.1056/nejmoa0908492

4- Rolland, M., & Edlefsen, P. T. (n.d.). Increased HIV-1 vaccine efficacy against viruses with genetic signatures in Env-V2. doi: 10.3897/bdj.4.e7720.figure2f

5- German Advisory Committee Blood (Arbeitskreis Blut). (2016). Human Immunodeficiency Virus (HIV). Retrieved from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4924471/pdf/tmh-0043-0203.pdf

6- Ruimin Pan,a Yali Qin,b Marisa Banasik,b William Lees,c Adrian J. Shepherd,c Michael W. Cho,b Xiang-Peng Konga. (2017). Increased Epitope Complexity Correlated with Antibody Affinity Maturation and a Novel Binding Mode Revealed by Structures of Rabbit Antibodies against the Third Variable Loop (V3) of HIV-1 gp120. Retrieved from: https://www.ncbi.nlm.nih.gov/pubmed/29343576file:///C:/Users/Riham/Documents/e01894-17.pdf

7- Catarina E. Hioe, Rajnish Kumar. (2018). Modulation of Antibody Responses to the V1V2 and V3 Regions of HIV-1 Envelope by Immune Complex Vaccines. Retrieved from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6212562/

8- Penny L. Moore, Elin S. Gray.(2011). Potent and Broad Neutralization of HIV-1 Subtype C by Plasma Antibodies Targeting a Quaternary Epitope Including Residues in the V2

Loop. Retrieved from:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3067856/?report=reader

9- Mangala Rao, Kristina K. Peachman, [...], and Venigalla B. Rao. (2013).HIV-1 Variable Loop 2 and its Importance in HIV-1 Infection and Vaccine Development. Retrieved from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4086350/

10- von Bredow B, Andrabi R, Grunst M, Grandea AG 3rd, Le K, Song G, Berndsen ZT, Porter K, Pallesen J, Ward AB, Burton DR, Evans DT. (2019). Differences in the Binding Affinity of an HIV-1 V2 Apex-Specific Antibody for the SIVsmm/mac Envelope Glycoprotein Uncouple Antibody-Dependent Cellular Cytotoxicity from Neutralization.Retrived from: https://www.ncbi.nlm.nih.gov/pubmed/31266872

11- Shang-Rung Wu, Robin Loving, Birgitta Lindqvist, Hans Hebert, Philip J. B. Koeck, Mathilda Sjdberg, and Henrik Garoff.(2010). Single-particle cryoelectron microscopy analysis reveals the HIV-1 spike as a tripod structure. Retrieved from: file:///C:/Users/Riham/Downloads/HIV%20article%2014.pdf

12- Haoqing Wanga, Alexander A. Cohena, Rachel P. Galimidia, Harry B. Gristicka, Grant J. Jensena,b, and Pamela J. Bjorkmana,1 (2016). Cryo-EM structure of a CD4-bound open HIV-1 envelope trimer reveals structural rearrangements of the gp120 V1V2 loop. Retrieved from: file:///C:/Users/Riham/Downloads/HIV%20article%207.pdf

13- Russell ND, Marovich MA, Pox-Protein Public Private Partnership program and upcoming HIV vaccine efficacy trials. Current Opinion in HIV and AIDS. 2016 November 6; volume 11.

14- Maxime Moulard, Sanjay K. Phogatt, Yuuei Shut, Aran F. La Mei-Yun Zhang, Igor A. Sidorov, Christopher C. Broder, James Dennis R. Burton, and Dimiter S. Dimitr. (2001). Broadly cross-reactive HIV-1- neutralizing human monoclonal Fab selected for binding to gp120-CD4-CCR5 complexes. Retrieved from: file:///C:/Users/Riham/Downloads/HIV%20article%2013.pdf

15- Daniela Fera3, Aaron G. Schmidt3, Barton F. Haynes , Feng Gao , Hua-Xin Liao , Thomas B. Keplerc, and Stephen C. Harrison (2014). Affinity maturation in an HIV broadly neutralizing B-cell lineage through reorientation of variable domains. Retrieved from: file:///C:/Users/Riham/Downloads/HIV%20article%2012.pdf

16- Hui Lia, Shuyi Wanga, Rui Konga, Wenge Dinga, Fang-Hua Leea, Zahra Parkera, Eunlim Kima, Gerald H. Learna, Paul Hahna, Ben Policicchiob, Egidio Brocca-Cofanob, Claire Deleagec et. (2016). Envelope residue 375 substitutions in simian–human immunodeficiency viruses enhance CD4 binding and replication in rhesus macaques. Retrieved from: file:///C:/Users/Riham/Downloads/HIV%20article%2010.pdf

17- Jean-Philippe Juliena,b,c,1, Jeong Hyun Leea, Gabriel Ozorowskia, Yuanzi Huaa, Alba Torrents de la Peñad, Steven W. de Taeyed, Travis Nieusmaa, Albert Cupoe, Anila Yasmeene, Michael Golabeke, Pavel Pugache et.(2015). Design and structure of two HIV-1 clade C SOSIP.664 trimers that increase the arsenal of native-like Env immunogens. Retrieved from: file:///C:/Users/Riham/Downloads/HIV%20article%2011.pdf

18- S. Munir Alam, S. Moses Dennison, Baptiste Aussedatd, Yusuf Vohrad, Peter K. Parkd, Alberto Fernéndez-Tejadad Shelley Stewart, Frederick H. Jaeger, Kara Anasti, Julie H. Blinn, Thomas B. Kepler, Mattia Bonsignori. (2013). Recognition of synthetic glycopeptides by HIV-1 broadly neutralizing antibodies and their unmutated ancestors. Retrieved from: file:///C:/Users/Riham/Downloads/HIV%20article%208.pdf

19- TIMOTHY K. HARTt, RICHARD KIRSHt, HARMA ELLENSt, RAYMOND W. SWEET?, DENNIS M. LAMBERT?, STEPHEN R. PETTEWAY, JR.?, JEFFRY LEARY?, AND PETER J. BUGELSKI. (1990). Binding of soluble CD4 proteins to human immunodeficiency virus type 1 and infected cells induces release of envelope glycoprotein gp120. Retrieved From: file:///C:/Users/Riham/Downloads/HIV%20article%209.pdf

20- James M. Kovacsa, Joseph P. Nkololac, Hanqin Penga, Ann Cheungc, James Perryc, Caroline A. Miller, Michael S. Seaman, Dan H. Barouch, and Bing Chena. (2012). HIV-1 envelope trimer elicits more potent neutralizing antibody responses than monomeric gp120. Retrieved From: file:///C:/Users/Riham/Downloads/HIV%20article%206.pdf

21- Saunders, C. J., McCaffrey, R. A., Zharkikh, I., Kraft, Z., Malenbaum, S. E., Burke, B., … Stamatatos, L. (2005, July 1). The V1, V2, and V3 Regions of the Human Immunodeficiency Virus Type 1 Envelope Differentially Affect the Viral Phenotype in an Isolate-Dependent Manner. Retrieved from https://jvi.asm.org/content/79/14/9069#abstract-1

22- Huang, C.-chin, Tang, M., Zhang, M.-Y., Majeed, S., Montabana, E., Stanfield, R. L., … Kwong, P. D. (2005, November 11). Structure of a V3-containing HIV-1 gp120 core. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2408531/

23- Landais, E., & Moore, P. L. (2018, September 5). Development of broadly neutralizing antibodies in HIV-1 infected elite neutralizers. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6125991/

24- Liu, L., Cimbro, R., Lusso, P., & Berger, E. A. (2011, December 13). Intraprotomer masking of third variable loop (V3) epitopes by the first and second variable loops (V1V2) within the native HIV-1 envelope glycoprotein trimer. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3250183/

25- McCaffrey, R. A., Saunders, C., Hensel, M., & Stamatatos, L. (2004, April). N-linked glycosylation of the V3 loop and the immunologically silent face of gp120 protects human immunodeficiency virus type 1 SF162 from neutralization by anti-gp120 and anti-gp41 antibodies. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC371088/

26- Hioe, C. E., Wrin, T., Seaman, M. S., Yu, X., Wood, B., Self, S., … Zolla-Pazner, S. (2010, April 21). Anti-V3 monoclonal antibodies display broad neutralizing activities against multiple HIV-1 subtypes. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2858080/

27- Phillips, A. N., Cambiano, V., Nakagawa, F., Ford, D., Lundgren, J. D., Roset-Bahmanyar, E., … Van Effelterre, T. (2014, September 10). Potential future impact of a partially effective HIV vaccine in a southern African setting. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/25207973

28- Susan Zolla-PaznerPing ZhongKathy ReveszBarbara VolskyConstance WilliamsPhillipe NyambiMiroslaw K. Gorny, Zolla-Pazner, S., Zhong, P., Revesz, K., Volsky, B., Williams, C., … Felsövályi, K. (2004, November 19). The Cross-Clade Neutralizing Activity of a Human Monoclonal Antibody Is Determined by the GPGR V3 Motif of HIV Type 1. Retrieved from https://www.liebertpub.com/doi/abs/10.1089/aid.2004.20.1254

29- Walker, L. M., Huber, M., Doores, K. J., Falkowska, E., Pejchal, R., Julien, J.-P., … Poignard, P. (2011, September 22). Broad neutralization coverage of HIV by multiple highly potent antibodies. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/21849977

30- Rubens, M., Ramamoorthy, V., Saxena, A., Shehadeh, N., & Appunni, S. (2015). HIV Vaccine: Recent Advances, Current Roadblocks, and Future Directions. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4633685/#B58

31- Zolla-Pazner, S. (2014, July 11). A critical question for HIV vaccine development: which antibodies to induce? Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/25013066