

# Conceptual Framework and Strategy for Designing Population-specific Epitope-based HIV-1 CTL Vaccine- a Proposal for the Nigerian Population

## Abstract

HIV infection has remained a global health concern since its discovery over forty years ago. Although antiretroviral (ARV) drugs have changed the initial damning prognosis, the need to prevent new infection is still as pressing as ever because individuals who have achieved viral suppression on Antiretroviral therapy have an additional concern of more susceptibility to non-AIDS-defining illnesses than the general population. This is attributable to the progressive immunological dysfunction in these individuals, a pathology that current therapeutic options for HIV management cannot ameliorate. Vaccination, which is the game-changing intervention for most communicable diseases, is constrained by the peculiar molecular bottleneck created by HIV hypermutability, making vaccine development quite challenging. This article reviews the dynamics of these challenges and makes a case for a customized vaccine development approach that leverages the insights gained from the *HLA* profiles of the HIV elite controllers. It then suggests a conceptual framework for developing a population-based CTL epitope-based HIV-1 vaccine using the Nigerian population as a model.

**Keywords:** HIV-1 vaccine, Human leucocyte antigen (HLA), genetic diversity, epitope-based vaccine

## Introduction

Human Immunodeficiency Virus (HIV) primarily infects the CD4<sup>+</sup> cells (T-lymphocytes, macrophages, and dendritic cells), critical to effective immune responses. The ensuing depletion of the CD4<sup>+</sup> T-cell pool leads to progressive and ultimately profound immune suppression (Fields et al., 2007). The advent of effective combination antiretroviral therapy (cART) has significantly slowed down the progression of the disease and improved the quality of life of most affected individuals.

However, resistance to ART is an ongoing concern, necessitating identifying new treatment targets and therapeutic options, including the presently unrewarding efforts to design an effective vaccine. Several obstacles stand in the way of developing a protective vaccine against HIV-1 infection. Firstly, because natural immunity fails to prevent or eradicate the infection, there are no clear immune correlates of protection in human studies that can provide a template for vaccine development (Pantaleo & Koup, 2004; Plotkin, 2008). Specifically, it is still unclear which type of immune response is more significant for preventing infection: induction of HIV-neutralizing antibodies (systemic vs. mucosal compartments), CD4+ T-helper cells, cytotoxic CD8+ T-cells (Cytotoxic T Lymphocyte [CTL]), innate immunity, or a combination of all forms. Furthermore, immunogens that elicit neutralizing antibodies (NAb) have been elusive due to epitope sequestration in the lipid membrane (Sun *et al.*, 2008) and the fact that epitopes are only transiently exposed during viral entry (Frey *et al.*, 2008). As a result, novel approaches are continuously being employed in HIV-vaccine development.

The exceptional molecular diversity of the virus is the most formidable obstacle to vaccine development. No two HIV isolates are identical. Even isolates from a single individual show significant heterogeneity (Fields *et al.*, 2007). Specifically, viral genomic sequences within a single individual can differ by up to 10% (Korber *et al.*, 2001). Within a few months of infection, transmission founder (TF) viruses are almost completely replaced by viruses differing at several highly selected genomic loci. This phenomenon is predominantly mediated by CTL-driven immune escape (Goonetilleke *et al.*, 2009). This extremely high rate of evolution is underpinned by the error-prone reverse transcription step of the virus replication process, the high recombination frequencies in the virus, and the high turnover of progeny virions (Esbjörnsson *et al.*, 2010). Together, these produce many mutant viruses with immune-escape potential (Mascola & Montefiori, 2010), serving as moving targets for vaccines.

### **Molecular evolution of HIV-1 and Inter-individual variability in HLA genes in the context of vaccine development**

A high level of molecular evolution of HIV-1 has resulted in the diversification of HIV-1 into genetically distinct subtypes or clades and numerous circulating recombinant forms (CRFs)

showing distinct geographic distributions (Esbjörnsson *et al.*, 2010). These strains are classified into four groups: the "major" group M, the "outlier" group O, the "new" or non-M, non-O group N, and the P group (Abecasis *et al.*, 2013). More than 90% of HIV-1 infections belong to HIV-1 group M, within which there are nine genetically distinct subtypes (or clades), namely, A, B, C, D, F, G, H, J, and K. Furthermore, the HIV-1 group M is diversified globally into clades (A-K). Still, interclade viral recombination has given rise to "circulating recombinant forms" or CRFs, which account for 10% of circulating HIV-1 strains commonly found in geographic areas such as Africa, South America, and Southeast Asia, where multiple subtypes co-exist (Albert *et al.*, 2013).

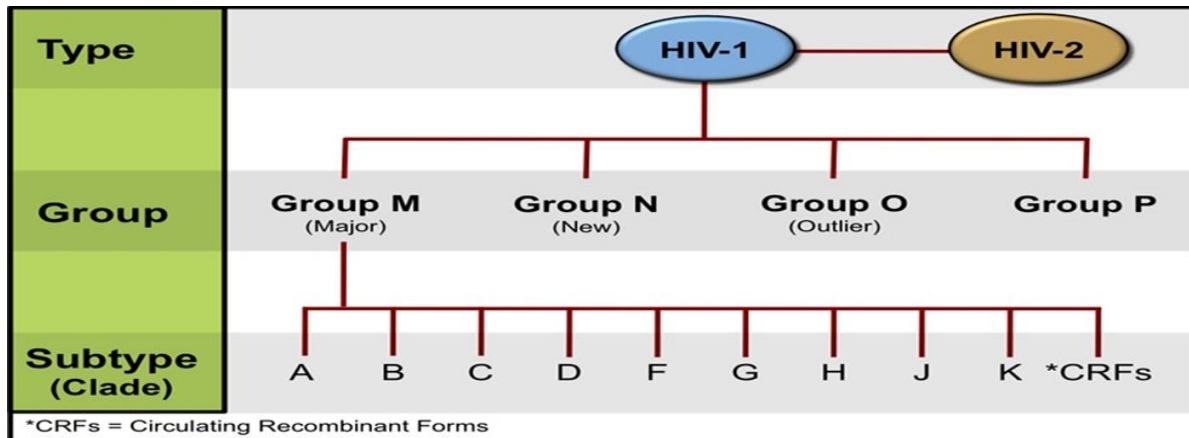


Figure 1. HIV-1 groups and subtypes (clades) distributed in different geographical regions

Most HIV-1 recombinants have arisen from Africa, and most contain segments originally derived from clade-A viruses (Tongo *et al.*, 2015). HIV-1 CRF02\_AG (recombinant of subtype A and G) and subtype G (HIV-1 G) account for most HIV infections in Nigeria (Ajoge *et al.*, 2011). Njoku *et al.* (2016), in a study in Nigeria involving six sites (Makurdi, Abuja, Enugu, Kaduna, Tafa, and Ojo-Lagos), showed the distribution of the major HIV-1 subtypes across these six sites as: 37.5% CRF02\_AG, 27.7% G, and 25.9% G/CRF02\_AG, while 8.9% could not be typed. Such massive genetic diversity lays bare the difficulty of developing a vaccine that is immunologically effective across divergent strains (Stephenson & Barouch, 2013)

Mirroring the diversity in HIV-1, numerous studies have shown extensive inter-individual variability in susceptibility to infection and disease progression (Winkler *et al.*, 2004; Fellay *et al.*, 2007; Aziz *et al.*, 2019). At one end of the spectrum, individuals develop AIDS within 6-12 months after infection; at the other, they could remain asymptomatic without treatment for over 25 years. Some individuals even remain free of infection despite repeated exposure to the virus (Fields *et al.*, 2007).

Some of the widely studied and well-characterized genetic variants underlying inter-individual variability in the susceptibility to HIV infection and disease outcomes are found in genes encoding HIV entry receptors (CCR5, CCR2) and their ligands (Modi *et al.*, 2006); genes involved in the innate immune response to the initial infection, including dendritic cell-specific intracellular adhesion molecule-3-grabbing non-integrin [DC-SIGN], killer cell immunoglobulin-like receptors [KIR]; genes encoding viral restriction proteins (ABOBE3G, tetherin, TRIM5alpha, and SAMHD1) (Kaur & Mehra, 2009); and genes encoding human leukocyte antigen (HLA) class I molecules (Baker *et al.*, 2009). Variability in the latter seems to confer the most robust and most consistent effects on the outcome of the effort of the human immune system to contain the virus, and numerous reports describe the associations of HLA class I polymorphism with clinical, virologic, and immunologic endpoints in the course of HIV-1 infection.

### **HLA class I and HIV control**

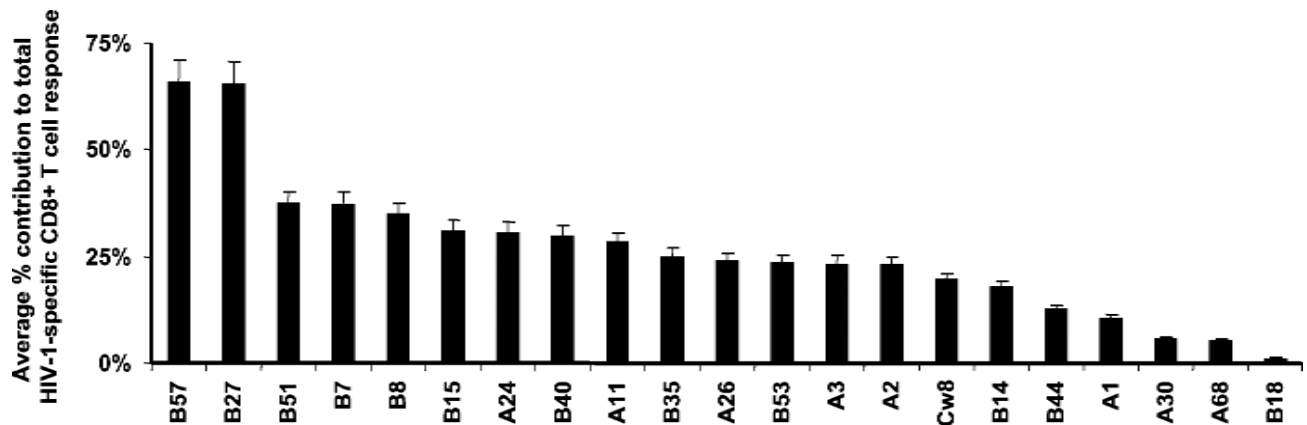
Canonically, CD8<sup>+</sup> T lymphocytes recognize and destroy cells expressing non-self-peptides in HLA class I molecules on the surface of nucleated cells. The effectiveness of this response is linked to the individual's immunogenetic profile, including HLA class I expression. In HIV, the viral set-point (VSP) (steady state viral replication following acute HIV-1 infection) reflects virus-host interplay in which the CTL response (driven by HLA class I interaction with viral antigen) plays a central role (Tang *et al.*, 2002). This is corroborated by the study showing that the first appearance of HIV-1-specific CTL in peripheral blood coincides with the initial decline of VSP and by the failure of the decline of viremia in SIV (simian immune deficiency virus)-infected macaques that have been depleted of CD8<sup>+</sup> T cells (Altfeld *et al.*, 2006).

At least three other lines of evidence support this pivotal role of CD8+ T cells in the control of HIV infection: (1) specific HLA class I alleles show a consistent association with either favorable or unfavorable HIV disease outcomes. *HLA- B\*27 and -B\*57* are the two most dominant alleles associated with favorable disease outcomes (Carrington *et al.*, 1999; Lunardi *et al.*, 2021) and are known to induce CTL responses against conserved HIV-1 epitopes (Tang *et al.*, 2002). These epitopes are derived from the capsid protein, which is essential in viral assembly. Therefore, its preservation is crucial to the survival of the virus, and an immune response directed at it tends to correlate with a better prognosis (Rihn *et al.*, 2013).

The *-B\*57* allele, in particular, appears to be the most favorable, regardless of the human population or the clade of the virus (Tang *et al.*, 2002), and together with the *-B\*27*, constitutes the major contributor to the HLA-mediated HIV-1 specific CTL response. (Table 1). On the other hand, *-B\*35* and *-Cw\*04* are linked with rapid progression to AIDS (Carrington *et al.*, 1999).

HLA CLASS I ALLELE	EPITPOPE (PEPTIDE IDENTIFICATION)	EPITOPE (PEPTIDE SEQUENCE)
<i>B*57</i>	B57-TW10(CA)	TSTLQEQIGW
<i>B*27</i>	B27-KK10(CA)	KRWIIGLNK
<i>B*51</i>	B51-LI9(IN)	LPPVVAKEI
<i>B*7</i>	B7-KK10(GP41)	IPRRIRQGL
<i>A*24</i>	A24-RW8(NEF)	RYPLTFGW
<i>CW*8</i>	CW8-AL9(NEF)	AAVDLSHFL
<i>B*35</i>	B35-VY8(NEF)	VPLRPMTY

Table 1: Epitopes presented by various HLA class I alleles. Selected HLA class I alleles and the epitopes they present to CD8+ T cells, the viral origin of the epitopes (in brackets), and their amino acid sequence have been shown. The epitopes presented by the products of *B\*27* and *B\*57* alleles are derived from the viral C.A. (known to be from a conserved domain) Rihn *et al.*, 2013.



**Figure 2: Percentage contribution of various HLA class I alleles to HIV-1-specific CD8+ T-cell(CTL) response-** The various HLA class I alleles that present HIV-1 epitopes for CTL recognition. B57 and B27 contribute 66% and 64.5%, respectively to the total HIV-1-specific CTL response in individuals expressing them, making them the two most favourable alleles for the outcome of HIV-1 infection (Adapted from Altfield *et al.*, 2006)

Another piece of evidence comes from studies in non-human primates, where it was shown that the two rhesus macaque MHC class I alleles (Mamu-B\*08 and Mamu-B\*17) that are associated with control of SIV replication have peptide-binding motifs that resemble those of the two HLA molecules, HLA B\*27 and HLA B\*57(Gouder & Watkins, 2008; Loffredo *et al.*, 2009).

The HLA genes have extraordinary polymorphism, which allows them to present a vast repertoire of antigenic peptides, and because the alleles are co-dominant, heterozygotes for a given allele present twice as many antigens than homozygotes, the result being a more efficient immune response against pathogens (Carrington *et al.*, 1999). There, maximum heterozygosity at HLA class I loci (*HLA-A*, *HLA-B*, and *HLA-C*) is strongly associated with delayed progression to AIDS. This selective advantage is believed to maintain the extensive allelic diversity in these genes (Carrington *et al.*, 1999; Tang *et al.*, 1999).

Finally, the appearance of viral mutants escaping CD8+ T-cell recognition is temporarily associated with losing immune control of the infection, suggesting that the main obstacle to viral replication is the HIV-specific CD8+ response (Garcia & Regoes, 2015).

### **Virus and host genetic diversity in the context of vaccine development**

The dynamic created by the molecular interaction between HIV-1 genomic diversity and the inter-individual genetic variations of the determinants of host control, particularly HLA-1 genes, seems central to disease outcomes in HIV infection. This is brought to the fore in HIV-1 elite controllers (ECs), a small subset of individuals with a rare ability to spontaneously maintain an undetectable V.L. in the absence of therapeutic intervention. The E.C.s have been shown to have a prevalence of HLA-B alleles, such as *B\*27*, *B\*57*, and *B\*14*, associated with enhanced virological control. The HLA-B alleles are more protective against HIV-1 infection than HLA-A due to their ability to resist Nef-mediated downregulation. This “viral epitope-HLA interaction” forms a reasonable immune correlate of the host’s control of HIV-1 and could be fed into a conceptual framework for developing an HIV-1 vaccine.

Nevertheless, the two-prong molecular complexity of the virus and the host still leaves an enduring obstacle in designing a globally effective HIV vaccine capable of eliciting immune responses against the diverse HIV-1 subtypes. For one, it is known that certain HIV-1 subtypes are associated with specific demographics, suggesting compartmentalized epidemics characterized by subtype/clade specificities (Abecasis *et al.*, 2013), table 1. The implication is obvious: For designing and developing an effective HIV-1 vaccine, one size will most likely not fit all. Regions with subtype specificities and specific HLA profiles would need customized vaccine design and development.

Table 2: HIV-1 CRF02\_AG (recombinant of subtype A and G) and HIV-1 G account for most (approx. 80%) HIV-1

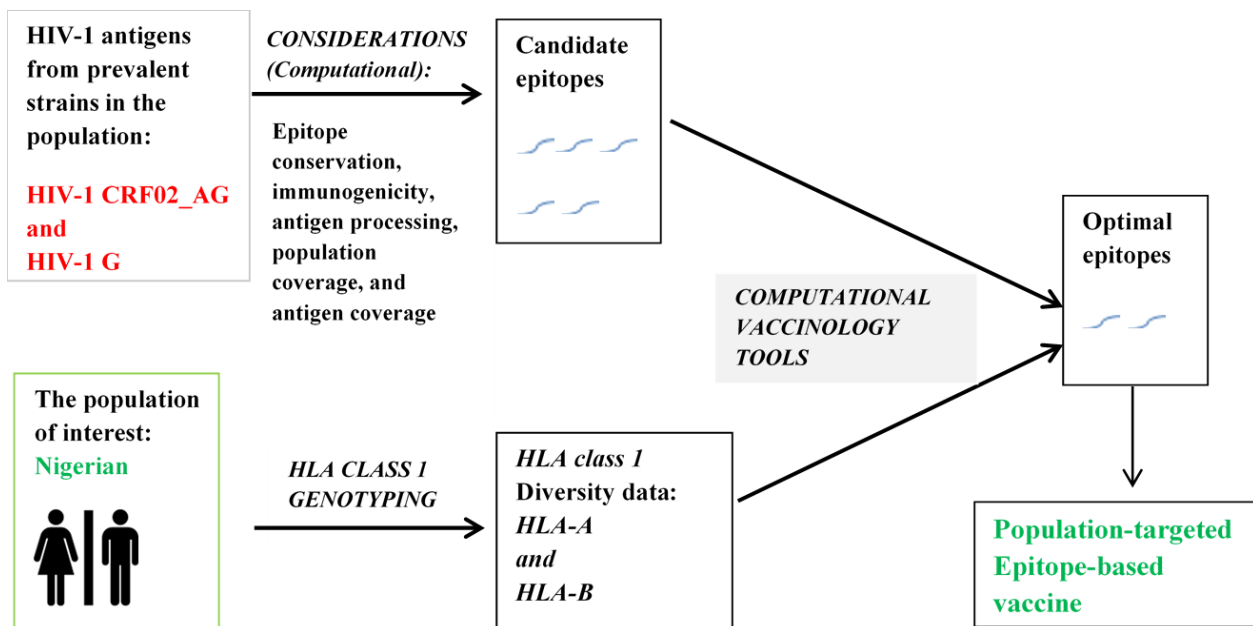
HIV-1 Subtypes	Percentage contribution to HIV-1 infections worldwide	HIV-1 Subtypes	Percentage contribution to HIV-1 infections in the African population	HIV-1 Subtypes	Percentage contribution to HIV-1 infections in the Nigerian population
CRF 01_AE	5.8	CRF02_AG	7.6	<b>CRF 02_AG</b>	<b>38.3</b>
CRF02_AG	2.7	<b>A</b>	<b>19.2</b>	CRF06_cpx	2.7
CRF07_BC	1.1	A1C	1.09	02G	6.0
A	5.8	A1D	1.6	A	1.6
A6	1.0	B	0.9	A3	1.3
<b>B</b>	<b>55.9</b>	<b>C</b>	<b>49.2</b>	C	1.3
<b>C</b>	<b>16.8</b>	D	11.4	<b>G</b>	<b>39.4</b>
D	3.2	G	1.7	Other	8.6
F	0.9	Total	100	Total	100
G	01				
Other	5.8				
Total	100				

infections in Nigeria (Ajoge *et al.*, 2011; Bhosa *et al.*, 2019); other strains predominate elsewhere)

In considering the Nigerian situation, the HIV-1 subtypes driving the Nigerian epidemic have been characterized. The next step is identifying immunogenic T-cell epitopes among these HIV-1 subtypes capable of inducing long-lasting and potent CTL response against HIV-1. Relatedly, these epitopes must be recognized by the HLA class 1 prevalent in the Nigerian population, to

which the vaccine would be targeted (a promising HIV-1 vaccine design for a given population must consider the HLA class I alleles prevalent in that geographic region and the CTL epitopes restricted by the prevalent HLA class I alleles in that population).

Unfortunately, there is a paucity of data on HLA diversity, especially in the Nigerian population. Therefore, large-scale HLA-1 genotyping data from the diverse ethnic groups in Nigeria would be a necessary piece of the vaccine design puzzle. Epitope selection could be approached computationally, using computational vaccinology tools, which make epitope selection based on the essential requirements for a suitable vaccine: immunogenicity, effective proteasomal cleavage, epitope processing, and epitope conservation. The derived epitopes can then be concatenated to produce the vaccines (Moise et al., 2015).



**Figure 3: Conceptual framework:** The target antigens derived from the most dominant HIV-1 strains in Nigeria are screened with respect to the considerations for a good vaccine. These antigens are parsed against the HLA-I data from the target population with the aid of computational vaccinology tools to determine an optimal set of immunogenic epitopes. The latter is then utilized in the design of a CTL epitope-based vaccine specific to the Nigerian population (Toussaint et al., 2008; Moise et al., 2015)

## Conclusions

A cross-strain and globally effective HIV vaccine is the desire of all vaccine developers worldwide. However, the molecular peculiarities of the virus and global diversity in the distribution of the infecting strains have made progress towards this goal quite slow and insignificant. Consequently, it has become apparent that novel approaches to vaccine development need to be explored. One such option lies in trying to leverage the understanding of the regional diversity of HIV-1 strains and *HLA* profiles (the two core determinants of the effectiveness of a CTL vaccine) to customize CTL vaccines for different populations. With improvements in computing power and the availability of more genotyping data for both virus and host, this approach could take giant strides in the next few years if the interest of researchers can be drawn in this direction.

## References

1. Abecasis *et al.* (2013). HIV-1 subtype distribution and its demographic determinants in newly diagnosed patients in Europe suggest highly compartmentalized epidemics. *Retrovirology* 7(10)
2. Ajoge, H.O, Gordon, M.L, de Oliveira, T., Green, T.N., Ibrahim, S., et al. (2011). Genetic Characteristics, Co-receptor Usage Potential and Evolution of Nigerian HIV-1 Subtype G and CRF02\_AG Isolates. *PLoS ONE* 6(3):
3. Altfeld, M., Kalife, E. T., Qi, Y., Streeck, H., Lichterfeld, M., Johnston, M. N., Burgett, N., Swartz, M. E., Yang, A., Alter, G., Yu, X. G., Meier, A., Rockstroh, J. K., Allen, T. M., Jessen, H., Rosenberg, E. S., Carrington, M., & Walker, B. D. (2006). HLA Alleles Associated with Delayed Progression to AIDS Contribute Strongly to the Initial CD8+ T Cell Response against HIV-1. *PLoS Medicine*, 3(10). <https://doi.org/10.1371/journal.pmed.0030403>
4. Aziz, N., Jamieson, B. D., Quint, J. J., Martinez-Maza, O., Chow, M., & Detels, R. (2019). Longitudinal Intra- and Inter-individual variation in T-cell subsets of HIV-infected and uninfected men participating in the LA Multi-Center AIDS Cohort Study. *Medicine*, 98(41), e17525. <https://doi.org/10.1097/MD.00000000000017525>
5. Baker, B.M., Block, B.L., Rothchild, A..., Walker, B.D. (2009). Elite control of HIV infection: implications for vaccines and treatments. *Expert Opin Biol Ther* 9: pp. 55–69.
6. Bbosa, N., Kaleebu, P., & Ssemwanga, D. (2019). HIV subtype diversity worldwide. *Current opinion in HIV and AIDS*, 14(3), 153–160. <https://doi.org/10.1097/COH.0000000000000534>

7. Carrington, M., et al. (1999). HLA and HIV-1 Heterozygote advantage and B\*35-Cw\*04 disadvantage. *Science*, 283:1748–1752.
8. Esbjörnsson, J., Månsson, F., Martínez-Arias, W., Vincic, E., Biague, A. J., da Silva, Z. J., Fenyö, E. M., Norrgren, H., & Medstrand, P. (2010). Frequent CXCR4 tropism of HIV-1 subtype A and CRF02\_AG during late-stage disease-an indication of an evolving epidemic in West Africa. *Retrovirology*, 7, 23. <https://doi.org/10.1186/1742-4690-7-23>
9. Fellay, J., Shianna, K.V., Ge, D., Colombo, S., Ledergerber, B., Weale, M., Zhang, K., et al. (2007). A Whole-Genome Association Study of Major Determinants for Host Control of HIV-1. *Science*, 317(944)
10. Frey, G., Peng, H., Rits-Volloch, S., Morelli, M., Cheng, Y., Chen, B. (2008). A fusion-intermediate state of HIV-1 gp41 targeted by broadly neutralizing antibodies. *Proc Natl Acad Sci* 105: 3739–3744
11. Fields, B. N., Knipe, D. M., & Howley, P. M. (2007). *Fields' virology* (5th ed.). Wolters Kluwer Health/Lippincott Williams & Wilkins.
12. Garcia, V., & Regoes, R. R. (2015). The Effect of Interference on the CD8+ T Cell Escape Rates in HIV. *Frontiers in Immunology*, 5, 121660. <https://doi.org/10.3389/fimmu.2014.00661>
- 13.
14. Goulder, P., Walker, B. (2012). HIV and HLA class I: An evolving relationship. *Immunity*; (37)426–440.
15. Goulder, P. J., & Watkins, D. I. (2008). Impact of MHC class I diversity on immune control of immunodeficiency virus replication. *Nat. Rev. Immunology*. 8:619-630.
16. Kaur, G. & Mehra, N. (2007). Genetic determinants of HIV-1 infection and progression to AIDS: susceptibility to HIV infection. *Tissue Antigens* (73)289–301.
17. Korber, B., Gaschen, B., Yusim, K., Thakallapally, R., Kesmir, C., Detours, V. (2001). Evolutionary and immunological implications of contemporary HIV-1 variation. *Br Med Bull.*; (58)19–42.
18. Lunardi, L. W., Bragatte, M. A. D. S., & Vieira, G. F. (2021). The influence of HLA/HIV genetics on the occurrence of elite controllers and a need for therapeutics geotargeting view. *The Brazilian Journal of Infectious Diseases*, 25(5), 101619. <https://doi.org/10.1016/j.bjid.2021.101619>

19. Mascola, J.R., Montefiori, D.C. (2010). The role of antibodies in HIV vaccines. *Annu Rev Immunol.* 2010; 28:413-44. doi: 10.1146/annurev-immunol-030409-101256. PMID: 20192810
20. Modi, W.S., Lautenberger, J., An, P. *et al.* (2006). Genetic variation in the CCL18-CCL3-CCL4 chemokine gene cluster influences HIV type 1 transmission and AIDS disease progression. *Am J Hum Genet.* 79: 120–8.
21. Moise, L., Gutierrez, A., Kibria, F., Martin, R., Tassone, R., Liu, R., Terry, F., Martin, B., & De Groot, A. S. (2015). IVAX: An integrated toolkit for the selection and optimization of antigens and the design of epitope-driven vaccines. *Human Vaccines & Immunotherapeutics*, 11(9), 2312-2321. <https://doi.org/10.1080/21645515.2015.1061159>
22. Njoku, O.S., Manak, M.M., O’Connell, R.J., Shutt, A.L.W., Malia, J.A, *et al.* (2016). An Evaluation of Selected Populations for HIV-1 Vaccine Cohort Development in Nigeria. *PLOS ONE* 11(12)
23. Pantaleo, G., Koup, R.A. (2004). Correlates of immune protection in HIV-1 infection: what we know, what we don't know, what we should know. *Nat Med.*,10(8):806-10. doi 10.1038/nm0804-806. PMID: 15286782.
24. Plotkin, S.A. (2008). Vaccines: correlates of vaccine-induced immunity. *Clin Infect Dis.* 47(3):401-9. doi 10.1086/589862. PMID: 18558875.
25. Rihn, S.J., Wilson, S.J., Loman, N.J., Alim, M., Bakker, S.E., *et al.* (2013). Extreme Genetic Fragility of the HIV-1 Capsid. *PLoS Pathog* 9(6)
26. Stephenson, K. E., & Barouch, D. H. (2013). A global approach to HIV-1 vaccine development. *Immunological Reviews*, 254(1), 295–304. <https://doi.org/10.1111/imr.12073>
27. Sun, Z.Y., Oh, K.J., Kim, M., Yu, J., Brusica, V., Song, L., Qiao, Z., Wang, J.H., Wagner, G., Reinherz, E.L. (2008). HIV-1 broadly neutralizing antibody extracts its epitope from a kinked gp41 ectodomain region on the viral membrane. *Immunity* 28: 52–63
28. Tang, J *et al.* (1999). HLA class I homozygosity accelerates disease progression in HIV-1 infection. *AIDS Res Human Retroviruses*;(15)317–324

29. Tang, J., Tang, S., Lobashevsky, E., Myracle, A. D., Fideli, U., Aldrovandi, G., Allen, S., Musonda, R., & Kaslow, R. A. (2002). Favourable and Unfavorable HLA Class I Alleles and Haplotypes in Zambians Predominantly Infected with Clade C Human Immunodeficiency Virus Type 1. *Journal of Virology*, 76(16), 8276-8284. <https://doi.org/10.1128/JVI.76.16.8276-8284.2002>
30. Tongo, M., Dorfman, J. R., & Martin, D. P. (2015). High Degree of HIV-1 Group M (HIV-1M) Genetic Diversity within Circulating Recombinant Forms: Insight into the Early Events of HIV-1M Evolution. *Journal of virology*, 90(5), 2221–2229. <https://doi.org/10.1128/JVI.02302-15>
31. Toussaint, N.C., Dönnies, P., Kohlbacher, O. (2008) A Mathematical Framework for the Selection of an Optimal Set of Peptides for Epitope-Based Vaccines. *PLoS Comput Biol* 4(12): e1000246. <https://doi.org/10.1371/journal.pcbi.1000246>
32. Winkler, C., An, P., O'Brien, S.J. (2004). Patterns of ethnic diversity among the genes that influence AIDS. *Hum Mol Genet.* 2004 Apr 1;13 Spec No 1:R9-19. Doi: 10.1093/hmg/ddh075. Epub PMID: 14764621.