



**Broadly neutralising monoclonal antibodies display
heterogeneous neutralisation activity against HIV-1 subtype
C transmitter/founder viruses from infants**

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209528542

**Submitted in fulfilment of the requirements for the degree of
*Doctor of Philosophy in Virology***

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2025

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Manuscript 1: Neutralisation Profiles of Infant-Transmitted Founder HIV-1 Subtype C Viruses Against Broadly Neutralizing Monoclonal Antibodies

Author's: Nelisiwe Zikhali, Bongiwe Ndlovu, Kamini Gounder, Jane Millar, Thumbi Ndung'u, Philip Goulder

Manuscript 2: Identification of CXCR4-using HIV-1 subtype C transmitted/founder viruses in infants infected *in utero*

Author's: Nelisiwe Zikhali, Bongiwe Ndlovu, Kamini Gounder, Katlego Sojane, Thumbi Ndung'u, Philip Goulder

Manuscript 3: Variations in neutralisation sensitivity of HIV-1 subtype C in mother-infant pairs

Author's: Nelisiwe Zikhali, Bongiwe Ndlovu, Kamini Gounder, Jane Millar, Thumbi Ndung'u, Philip Goulder

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3 PRESENTATIONS

Oral Presentations

1. Nelisiwe Zikhali, Bongiwe Ndlovu, Kamini Gounder, Jane Millar, Thumbi Ndung'u, Philip Goulder. Broadly neutralising monoclonal antibodies display heterogeneous neutralisation activity against HIV-1 subtype C transmitter/founder viruses from infants. SANTHE (Sub-Saharan African Network for TB/HIV Research Excellence) 2023 Annual Consortium Meeting at the Ciêla Resort, Lusaka, Zambia 12-14 July.

Poster Presentations

1. Nelisiwe Zikhali, Bongiwe Ndlovu, Kamini Gounder, Katlego Sojane, Thumbi Ndung'u, Philip Goulder. Determination of coreceptor usage of human immunodeficiency type 1 subtype C viruses from infants infected in utero to the 18th International Congress of Immunology (IUIS 2023), Cape Town, South Africa from 27 November to 2 December 2023.
2. Nelisiwe Zikhali, Bongiwe Ndlovu, Kamini Gounder, Jane Millar, Thumbi Ndung'u, Philip Goulder. Broadly neutralising monoclonal antibodies display heterogeneous neutralisation activity against HIV-1 subtype C transmitter/founder viruses from infants. 2024 Collaboration for AIDS Vaccine Discovery (CAVD) Annual Meeting - 13th Norman L. Letvin Early Career Investigator Presentations. February 6-8, 2024, at the Seattle Campus of the Bill and Melinda Gates Foundation.

4 DEDICATIONS

First and foremost, I dedicate this thesis to myself, a testament to my unwavering perseverance and personal growth throughout this academic journey. Through moments of uncertainty and challenge, I discovered depths of resilience I never knew I possessed. This achievement represents countless hours of dedication, sleepless nights, and an unyielding commitment to my dreams. I am proud of the scholar and individual I have become through this transformative process.

This work is equally dedicated to my beloved family, who have been my foundation through life's most challenging seasons. Your unwavering love, endless patience, and steadfast belief in my abilities have given me the courage to persevere. You have walked beside me through every obstacle and triumph, offering strength when mine faltered and joy when the path seemed dark. Your sacrifices and support have made this achievement possible, and it is as much yours as it is mine.

To those who dreamed this dream with me, lifted me when I stumbled and celebrated every small victory along the way, this work is a testament to your boundless love and faith in me. May this accomplishment honour the countless ways you have enriched my life and reflect the values of perseverance and dedication you have instilled in me.

5 ACKNOWLEDGEMENTS

My PhD journey has been an incredible learning experience that has enriched me with invaluable skills in teamwork, academic writing, and scientific inquiry. I am deeply indebted to the many individuals who helped bring this book of knowledge to fruition. Words cannot fully express my gratitude to my PhD supervisors, Prof. Philip Goulder, Prof Thumbi Ndung'u and Dr. Bongiwe Ndlovu, whose guidance and support were instrumental throughout this journey. Dr. Ndlovu seamlessly fulfilled multiple roles as a mentor, supervisor, sister, and a friend - thank you for always knowing exactly what I needed, whether professional guidance or personal support.

I extend my deepest gratitude to my two cherished families: the esteemed **Nxele lineage** - oSeme, oNongalo, oMnyambo kaZaza, oMazala Nkosi, wena owazala uJobe, wazala uKhubase waze wazala noDingiswayo; and the noble **Zikhali lineage** - oHlongwane, oNgwane, oNgwenduna, oSangweni, oMasumpa, oZikhali ezingafani nezabafokazane. You have been my unwavering pillar of support. When doubt crept in and I contemplated giving up, you reminded me of my passion for this work and how far I had come, giving me the strength to persevere.

I am truly grateful to the staff and students at HPP, and to Dr. Ndlovu's lab BNAb group, for the constant support and sisterhood that I needed to face and share the challenges along the way. Your encouragement, laughter, and friendship carried me through the hardest days and turned this journey into something lighter and more meaningful than I could have imagined. Finally, my heartfelt thanks go to the study participants for selflessly donating their samples and making this work possible.

Philippians 4:13

“I can do all this through him who gives me strength.”

2 Chronicles 15:7

“But as for you, be strong and do not give up, for your work will be rewarded.”

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8 AMINO ACID

<u>Abbreviations</u>		<u>Name</u>	<u>Side chain</u>
A	Ala	Alanine	Hydrophobic
C	Cys	Cysteine	Hydrophobic
D	Asp	Aspartic acid	Negative
E	Glu	Glutamic acid	Negative
F	Phe	Phenylalanine	Hydrophobic
G	Gly	Glycine	Hydrophobic
H	His	Histidine	Positive
I	Ile	Isoleucine	Hydrophobic
K	Lys	Lysine	Positive
L	Leu	Leucine	Hydrophobic
M	Met	Methionine	Hydrophobic
N	Asn	Asparagine	Polar
P	Pro	Proline	Polar
Q	Gln	Glutamine	Polar
R	Arg	Arginine	Positive
S	Ser	Serine	Polar
T	Thr	Threonine	Polar
V	Val	Valine	Hydrophobic
W	Trp	Tryptophan	Hydrophobic
Y	Tyr	Tyrosine	Polar

9 LIST OF ABBREVIATIONS

<	Smaller than
>	Greater than
µg	Microgram
µM	Micromolar
µl	Microliter
°C	Degrees Celsius
AA	Amino acid
ADCC	Antibody-dependent cellular cytotoxicity
ADCP	Antibody-dependent cellular phagocytosis
AIDS	Acquired immune deficiency syndrome
AID	Activation-induced cytidine deaminase
APC	Antigen-presenting cells
ART	Antiretroviral therapy
ATV	Atazanavir
AZT	Zidovudine
BCR	B cell receptor
bNAb	Broadly neutralising antibody
CA	Capsid protein
cART	combination Antiretroviral therapy
CCR5	C-C chemokine receptor type 5
cDNA	Complementary deoxynucleic acid
CD4-bs	CD4- binding site
C1-5	Conserved region 1 – 5
CTL	Cytotoxic T lymphocyte
CRF	Circulating recombinant form
CXCR4	C-X-C chemokine receptor type 4
EFV	Efavirenz
Env	Envelope
Fab	Antigen-binding fragment
Fc	Fragment crystallisable
FP	Fusion peptide

Gp41	HIV-1 Env glycoprotein 41 subunit
Gp120	HIV-1 Env glycoprotein 120 subunit
Gp160	HIV-1 Env glycoprotein 160
gRNA	genomic RNA
HEK	Human embryonic kidney
HeLa	Human cervical epithelial cells
HIV	Human Immunodeficiency Virus
HR	Heptad Repeat region
IFN	Interferon
IC50	50% inhibitory concentration
IN	Integrase
LTR	Long terminal repeat
MA	Matrix protein
mAbs	Monoclonal antibodies
MHC	Major Histocompatibility Complex
mL	milliLitre
M-I pairs	Mother-Infant pairs
MPER	Membrane Proximal External Region
mRNA	messenger RNA
NaCl	Sodium Chloride
NaOAc	Sodium acetate
NC	Nucleocapsid protein
NK cells	Natural Killer cells
NPC	Nuclear Pore Complex
NVP	Nevirapine
PBS	Phosphate-Buffered Saline
PBMCs	Peripheral Blood Mononuclear Cells
PCR	Polymerase Chain Reaction
pDCs	plasmacytoid Dendritic Cells
PPTs	Polypurine tracts
PR	Protease
RNA	Ribonucleic acid

RNaseOUT	Recombinant RNase Inhibitor
RT	Reverse Transcriptase
RT-PCR	Reverse transcriptase polymerase chain reaction
RRE	Rev response element
SHIV	Simian/Human Immunodeficiency Virus
SIV	Simian Immunodeficiency Virus
Tat	Trans-activator of transcription
TAR	Transactivation response element
TCR	T cell receptor
TF	Transmitter/founder
Tfh	T follicular helper cells
TM	Transmembrane protein
Treg	regulatory T cells
HIV	Human Immunodeficiency Virus
HIV-1	Human Immunodeficiency Virus type 1
HIV-2	Human Immunodeficiency Virus type 2
SIV	Simian immunodeficiency virus
SU	Surface protein
U/ μ L	Units per microliter
URF	Unique recombinant form
V1-5	Variable 1 – 5
Vif	Virion infectivity factor
Vpr	Viral protein R
Vpu	Viral protein U gene
Vpx	Viral protein X

ABSTRACT

Introduction: Vertical transmission of HIV-1 remains a global health challenge, especially in resource-limited settings due to drug resistance and mothers' non-adherence to antiretroviral therapy. The aim of the study was to determine the characteristics of HIV-1 subtype C envelope in infants and their mothers, determine their sensitivity to broadly neutralizing antibodies (bNAbs) and coreceptor usage.

Methods: We characterized 21 transmitted/founder (TF) pseudoviruses produced from infants and a subset of mothers and evaluated their susceptibility to clinically relevant broadly neutralizing antibodies (bNAbs). We also analysed amino acid signatures within bNAb epitopes to identify bNAb resistance mechanisms. Bioinformatics tools and tissue culture methods were used to determine the coreceptor usage in infants and mothers. This was followed by comparing the neutralization sensitivity and coreceptor usage between mother-infant pairs.

Results: VRC07-523LS, 10-1074, and 10E8 demonstrated the highest neutralisation coverage in infants, while resistance was frequently observed against CAP256-VRC26, PGDM1400, and PGT151. Key escape mutations were identified in various epitope regions, particularly in the CD4 binding site, V3 glycan and V2-apex regions. In addition, 71% of infants isolates utilised CCR5, while 29% used CXCR4 or both CCR5/CXCR4, representing a higher frequency of CXCR4 usage than previously reported in subtype C infections. Analysis of mother-infant pairs showed predominantly concordant coreceptor usage, though notable exceptions were observed. Mother-infant pairs revealed distinct patterns in neutralization sensitivity, infants isolates showing enhanced sensitivity to CD4 binding site antibodies but increased resistance to antibodies targeting the V2 apex-targeting and gp120-gp41 interface. Sequence analysis of the V3 loop identified specific determinants of coreceptor tropism, including the N-linked glycosylation patterns, loop length, and crown motif substitutions.

Conclusion: These findings suggest either the transmission of minor maternal viral populations or rapid post-transmission adaptation. Structural analysis identified variations in variable loop lengths and glycosylation patterns between maternal and infant isolates, suggesting complex selective pressures during transmission events. Prevention of vertical HIV transmission may require the combination of broadly neutralizing antibodies that target different epitopes, and potentially with entry inhibitors. The higher frequency of CXCR4-using viruses in this population suggests prevention strategies must be tailored to local viral characteristics, highlighting the need for multi-target approaches in effective HIV prevention.

CHAPTER 1

1.1 General Introduction

Human Immunodeficiency Virus (HIV) continues to be a significant global health challenge; approximately 40.8 million people were living with HIV worldwide in 2024 (UNAIDS, 2024a). Despite advancements in prevention and treatment, an estimated 630,000 people died due to AIDS-related illnesses in the same year (UNAIDS, 2024b). Sub-Saharan Africa remains disproportionately affected, accounting for nearly two-thirds of all new HIV infections globally. (UNAIDS, 2024b). South Africa bears a heavy burden, with approximately 8.2 million people living with HIV as of 2022 (AVAC report, 2023). This figure includes 7.8 million adults aged 15 and about 400,000 children aged 0-14 years (UNAIDS, 2024a). The prevalence of HIV varies significantly across South African provinces, with KwaZulu-Natal consistently reporting the highest prevalence rate at 27.0% (Simbayi *et al.*, 2019).

Prevention tools and treatment options for HIV have expanded significantly over the past decades. Pre-exposure prophylaxis (PrEP) has emerged as a highly effective prevention method, reducing the risk of HIV acquisition by up to 99% when taken as prescribed (CDC, 2022). While antiretroviral therapy (ART) has been remarkably successful in the treatment of HIV infection, people living with HIV who adhere to ART have achieved undetectable viral loads, effectively eliminating the risk of sexual transmission. (Cohen *et al.*, 2011). However, there are significant challenges, such as non-adherence, drug resistance and drug-induced toxicity. Drug resistance remains an important concern; approximately 10% of people starting ART in low- and middle-income countries were infected with drug-resistant strains (WHO, 2024). Additionally, the long-term use of ART may lead to serious side effects such as cardiovascular diseases, liver and kidney diseases, and bone density loss. (Deeks, Lewin and Havlir, 2013).

The limitations of current prevention strategies have spurred research into alternative approaches, particularly passive infusion of broadly neutralising antibodies (bNAbs) and germline targeting vaccines. The landmark IMPAACT P1112 trial in Botswana demonstrated that passive administration of VRC01 monoclonal antibody that targets the CD4 binding site in HIV-exposed infants was safe and well-tolerated for viral suppression, with no serious adverse events reported (Shapiro *et al.*, 2020). This study showed that single and multiple infusions of VRC01 achieved target concentrations that could potentially suppress viral replication, maintaining therapeutic levels for up to 16 weeks in infants (Shapiro *et al.*, 2020). In addition, the pivotal AMP (Antibody-Mediated Prevention) trials provided the first proof-of-concept evidence that passive administration

of bNAbs can prevent HIV acquisition in humans (Corey *et al.*, 2021). These two randomized, double-blind, placebo-controlled trials (HVTN 704/HPTN 085 and HVTN 703/HPTN 081) evaluated VRC01 for HIV prevention in high-risk populations, demonstrating that VRC01 was effective against VRC01-sensitive strains but showed limited overall efficacy due to the high prevalence of resistant viruses in the study populations. Additional studies have shown promising results with other bNAbs. The phase 1 trial of 3BNC117 demonstrated potent viral suppression in HIV-infected adults, with a single infusion maintaining viral suppression for up to 28 days (Caskey *et al.*, 2015). Similarly, passive administration of 3BNC117 and 10-1074 showed sustained viral suppression in HIV-infected individuals, with some participants maintaining viral control for several months after antibody administration (Bar-On *et al.*, 2018a).

However, the main challenge in the development of antibody-based interventions is the genetic diversity of HIV-1 group M, which encompasses multiple subtypes and circulating recombinant forms (CRFs), each with distinct antigenic properties (Hemelaar, 2012). Another significant challenge is the increasing prevalence of escape mutations in currently circulating viruses, which leads to substantial resistance against available bNAbs. Recent surveillance studies have documented concerning levels of bNAb resistance across different geographical regions. For instance, analysis of circulating strains showed frequent resistance to several promising bNAbs, including CAP256-VRC26, PGDM1400, and PGT151, with escape mutations identified in key epitope regions such as the V2-apex, V3-glycan supersite, and gp120-gp41 interface (Mkhize *et al.*, 2023). Similarly, longitudinal studies have revealed the emergence of resistance mutations over time, with some bNAbs showing decreased neutralization potency against contemporary isolates compared to earlier strains (Cohen *et al.*, 2025). Subtype-specific resistance patterns have been observed, with subtype C viruses showing distinct escape mechanisms compared to other subtypes, particularly in the context of CD4 binding site-targeting antibodies (Sutar *et al.*, 2025).

Furthermore, HIV-1 prevention strategies are also influenced by HIV-1 coreceptor tropism, as the majority of HIV-1 subtype C transmitted/founder viruses use CCR5 coreceptor to establish infection, though the frequency of transmitted/founder viruses that use CXCR4 coreceptors is increasing over time (Ping *et al.*, 2013). Entry inhibitors, particularly CCR5 antagonists like maraviroc, have been extensively evaluated primarily in treatment studies for people living with HIV-1, with the MOTIVATE trials demonstrating viral suppression in treatment-experienced adults (Gulick *et al.*, 2008). However, entry inhibitors have shown limited application in prevention studies, with most research focusing on therapeutic rather than prophylactic use (Westby and Ryst, 2010). The clinical utility of maraviroc is restricted to individuals with confirmed CCR5-tropic

virus, and treatment failure often occurs through the emergence of pre-existing CXCR4-using variants or viral adaptation to use drug-bound CCR5 receptors, with over 50% of patients experiencing virological failure on maraviroc therapy showing CXCR4-using virus at the time of failure (Raymond *et al.*, 2015). Despite approval for clinical use since 2007, maraviroc's optimal application in prevention strategies remains poorly defined, and its effectiveness is limited by the requirement for tropism testing and the potential for coreceptor switching during treatment (Gulick *et al.*, 2008; Westby and Ryst, 2010).

However, significant research gaps remain in understanding the role of bNAbs, and the selection of effective bNAbs particularly for the paediatric population. While the initial safety data from the IMPAACT P1112 trial is encouraging and suggest that bNAbs have a great potential in suppressing viraemia in children, more comprehensive studies are needed to define bNAbs that are effective in various African populations and their long-term effects on infants. Additional studies are required to determine the role of bNAbs in the prevention of *in utero*, intrapartum, and breastmilk vertical transmission and optimal dosing strategies. Furthermore, limited data exists on infant viral coreceptor usage patterns, crucial for optimising entry inhibitor-based interventions. Recent studies have highlighted the importance of understanding viral tropism in mother-infant pairs, as this could impact both transmission risk and treatment efficacy (Kumar *et al.*, 2021).

1.1.1 Aims and Objectives

To determine the characteristics of HIV-1 subtype C envelope in infants that acquired HIV-1 in utero and determine their sensitivity to broadly neutralizing antibodies and their coreceptor usage in mother-infant pairs.

The specific objectives are to:

1. Determine the neutralization sensitivity of infant transmitter/founder viruses to a panel of broadly neutralizing monoclonal antibodies targeting distinct epitopes on the HIV-1 envelope glycoprotein.
2. Compare the HIV-1 envelope characteristics and neutralization sensitivity profiles between matched infant transmitter/founder viruses and corresponding maternal viruses from mother-infant transmission pairs.
3. Characterize the coreceptor usage patterns of HIV-1 subtype C viruses from in utero infected infants and their mothers to assess tropism differences between transmitted and circulating maternal variants.

1.2 Literature Review

1.2.1 Origin, History and Classification of HIV

Human Immunodeficiency Virus (HIV) is classified within the *Retroviridae* family, specifically in the *Orthoretrovirinae* subfamily and *Lentivirus* genus (Coffin, Hughes and Varmus, 1997). Previous studies have shown that HIV infection progresses into acquired immunodeficiency syndrome (AIDS). In 1981, the first cases of acquired immunodeficiency syndrome (AIDS) were identified in the United States (Barré-Sinoussi *et al.*, 1983). In 1983, HIV was isolated from infected humans (Barré-Sinoussi *et al.*, 1983), (Gallo *et al.*, 1984). HIV evolved from the Simian Immunodeficiency Virus (SIV), HIV-1 originated from chimpanzees (*Pan troglodytes troglodytes*) in Southern Cameroon, and HIV-2 originated from sooty mangabeys (*Cercocebus atys*) in Western Africa (Sharp and Hahn, 2011). The earliest confirmed HIV-1 infection was identified in a blood sample collected in 1959 from Kinshasa, Democratic Republic of Congo, suggesting that the virus had been circulating in human populations decades before its clinical recognition (Worobey *et al.*, 2008).

HIV-1 and HIV-2 are two distinct HIV types; however, they share fundamental taxonomic characteristics while exhibiting significant genetic and pathogenic differences (Gao *et al.*, 1999; Reeves and Doms, 2002). HIV-1 is more virulent and pathogenic than HIV-2. It is distributed all over the world and responsible for the global HIV/AIDS pandemic, while HIV-2 is primarily found in West Africa (Clavel *et al.*, 1986; Guyader *et al.*, 1987). HIV-1 is further classified into four distinct groups: M (main), N (non-M, non-O), O (outlier), and P (Simon *et al.*, 1998; Robertson *et al.*, 2000). Group M is the most dominant subtype, while the other subtypes, groups N, O and P, have not been extensively distributed and only account for ~ 1 – 2% of all HIV infections (Simon *et al.*, 1998; Robertson *et al.*, 2000; Vallari *et al.*, 2011). Group M accounts for 95% of all isolated strains and can be further subdivided into several subtypes or clades, as demonstrated in Figure 1 (A, B, C, D, F, G, H, J, and K) (Robertson *et al.*, 2000; Perrin, Kaiser and Yerly, 2003; Geretti, 2006).

Subtype C is prevalent in sub-Saharan Africa, especially in South Africa, Botswana, Zimbabwe, Zambia, Tanzania and Ethiopia, where it accounts for over 90% of infections in these regions (de Oliveira *et al.*, 2003; Kiwanuka *et al.*, 2008; Novitsky *et al.*, 2010). Studies have demonstrated that subtype C dominates the southern African epidemic, with phylogenetic analyses showing multiple viral introductions into South Africa primarily from Zambia, Botswana, Malawi and Zimbabwe (de Oliveira *et al.*, 2016; Faria *et al.*, 2014). Subtype C is also dominant in India, it is responsible for more than 50% of HIV infections globally (Buonaguro, Tornesello and Buonaguro, 2007). In contrast, Subtype B is dominant in Europe, North and South America, and Australia (Spira *et al.*, 2003; Taylor *et al.*, 2008), representing another significant genetic variant. Subtype A shows a broad distribution pattern, prevalent

in East Africa, particularly Kenya, Rwanda and Uganda as well as Eastern Europe and Central Asia (Novitsky *et al.*, 2010; Hemelaar *et al.*, 2019). Subtype D remains concentrated in East African regions, especially Uganda and the Democratic Republic of the Congo (Spira *et al.*, 2003). Subtype G demonstrates a notable presence in West and Central African countries, with significant representation in Nigeria and Cameroon (Taylor *et al.*, 2008; Novitsky *et al.*, 2010). The viral genetic landscape is further complicated by circulating recombinant forms, with CRF02_AG primarily found in Cameroon and CRF01_AE concentrated in Asian countries including Thailand, Japan, Singapore, and China (Fig 1.1) (Lau and Wong, 2013; Hemelaar *et al.*, 2019). These recombinant forms highlight the remarkable adaptive capabilities of HIV-1, demonstrating high genetic diversity through complex molecular mechanisms.

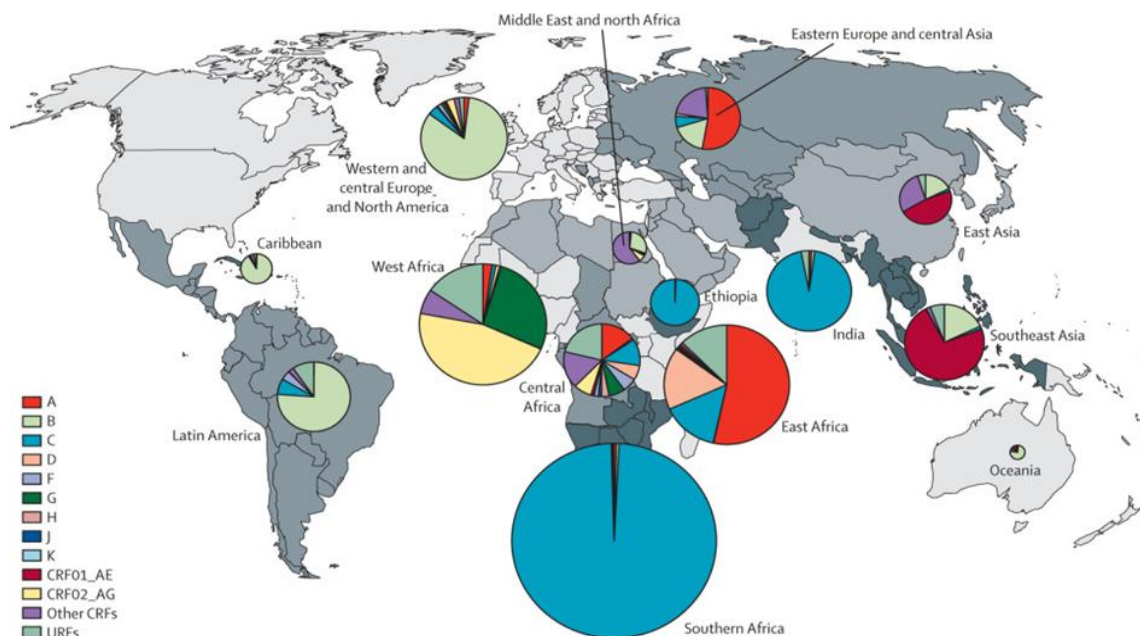


Figure 1.1: Regional distributions of HIV-1 group M subtypes, CRFs, and URFs (Hemelaar *et al.*, 2019). Countries forming a region are shaded in the same colour on the world map. The surface area of each pie chart corresponds to the number of people living with HIV in each region: CRF=circulating recombinant form and URFs=unique recombinant forms. HIV subtypes are shown in different colours to highlight the distribution, as shown in the key on the left.

1.2.2 HIV-1 Structure and Genome Organization

1.2.2.1 Virion Structure

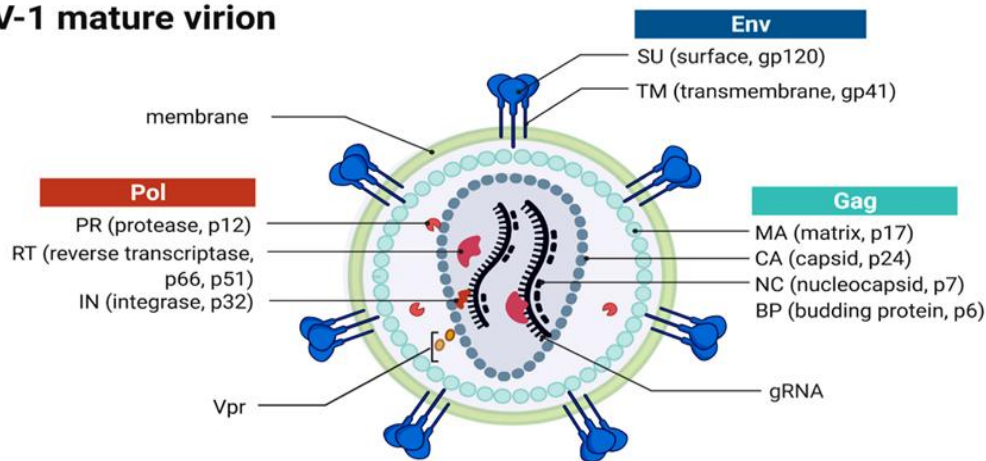
A mature HIV-1 virion is approximately 120 nm in diameter and contains a lipid bilayer membrane studded with trimeric Env spikes. (Zennou *et al.*, 2000; Briggs and Kräusslich, 2011). Beneath the membrane lies a matrix (MA) layer composed of p17 protein, which primarily functions in viral assembly and release. The viral core consists of a conical capsid (CA) made of p24 protein molecules that encase two copies of the viral RNA genome along with essential viral enzymes, including reverse transcriptase (RT), integrase (IN), and protease (PR) (Fig 1.2a) (Zhu *et al.*, 2006).

1.2.2.2 Genome Organization

The HIV-1 genome is approximately 9.7 kb in length and efficiently encodes multiple proteins essential for viral replication and survival (Freed, 2001; Engelman and Cherepanov, 2012). The genome structure features Long Terminal Repeat (LTR) sequences at both ends, with the 5' LTR region functioning as the promoter for viral gene transcription and containing the Transactivation Response (TAR) element (Fig 1.2b) (Ott, Geyer and Zhou, 2011). The viral genome is organised from the 5' to 3' direction and contains three major genes: *gag*, *pol*, and *env*. The *gag* gene encodes structural proteins, including the matrix protein (MA, p17), capsid protein (CA, p24), and nucleocapsid proteins (NC, p6 and p7). The *pol* gene encodes essential enzymes: protease (PR, p12), reverse transcriptase (RT, p51 and p66), RNase H (p15), and integrase (IN, p31). The *env* gene encodes two critical envelope glycoproteins: gp120 (surface protein, SU) and gp41 (transmembrane protein, TM) (Fig 1.2b).

The HIV genome also contains several regulatory genes (Table 1.1), including *tat*, which codes for Tat (transactivator protein) and *rev*, which codes for Rev (RNA splicing-regulator) protein, both crucial for viral replication (Craigie and Bushman, 2012a; Karn and Stoltzfus, 2012). Additional accessory genes include *nef* (negative regulating factor), *vif* (viral infectivity factor), *vpr* (virus protein R), and *vpu* (virus protein U), which are involved in viral replication, budding, and pathogenesis (Levy, 2007; Sauter *et al.*, 2012). Notably, HIV-2 differs from HIV-1; it contains Vpx (virus protein X6) instead of Vpu, this contributes to its reduced pathogenicity (Vicenzi and Poli, 2013). Beyond the coding regions, the HIV-1 genome harbours critical regulatory elements, including the Psi (Ψ) sequence for genomic RNA packaging, the Rev Response Element (RRE) for mRNA export, and polypurine tracts (PPTs) for reverse transcription (Lever, 2007).

HIV-1 mature virion



HIV-1 genome



Figure 1.2: Schematic overview of the HIV-1 virion structure (A) and the genome organization (B) (van Heuvel *et al.*, 2022).

Table 1.1: Overview of HIV proteins and their function

Gene	Size*	Protein	Function
<i>gag</i>	p24 p17 p7 p6	Pr55Gag capsid protein (CA) matrix protein (MA) nucleoprotein (NC)	precursor of the inner structural proteins formation of conical capsid myristilated protein, forming the inner membrane layer formation of the nucleoprotein/RNA complex involved in virus particle release
<i>pol</i>	p10 p51 p15 (66) p32	Pr160GagPol protease (PR) reverse transcriptase (RT) RNase H integrase (IN)	precursor of the viral enzymes proteolytic cleavage of Gag (Pr55) and Gag-Pol (Pr160GagPol) precursor protein; release of structural proteins and viral enzymes transcription of HIV RNA in proviral DNA degradation of viral RNA in the viral RNA/DNA replication complex integration of proviral DNA into the host genome
<i>env</i>	gp120 gp41	PrGp160 surface glycoprotein (SU) transmembrane protein (TM)	precursor of the envelope proteins SU and TM, cleavage by cellular protease attachment of virus to the target cell anchorage of gp120, fusion of viral and cell membrane
<i>tat</i>	p14	transactivator protein	activator of transcription of viral genes
<i>rev</i>	p19	RNA splicing regulator	regulates the export of non-spliced and partially spliced viral mRNA
<i>nef</i>	p27	negative regulating factor	myristilated protein, influence on HIV replication, enhancement of infectivity of viral particles, downregulation of CD4 on target cells and HLA cells on target
<i>vif</i>	p23	viral infectivity protein	critical for infectious virus production in vivo
<i>vpr</i>	p15	virus protein r	component of virus particles, interaction with p6, facilitates virus infectivity, effect on the cell cycle
<i>vpu</i>	p16	virus protein unique	efficient virus particle release, control of CD4 degradation, modulates intracellular trafficking
<i>vpx</i>	p15	virus protein x	interaction with p6 in virus particles, involved in early steps of virus replication of HIV-2, component of virus particles
<i>tat/rev</i>	p26	tat/rev protein	Tat-Env-Rev fusion protein, regulates the activity of Tat and Rev in nucleus

*Numbers correspond to the size of the proteins (p) or glycoproteins (gp) in 1,000 Da.

1.2.3 HIV-1 Envelope Structure and Function

Env is synthesised as a trimeric gp160 precursor protein, which undergoes cleavage by furin protease into gp120 and gp41 subunits that remain covalently linked by disulfide bonds (Chan *et al.*, 1997a; Checkley, Luttge and Freed, 2011). This cleavage is crucial for Env to adopt a metastable prefusion state required for receptor-triggered activation. Uncleaved gp160 is capable of CD4 receptor binding; however, it fails to facilitate viral and host membrane fusion (Wyatt and Sodroski, 1998). The HIV-1 envelope (Env) trimer exhibits remarkably high genetic diversity due to high mutation rates and selective immune pressure, with distinct levels of variability observed at different taxonomic levels of HIV-1 classification. At the highest level, nucleotide diversity reaches approximately 50% between HIV-1 and HIV-2, while between different HIV-1 groups (M, N, O, P), the diversity is approximately 38% (Li *et al.*, 2015). Within HIV-1 group M, which accounts for over 90% of global HIV infections, there is an estimated 25% (range 20-36%) sequence variation in envelope protein between different subtypes (A-D, F-H, J-L), while within individual subtypes the variation is approximately 17% (range 4-30%) (Bbosa, Kaleebu and Ssemwanga, 2019; Nair *et al.*, 2024). The HIV-1 Env glycoproteins can differ by around 35% between subtypes, particularly in the variable regions such as V3, which can show up to 40% nucleotide sequence dissimilarity, while even within subtypes these differences can exceed 20% (van Opijnen *et al.*, 2004; Lynch *et al.*, 2009). This extensive genetic diversity, largely driven by immune pressure from neutralizing antibodies, represents one of the greatest challenges for HIV vaccine development and therapeutic intervention (Gaschen *et al.*, 2002).

The gp120 subunit consists of an inner domain, outer domain, and bridging sheet, organised into five variable regions (V1-V5) and five conserved regions (C1-C5) (Kwong *et al.*, 1998). The variable regions exhibit high genetic variability and extensive glycosylation, forming a glycan shield that comprises approximately 50% of the protein's molecular mass (Behrens *et al.*, 2016a; Stewart-Jones *et al.*, 2016). The V1/V2 loops contain multiple N-linked glycosylation sites and play a crucial role in trimer stability, and it is an important target epitope for broadly neutralising antibodies (Ward and Wilson, 2017). The V3 loop binds to CCR5 and CXCR4 coreceptors and primarily determines viral tropism. In addition, the V3 loop is a target epitope for bNAbs that target high mannose glycans at position 301 and 332 (Mouquet *et al.*, 2012; Kong *et al.*, 2015). While the V4 and V5 regions harbour dense clusters of N-linked glycans that help shield underlying epitopes from antibody recognition, the V5 loop also interacts with CD4 binding site bNAbs (Leonard *et al.*, 1990; Wei *et al.*, 2003). In contrast, the conserved regions (C1-C5) maintain structural integrity and contain functionally critical sites, including the CD4 binding site (Wyatt and Sodroski, 1998).

The gp41 subunit consists of an ectodomain, transmembrane and cytoplasmic domains (Fig 1.3). The ectodomain is subdivided into the fusion peptide, two heptad repeat regions (HR1 and HR2), and membrane-proximal external region (MPER) (Chan *et al.*, 1997a; Parker Miller *et al.*, 2021). The ectodomain facilitates the fusion of host and viral membranes and facilitates viral entry, while MPER interacts with bNAbs. Unlike gp120, gp41 contains only four N-linked glycosylation sites, primarily located in the ectodomain, and these glycans interact with gp120-gp41 interface bNAbs (Johnson, Sauvron and Desrosiers, 2001). The transmembrane domain influences Env incorporation into virions, while the cytoplasmic tail interacts with the matrix protein and contains trafficking signals for immune evasion (Checkley, Luttge and Freed, 2011). Gp41 shows less overall genetic variability than gp120, although it exhibits notable sequence diversity in the MPER and cytoplasmic tail regions (Zwick *et al.*, 2001; Montero *et al.*, 2008).

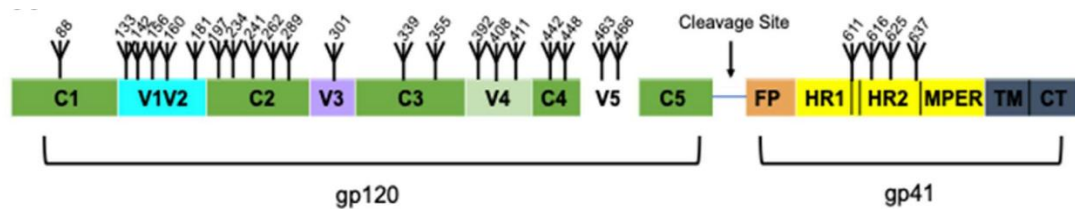


Figure 1.3: HIV-1 Env structure showing the structural components of gp120 and gp41, highlighting the regions responsible for viral entry and fusion with host cells (Parker Miller *et al.*, 2021).

1.2.4 HIV Tropism and coreceptor usage

HIV-1 tropism refers to the preferential infection of specific cell types, which is primarily determined by the interaction between the viral envelope glycoprotein gp120 and cellular coreceptors alongside the primary CD4 receptor (Berger, Murphy and Farber, 1999). The discovery of CCR5 and CXCR4 as the major coreceptors for HIV-1 entry represented a pivotal breakthrough in understanding viral pathogenesis and has since become fundamental to therapeutic strategies (Alkhatib *et al.*, 1996; Feng *et al.*, 1996). HIV-1 strains are classified based on their coreceptor usage: R5 viruses utilize CCR5, X4 viruses use CXCR4, and dual-tropic (R5X4) viruses can employ both coreceptors for cellular entry (Berger *et al.*, 1998). This classification system has profound implications for disease progression, as R5 viruses typically predominate during early infection and are responsible for sexual transmission, while X4 variants often emerge during advanced disease stages and are associated with accelerated CD4⁺ T cell depletion and faster progression to AIDS (Connor *et al.*, 1997; Schuitemaker, van 't Wout and Lusso, 2011).

The molecular basis of tropism lies within the third variable (V3) loop of the gp120 envelope protein, which serves as the primary determinant of coreceptor specificity (Hwang *et al.*, 1991; De Jong *et al.*, 1992). Structural analyses have revealed that specific amino acid sequences and charge distributions within the V3 loop dictate binding affinity to either CCR5 or CXCR4, with positively charged residues typically favouring CXCR4 usage and more neutral or negatively charged regions associated with CCR5 binding (Fouchier *et al.*, 1992; Shioda, Levy and Cheng-Mayer, 1992). The conformational changes in gp120 following CD4 binding expose the coreceptor binding site, allowing for sequential interactions that ultimately lead to membrane fusion through activation of the gp41 fusion protein (Trkola *et al.*, 1996; Wu *et al.*, 1996). Understanding these molecular interactions has been crucial for the development of entry inhibitors targeting HIV-1 coreceptors, most notably maraviroc (UK-427,857), a selective CCR5 antagonist that functions as an allosteric inhibitor by binding to CCR5 and preventing the conformational changes necessary for viral entry (Dorr *et al.*, 2005). Maraviroc represents the first clinically approved entry inhibitor and has demonstrated significant efficacy in treatment-experienced patients harbouring R5-tropic virus, leading to its regulatory approval and integration into clinical practice (Gulick Roy M. *et al.*, 2008). Complementing research into CCR5 targeting, AMD3100 (plerixafor) was developed as a selective CXCR4 antagonist and has proven invaluable for understanding CXCR4-mediated viral entry mechanisms, though its clinical application for HIV treatment has been limited due to the essential physiological roles of CXCR4 in hematopoiesis and potential cardiovascular side effects (Donzella *et al.*, 1998; Hendrix *et al.*, 2004).

1.2.5 HIV-1 Replication Cycle

HIV-1 replication cycle begins when a mature virion encounters a susceptible host cell, primarily CD4⁺ T cells, macrophages, or dendritic cells (Wilén, Tilton and Doms, 2012). The first step is viral entry into the host cell, this process initiates during the CD4 receptor binding to the viral envelope gp120 glycoproteins (1) (Fig. 1.4). This interaction triggers conformational changes that expose the V1/V2 and subsequently the V3 loops (Wu *et al.*, 1996). The V3 loop then engages CCR5 or CXCR4 coreceptors, leading to further conformational changes in gp41 that facilitate fusion (2). The fusion peptide inserts into the target cell membrane, followed by the formation of a six-helix bundle structure that brings the viral and cellular membranes into proximity, ultimately leading to membrane fusion (Chan *et al.*, 1997b; Melikyan *et al.*, 2000).

Following fusion and entry, the viral core is released into the cytoplasm and undergoes uncoating. Reverse transcription begins in the cytoplasm where reverse transcriptase initiates the conversion of the single-stranded RNA genome into double-stranded DNA; this process is prone to errors contributing to HIV genetic diversity (Mansky and Temin, 1995; Hu and Hughes, 2012). The viral RNA genome and enzymes are transported through nuclear import (3) via the nuclear pore complex (NPC) into the nucleus (Arhel *et al.*, 2007). In the nucleus, reverse transcription continues, this process is prone to errors contributing to HIV genetic diversity (Mansky and Temin, 1995; Hu and Hughes, 2012). Afterwards, integration process (5) takes place as integrase integrates the viral DNA into the host chromosome to form a provirus (Craigie and Bushman, 2012b). The integrated provirus serves as a template for viral gene expression during transcription (6); viral DNA is converted into messenger RNA and genomic RNA (gRNA). This process is facilitated by the host RNA polymerase II. After transcription, mRNA molecules are transported to the cytoplasm during nuclear export (7), and Tat and Rev proteins facilitate this process. In the cytoplasm, mRNA molecules undergo translation (8a, 8b) into viral proteins; some proteins are formed as inactive precursor proteins, including Gag, Gag-Pol and Env. Single or double-spliced mRNA codes for Vif, Vpu, Vpr, Nef, Tat, and Rev proteins (Malim *et al.*, 1989; Cullen, 1992; Wei *et al.*, 1998). The next step is viral assembly (9), which occurs at the plasma membrane, where the Gag polyprotein coordinates the gathering of viral components, including dimerised gRNA, viral enzymes, and envelope glycoproteins and this results in the formation of immature virions. This is followed by viral budding (10) from the cell surface with the help of the ESCRT (Endosomal Sorting Complexes Required for Transport) machinery, which facilitates the membrane scission process necessary for viral particle release (11). The final step is maturation (12), the viral protease cleaves Gag, Gag-Pol, and Env polyproteins into functional subunits, leading to structural rearrangements that produce infectious virions (Briggs *et al.*, 2004; Konvalinka, Kräusslich and Müller, 2015).

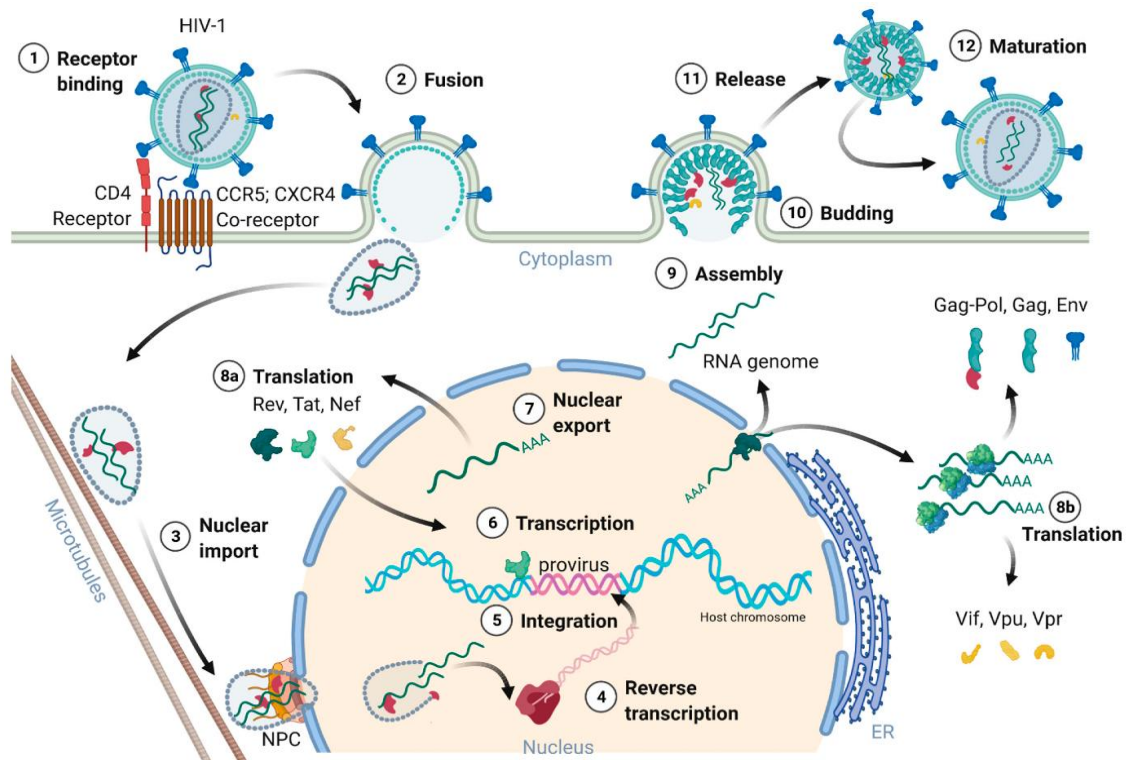


Figure 1.4. Schematic representation of the HIV-1 replication cycle, highlighting the different steps such as (1) receptor binding, (2) fusion, (3) nuclear import, (4) reverse transcription, (5) integration, (6) transcription, (7) nuclear export, (8) translation, (9) assembly, (10) budding, (11) release and (12) maturation (van Heuvel *et al.*, 2022).

1.2.6 Innate Immune Responses

The innate immune system provides the initial defence against HIV infection through various cellular and molecular mechanisms. Upon viral entry, plasmacytoid dendritic cells (pDCs) and macrophages produce Type I interferons, particularly IFN- α , which play a crucial role in limiting viral replication and spread (McNab et al., 2015). This is followed by the secretion of pro-inflammatory cytokines, including IL-15, IP-10, and TNF- α (Stacey et al., 2009; Mogensen et al., 2010). Subsequently, regulatory T cells and macrophages release anti-inflammatory cytokines in chronological order: IL-8, IL-10, IL-16, and IL-18, to maintain immune homeostasis (Stacey et al., 2009; Shankar et al., 2011).

Furthermore, natural killer (NK) cells are essential in the innate immune response through direct cytotoxicity and antibody-dependent cell-mediated cytotoxicity (ADCC). These cells utilise various activating and inhibitory receptors to identify virus-infected cells (Alter *et al.*, 2011). Upon activation, NK cells secrete cytotoxic granules containing perforin and granzymes, triggering apoptosis in infected cells while simultaneously producing IFN- γ and TNF- α (Jost and Altfeld, 2013). Research has shown that enhanced NK cell function, characterised by increased cytotoxicity and anti-viral cytokine production, correlates with slower disease progression in HIV-infected individuals (Martin *et al.*, 2007; Carrington and Alter, 2012). Macrophages and dendritic cells serve dual roles as target cells for HIV infection and crucial components of immune defence. Professional antigen-presenting cells (APCs) phagocytose HIV particles and process them through endosome proteolytic degradation. The resulting antigenic peptides bind to the Major Histocompatibility Complex (MHC) molecules and are presented on the cell surface for recognition by T cells (Koppensteiner, Brack-Werner and Schindler, 2012).

1.2.7 Adaptive Immune Responses

The adaptive immune response against HIV infection involves sophisticated cellular and humoral mechanisms with immunological memory, making it particularly significant for vaccine development (McMichael and Haynes, 2012). CD4⁺ T cell activation occurs when T cell receptors (TCRs) recognise HIV peptides presented by MHC class II molecules, along with costimulatory signals from CD28-B7 interactions and cytokine stimulation (Chen and Flies, 2013). These activated CD4⁺ T cells differentiate into distinct subsets under the guidance of specific transcription factors: T-bet for TH1, GATA3 for TH2, and ROR γ t for TH17 cells (Zhu, Yamane and Paul, 2010). The TH1 subset produces interferon gamma (IFN- γ), tumor necrosis factor-alpha (TNF- α) and IL-2, which activate macrophages and dendritic cells and enhance antigen presentation to CD8⁺ T cells. TH2 cells secrete IL-4, IL-5, IL-10, and IL-13, promoting B cell differentiation and antibody production (Zhu and Paul, 2008; Crotty, 2014). The TH17 subset produces IL-17 and IL-22, which enhance mucosal immunity and maintains barrier integrity (Okoye and Picker, 2013).

In contrast, CD8⁺ T cells differentiate into cytotoxic T lymphocytes (CTLs) and memory cells following antigen recognition and appropriate cytokine signals (Kaeche and Cui, 2012). While CTLs predominantly target HIV Gag peptides, they also recognise conserved epitopes within Pol and Nef proteins (Matthews *et al.*, 2012; Ndhlovu *et al.*, 2015). Cytotoxic T-cell responses against Gag are particularly effective as mutations in these regions often compromise viral fitness (Radebe *et al.*, 2015; Koofhethile *et al.*, 2016). They eliminate infected cells through the secretion of perforin and granzymes while also producing antiviral cytokines, including IFN- γ and TNF- α (Migueles *et al.*, 2008; Hersperger *et al.*, 2010). However, HIV employs various evasion strategies, including Nef-mediated downregulation of MHC-I molecules and rapid mutation of CTL epitopes (Collins *et al.*, 1998; Goulder and Walker, 2012). Chronic HIV infection leads to T cell dysfunction and exhaustion, characterised by elevated expression of inhibitory receptors, including PD-1, CTLA-4, and LAG-3, resulting in decreased proliferative capacity and cytokine production (Day *et al.*, 2006). This dysfunction is exacerbated by chronic immune activation and inflammation, posing significant challenges for therapeutic vaccination approaches. Additionally, regulatory T cells (Tregs) may impact vaccine efficacy by suppressing HIV-specific responses through inhibitory cytokines like IL-10 and TGF- β (Chevalier and Weiss, 2013).

1.2.8 Humoral Immune responses

During acute HIV infection, B cell activation is primarily initiated when B cells interact with extracellular antigens, including the HIV envelope glycoprotein trimer in its various conformational states (pre-fusion and post-CD4 binding), via the B cell receptor (BCR) complex (Kwong *et al.*, 1998). Following this interaction, B cells internalize and process these antigens into antigenic peptides, which are then presented on their surface through MHC class II molecules for recognition by CD4⁺ T cells via their T cell receptors (Moir and Fauci, 2009). B cell activation is further enhanced through interactions with T helper cells, particularly T follicular helper (Tfh) cells, which recognize viral antigens presented by antigen-presenting cells (APCs) and provide critical co-stimulatory signals (Rastogi *et al.*, 2022). This cognate interaction involves the engagement of CD40 ligand (CD40L) on activated T cells with CD40 receptor on B cells, a crucial signal that promotes B cell survival, proliferation, and differentiation (Banchereau *et al.*, 1994). Activated Tfh cells subsequently secrete Th2 cytokines, including IL-4 and IL-21, which drive B cell differentiation into antibody-secreting plasma cells and memory B cells, ultimately leading to the production of HIV-specific antibodies that target various epitopes on the envelope trimer in different conformational states (Fig. 1.5) (Moir and Fauci, 2009; Crotty, 2011). The initial antibody responses to HIV follows a predictable temporal pattern; Initial IgM antibodies target the gp41 envelope protein, these are followed by gp120-specific and p24-specific IgG antibodies. The early response consists of non-specific IgM antibodies, which evolve through class-switching to produce HIV strain-specific IgG and IgA antibodies with increasing affinity (Tomaras *et al.*, 2008). These antibodies exhibit multiple effector functions: antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and complement-mediated viral neutralisation (Brown *et al.*, 2008).

Broadly neutralising antibodies (bNAbs) emerge after several years of infection in 10-20% of individuals (Simek *et al.*, 2009; Burton and Hangartner, 2016a; Landais *et al.*, 2016; Ndlovu *et al.*, 2020). These antibodies are characterised by extensive somatic hypermutation, unusually long CDRH3 regions, and polyreactivity (Derdeyn, Moore and Morris, 2014). They target conserved epitopes on the HIV envelope, including the CD4 binding site, V1/V2 loops, V3 glycan supersite, and the membrane-proximal external region (MPER) (Sok and Burton, 2018). The development of bNAbs is influenced by multiple factors, including high viral load, viral diversity, and host genetic factors such as HLA-B*57 alleles and RAB11FIP5 gene variants (Bradley *et al.*, 2018; Illing *et al.*, 2018; Rahmouni *et al.*, 2023). Notably, Fc receptor polymorphisms, particularly in FcγRIIa and FcγRIIIa, affect antibody-mediated effector functions and correlate with enhanced bNAb development (McLaren and Carrington, 2015; Richardson *et al.*, 2018). Recent studies have shown that certain bNAbs, such as BG18 and BG1 antibodies may contribute to viral control (Freund *et al.*, 2017), we also reported weak associations between neutralization potency and slower disease progression (Moyano *et al.*, 2023). Furthermore,

passive administration or elicitation of bNAbs offers promising therapeutic and preventive strategies (Baba *et al.*, 2000; Doria-Rose *et al.*, 2014). These investigations underscore the complex relationship between HIV and the human immune system, highlighting the remarkable adaptability and limitations of our immune response to this challenging pathogen.

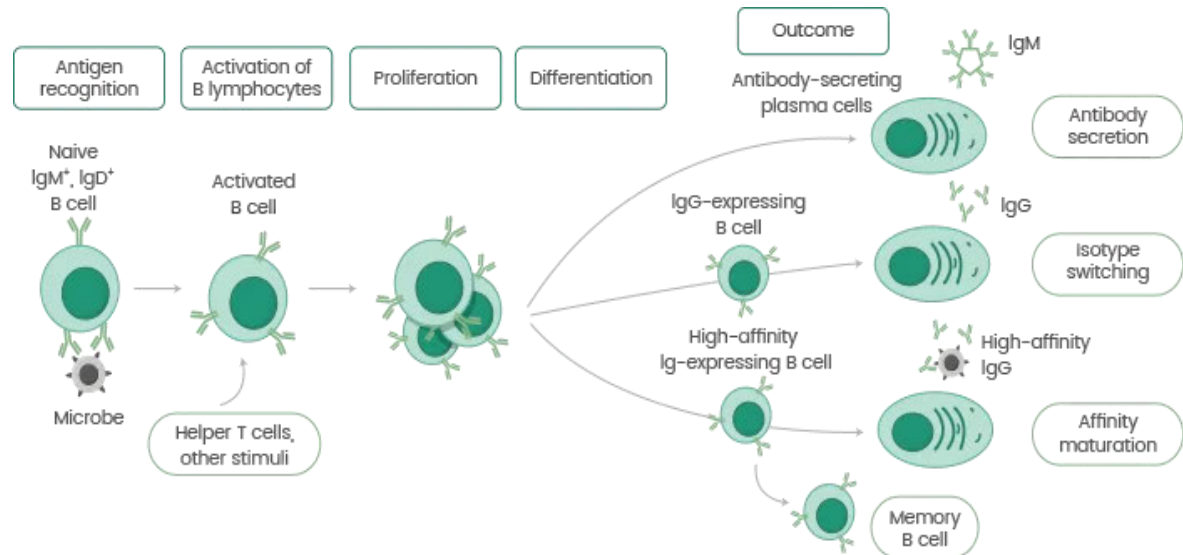


Figure 1.5: The process of antibody production during humoral immune response. This figure depicts the humoral immune response, where naïve B cells recognise antigens, activate, and proliferate. They differentiate into antibody-secreting plasma cells (producing IgM or switched isotypes like IgG), high-affinity B cells via affinity maturation, and memory B cells for long-term immunity. These processes ensure efficient and specific pathogen targeting (Lu *et al.*, 2020).

1.2.9 Target epitopes for Broadly neutralising antibodies:

1.2.9.1 CD4 binding site

The CD4 binding site (CD4bs) (highlighted in red in Fig 1.6) is a conformational epitope on the HIV envelope glycoprotein gp120 that spans approximately 145 amino acids (using HXB2 numbering). This complex binding site incorporates discontinuous regions of gp120, including the inner domain, outer domain, bridging sheet and parts of the V5 loop of gp120, which are brought into proximity through protein folding to form the CD4 receptor binding interface (Kwong *et al.*, 1998). Broadly neutralising antibodies targeting this site demonstrate extensive neutralisation breadth, with some capable of neutralising 70-90% of diverse HIV-1 strains (Wu *et al.*, 2010). These antibodies are categorised into three primary classes: VRC01-class bNAbs, VH1-46-derived bNAbs, and CDRH3 binder bNAbs. VRC01-class bNAbs represent the broadest and most potent group, neutralizing approximately 90% of heterologous HIV-1 strains. They arise from the *VH1-2* germline gene segment and acquire unique structural adaptations that enable precise targeting of the HIV-1 CD4 binding site. A defining hallmark of this lineage is a five-amino acid long CDRL3 region, coupled with a flexible CDRL1 loop that accommodates gp120 and functionally mimics CD4 receptor engagement (Scheid *et al.*, 2011; Jardine *et al.*, 2016). These antibodies typically take several years of chronic infection and extensive somatic hypermutation to develop, ultimately achieving broad and potent neutralization capacity that distinguishes them from other bNAb classes. In contrast, VH1-46-derived bNAbs target a distinct epitope and do not mimic the CD4 receptor interactions, and they neutralise 40-80% of tested strains (Huang *et al.*, 2016; Zhou and Xu, 2018). The CDRH3 binder bNAbs use their long CDRH3 loops to access conformationally constrained envelope trimers, though they demonstrate more limited neutralisation breadth, they only neutralise 30-50% of heterologous strains (Scharf *et al.*, 2014; Bonsignori *et al.*, 2016). This diverse array of antibody responses highlights the sophisticated adaptive mechanisms of the human immune system in responding to highly variable viral pathogens (Zhou *et al.*, 2015).

1.2.9.2 V2 apex

The V2 apex (highlighted in blue in Fig 1.6) is located on top of the Env trimer (approximately residues 127-195 in the HXB2 numbering system). This is the only epitope formed on the intact pre-fusion envelope trimer (Walker *et al.*, 2009; McLellan *et al.*, 2011). A dense glycan coat, including the high-mannose N160 glycan, shields this conserved quaternary structure (McLellan *et al.*, 2011; Julien, Lee, *et al.*, 2013). BNABs that target the V2 apex are characterised by exceptionally long CDRH3 loops of 24-39 amino acids and are detected in approximately 10% of broad neutralisers particularly in subtype C settings (Doria-Rose *et al.*, 2016; Landais *et al.*, 2016). V2 apex bNAbs are sub-divided into PGDM1400-like and CAP256-VRC26-like antibodies. CAP256-VRC26-like bNAbs are particularly sensitive to the deletion of lysine at positions 166 and 169, while the PGDM1400-like bNAbs are

dependent on N-linked glycan at position 160 and lysine at position 169 (Walker *et al.*, 2011; Doria-Rose *et al.*, 2014; Andrabi *et al.*, 2015; Sok and Burton, 2018).

1.2.9.3 V3 Glycan supersite

The V3 loop (highlighted in purple in Fig 1.6) represents a critical region within the HIV envelope glycoprotein, characterised by its conserved nature and complex glycosylation patterns (Pantophlet and Burton, 2006; Jiang *et al.*, 2010). Located at the base of the V3 loop (approximately residues 295-330 in HXB2), the V3 glycan supersite is a key target for bNAbs that interact with specific glycosylation sites and protein motifs (Landais *et al.*, 2016). Broadly neutralising antibodies targeting the V3 glycan supersite have limited neutralisation breadth with a strong potency; they are classified into three primary families: PGT121, 10-1074, and BG18 families (Burton and Hangartner, 2016b; Kwong and Mascola, 2018a; Sok and Burton, 2018). The PGT121 family demonstrates unique characteristics by engaging multiple glycans, including N301 and N156, beyond the traditional N332 glycan (Julien, Cupo *et al.*, 2013; Garces *et al.*, 2014). In contrast, the 10-1074 family relies heavily on the N332 glycan for binding and neutralisation. However, it is more potent than the PGT121 family (Mouquet *et al.*, 2012; Garces *et al.*, 2014). The BG18 family emerges as one of the most potent neutralising antibodies, engaging an extensive glycan array including N332, N392, and N386. The BG18 family has less somatic hypermutation with reduced dependency on the V3 loop protein surface, and it is an interesting candidate for vaccine development (Freund *et al.*, 2017; Barnes *et al.*, 2018).

1.2.9.4 Gp120/41 interface

The gp120/gp41 interface (highlighted in olive-green in Fig 1.6) represents another critical structural element in HIV entry. This non-covalent interface is formed by interactions between the C-terminal region of gp120 and the N-terminal ectodomain of gp41, which plays a crucial role in viral fusion (Pancera *et al.*, 2013). The interface exists in a meta-stable state, transiently exposed during viral entry and strategically positioned to avoid competition with other broadly neutralising antibodies (Tran *et al.*, 2012). However, only a limited number of interface bNAbs have been identified, including PGT151, 8ANC195, 35022, 3BC196, and 3BC315 (Walker *et al.*, 2009; Huang *et al.*, 2014; Sok *et al.*, 2016). PGT151 is the most broad and potent bNAb in this class, and it interacts with N-linked glycans at positions N611, N637, and N197 (Blattner *et al.*, 2014; Falkowska *et al.*, 2014). These antibodies highlight the complex mechanisms of viral immune evasion and the sophisticated targeting strategies of the immune system.

1.2.9.5 Membrane-proximal external region (MPER)

The membrane-proximal external region (highlighted in light peach in Fig 1.6) is a critical 23-amino acid linear region (660-683 on HXB2) located at the C-terminal end of the gp41 ectodomain that plays a pivotal role in HIV membrane fusion (Salzwedel, West and Hunter, 1999; Suárez *et al.*, 2000; Montero *et al.*, 2008; Gach *et al.*, 2011). Several bNAbs targeting this epitope have been isolated, with 10E8 emerging as the most effective, demonstrating exceptional neutralisation breadth (97-99%) and strong potency when tested against multi-clade virus panels, including the 208-virus Seaman global panel commonly used for bNAb assessment (Huang *et al.*, 2012; Eroshkin *et al.*, 2014; Wagh *et al.*, 2018; Krebs *et al.*, 2019). The 10E8 antibody primarily recognizes conserved residues within the distal MPER region including W672, F673, W676, T676, and R683, making it a promising candidate for therapeutic development (Huang *et al.*, 2012).

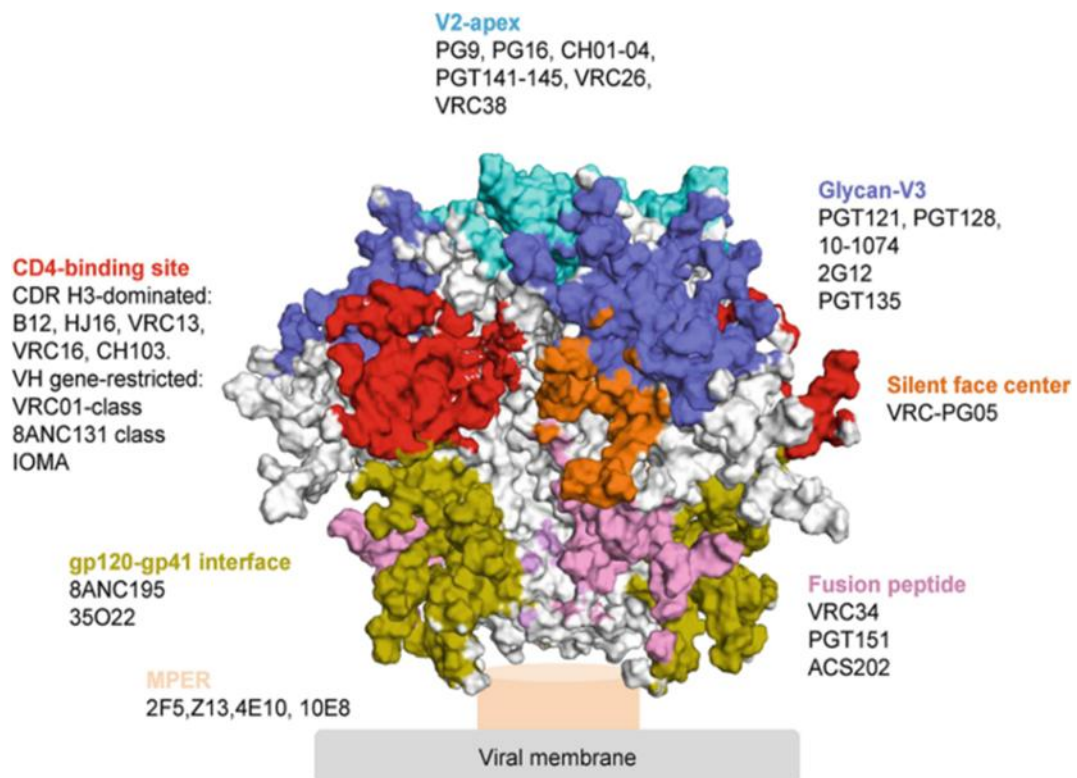


Figure 1.6: Schematic representation of the HIV-1 env glycoprotein trimer (Zhou and Xu, 2018). HIV-1 bNAbs epitopes are highlighted; bNAb epitopes located within gp120 epitopes involve the CD4 binding site (red), V2-apex (blue), V3-glycan (purple), Silent face centre (orange), while bNAb epitopes located within gp41 involve the membrane-proximal external region (MPER) (peach) and fusion peptide (pink). The gp120-gp41 (olive green) interface is in the junction that connects the gp120 and gp41 subunits.

1.2.10 HIV Antibody-based Vaccine Trials

Multiple HIV vaccine efficacy trials have been conducted over the past decades, with varying degrees of success and failure. The first vaccine clinical trials used recombinant monomeric HIV-1 envelope proteins, these include the VAX004 in North American and European populations and VAX003 in Thai injection drug users. They evaluated AIDSVAX recombinant B/B and AIDSVAX recombinant B/E respectively, however they both failed to demonstrate protective efficacy despite inducing high antibody titers (Flynn *et al.*, 2005; Pitisuttithum *et al.*, 2006). A modest breakthrough was achieved through the RV144 trial in Thailand, which achieved 31.2% vaccine efficacy through a prime-boost regimen combining ALVAC-HIV (canarypox vector) with AIDSVAX B/E gp120 (Rerks-Ngarm *et al.*, 2009). Follow-up analyses of the RV144 participants identified several correlates of reduced infection risk, including high levels of V1/V2-specific IgG3 antibodies that waned rapidly post-vaccination (Yates *et al.*, 2014), distinct polyfunctional Fc-effector profiles compared to VAX003 (Chung *et al.*, 2014), and polyfunctional HIV-specific antibody responses correlating with viral control (Ackerman *et al.*, 2016). Building on these findings, subsequent trials were launched but with disappointing outcomes: HVTN 505, which tested a DNA prime and recombinant adenovirus serotype 5 vector boost, was terminated early due to lack of efficacy and concerns about increased HIV acquisition risk (Hammer *et al.*, 2013). In addition, the HVTN 702 (Uhambo) trial, a subtype C-adapted modification of RV144 using ALVAC-HIV with a bivalent gp120/MF59 boost, was halted for futility (Gray *et al.*, 2019). These vaccines failed to elicit broadly neutralizing antibodies because they used monomeric gp120 forms and bNAbs require a trimeric Env structure. Furthermore, Imbokodo study (HVTN 705/HPX2008), which evaluated a mosaic adenovirus vector-based vaccine in Southern African women, also failed to demonstrate significant protection (Gray *et al.*, 2024). Collectively, these trials highlight the persistent challenges of HIV vaccine development but continue to provide valuable insights for refining future strategies.

Significant advancements were made in structure-based vaccine immunogen design by developing stabilised HIV envelope trimers, with the concept first introduced through the SOS gp140 (Sanders *et al.*, 2002), leading to the creation of BG505 SOSIP.664 (Sanders *et al.*, 2013). This trimer was engineered through specific modifications, including an intermolecular disulfide bond between residues 501 and 605 (SOS), isoleucine to proline substitution at position 559 (IP), and truncation at residue 664 (Sanders *et al.*, 2013). In non-human primate studies, BG505 SOSIP.664 trimer induced neutralising antibodies against tier 2 viruses, while human trials demonstrated both safety and immunogenicity with robust autologous neutralising responses (Sanders *et al.*, 2015; Pauthner *et al.*, 2019). Germline targeting vaccines has emerged as an innovative vaccine approach, activating specific naive B cell precursors through carefully designed immunogens that bind to germline B cell receptors (Jardine *et al.*, 2016; Leggat *et al.*, 2022). The IAVI G001 trial, conducted at multiple sites in the United States,

demonstrated this strategy's potential when participants received two doses of eOD-GT8 60mer nanoparticle, successfully activating VRC01-class B cell precursors in 97% of vaccine recipients (Cohen *et al.*, 2023). Another alternative vaccine strategy is passive immunisation, which involves the direct administration of bNAbs in participants living with or without HIV. The Antibody Mediated Prevention (AMP) trial evaluated VRC01 monoclonal antibody, demonstrating 75% prevention efficacy against highly sensitive viral strains but no protection against resistant strains (Corey *et al.*, 2021). A separate clinical trial investigating 3BNC117 and 10-1074 combination therapy in chronically infected individuals showed sustained viral suppression during analytical treatment interruption (Mendoza *et al.*, 2018).

Despite these advances, significant challenges persist in HIV vaccine development. The extraordinary genetic diversity, heavy glycosylation of the HIV-1 envelope, and complex immune evasion mechanisms impede comprehensive protection. Future research must address these limitations, focusing on developing immunogens that can generate broadly neutralising antibodies capable of targeting conserved viral epitopes across diverse viral strains.

1.2.11 HIV-1 Vertical Transmission: Mechanisms, Challenges, and Transmitted/Founder Virus Characteristics

Vertical transmission of HIV-1 represents the predominant mechanism of paediatric HIV acquisition globally, accounting for approximately 90% of all childhood HIV infections (De Cock *et al.*, 2000a; UNAIDS, 2024). The natural history of perinatal HIV-1 transmission without therapeutic intervention demonstrates transmission rate ranges from 15-45%, with considerable variation observed across different geographic regions and HIV-1 subtypes (Connor *et al.*, 1994; Athena P. Kourtis *et al.*, 2006). Vertical transmission can occur during three distinct gestation periods: in utero (30-40% of infections), intrapartum (50-65%), and postnatally through breastfeeding (10-20%) (Fowler, Simonds and Roongpisuthipong, 2000; Magder *et al.*, 2005). Despite remarkable progress achieved through comprehensive prevention of vertical transmission programs, an estimated 150,000-160,000 new paediatric HIV infections continue to occur annually worldwide (UNAIDS, 2024). Sub-Saharan Africa bears the greatest burden, with 78 % of children aged 0-14 living with HIV residing in this region (UNAIDS, 2024). This persistent global burden underscores an urgent need for innovative prevention strategies, particularly in resource-limited settings where implementation challenges remain substantial.

The implementation of combination antiretroviral therapy (cART) during pregnancy, labor, and the postpartum period has revolutionized vertical transmission outcomes, with landmark studies such as ACTG 076 demonstrating the first significant reduction in transmission rates through zidovudine monotherapy (Connor *et al.*, 1994), subsequently followed by more potent combination regimens (Cooper *et al.*, 2002; Townsend *et al.*, 2014). Contemporary vertical transmission guidelines recommend immediate initiation of cART for all pregnant women living with HIV, regardless of CD4+ T cell count or viral load, alongside elective caesarean delivery for women with detectable viral loads and avoidance of breastfeeding in resource-rich settings (WHO, 2022). However, significant implementation barriers such as the late antenatal care presentation, inadequate healthcare infrastructure, stock-outs of antiretroviral medications, poor adherence to treatment regimens, and socioeconomic factors that limit sustained engagement with care (Nachega *et al.*, 2012b). Furthermore, breakthrough transmissions continue to occur even in optimal settings, suggesting that additional mechanisms beyond viral suppression may be required to achieve the global goal of eliminating paediatric HIV infections (Sibiude *et al.*, 2014; Peters *et al.*, 2017). This highlights an urgent need for alternative vertical transmission prevention and therapy strategies such as passive administration of bNAbs. However, very few studies evaluated the role of bNAbs in prevention or treatment of paediatric populations.

The characterization of HIV-1 Transmitter/Founder (T/F) viruses in the context of mother-to-child transmission has revealed critical insights into the selective pressures that govern perinatal HIV-1 transmission. Single-genome amplification and next-generation sequencing technologies have demonstrated that infant T/F viruses represent a genetically restricted subset of the diverse maternal viral population, typically consisting of one to three transmitted variants that establish productive infection in the infant (Luo *et al.*, 2005; Zhang *et al.*, 2010; Russell *et al.*, 2013). These T/F viruses exhibit distinct phenotypic characteristics that differentiate them from the broader maternal viral quasispecies. Notably, the infants T/F viruses demonstrated enhanced resistance to autologous maternal plasma neutralization, with studies showing that 85% of infant envelope pseudoviruses resist neutralization by paired maternal plasma samples (Milligan *et al.*, 2015; Fouda *et al.*, 2018). This resistance pattern suggests that maternal neutralizing antibody responses exert selective pressure during transmission, favouring variants that have evolved mechanisms to escape autologous neutralization (Bunnik *et al.*, 2008; Permar *et al.*, 2015). However, despite resistance to maternal antibodies, infant T/F viruses frequently retain sensitivity to heterologous neutralizing antibodies and broadly bNAbs, indicating that the escape mechanisms are specifically adapted to evade maternal immune responses rather than conferring broad neutralization resistance (Fouda *et al.*, 2018).

1.2.12 TZM-bl Neutralization Assay

The TZM-bl neutralisation assay is a widely used technique for measuring the neutralising activity of antibodies or compounds against HIV-1; it was developed at Duke University in the late 1990s (Platt *et al.*, 1998). This assay involves the use of TZM-bl HeLa cell line engineered to express CD4 receptors, CXCR4 and CCR5 coreceptors, which are essential for HIV-1 entry into host cells (Platt *et al.*, 1998; Wei *et al.*, 2003). This cell line contains integrated copies of luciferase and β -galactosidase reporter genes under the control of the HIV-1 LTR promoter (Wei *et al.*, 2003). It has undergone several modifications over time to improve its sensitivity and reliability. The main modification was the introduction of Env-pseudotyped viruses instead of replication-competent strains (Li *et al.*, 2005; David C Montefiori, 2009). Pseudoviruses have a single round of infection, which allows the measurement of the neutralisation activity, enhances safety and ease of handling of viruses (Wei *et al.*, 2003).

The TZM-bl neutralisation assay has become invaluable in HIV vaccine research and development. It is critical to screen vaccine-induced neutralising antibodies, evaluate the neutralisation sensitivity of diverse HIV-1 strains, and identify individuals who develop bNAbs (Mascola *et al.*, 2005). This assay is also used in epitope mapping of neutralising antibodies, essential for understanding the molecular mechanisms of neutralisation and guiding immunogen design (Walker *et al.*, 2009). Additionally, the

TZM-bl assay has been instrumental in large-scale screening efforts to discover novel HIV-1 inhibitors and assess the neutralisation breadth and potency of antibodies isolated from HIV-1-infected individuals (Doria-Rose *et al.*, 2009). Other cell lines, such as A3R5 or A3R5.7, were explored for the neutralisation assay to improve the neutralisation sensitivity (Sarzotti-Kelsoe *et al.*, 2014). However, these cell lines did not significantly outperform the TZM-bl cells in detecting neutralising antibodies. Standardisation of assay protocols, reagents, and data analysis methods has been crucial for ensuring consistency and reproducibility across different laboratories (Todd *et al.*, 2012).

1.2.13 Coreceptor Usage

Coreceptor usage detection assays play a crucial role in HIV research and treatment; they determine viral tropism and guide the selection of appropriate antiretroviral therapy. These assays include bioinformatics tools and tissue culture-based assays (Jensen *et al.*, 2003; Kuiken, Korber and Shafer, 2003). Bioinformatic tools employ computational algorithms to analyse genetic sequences of HIV env and predict their coreceptor usage based on specific genetic signatures. In the early 2000s, researchers developed various tools such as Web-based Position Specific Scoring Matrices (Web-PSSM), Geno2Pheno, and PSSM-X4/R5, which analyse the V3 loop of gp120 and predict coreceptor usage (Jensen *et al.*, 2003; Kuiken, Korber and Shafer, 2003; Lengauer *et al.*, 2007a). Additionally, advancements in computational power have enabled more accurate structural modelling of the env protein and its interactions with coreceptors (Chuang *et al.*, 2008; Tamamis and Floudas, 2014).

In contrast, tissue culture assays provide direct phenotypic evidence of coreceptor tropism and are often used to validate bioinformatic predictions. These assays include pseudovirus entry assays, replication-competent virus assays, and single-cycle entry assays. Various assays have been designed, including the TZM-bl assay, Ghost cell assays, and Cf2Th cell assays. These assays include the production of HIV pseudoviruses carrying reporter genes to infect cell lines expressing different coreceptors (David C. Montefiori, 2009; Chikere *et al.*, 2013). Meanwhile, replication-competent virus assays, including the MT-2 cell assay, PBMC assay, and U87 cell assay, have been refined over time with improved detection methods and biosafety protocols (Björndal *et al.*, 1997; Yi *et al.*, 1999).

Recently, fluorescence-based assays such as the BlaM-Vpr assay, virion-based fusion assay, and FRET-based assays have emerged as powerful tools for real-time monitoring of virus-cell interactions. These assays provide insights into the dynamics of coreceptor engagement and membrane fusion (Cavrois, De Noronha and Greene, 2002; Melikyan, 2014). Choosing a particular coreceptor usage detection method often depends on the specific research question, available resources, and the need for phenotypic

confirmation. Increasingly, researchers are employing a combination of bioinformatics and phenotypic assays to obtain a comprehensive understanding of coreceptor usage and its implications for HIV pathogenesis and treatment.

1.2.14 Sequencing

The evolution of DNA sequencing technology has dramatically transformed genomics research, with each generation of techniques offering unique advantages and applications. First-generation methods, primarily Sanger sequencing, continue to serve as the gold standard for targeted studies and clinical diagnostics due to their high accuracy (>99%) and read lengths up to 1000 base pairs (Smith *et al.*, 1986; Shendure and Ji, 2008). Despite the advent of newer technologies, Sanger sequencing remains widely used, especially in resource-limited settings, owing to its simplicity, lower infrastructure demands, and suitability for small-scale projects, teaching, and validating critical findings (Erlich *et al.*, 2009; Kulkarni and Frommolt, 2017). Sequence analysis is simple, smaller data files, and lower power and internet requirements make it advantageous in labs with budget constraints, limited bioinformatics expertise, or unreliable infrastructure (Helmy, Awad and Mosa, 2016; Schwarze *et al.*, 2018).

The next-generation sequencing (NGS) techniques revolutionised the field by simultaneously enabling massively parallel sequencing of millions to billions of DNA fragments (Margulies *et al.*, 2005; Bentley *et al.*, 2008). This technological leap dramatically reduced costs, making \$1000 human genomes a reality, and increased speed, allowing a human genome to be sequenced in days (Dijk *et al.*, 2014). NGS opened doors to population-scale studies and fields like metagenomics despite having lower accuracy (98-99.9%) and shorter read lengths (typically 100-600 bp) compared to Sanger sequencing (Glenn, 2011; Quince *et al.*, 2017). However, the high throughput and cost-effectiveness of NGS have made it invaluable for large-scale genomic projects and have significantly accelerated research in various fields of biology and medicine.

The choice of sequencing techniques depends on the specific research requirements, available resources, and the scale of the project. While NGS has revolutionised large-scale genomic studies, Sanger sequencing is crucial in targeted analyses, validation studies, and settings where high accuracy and simplicity are paramount. As sequencing technologies continue to evolve, researchers can leverage the strengths of both approaches to advance our understanding of genetic information and its implications for health, disease, and biological processes (Slatko, Gardner and Ausubel, 2018).

1.3 Study Rationale/ Research Problem.

The limitations of current ART, including long-term toxicity, drug resistance, and the demanding regimen of daily lifelong adherence, underscore the urgent need for alternative therapeutic approaches in HIV treatment. While combination ART has dramatically transformed HIV from a fatal disease to a manageable chronic condition, it remains effective but not curative, and no successful preventive vaccine is currently available. In this therapeutic landscape, bNAbs have emerged as a promising new frontier, offering potential solutions for both HIV prevention and treatment through their ability to neutralize diverse HIV variants by targeting conserved viral epitopes.

Despite compelling evidence from early clinical trials demonstrating that bNAbs are safe, well-tolerated, and capable of significantly reducing HIV RNA levels in adults, a critical knowledge gap persists regarding their application in paediatric populations and mother-infant pairs. This gap is particularly concerning given that children represent one of the most vulnerable populations affected by HIV, with unique physiological characteristics and immune system dynamics that may profoundly influence therapeutic outcomes.

The paediatric HIV population presents distinct challenges that demand specialized investigation. Recent breakthrough studies have shown that monthly treatment with neutralizing antibodies can maintain HIV suppression in nearly half of children who received early antiretroviral treatment, particularly those with favorable viral reservoir characteristics. However, children's rapidly developing immune systems, different pharmacokinetic profiles, and varying viral dynamics compared to adults raise fundamental questions about optimal dosing, efficacy, and safety profiles of bNAb therapy in this population.

Furthermore, the complexity of HIV coreceptor usage adds another layer of therapeutic consideration. The virus's ability to switch from using CCR5 to CXCR4 as its primary entry coreceptor represents a significant escape mechanism that can lead to treatment failure, particularly with entry inhibitors like maraviroc. This evolutionary pressure becomes especially relevant during chronic infection phases, where coreceptor switching often coincides with accelerated disease progression and declining CD4⁺ T-cell counts. Understanding these dynamics is crucial for optimizing bNAb therapy, as different antibody targets may exhibit varying efficacy profiles depending on viral coreceptor preferences.

These findings could have implications for shaping HIV prevention policies and determining the feasibility of administering bNAbs to both mothers and infants within the same treatment regimen. This study could provide valuable insights into the potential use of bNAbs for paediatric HIV treatment and prevention of mother-to-child transmission, as well as identify any unique considerations or challenges that may arise in these populations.

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CHAPTER 2

Neutralisation Profiles of Infant-Transmitted Founder HIV-1 Subtype C Viruses against Broadly Neutralizing Monoclonal Antibodies

2.1 Introduction

Vertical HIV transmission remains a critical global health challenge, with approximately 160,000 children newly infected annually, predominantly in resource-limited settings of sub-Saharan Africa (UNAIDS, 2023). Despite significant advances in the prevention of vertical transmission (PVT) programs, infants continue to face substantial risks of HIV acquisition during pregnancy, childbirth, and breastfeeding (De Cock *et al.*, 2000b; WHO, 2024). Vertical transmission of HIV can occur at multiple stages: in utero (30-50% of infections), during labour and delivery (10-20%), and through breastfeeding (10-20%), with transmission rates varying based on maternal viral load, antiretroviral treatment, and infant immune system characteristics (Thorne and Newell, 2004; Athena P Kourtis *et al.*, 2006).

Current vertical transmission prevention strategies primarily rely on antiretroviral (ART) administration during pregnancy, labour, and postpartum periods, alongside comprehensive maternal care and infant prophylaxis settings (WHO, 2024). However, these approaches face significant challenges, including adherence issues, drug resistance, and limited accessibility in resource-constrained settings (Sibanda *et al.*, 2013). In response to these limitations, broadly neutralizing antibodies (bNAbs) have emerged as a promising alternative approach in HIV-1 prevention and treatment strategies. They have a longer half-life and could be administered intravenously twice a year, they also have lower toxicity and side effects compared to ART drugs. These antibodies target multiple regions of the HIV-1 Env trimer, including the CD4 binding site, V1/V2 loop, V3 glycan patch, MPER, the interface between gp120 and gp41 subunits (Gama & Koup, 2018; Sok & Burton, 2018; Walker & Burton, 2018), the fusion peptide (Falkowska *et al.*, 2014; Kong *et al.*, 2016; van Gils *et al.*, 2016), and the silent face (Schoofs *et al.*, 2019). However, they have unique features such as a high frequency of somatic hypermutation (CD4 binding site bNAbs), long CDRH3 loops (V2 apex and V3 glycan bNAbs), and polyreactivity or autoreactivity (MPER and CD4bs bNAbs). These unique features are difficult to elicit in vaccine settings, hence no vaccine has successfully elicited bNAbs.

Animal studies have demonstrated the potential of passive immunization of bNAbs in preventing simian-human immunodeficiency virus SHIV infection across various animal models (Hessell *et al.*, 2016; Julg *et al.*, 2017; Xu *et al.*, 2017). These studies showed that bNAbs could protect macaques

against intrarectal SHIV challenge at serum concentrations achievable by vaccination (Moldt *et al.*, 2012; Shingai *et al.*, 2014). Another landmark study demonstrated the potential of bNAbs in preventing vertical transmission by successfully halting SHIV infection in all infant macaques who were treated with neutralizing monoclonal antibodies (nMAbs) (Hessell *et al.*, 2016). Human clinical trials have demonstrated the safety and efficacy of bNAbs in HIV-1 prevention and treatment (Scheid *et al.*, 2016; Bar-On *et al.*, 2018b; Caskey, Klein and Nussenzweig, 2019).

Landmark clinical trials have demonstrated the potential of bNAbs in HIV prevention and treatment. The AMP trial evaluated VRC01's efficacy in preventing HIV-1 acquisition, showing promise against sensitive viral strains (Corey *et al.*, 2021). The IMPAACT P1112 trial investigated the safety and pharmacokinetics of VRC01 in HIV-exposed newborn infants, showing that it was well tolerated and achieved plasma concentrations comparable to adults, thereby supporting its potential role in preventing vertical transmission (Cunningham *et al.*, 2020). VRC01 has demonstrated a favourable safety profile in clinical trials, with exceptional safety of intravenous administration and infusion reactions being uncommon and typically mild or moderate (Edupuganti *et al.*, 2021; Takuva *et al.*, 2022). This safety data supports its potential use in vulnerable populations, including pregnant women, pending dedicated pregnancy studies. The dual combination of 3BNC117 and 10-1074 and triple combination of 3BNC117, 10-1074 and PGT121 maintained viral suppression for extended periods and significantly reduced the emergence of viral escape variants compared to single antibody treatment regimens (Bar-On *et al.*, 2018a; Mendoza *et al.*, 2018). These multi-specific antibody combinations target different epitopes on the HIV envelope protein, thereby creating higher barriers to viral resistance and improving overall therapeutic efficacy.

Research examining vertical HIV transmission has revealed unique aspects of infant immune responses and viral escape mechanisms (Milligan & Overbaugh, 2014). Studies have shown that infants develop broader and more potent HIV-specific antibody responses compared to adults, with some infants demonstrating cross-clade neutralising antibodies as early as one year of age (Goo *et al.*, 2014). However, HIV can effectively evade these early immune responses through sophisticated conformational masking of epitopes (Goo *et al.*, 2014). Analysis of TF viruses from vertically infected infants has revealed complex neutralisation patterns, with some viruses showing resistance to CD4 binding site antibodies, V1/V2-directed antibodies, and glycan-dependent antibodies (Kumar *et al.*, 2021). Additional studies have identified specific molecular signatures in infant TF viruses that contribute to neutralisation resistance, including unique glycan patterns and conformational epitopes that differ from adult-derived viruses (Ditse *et al.*, 2018; Kumar *et al.*, 2021). Despite these advances, significant knowledge gaps remain regarding infant immune responses and viral adaptation during

vertical transmission, highlighting the need for continued research. This study assessed the neutralisation sensitivity of *in utero* infected infants with TF viruses to various bNAbs. Env-pseudotyped TF viruses were generated from *in utero* infected infants and evaluated against a panel of nine bNAbs targeting five different epitopes (CD4bs, V1/V2 loop, V3 glycan, MPER and gp120/41 interface). HIV-1 *env* was amplified from plasma samples, cloned into TOPO 3.1 expression vector and sequenced by Sanger sequencing. Signature amino acid sequences were potentially linked to neutralisation sensitivity or resistance to various bNAbs.

2.2 Materials and Methods

2.2.1 Study population

Ucwaningo Lwabantwana (Baby Cure) cohort study was conducted in KwaZulu-Natal, South Africa, from 2015 to date, with the primary aim of assessing the impact of early antiretroviral therapy (ART) initiation in infants with in utero infection (Millar *et al.*, 2020). In this sub-study, 21 infants (15 females and six males) were recruited from four regional hospitals: Queen Nandi Regional, Mahatma Gandhi Memorial, Edendale, and Stanger hospitals. The study implemented a structured longitudinal follow-up design with monthly clinical visits and blood sample collection during the first six months, followed by quarterly visits thereafter. In 17 infants, blood samples were collected at birth, while in four infants, samples were collected at one month after birth. The study focused on high-risk infants, which included three specific groups: those born to mothers who seroconverted during pregnancy, infants born to HIV-positive mothers that were ART-naive, and infants born to HIV-positive mothers on ART who were either non-adherent or experienced treatment interruptions. To ensure accurate HIV-1 detection, all participants underwent comprehensive testing using the GeneXpert HIV-1 Qual Assay (Cepheid AB, Sunnyvale, CA, USA). Diagnostic testing was performed using dried blood spots collected from the participants.

In alignment with the South African National Prevention of Vertical transmission Guidelines, the study implemented a comprehensive ART protocol for HIV-exposed infants, beginning immediately after birth. All infants received daily nevirapine (NVP); in high-risk cases, they received additional twice-daily zidovudine (AZT). Upon confirming a positive HIV-1 test, the research team initiated a structured combination antiretroviral therapy (cART) using oral suspensions of zidovudine (AZT), lamivudine (3TC), and nevirapine (NVP). The treatment strategy was dynamically adapted based on infant characteristics, with infants under 2.5 kg switching to ritonavir-boosted lopinavir (LPV/r), 3TC, and abacavir (ABC) at 42 weeks corrected gestational age, while other infants transitioned to the same combination at one month of age. Concurrently, transmitting mothers received Tenofovir/Emtricitabine/Efavirenz (TDF/FTC/EFV) to support comprehensive viral management.

2.2.2 RNA Extraction and Complementary DNA (cDNA) Synthesis

Viral RNA was extracted from plasma only at birth or one-month after birth using the QIAamp Viral RNA Mini Kit (Qiagen, Milan, Italy) according to the manufacturer's instructions. Viral RNA was reverse transcribed into double-stranded cDNA using the SuperScript IV kit according to the manufacturer's instructions (Invitrogen Life Technologies, Carlsbad, CA). The reverse transcription reaction was initiated by combining 9 μ L viral RNA with deoxynucleoside triphosphates 1 μ L at 0.5 mM each and 0.25 μ M OFM19 primer 1 μ L (5' GCACTCAAGGCAAGCTTTATTGAGGCTTA 3'), followed by incubation at 65°C for 5 minutes to denature RNA secondary structures. First-strand cDNA synthesis was performed using a reaction mixture containing 4 μ L of 1 $\times$ reverse transcriptase buffer with 5 mM dithiothreitol, 1 μ L RNase inhibitor (RNaseOUT), and 1 μ L SuperScript IV reverse transcriptase (200 U/ μ L), with incubation at 55°C for 10 minutes. The cDNA was subsequently heat-inactivated at 80°C for 10 minutes, followed by RNase H digestion at 37°C for 20 minutes (Invitrogen Life Technologies, Carlsbad, CA), and the resulting cDNA was either used immediately for PCR amplification or stored at -80°C for subsequent analysis.

2.2.3 Amplification and cloning of the HIV-1 *env*

As previously described, the bulk or single genome amplification (SGA) of full-length HIV-1 *env* was amplified using a nested PCR approach with forward and reverse primers (Salazar-Gonzalez et al., 2008). Single genome amplification samples were diluted to a point where each reaction contained only one template molecule (where the PCR product was not more than 30%). First-round PCR was performed using primers OMF19 and VIF 1 (5' GGGTTTATTACAGGGACAGCAGA 3') and the Platinum Taq DNA Polymerase High Fidelity enzyme (Invitrogen, Carlsbad, CA, USA) with the following cycling conditions: initial denaturation at 94°C for 4 minutes, 35 cycles at 94°C for 15 seconds, annealing at 55°C for 30 seconds, and extension at 68°C for 4 minutes, with a final extension at 68°C for 20 minutes. Second-round PCR was performed using primers Env1A (5' CACCGGCTTAGGCATCTCCTATGGCAGGAAGAA3') forward and reverse primer Env1M (5' TAGCCCTTCCAGTCCCCCTTTTCTTTTA 3'), the first-round PCR product as a template, Phusion High Fidelity DNA polymerase (Thermo Fisher Scientific, Waltham, MA, USA). Reactions were incubated in the following cycling conditions: initial denaturation at 98°C for 30 seconds, 35 cycles of denaturation at 98°C for 10 seconds, annealing at 65°C for 30 seconds, and extension at 72°C for 4 minutes, with a final extension at 72°C for 10 minutes. The final PCR products were analysed on 1% agarose gel electrophoresis, and products of the predicted size (~3.2 kb) were ligated into the pcDNA3.1. V5-His TOPO TA vector (Invitrogen Life Technologies, Carlsbad, CA) according to the manufacturer's instructions. The ligated vector was transformed into the Top 10 competent cells at 42°C for 25 seconds (Invitrogen Life Technologies, Carlsbad, CA).

Transformed reaction mixtures were inoculated on LB-Amp (ampicillin; 100 µg/ml) plates and cultured overnight at 37°C. Positive colonies were picked and grown in LB broth (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with ampicillin (100 µg/ml) (Sigma-Aldrich, St. Louis, MO, USA), and plasmid DNA was isolated using a QIAprep spin miniprep kit (Qiagen, Valencia, CA) from all positive clones.

2.2.4 Sanger sequencing

HIV-1 *env* clones were sequenced using the BigDye Terminator kit (Applied Biosystems, Foster City, CA) following the manufacturer's instructions. The sequences (appendix) were determined using an ABI 3500xl genetic analyser (Applied Biosystems, Foster City, CA) and edited using the Sequencher program, version v5.4.1 (Gene Codes, Ann Arbor, MI). All chromatograms were carefully inspected for sites of ambiguous sequence (double peaks), and those that contained one or more positions of mixed bases were excluded from further analysis.

2.2.5 Next-generation Sequence (NGS) Sequencing

Next-generation sequencing was performed on plasma samples from transmitting mothers. Total viral RNA was extracted from maternal plasma samples (n = 7) using the NucliSens easyMAG system (bioMérieux, Marcy-l'Étoile, France) following the manufacturer's protocols, with subsequent RNA concentration performed using Agencourt RNAClean XP beads (Beckman Coulter, Pasadena, CA, USA) to achieve sufficient input material for library preparation. Strand-specific cDNA libraries were prepared using the SMARTer Stranded Total RNA-Seq Kit v2 - Pico Input Mammalian (Takara Bio, Kyoto, Japan) with specific modifications to the manufacturer's protocol as previously described (Bonsall *et al.*, 2020). Deep sequencing was performed using the Illumina MiSeq platform with the MiSeq Reagent Kit v3 (600-cycle) (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. Raw sequencing reads underwent quality control filtering using FastQC v0.11.9, followed by alignment against the Los Alamos HIV Sequence Database (<http://www.hiv.lanl.gov/>) "Web Alignments 2017" reference sequences using BLAST analysis to determine HXB2 coordinate positions for each read.

2.2.6 Phylogenetic and sequence analyses

Following both Sanger and NGS sequencing, overlapping DNA fragments were assembled and edited using Sequencher Program v8.1.2 (Gene Codes Corporation, Michigan, USA) and aligned using ClustalW. Maximum-likelihood phylogenetic trees were constructed using Geneious Prime version 2024.0.5 to evaluate the clustering of sequences with each other and with subtype reference

strains obtained from the Los Alamos HIV sequence database. HIV *env* nucleotide sequences (within amino acid positions 6348 and 8478 relative to the HXB2 strain of HIV-1) were aligned against the corresponding regions of a panel of reference sequences from different HIV-1 subtypes (obtained from the Los Alamos HIV Database) using ClustalW. Phylogenetic analysis was conducted using the Neighbour-joining method using Figtree software. Epitope analysis focused on five known bNAb target sites, including the CD4 binding site, V1/V2 apex, V3-glycan supersite, the membrane-proximal external region (MPER) and the gp120/gp41 interface (Burton and Hangartner, 2016a). HIV *env* nucleotide sequences were manually curated in Geneious Prime v.2023 with env HXB2 for numbering.

2.2.7 Sequence Logo Plots

Logo plot were generated with sequences produced from both Sanger and NGS sequence alignment using the AnalyzeAlign tool from the Los Alamos HIV Sequence Database (https://www.hiv.lanl.gov/content/sequence/ANALYZEALIGN/analyze_align.html) using position-based sequence weights and background frequencies calculated from the input alignment. Stack heights were measured in bits using Shannon entropy, with the Y-axis scaled from 0 to 1 bit. The amino acid colour scheme was employed (Red: resistant, Blue: sensitive, Green: intermediate/undetermined, Black: uncharacterised). Each position in the logo plot represents the sequence conservation at that site, where letter height is proportional to nucleotide frequency and overall stack height indicates sequence conservation measured in bits.

2.2.8 Maintenance of Cells

The TZM-bl cell line was obtained from the NIH AIDS Research Centre (NIH, AIDS Reagent Program, Bethesda, MD, USA). In contrast, the human embryonic kidney (HEK) 293T cell line was obtained from the American Type Culture Collection (ATCC, Manassas, CA, USA). The cell lines were maintained in Dulbecco's Modified Eagle's Medium (DMEM) (Life Technologies, Carlsbad, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum (Gibco BRL Life Technologies, Carlsbad, California, USA), 25 mM HEPES (Life Technologies, Taiwan) and 50 µg/ml gentamicin (Sigma Aldrich, St Louis, MD, USA). The cells were incubated at 37 °C with 5% CO₂, and the growth media were replaced every two days. The medium was decanted, and monolayers were rinsed with sterile phosphate-buffered saline (PBS) and trypsinised with 0.25% trypsin-ethylenediaminetetraacetic acid (EDTA) (Invitrogen, Grand Island, NY, USA). The cells were re-suspended in a growth medium and counted with trypan blue and a hemocytometer TC20 Automated Cell Counter (Bio-Rad, Hercules, California, USA).

2.2.9 Preparation and Titration of Env-pseudotyped Viruses

Env-pseudotyped viruses were prepared by transfecting exponentially dividing HEK293T cells (5×10^6 cells in 15 ml growth medium in a T-75 culture flask) with 4 µg of *env* expression plasmid and 8 µg of an *env*-deficient HIV-1 backbone vector (pSG3Δ^{env}), using X-tremeGENE 9 DNA Transfection Reagent as described by the manufacturer. The transfection complexes were allowed to incubate for 30 minutes at room temperature. Afterwards, they were added into HEK293T cells and incubated for 48 hrs with a change of growth media after 3-8 hrs following incubation. Virus-containing cultures were filtered through a 0.45µm filter to eliminate cell debris. Fetal Bovine Serum was adjusted to a final concentration of 20% before storing the viral stocks at -80°C in 1.5 ml sterile screw cap polypropylene vials. Env-pseudotyped viral stocks were titrated by performing serial 4-fold dilutions in quadruplicate in a growth medium in 96-well culture plates. Freshly trypsinised TZM-bl cells (10,000 cells) were added to each well in a growth medium containing DEAE-dextran. Following a 48-hour incubation, the culture medium was removed from each well and replaced with Bright-Glo™ Luciferase Assay System (Promega, Madison, Wisconsin, United States). After incubation, the cell lysate was transferred to 96-well black solid plates for luminescence measurements using a Victor Nivo plate reader (Perkin-Elmer, Revvity Health Science, Canada).

2.2.10 Neutralization assay

We assessed the neutralization sensitivity of TF viruses from all 21 HIV-infected infants using a single-round luciferase reporter gene assay in TZM-bl cells, as previously described (Wei *et al.*, 2002; David C. Montefiori, 2009). For each infant, sensitivity was measured at a single time-point, with one representative *env* sequence selected per infant to capture the predominant viral variant present at the time of sampling. We evaluated a panel of nine bNAbs targeting different envelope trimer epitopes: CD4-binding site (VRC01, VRC07-523LS, 3BNC117), V2-apex (CAP256-VRC26.25, PGMD1400), V3-glycan supersite (PGT121, 10-1074), gp120-gp41 interface (PGT151), and MPER (10E8). Broadly neutralizing monoclonal antibodies were diluted in growth media, with a starting concentration of 10 µg/ml, three-fold dilutions were performed in 96-well plates and antibodies were incubated with Env-pseudotyped viruses. Freshly trypsinised TZM-bl cells (10,000 per well) were added to a growth medium with DEAE-dextran. After 48-hour incubation, the culture medium was replaced with Bright-Glo™ Luciferase Assay System, and cell lysates were transferred to black solid plates for luminescence measurement using a Victor Nivo plate reader. The 50% inhibitory concentration (IC₅₀) was calculated as the antibody concentration causing a 50% reduction in relative light units (RLU) compared to virus control wells, with background RLU subtraction. Control wells included virus controls and cell-only background controls.

2.2.11 Statistics

Statistical analysis was performed using GraphPad Prism version 10.2 software (San Diego, CA, USA). Comparisons between infants and maternal viral load were performed using the Mann-Whitney test for comparisons and Spearman rank correlation coefficient to assess association, with $P < 0.05$ considered statistically significant.

2.3 Results

2.3.1 Clinical characteristics

The median absolute CD4⁺ T-cell count was 1,952 cells/mm³, with a median CD4% of 38% in infants (Table 2.1). Infant viral loads ($\log_{10}$ VL = 4.7) were significantly higher than maternal viral loads ($\log_{10}$ VL = 3.9) ($p = 0.02$) (Fig. 2.1A). However, a positive correlation was observed ($r = 0.52$, $p = 0.02$) between maternal and infant viral loads (Fig 2.1B). The correlation emphasises the critical role of maternal viral suppression in preventing and mitigating vertical HIV transmission.

Table 2.1: Clinical characteristics of the Baby Cure study participants, including the time points of sample collection, viral load, CD4⁺ T-cell count and CD4⁺ T-cell % and transmitting mother's viral load and CD4⁺ T-cell count.

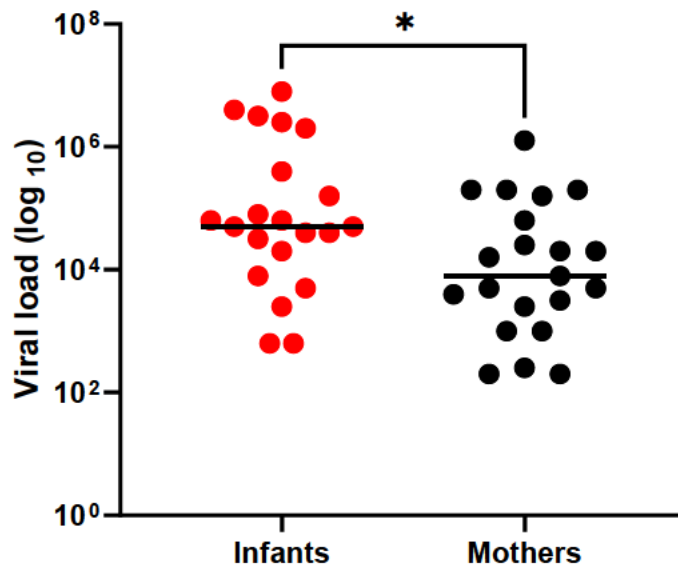
Infants								Mothers			
	PID's	Time point	Gender	Viral Load (Log ₁₀)	CD4 %	CD4 count (cell/mm ³)	Treatment Reg	PID's	Viral Load (Log ₁₀)	CD4 count (cell/mm ³)	Treatment Reg
1	50-2178-1	1 month	F	4,9	22	642	AZT/3TC/NVP	50-2178-0	6,1	369	EFV/TDF/FTC
2	50-2285-1	Birth	F	2,8	35	3498	AZT/3TC/NVP	50-2285-0	3,0	254	EFV/TDF/FTC
3	*60-0048-1	Birth	F	6,6	38	831	AZT/3TC/NVP	60-0048-0	5,3	5	EFV/TDF/FTC
4	60-0902-1	Birth	F	4,7	44	3129	AZT/3TC/NVP	60-0902-0	3,5	1201	EFV/TDF/FTC
5	60-1778-1	Birth	M	4,6	37	2397	AZT/3TC/NVP	60-1778-0	3,7	1081	EFV/TDF/FTC
6	*60-5055-1	Birth	F	4,8	55	2114	AZT/3TC/NVP	60-5055-0	5,3	588	EFV/TDF/FTC
7	60-4063-1	Birth	F	6,5	38	1952	AZT/3TC/NVP	60-4063-0	4,8	350	EFV/TDF/FTC
8	*60-4082-1	Birth	M	4,7	28	717	AZT/3TC/NVP	^60-4082-0	4,3	350	EFV/TDF/FTC
9	60-6027-1	1 month	F	4,8	24	1748	AZT/3TC/NVP	60-6027-0	4,2	558	EFV/TDF/FTC
10	*60-6041-1	Birth	F	4,3	43	1304	AZT/3TC/NVP	60-6041-0	3,6	611	EFV/TDF/FTC
11	*60-6082-1	Birth	F	6,3	69	2272	AZT/3TC/NVP	^60-6082-0	5,2	407	EFV/TDF/FTC
12	*60-8084-1	Birth	F	5,6	42	2212	AZT/3TC/NVP	60-8084-0	5,3	652	EFV/TDF/FTC
13	*60-8195-1	Birth	F	3,4	51	1625	AZT/3TC/NVP	60-8195-0	3,4	576	EFV/TDF/FTC
14	60-8171-1	1 month	F	3,9	37	1720	AZT/3TC/NVP	60-8171-0	3,7	1736	EFV/TDF/FTC
15	60-8277-1	Birth	M	4,6	51	4486	AZT/3TC/NVP	60-8277-0	2,3	693	EFV/TDF/FTC
16	*70-0239-1	Birth	F	6,9	23	500	AZT/3TC/NVP	^70-0239-0	4,4	509	EFV/TDF/FTC
17	70-0758-1	Birth	M	3,7	33	924	AZT/3TC/NVP	^70-0758-0	3,9	241	EFV/TDF/FTC
18	*70-0778-1	Birth	F	5,2	35	1680	AZT/3TC/NVP	70-0778-0	3,0	707	EFV/TDF/FTC
19	80-0180-1	Birth	M	4,5	46	2444	AZT/3TC/NVP	80-0180-0	4,3	365	EFV/TDF/FTC
20	80-0204-1	Birth	M	2,8	51	4084	AZT/3TC/NVP	80-0204-0	2,4	446	EFV/TDF/FTC
21	80-0215-1	1 month	F	6,4	27	2224	AZT/3TC/NVP	80-0215-0	2,3	877	EFV/TDF/FTC
Median				4,7	38,0	1952			3,9	558	

AZT = Zidovudine, **3TC** = Lamivudine, **NVP** = Nevirapine, **EFV** = Efavirenz, **TDF** = Tenofovir, **FTC** = Emtricitabine

*Birth means blood taken within 48 hours

^Mother's VL taken at birth

A



B

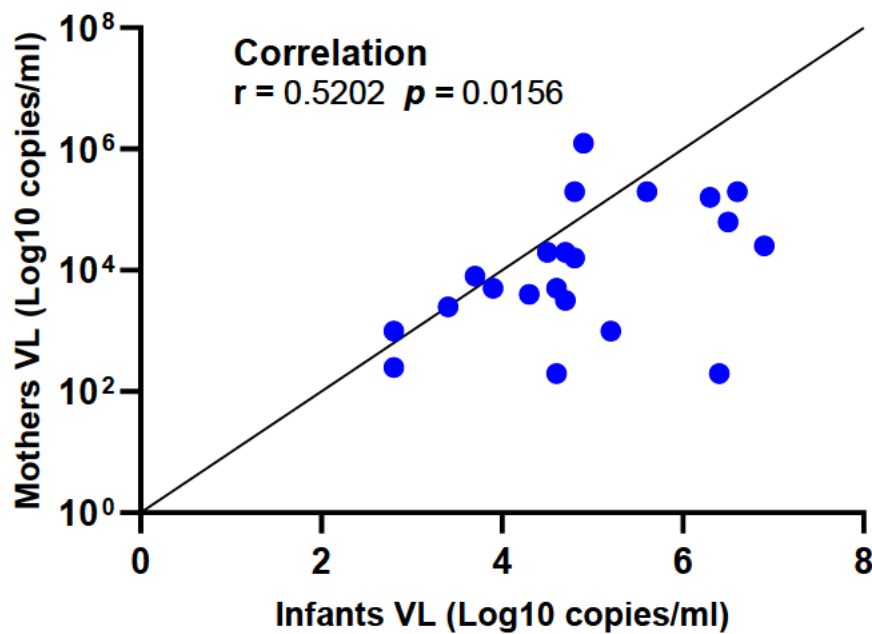


Figure 2.1: HIV-1 Viral load comparison and correlation in mother-infant pairs

A). Comparison of median viral loads between transmitting mothers and their infants. B). Scatter plot showing the correlation between maternal and infant log viral loads. Each point represents a mother-infant pair, with the mother's viral load on the x-axis and the infant's viral load on the y-axis. Statistical analysis was performed using the Mann-Whitney test for comparisons and Spearman rank correlation coefficient to assess association, with $P < 0.05$ considered statistically significant.

2.3.2 Phylogenetic analysis of HIV-1 *env* sequences from mother-infant pairs reveals predominant subtype C transmission with limited sequence diversity

Due to limited sample availability, only a total of fifty-three HIV-1 transmitted/founder (TF) *env* clonal sequences were produced from bulk PCR amplicons from twenty-one HIV-1-infected infants. Seven additional sequences were produced from mothers by single-genome amplification (SGA) (n=3) and next-generation sequencing (NGS) (n=5) (Fig. 2.2). The median number of full-length HIV-1 *env* clonal sequences from both infants and mothers was one sequence. Infants *env* sequences clustered with their transmitting mother-pair SGA or NGS sequences, highlighting the strong genetic relatedness between mother-infant viral populations (Fig 2.2). Most of the infant HIV-1 *env* sequences clustered within HIV-1 subtype C reference strain, a dominant HIV-1 subtype in South Africa (Hemelaar *et al.*, 2019; Mkhize *et al.*, 2023). However, one infant was infected with an HIV-1 subtype AC recombinant (0215-1), and two infants (4082-1 and 0204-1) were infected with HIV-1 subtype BC recombinants, respectively (Fig 2.2). We could not determine the level of diversity or multiplicity of infection due to the inability to generate single-genome amplicons.

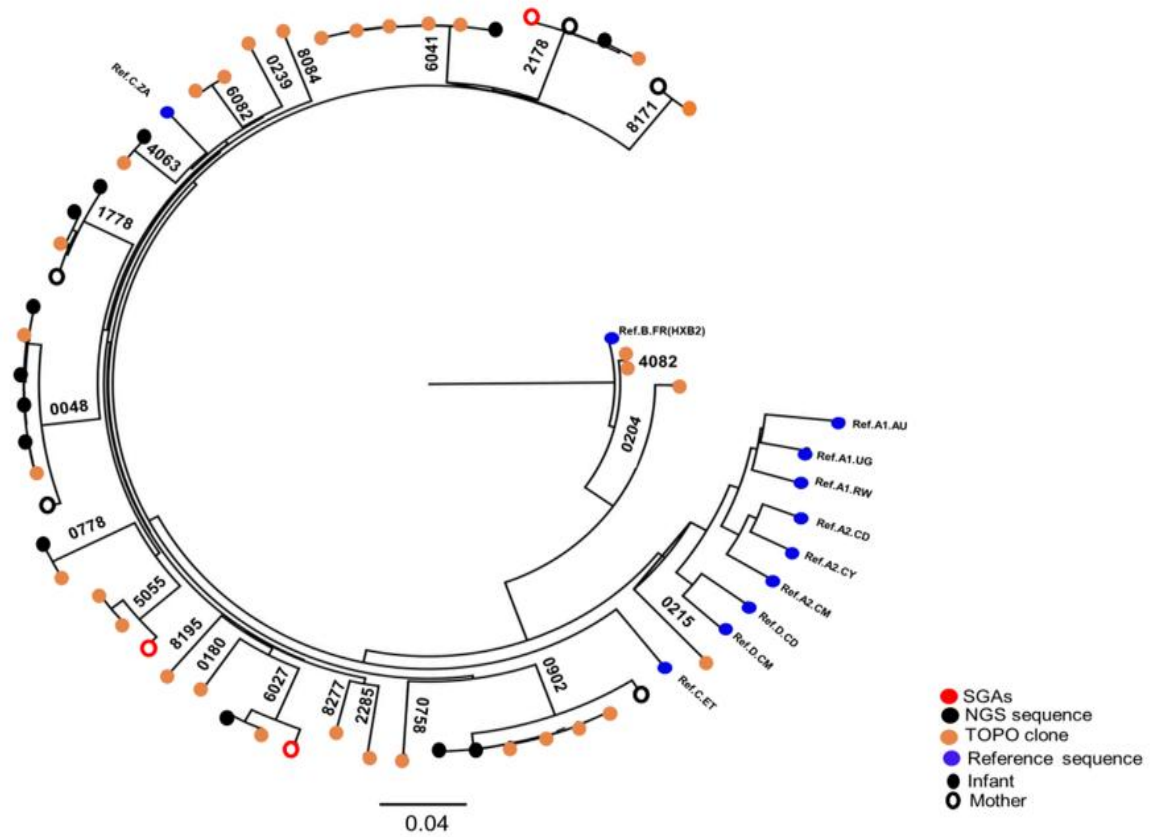


Figure 2.2: Maximum likelihood phylogenetic tree of HIV-1 env sequences from mother-infant pairs. A maximum likelihood phylogenetic tree was constructed using 53 genome sequences, comprising 45 from infants and eight from transmitting mothers. The sequences were obtained through various methods and are represented as follows: Single Genome Amplification (SGA) sequences are depicted as red circles (n = 3), TOPO clones as orange circles (n = 32), and Next-Generation Sequencing (NGS) derived sequences as black circles (n = 18). Closed circles denote infant sequences, while open circles indicate mother sequences. The tree also includes HIV-1 reference sequences (blue circles) representing various genetic subtypes obtained from the Los Alamos National Laboratory HIV Sequence Database. These reference sequences include Ref. A1.RW (**BAF31340.1**), Ref. A1.UG (**BAF31412.1**), Ref. A2.CM (**ADA83368.1**), Ref. A2.CD (**AF286238**), Ref. D.CD (**K03454**), Ref. D.CM (**AY371157**), Ref. B.FR (**NP057856.1**), and Ref. C.ZA (**AAV41353.1**), encompassing the major HIV-1 subtypes relevant to this analysis.

2.3.3 VRC07-523LS, 10-1074, and 10E8 had the highest neutralization coverage and high potency against infant HIV-1 subtype C TF viruses

All cloned *env* genes were successfully expressed in the pseudovirus system, as evidenced by infectious titers ranging from 20 000 to 50 000 RLU/mL. The ability of these pseudoviruses to mediate entry was further confirmed by neutralization assays with broadly neutralizing antibodies (Fig. S3), demonstrating that the Env proteins were functional. To determine the neutralization sensitivity of infant viruses to bNAbs, we employed a TZM-bl-based pseudovirus neutralization assay in which 21 transmitted/founder (TF) pseudoviruses produced from *in utero* infected infants were tested against a panel of 9 bNAbs targeting the CD4 binding site (VRC01, VRC07-523LS, 3BNC117), V2-apex (CAP256-VRC26.25, PGDM1400), V3-glycan supersite (PGT121, 10-1074), MPER (10E8), and gp120-gp41 interface (PGT151). Most infants TF pseudoviruses were susceptible to VRC07-523LS (95% neutralisation coverage, geometric mean IC_{50} titer of 0.04 μ g/mL) (Fig. 2.3, Fig. 2.4A- B). These pseudoviruses were also moderately sensitive to VRC01 and 3BNC117; VRC01 neutralised 76% of pseudoviruses with a geometric mean IC_{50} titer of 0.71 μ g/mL, while 3BNC117 neutralised 71% of viruses with a geometric mean IC_{50} of 0.19 μ g/mL. However, one of the pseudoviruses (PID 2285-1) was resistant to all CD4 binding site antibodies, while the other three viruses (758-1, 5055-1, and 902-1) were only resistant to 3BNC117 and VRC01.

Interestingly, we found a moderate neutralisation coverage for V2-apex bNAbs CAP256-VRC26.25 and PGDM1400 (57% and 67% coverage respectively). Nevertheless, V2-apex bNAbs (PGDM1400 and CAP256-VRC26.25) demonstrated the highest neutralisation potency against sensitive pseudoviruses, with geometric mean IC_{50} titer of 0.08 μ g/mL and 0.01 μ g/mL, respectively. However, five pseudoviruses (4082-1, 8195-1, 5055-1, 0758-1, and 2285-1) were resistant to both PGDM1400 and CAP256-VRC26.25, while four pseudoviruses (0204-1, 8277-1, 4063-1 and 6027-1) were only resistant to CAP256-VRC26.25, and two viruses (0239-1 and 2178-1) were only resistant to PGDM1400. The V3-glycan supersite bNAbs (10-1074 and PGT121) demonstrated good neutralisation coverage against the pseudoviruses, neutralising 76% and 71% of pseudoviruses, respectively, with geometric mean IC_{50} titers of 0.07 μ g/mL and 0.18 μ g/mL, respectively (Fig 2.3). However, five pseudoviruses (0204-1, 4082-1, 4063-1, 2178-1, and 6027-1) were resistant to both PGT121 and 10-1074, while 8171-1 was resistant only to PGT121.

The MPER-specific bNAb 10E8 exhibited complete neutralisation coverage (100%) but with relatively low potency (geometric mean IC_{50} titer of 0.42 μ g/mL) (Fig. 2.3, Fig. 2.4A-B). In

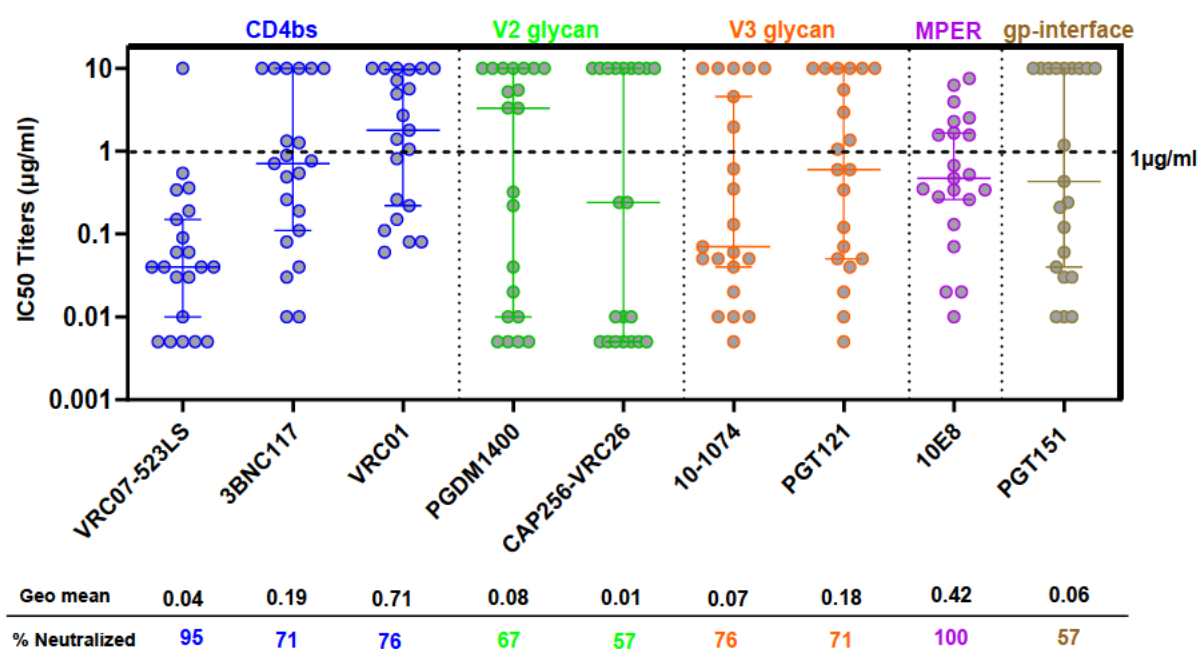
contrast, the glycan-dependent gp120-gp41 interface bNAb PGT151 showed lower coverage of only 57% despite demonstrating high potency (geometric mean IC_{50} of 0.06 $\mu\text{g/mL}$). Overall, VRC07-523LS (CD4 bs), 10-1074 (V3-glycan), and 10E8 (MPER) (Fig. 2.4A) were the most effective against infant's subtype C TF pseudoviruses, they all achieved greater than 75% coverage. Meanwhile, CAP256-VRC26.25 and PGT151 had the lowest overall performance against this panel of infant-derived pseudoviruses.

PID's	CD4-binding site			V1V2-Loop		V3 Glycan		MPER	Gp120-gp41
	VRC07-523LS	VRC01	3BNC117	PGDM1400	CAP256-VRC26.25	10-1074	PGT121	10E8	PGT151
60-6041-1	0,005	0,08	0,08	0,01	0,005	0,01	0,01	0,52	>10
80-0204-1	0,005	0,06	0,01	5,46	>10	>10	>10	0,01	0,24
60-6082-1	0,005	0,26	0,49	0,02	0,005	0,04	5,50	0,28	>10
60-4082-1	0,005	0,08	0,03	>10	>10	>10	>10	0,02	0,43
60-8195-1	0,005	0,11	0,01	>10	>10	0,005	0,60	1,56	0,01
80-0215-1	0,06	0,15	0,04	0,005	0,005	1,95	2,94	0,02	0,03
70-0778-1	0,06	0,22	0,11	0,22	0,01	0,07	0,60	1,56	0,01
60-8171-1	0,03	0,81	0,19	0,32	0,24	0,61	>10	1,65	0,06
60-8277-1	0,03	1,05	0,26	0,04	>10	0,01	0,005	2,28	>10
70-0239-1	0,04	1,4	0,89	>10	0,01	0,13	0,12	0,47	>10
50-2178-1	0,04	2,7	0,54	>10	0,005	>10	>10	6,24	>10
60-0902-1	0,04	>10	>10	0,005	0,005	0,06	1,05	3,94	>10
60-1778-1	0,04	1,79	0,76	0,005	0,005	0,05	0,02	0,26	>10
60-0048-1	0,09	4,9	>10	3,3	0,24	0,01	0,05	0,07	0,03
60-5055-1	0,01	>10	>10	>10	>10	0,35	0,07	0,34	0,04
80-0180-1	0,19	>10	0,71	0,005	0,005	0,05	0,05	0,13	1,18
60-4063-1	0,34	7,14	1,32	5,21	>10	>10	>10	0,34	>10
60-6027-1	0,54	9,64	1,26	3,31	>10	>10	>10	0,35	>10
70-0758-1	0,36	>10	>10	>10	>10	4,56	0,34	7,52	0,21
60-8084-1	0,15	5,66	>10	0,01	0,01	0,02	1,36	0,67	0,01
50-2285-1	>10	>10	>10	>10	>10	0,05	0,04	2,52	0,12
Geo mean	0,04	0,71	0,19	0,08	0,01	0,07	0,18	0,42	0,06
IC ₅₀ Breath	95	76	71	67	57	76	71	100	57

IC ₅₀ titers µg/ml	
	<0,1
	0,1-0,9
	1,0-3,0
	3,1-9,9
	>10

Figure 2.3: Neutralization heatmap IC₅₀ titers of HIV-1 TF pseudoviruses from infants against bNAbs. The heatmap illustrates IC₅₀ titers of infant transmission founder (TF) pseudoviruses (n = 21) against nine broadly neutralising antibodies (bNAbs) targeting key HIV-1 envelope regions: CD4 binding site, V1/V2 loop, V3 glycan, membrane-proximal external region, and gp120/gp41 interface. Colour-coded tiers represent neutralisation sensitivity: red (<0.1 µg/mL) indicates high sensitivity, orange and light-yellow show moderate sensitivity, light yellow represents minimal sensitivity, and white denotes complete resistance.

A



B

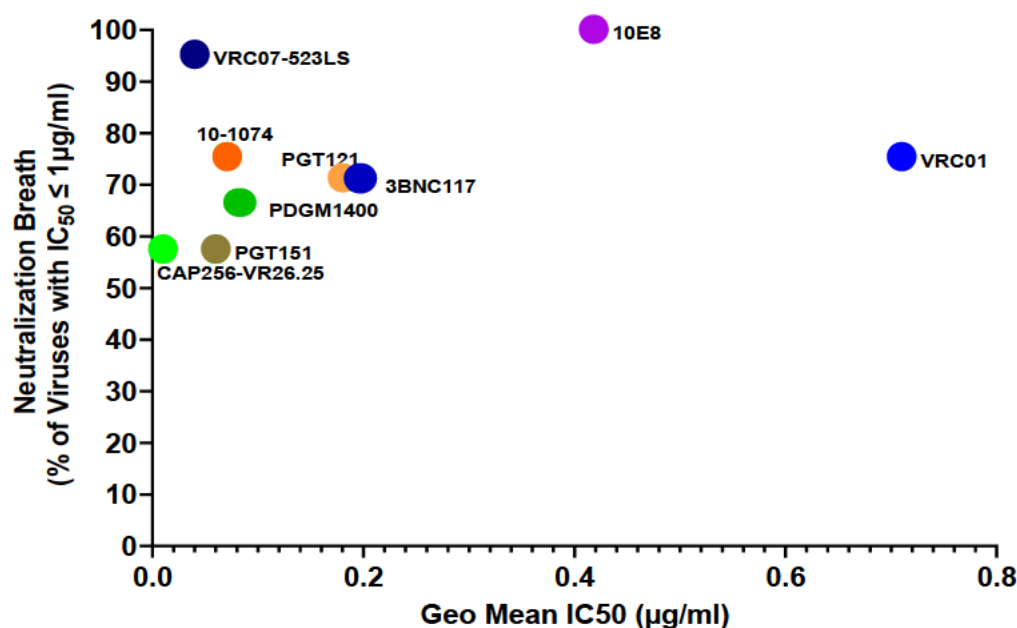


Figure 2.4 A-B: Neutralization sensitivity of HIV-1 pseudoviruses from in utero infected infants. Neutralisation titers (IC_{50}) of individual infant pseudoviruses against nine bNAbs. The solid line denotes the median IC_{50} neutralisation titer of sensitive pseudoviruses (A). Average percentage of neutralisation breadth and geometric mean titers (potency) (B). The epitope specificity is indicated by colour, with antibodies specific for the CD4 binding site depicted in different shades of blue, V1/V2 apex-specific antibodies in shades of green, antibodies binding to V3 Glycan-specific in shades of orange, antibodies binding to the gp120-gp41 interface in brown, and MPER-specific antibody in purple.

2.3.4 Amino Acid Signature Mutations

To investigate HIV-1 *env* determinants of neutralisation sensitivity, we analysed *env* clonal sequences from infants, focusing on the CD4 binding site (Fig. 2.5A), loop D, and V5 loop of gp120 subunits. The N-linked glycans at positions 197, 234, and 276 were highly conserved in loop D. While amino acid variations at positions 279 and 281 revealed complex neutralisation patterns. At position 279, 67 % of *env* sequences contained aspartic acid (279D) associated with sensitivity, while 29% of sequences had asparagine (279N) found in both sensitive and resistant viruses. However, three sequences (0215-1, 8171-1, and 2178-1) containing asparagine at position 279 (279N) demonstrated reduced sensitivity to some CD4bs antibodies compared to sequences with 279D (Fig. S3A). In comparison, one sequence (0180-1) with 279N showed resistance specifically to VRC01 but remained sensitive to other CD4bs antibodies. Three sequences (0902-1, 5055-1, and 0758-1) containing aspartic acid at position 279 (279D) were sensitive exclusively to the VRC07-523LS antibody. A notably unique sequence from one infant (2285-1) featured a rare glutamic acid mutation at position 279 (N279E), which may have conferred complete resistance to all three CD4bs antibodies (Fig. S3a). In the β 20- β 24 region, an arginine (R) at position 456 was highly conserved in 95% of sequences, with a histidine mutation (R456H) in one infant clone (0902-1) that was resistant to VRC01 and 3BNC117. While glycine residues at positions 458 and 459 were highly conserved, consistent with previous associations with CD4 binding site antibody sensitivity (Diskin *et al.*, 2013; Bricault *et al.*, 2019).

2.3.5 V2-Apex Escape Mutations associated with V2-apex resistance

Our analysis of the V2-apex contact site revealed complex mutational patterns associated with antibody resistance. We first examined the N-linked glycans at positions 156 and 160, these are critical contact positions for CAP256-VRC26.25 and PGDM1400 (McLellan *et al.*, 2011). The N-linked glycan at position 156 was highly conserved (Fig. 2.5B), with one notable exception (2285-1) which had an arginine mutation (N156R) that may have contributed to resistance to both PGDM1400 and CAP256-VRC26.25. The N160 glycan was found in 86% of the *env* clones, while three distinct clones had serine, lysine, and tyrosine (N160S/K/Y) mutations. Among the three clones, two clones (8195-1 and 0758-1) had a serine and tyrosine mutations respectively and they were resistant to both PGDM1400 and CAP256-VRC26.25 antibodies, while the third clone (2178-1) had a lysine (N160K) mutation displayed resistance exclusively to PGDM1400 (Fig. S3B). We also analysed amino acid residues at positions 164, 166, 167, 168, and 169 of the V2 loop, these revealed complex viral resistance patterns. At position 164, glutamic acid (E) was predominant in 86% of clones, while two infant clones (0204-1 and 6027-1) had serine or valine mutations and were resistant to CAP256-VRC26.25. Contrary to previous studies associating position 164E with increased sensitivity to CAP256-VRC26.25 in adults (Doria-Rose *et al.*, 2014; Bricault *et al.*, 2019), 44% of clones with 164E in our study were resistant to either PGDM1400

or CAP256-VRC26.25. Arginine was predominant at position 166, however we identified three clones with variations: two clones (0758-1 and 5055-1) had threonine (T) and glycine (G) respectively, they were resistant to both CAP256-VRC26.25 and PGDM1400, while one clone (6027-1) had threonine at position 166 (166T), and was resistant to CAP256-VRC26.25 but remained sensitive to PGDM1400. Aspartic acid (D) residues were highly conserved at position 167 across all the viral sequences analysed, suggesting it may play a critical role in maintaining viral fitness and structural integrity (Pancera *et al.*, 2013; Sok, Doores, *et al.*, 2014a). Previous studies have shown that mutations occurring in highly conserved positions often result in reduced viral replication capacity or fitness costs that are deleterious to the virus (Russell *et al.*, 2011). Position 169 revealed the most striking pattern, with 52% of sequences containing lysine (commonly found in consensus subtype C) and 48% displaying mutations including glutamic acid (E), glutamine (Q), arginine (R), serine (S), or isoleucine (I). These mutant clones were resistant to CAP256-VRC26.25, with variable sensitivity to PGDM1400. The high frequency of substitutions at position 169 appears to be a primary driver of increased resistance in infant TF HIV clones.

2.3.6 Substitutions at the V3-Glycan Supersite

Previous studies have established the importance of N-linked glycans at positions 295, 301 and 332 as critical contact sites for broadly neutralising antibodies PGT121 and 10-1074 (Doores *et al.*, 2010; Garces *et al.*, 2014; Behrens *et al.*, 2016b). In our analysis of infant transmission founder (TF) clones, the N332 glycan was present in 86% of infant *env* clones. Although clone 4063-1 notably lacked the N332 glycan and contained an aspartic acid (D) substitution at position 301, demonstrating resistance to 10-1074 and PGT121 (Fig. S3C) (Fig 2.5C). Three clones (4063-1, 6027-1, and 2178-1) harboured mutations at position 332 (N332T/K/H), all resistant to both antibodies. Two clones (4063-1 and 6027-1) exhibited a glycan shift from position 332 to 334, further contributing to antibody resistance. Clone 2285-1 displayed a complex resistance pattern with multiple mutations: histidine at position 332 (N332H), aspartic acid at position 334 (N334D), and glutamic acid at position 295 (N295E), resulting in resistance to both antibodies. When analysing the glycan-dependent immunoreactivity (GDIR) motif, approximately 28.5% of clones presented an asparagine substitution at position 325 (D325N). Four *env* clones (8195-1, 6041-1, 1778-1, and 0215-1) with this mutation remained sensitive to PGT121 and 10-1074. Recent investigations have revealed that both GDIR and GNIR can exhibit variable neutralisation profiles depending on the specific genetic and structural context of the viral envelope (Cao *et al.*, 2017).

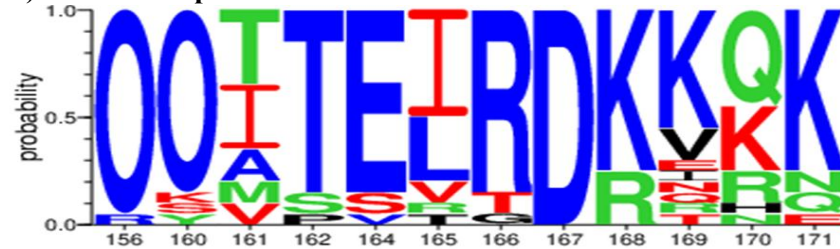
2.3.7 Lower frequency of escape mutations in MPER and gp120-gp41 interface epitopes

Our comprehensive analysis of the MPER of gp41 revealed a remarkably conserved genetic landscape. Focusing on critical contact positions (HXB2 position 671-683), we found minimal evidence of mutations associated with antibody resistance. Specifically, tryptophan (W) and phenylalanine (F) at positions 672 and 673 were conserved in 100% of env sequences (Fig. 2.5D), both residues previously linked to sensitivity to the 10E8 monoclonal antibody (Bricault *et al.*, 2019). While the K683R mutation has been previously associated with reduced sensitivity to MPER-targeting antibodies (Rademeyer *et al.*, 2016; Yin *et al.*, 2021), our analysis revealed an intriguing pattern: four infant clones (6082-1, 0239-1, 8171, and 0758-1) harbouring this mutation remained sensitive to the 10E8 bNAb. Examination of the gp120/gp41 interface unveiled consistently conserved glycosylation patterns, with N-linked glycans at positions 276, 611, and 637 present across all infant sequences.

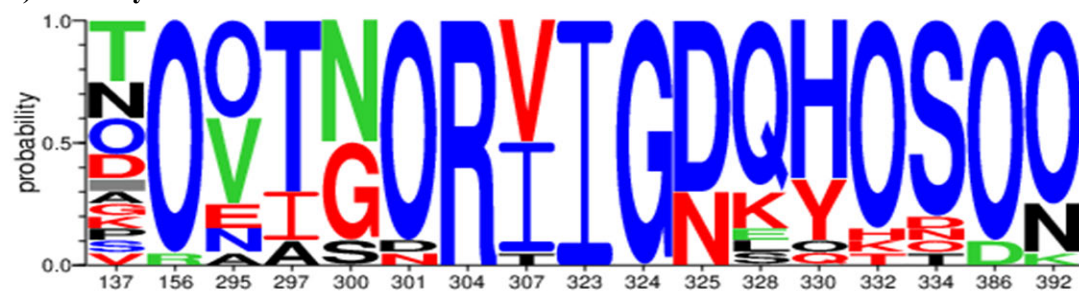
a) CD4 binding site



b) V1/V2 Loop



c) V3 Glycan



d) MPER and Gp120/gp41 interface

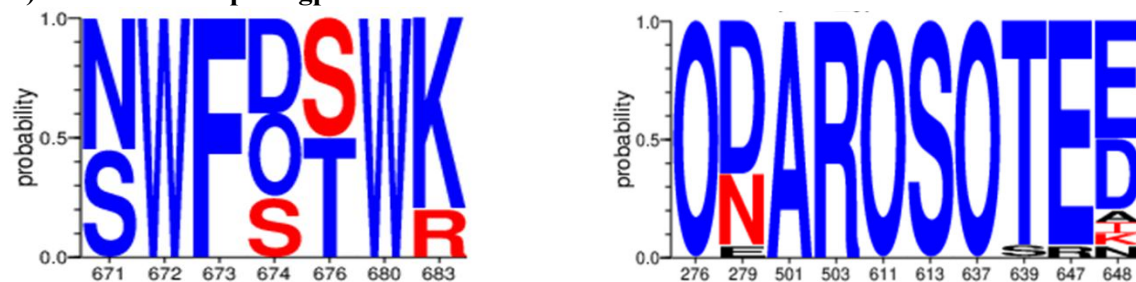


Figure 2.5 A-D: Sequence Logo Analysis of Key Epitope Residues in Infant HIV-1 Transmitted/Founder Viruses. Logogram of HIV-1 TF viruses from infants for five env epitopes. The amino acid frequency (y-axis) is shown for each residue. The size of each AA in the logogram is proportional to its frequency, and the “O” represents N-linked glycans. Residues associated with a sensitive neutralisation phenotype are in blue, while residues in red represent resistant residues. Residues that are associated with either resistance or sensitivity are in green, and residues in black are AA that we observed but have not yet been characterised

2.4 Discussion

This study characterised TF viruses from *in utero* HIV-1-infected infants in the Baby Cure cohort, providing comprehensive insights into the neutralisation profiles of infant HIV strains and their potential implications for vertical transmission prevention strategies. The neutralisation analysis revealed a complex landscape of antibody interactions, with notable variations in bNAb effectiveness. VRC07-523LS, 10-1074, and 10E8 demonstrated the highest neutralisation coverage and potency across the infant TF virus panel, suggesting their potential as promising therapeutic or prophylactic agents in paediatric HIV management. In contrast, CAP256-VRC26.25, PGDM1400, and PGT151 exhibited significant resistance, highlighting the challenges in developing universal neutralisation strategies for infant HIV transmission. The CD4bs analysis revealed remarkable conservation, critically important for viral functionality. Previous research has demonstrated the delicate balance between viral fitness and mutation tolerance (Liao et al., 2013). In our study, only a few escape mutations were identified in the CD4bs, glutamic acid at position 279 was associated with neutralisation resistance. This observation underscores the virus's limited capacity to modify this critical region without compromising its fundamental entry mechanisms.

The V2-apex region presented a particularly intricate neutralisation profile, including the loss of N-linked glycan at position 160, associated with resistance to PGDM1400 and CAP256-VRC26.25. Previous studies have demonstrated that lysine at position 169 (K169) is associated with increased neutralisation sensitivity to V2 apex bNAbs, particularly in subtype C viruses (Walker et al., 2009; Doria-Rose et al., 2014). Our cohort observed that 75% of the infant TF viruses maintained K169, which is thought to enhanced susceptibility to V2-apex targeting antibodies. The high frequency of mutations in the V2-apex region underscores the challenges in targeting this region for therapeutic or preventive interventions of vertical transmission. It also highlights the dynamic nature of this epitope and its role in viral escape from immune pressure.

Investigations into the V3 glycan region revealed critical insights into viral immune evasion mechanisms. The N332 glycan was present in 86% of infant viruses, with mutations previously associated with resistance to PGT121 and 10-1074 (Pejchal *et al.*, 2011; Wibmer *et al.*, 2013; Sok, Doores, *et al.*, 2014a; Gristick *et al.*, 2016; van den Kerkhof *et al.*, 2016). The glycosylation patterns demonstrated remarkable variability, suggesting a sophisticated evolutionary strategy for immune escape. While the gp120/41 interface exhibited high overall conservation, 43% of pseudoviruses from infants were resistant to PGT151. This resistance may be linked to polymorphisms in the gp120/gp41 interface, which could reduce the affinity of PGT151 without compromising viral fitness. These

findings underscore the dynamic interplay between antibody pressure and viral adaptation, which has been extensively documented in studies of HIV-1 immune escape mechanisms (Cao *et al.*, 2017). These mechanisms may sterically hinder or modify the epitope's accessibility, as described previously (Chuang *et al.*, 2013; Wibmer *et al.*, 2013; Garces *et al.*, 2015). The relatively high frequency of PGT151 resistance in infant TF viruses suggests that targeting the gp120/41 interface may present challenges in preventing or treating vertical transmission. This finding emphasises the need for a multifaceted approach to bNAb-based interventions in paediatric HIV.

The findings of this study have significant implications for HIV research and clinical interventions. The observed neutralisation sensitivity and resistance patterns can inform the design of more effective immunogens tailored for paediatric settings. Specifically, researchers can focus on conserved epitopes and develop immunogens that elicit antibodies capable of recognising diverse viral variants (Walker *et al.*, 2009; Doria-Rose *et al.*, 2014). The heterogeneity in neutralisation profiles suggests that combinations of bNAbs targeting different epitopes may be necessary to neutralise infant TF viruses comprehensively. This approach can guide the rational selection of bNAb combinations for future clinical trials, potentially improving therapeutic strategies for paediatric HIV management (Liao *et al.*, 2013; Martinez *et al.*, 2020). Understanding the neutralisation profiles of infant TF viruses provides critical insights into vertical transmission mechanisms. The research supports the development of advanced prevention strategies, including innovative approaches such as passive immunisation with bNAbs and targeted maternal vaccination protocols. These strategies could significantly reduce the risk of vertical transmission of HIV by addressing the unique characteristics of infant viral strains (Sok *et al.*, 2014; Wibmer *et al.*, 2013).

This study provides critical insights into a relatively understudied area of HIV transmission by characterising infant TF viruses. The comprehensive analysis reveals important neutralisation profiles and escape mutations, offering valuable data for the HIV research community while acknowledging several methodological limitations. The research's small sample size constrains its ability to fully capture neutralisation sensitivity across HIV-1 subtypes. Future investigations should expand cohort sizes to encompass a broader range of viral diversity, employing SGA and NGS to accurately represent circulating viral populations in infants and transmitting mothers. While the study provides a snapshot of TF viruses, it cannot capture the longitudinal evolution of neutralisation sensitivity. Crucial areas for future research include tracking viral changes from acute to chronic infection stages and exploring the specific mechanisms of viral adaptation in vertical transmission. The findings underscore the complexity of infant HIV transmission and highlight the need for more comprehensive, longitudinal

studies to develop targeted prevention and treatment strategies. Comparative analyses between infant and adult viral populations could further elucidate the unique characteristics of vertical transmission mechanisms.

In conclusion, this study provides critical insights into the neutralisation profiles of infant TF viruses, revealing both opportunities and challenges for bNAb-based interventions in the prevention of vertical transmission and paediatric HIV treatment. Identifying specific neutralisation escape mutations and characterising bNAb efficacy against infant TF viruses represent significant advances in our understanding of vertical HIV transmission. These findings have significant implications for the development of targeted interventions to prevent vertical transmission and treat paediatric HIV infections. The high efficacy of certain bNAbs against infant TF viruses offers promise for their use in prevention or treatment strategies. However, the observed heterogeneity in neutralisation profiles underscores the need for combination approaches and continued research to overcome viral escape mechanisms. The results of this study contribute to the growing body of knowledge on paediatric HIV and offer a foundation for future research and clinical interventions. By advancing our understanding of the unique characteristics of infant TF viruses, this work paves the way for more effective strategies to combat HIV in one of its most vulnerable target populations. Continued research in this area is crucial for developing tailored interventions that can significantly reduce the burden of HIV in paediatric populations worldwide.

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Chapter 3

Identification of CXCR4-using HIV-1 subtype C transmitted/founder viruses in infants infected *in utero*

3.1 Introduction

HIV infection initiates when the viral envelope glycoprotein 120 (gp120) binds to the CD4 receptor on host cells, inducing conformational changes that expose the V3 loop of gp120. This loop interacts with either CCR5 or CXCR4 coreceptors, facilitating viral and host membrane fusion, ultimately allowing viral entry (Alkhatib *et al.*, 1996; Deng *et al.*, 1996; Tamamis and Floudas, 2014). Entry inhibitors like Maraviroc and AMD3100 disrupt this process by targeting coreceptors. Maraviroc binds to CCR5 coreceptors and prevent the interactions between CCR5 and the V3 loop through conformational changes (Dorr *et al.*, 2005; Garcia-Perez *et al.*, 2011), and has demonstrated high prevention efficacy in phase I and II clinical trials (Fätkenheuer *et al.*, 2008; Gulick *et al.*, 2008). Conversely, AMD3100 blocks CXCR4 coreceptors and prevent its interaction with the V3 loop, and subsequently block infection by the X4 strains (Rosenkilde *et al.*, 2004; De Clercq, 2009). Despite their clinical promise, the application of entry inhibitors in preventing HIV transmission, particularly in African settings was not well accepted due to various challenges such as the lack of sufficient coreceptor usage data in the African population.

HIV-1 variants are categorised based on coreceptor usage into CCR5-using (R5), CXCR4-using (X4), and dual-tropic (R5/X4) viruses (Berger *et al.*, 1998). Subtype C, the most prevalent subtype in sub-Saharan Africa, predominantly utilises CCR5 during acute and early chronic infections (Tscherning *et al.*, 1998; Ndung'u *et al.*, 2006a; Matume, Tebit and Bessong, 2020a). However, in late chronic stages, some subtype C strains evolve to use CXCR4 either exclusively or alongside CCR5, a transition linked to disease progression and resistance to immune responses (Connor *et al.*, 1997; Coetzer *et al.*, 2007; Huang *et al.*, 2007; Matume, Tebit and Bessong, 2020b). This adaptive shift in coreceptor usage correlates with disease progression and represents a sophisticated viral strategy to maintain infectivity while evading both host immune responses and therapeutic interventions (Maphumulo and Gordon, 2024). Notably, some coreceptor switching events are driven by escape from V3-glycan-neutralizing antibodies, highlighting the complex interplay between viral evolution and immune pressure (Sok *et al.*, 2016).

Coreceptor usage patterns vary significantly among HIV-1 subtypes; subtypes B and D exhibit the highest CXCR4 usage (50–60%) during chronic infection, this is associated with increased virulence

and faster disease progression but reduced transmissibility (Huang *et al.*, 2007; Kiguoya *et al.*, 2017). In contrast, subtype C shows lower CXCR4 usage (15–30%) due to genetic barriers in the V3 loop that limit coreceptor switching, resulting in higher transmission efficiency but lower virulence (Cilliers *et al.*, 2003; Kaleebu *et al.*, 2007; Cavarelli & Scarlatti, 2011). Subtypes A and CRF01_AE display intermediate CXCR4 usage (30–50%) and exhibit moderate transmissibility and virulence (Utachee *et al.*, 2009; Ssemwanga *et al.*, 2012). Understanding these subtype-specific coreceptor usage differences is crucial for predicting disease progression, optimising antiretroviral therapy, and assessing CCR5 antagonist efficacy across diverse populations.

Research on vertical transmission of HIV-1 highlights that most infants acquire CCR5-using variants of subtype A (85–90%) and subtype C (90–95%), with only 2–5% of subtype C viruses utilising CXCR4 (Choge *et al.*, 2006; Zhang *et al.*, 2010). In addition, 10–15% of pregnant women harbour CXCR4-using variants, while dual-tropic variants are observed in 5% of pregnant women. While CXCR4 usage correlates with a higher transmission risk (30–35%) in European and American cohorts, there is limited data on African populations (Cavarelli and Scarlatti, 2011). Notably, subtype C has lower CXCR4 usage compared to other subtypes; however, significant gaps persist regarding longitudinal changes in coreceptor usage patterns among African infants and their implications for paediatric HIV progression (Cavarelli and Scarlatti, 2011; Matume *et al.*, 2018). In addition, phenotypic assays that utilise tissue culture remain the gold standard for determining HIV-1 coreceptor usage, yet very few studies used phenotypic assays (Hwang *et al.*, 1991; Lengauer *et al.*, 2007b). Computational tools offer a cost-effective alternative, using V3 loop sequences to predict coreceptor tropism. These methods show high concordance with phenotypic assays across multiple subtypes (Raymond *et al.*, 2008; Prosperi *et al.*, 2010; Cashin *et al.*, 2015) and are now widely applied in research and clinical contexts. This study seeks to characterise HIV-1 coreceptor usage in subtype C-infected infants and mother-infant pairs by combining computational prediction algorithms and tissue culture-based phenotypic assays. The findings aim to advance our understanding of coreceptor tropism in African populations and contribute to developing targeted interventions to prevent vertical transmission of HIV.

3.2 Materials and Methods

3.2.1 Study design

We conducted a comprehensive analysis of HIV-1 coreceptor tropism in the Baby Cure Study cohort, which comprised 21 HIV-infected infants and seven mother-infant transmission pairs at baseline. To determine viral tropism, we employed both genotypic and phenotypic approaches. The genotypic characterisation utilised three computational prediction tools: Geno2pheno, PhenoSeq, and PSSM-C, complemented by detailed analysis of V3 loop properties, including amino acid diversity, net charge, length, crown motif architecture, and N-linked glycosylation patterns. Meanwhile, phenotypic validation was performed using TZM-bl cell-based drug sensitivity assays with Maraviroc, CCR5 antagonist and AMD3100 CXCR4 antagonist. This dual methodological approach enabled robust assessment of HIV-1 coreceptor usage in both mothers and infants.

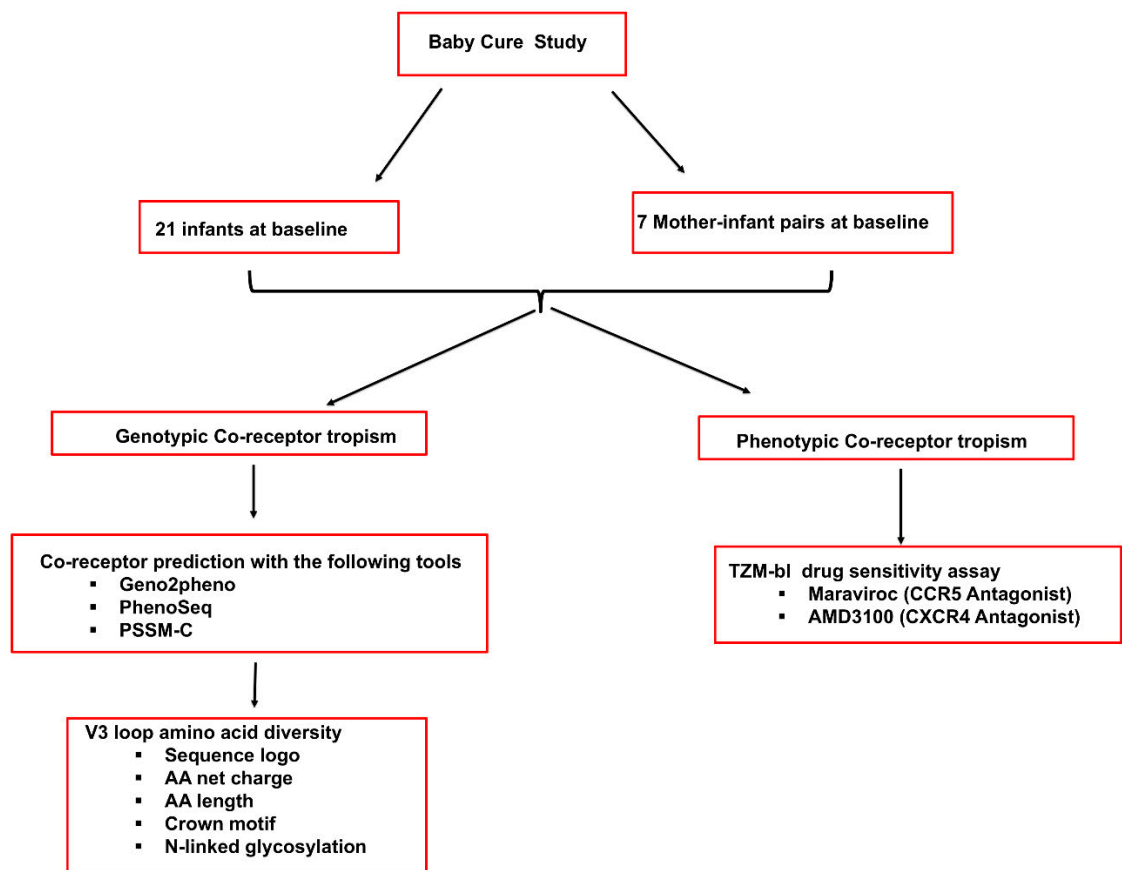


Figure 3.1: Study design and workflow for determining HIV-1 coreceptor tropism in the Baby Cure Study cohort.

3.2.2 Coreceptor prediction tools and algorithms

HIV-1 coreceptor usage was predicted using a comprehensive approach that combined multiple bioinformatics tools and sequence-based rules. The V3 loop sequences were analysed using three of the most commonly employed prediction algorithms: Geno2pheno (Lengauer *et al.*, 2007a), WebPSSM (Jensen *et al.*, 2003), and PhenoSeq (Cashin *et al.*, 2013). In addition to these computational methods, we also considered several sequence characteristics that are known to influence coreceptor tropism. These included the presence of potential N-linked glycosylation sites (PNGS), the application of the 11/25 rule (which examines amino acid changes at positions 11 and 25 of the V3 loop), and the calculation of the overall V3 loop net charge (Cashin *et al.*, 2015).

3.2.3 Phenotypic determination of coreceptor usage

CCR5 and CXCR4 coreceptor usage was determined using a single-round viral infectivity assay with TZM-bl cells, with modifications (Wei *et al.*, 2002). TZM-bl cells (10,000 cells/well) were seeded in 96-well tissue culture plates (Corning Costar, New York, USA) and incubated overnight at 37°C in 5% CO₂. The growth medium was then replaced with fresh media containing either the CCR5 antagonist Maraviroc (20 µM), the CXCR4 antagonist AMD3100 (2.4 µM), or a combination of both, followed by a 1-hour incubation at 37°C in 5% CO₂. Subsequently, the cells were exposed to the virus and incubated for 48 hours under the same conditions. After incubation, the culture medium was removed, replaced with 100 µL of the Bright-Glo™ reagent, and incubated for 2 minutes in the dark. Afterwards, the cell lysate was transferred into 96-well black solid plates and read using a Victor Nivo plate reader (Perkin-Elmer, Revvity Health Science, Canada). The 50% inhibitory dose (IC₅₀) was defined as the concentration of antagonist (Maraviroc or AMD3100) required to inhibit 50% of viral infection, as measured by relative light units (RLU) compared to virus control wells after background subtraction. TZM-bl cells alone served as a negative control, while three well-characterized HIV-1 strains were used as positive controls: YU2 (R5-tropic), NL4.3 (X4-tropic), and 89.6 (dual-tropic R5X4). Viral tropism was classified based on the inhibition patterns: viruses were defined as R5-tropic when inhibited by Maraviroc alone, X4-tropic when inhibited by AMD3100 alone, and dual/mixed (D/M) when complete inhibition required both Maraviroc and AMD3100.

3.2.4 Statistical analysis

Data was analysed using GraphPad Prism version 10 software (GraphPad Software, San Diego, California, USA). The median V3 length and net charge were compared between CCR5-tropic and CXCR4-tropic viral sequences using the Mann-Whitney *t*-test. Associations between V3 characteristics and coreceptor tropism were analysed. Data was statistically significant if the *p*-value ≤ 0.05.

3.3 Results

3.3.1 Most *in utero* infected infants harboured R5 variants

HIV-1 coreceptor usage was predicted in a cohort of 21 infants using multiple bioinformatics tools, with final predictions based on concordance between at least two tools: Geno2pheno, WebPSSM, and Phenoseq (Table: 3.1). Our findings revealed that 71% (15/21) of the transmitted founder variants exclusively used CCR5 (R5), while 29% (6/21) utilised either CXCR4 (X4) or displayed dual tropism (R5X4) (Huang *et al.*, 2005). Analysis of V3 loop features demonstrated that X4 variants consistently exhibited significantly higher net charges compared to R5 variants ($p < 0.001$) (Fig 3.2a). However, other V3 characteristics, including loop length, showed no significant differences between R5 and X4 variants ($p = 0.08$) (Fig. 3.2b), although there was a trend towards longer V3 loops in X4 variants (37 amino acids) compared to R5 variants (35 amino acids).

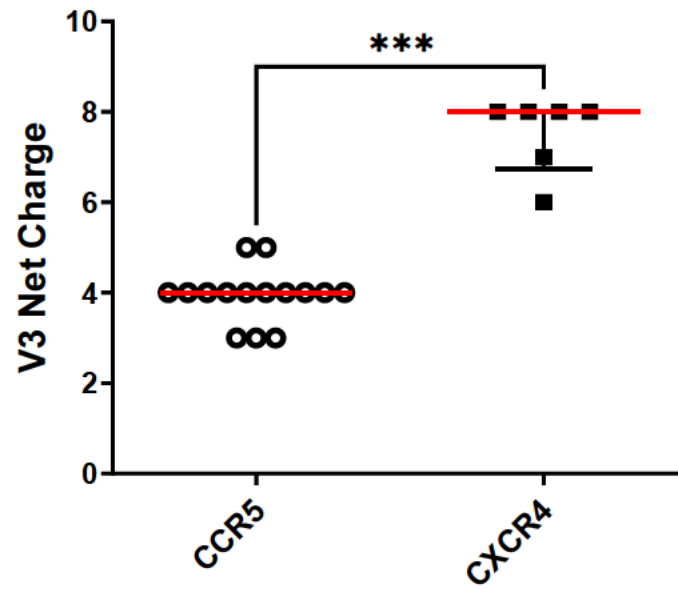
Analysis of the V3 loop demonstrated distinct amino acid signatures between CCR5- and CXCR4-tropic variants (Fig. 3.3). R5-tropic viruses predominantly contained a highly conserved GPGQ crown motif at positions 15-18 within a 35-amino acid V3 loop. In contrast, X4-tropic variants exhibited a GPGR motif at the same positions within a more extended 37-amino acid sequence. Previous studies have shown that substitution from a neutral Glutamine (Q) to a positively charged Arginine (R) in the crown motif is associated with a coreceptor change from the CCR5 to CXCR4 due to altered electrostatic interactions between the V3 loop and coreceptors (Huang *et al.*, 2005; Cardozo *et al.*, 2007).

Table 3.1: Characteristics of the V3 Loop in HIV-1 Subtype C in utero infected infants.

PID	Coreceptor prediction softwares				Net Charge	Pos 11/25	PNGS	Length
	Geno2pheno	PhenoSeq	Web-PSSM	Consensus				
50-2178-1	R5	R5	R5X4/X4	R5	4	TE	1	35
50-2285-1	R5	R5	R5	R5	5	SE	1	35
60-0048-1	R5	R5X4/X4	R5X4/X4	R5X4/X4	8	GA	1	35
60-0902-1	R5	R5	R5	R5	3	SE	1	35
60-1778-1	R5	R5	R5	R5	4	SE	1	35
60-4063-1	R5	R5	R5	R5	4	SD	1	35
60-4082-1	R5X4/X4	R5X4/X4	R5X4/X4	R5X4/X4	8	SK	1	36
60-5055-1	R5	R5X4/X4	R5X4/X4	R5X4/X4	7	SG	0	34
60-6027-1	R5	R5	R5	R5	3	SD	1	35
60-6041-1	R5	R5	R5	R5	5	SD	1	35
60-6082-1	R5	R5	R5X4/X4	R5	4	SN	1	34
60-8084-1	R5X4/X4	R5X4/X4	R5X4/X4	R5X4/X4	8	GA	1	35
60-8195-1	R5	R5	R5	R5	3	SE	1	35
60-8171-1	R5	R5	R5	R5	4	SD	1	35
60-8277-1	R5	R5	R5	R5	4	SD	1	35
70-0239-1	R5	R5	R5	R5	4	SE	1	35
70-0758-1	R5X4/X4	R5X4/X4	R5X4/X4	R5X4/X4	6	SK	1	34
70-0778-1	R5	R5	R5X4/X4	R5	4	SD	1	35
80-0180-1	R5	R5	R5	R5	4	SD	1	35
80-0204-1	R5X4/X4	R5X4/X4	R5X4/X4	R5X4/X4	8	SK	1	37
80-0215-1	R5X4/X4	R5	R5	R5	4	SE	1	34

The predicted coreceptor usage was determined using Geno2Pheno (with a false positive rate of 5%), PhenoSeq and WebPSSM subtype C sensi (PSSM-C). Consensus-predicted tropism was determined based on the agreement of three or two of three coreceptor usage prediction algorithms (CPAs). The amino acids occupying positions 11 and 25 (11/25 rule) were identified using PSSM-C. The number of potential N-linked glycosylation sites (PNGS) in the V3 sequence was counted using N-GlycoSite

A



B

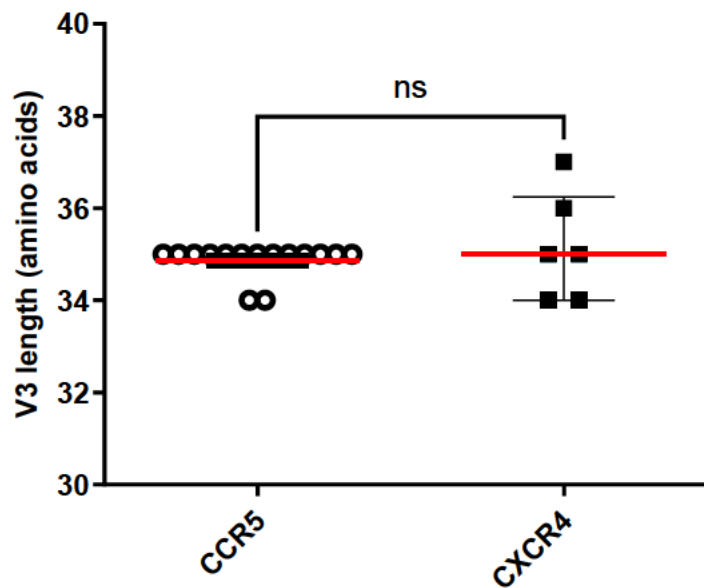


Figure 3.2. A) V3 net charge and B) V3 length (aa) between CCR5 and CXCR4 using variants from HIV-1 subtype C *in utero* infected infants. Each dot represents a net charge and V3 length of a unique *env* V3 loop sequence. The X-axis shows the *env* tropism, and the y-axis displays a calculated charge and V3 length. The values indicate an average from two independent experiments; the lines denote medians and interquartile ranges (p < 0.001).

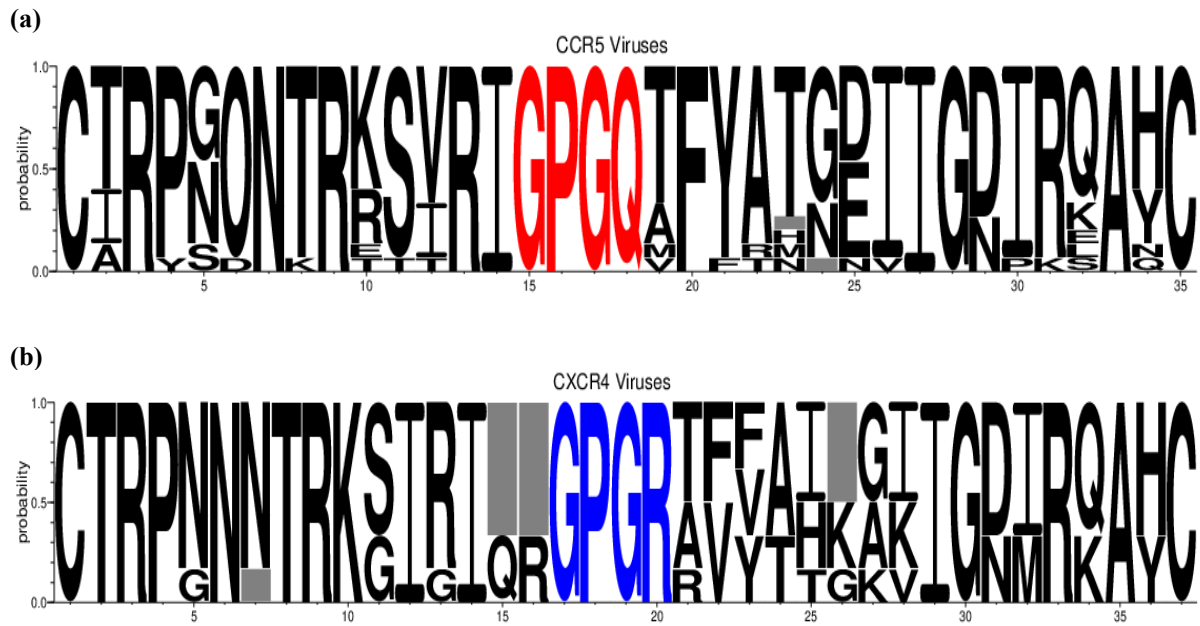


Figure 3.3. Sequence Variation in the V3 Loop of HIV-1 Envelope Glycoprotein by Coreceptor Tropism. The sequence variation in the hypervariable V3 loop of the HIV-1 envelope glycoprotein is depicted for (a) CCR5-tropic viruses and (b) CXCR4-tropic viruses. The sequence logos were generated using AnalyzeAlign, a tool available on the Los Alamos HIV Sequence Database. The x-axis represents the amino acid positions within the V3 loop. At the same time, the height of the symbols on the y-axis indicates the relative frequency of each amino acid at the corresponding position. The crown motif, a critical determinant of coreceptor tropism, is highlighted in red for CCR5-tropic viruses and blue for CXCR4-tropic viruses, illustrating the distinct sequence patterns associated with each tropism phenotype.

3.3.2 High frequency of CCR5 usage among *in utero* infected infants

We performed functional assays using TZM-bl cells with specific coreceptor antagonists to validate the predicted coreceptor usage of infant TF viruses. Consistent with coreceptor prediction algorithms, fifteen infant TF *env* sequences demonstrated exclusive CCR5 coreceptor usage, exhibiting complete inhibition of viral infectivity in the presence of Maraviroc (MVC) while maintaining infectivity when treated with AMD3100 (Fig. 3,4). This inhibition pattern mirrored the well-characterized CCR5-tropic YU2 subtype B control strain. Notably, the remaining six infants (29%) harboured viruses capable of utilising the CXCR4 coreceptor (Fig. 3,4), similar to the well-characterized CXCR4-tropic NL4.3 control strain. These findings reveal a higher frequency of CXCR4-tropic viruses in our cohort than previous studies that reported >90% CCR5-tropic viruses in mother-infant pairs, suggesting increased CXCR4 usage over time in *in utero* transmission. The presence of CXCR4-tropic viruses in maternal circulation increases the likelihood of transmitting these variants to the infant, potentially explaining the higher frequency observed in our study compared to earlier reports that may have focused on populations with more recent infections.

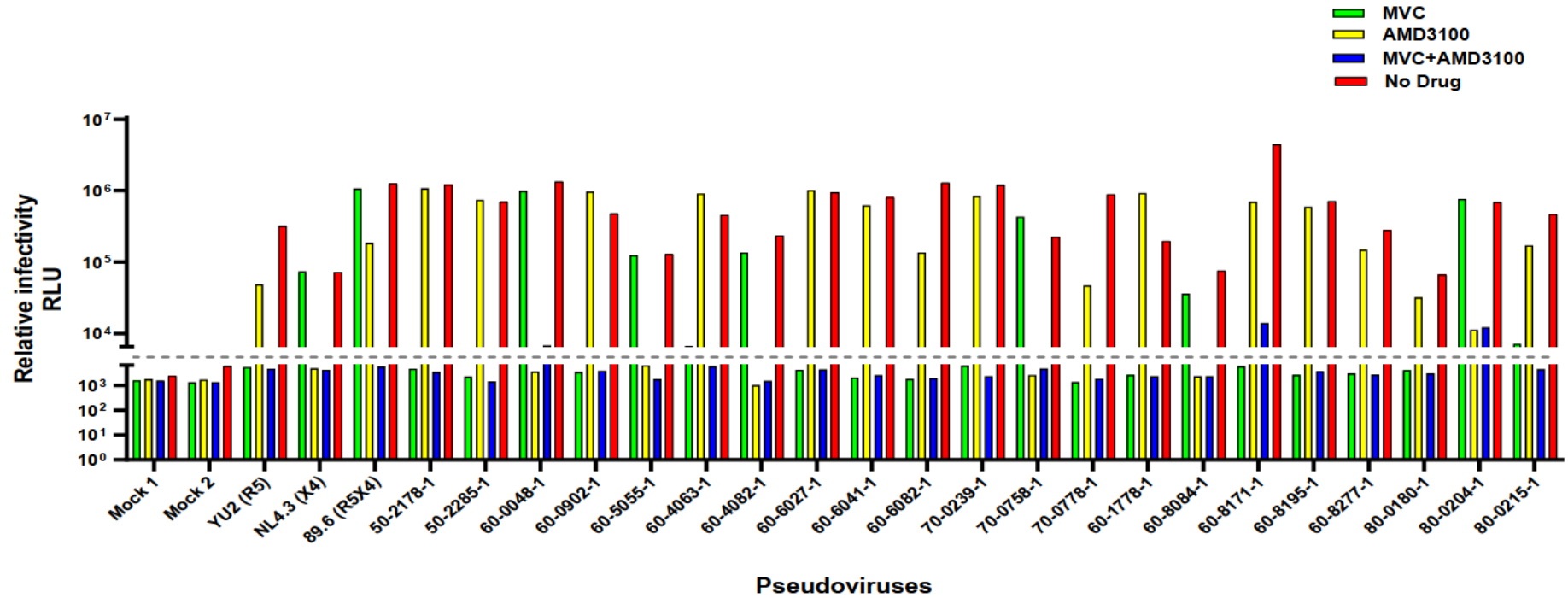


Figure 3.4: Coreceptor tropism of HIV-1 subtype C TF virus using the TZM-bl drug inhibition assay.

Coreceptor tropism of HIV-1 subtype C TF virus using the TZM-bl drug inhibition assay. TZM-bl cells were infected with patient-derived pseudovirus and virus control YU2 for R5-tropic, NL4-3 for X4-tropic and 89.6 for dual-tropic. Infection was indicated by a mean RLU which was statistically above background (infection of pseudovirus stock generated by HIV-1 backbone; NL4-3delEnv-LUC plasmid with a non-functional *env* gene) and suppression of 50% or more significant by an X4 or R5 inhibitor, AMD3100 or Maraviroc, respectively. A 3-fold increase in RLU compared to background was considered a successful entry.

3.3.3 Vertical transmission of CXCR4-tropic viruses in mother-infant pairs

We investigated coreceptor usage in seven HIV-1 transmitting mother-infant pairs using both computational algorithms and phenotypic confirmation via TZM-bl cells (Fig 3.4). The phenotypic assays confirmed that five mother-infant pairs (50-2178, 60-0902, 60-1778, 60-6027 and 60-8171) utilised CCR5 for HIV entry, while two pairs (60-0048 and 60-5055) showed CXCR4 usage (Table 3.1). Among the CXCR4-using pairs, the 60-0048 mother-infant pair displayed an interesting pattern where the mother's virus was CCR5-tropic. In contrast, the infant's virus utilised CXCR4, suggesting the transmission of a minor viral variant or a coreceptor switch during transmission. The second CXCR4-using pair (60-5055) exhibited concordant CXCR4 usage between mother and infant. Our study revealed strong concordance between computational predictions and TZM-bl assay results, suggesting that coreceptor algorithms may accurately predict CCR5 or CXCR4 coreceptor usage. This consistency between computational and experimental methods underscores the potential reliability of algorithmic approaches in determining HIV coreceptor tropism, which could have significant implications for both research and clinical applications in HIV treatment and management.

Table 3.2: Coreceptor prediction using sequences from HIV-1 subtype C mother-infant transmission pairs

Pairs		V3 Sequence	Length	Net charge	Predicted tropism			
					Geno2pheno	PSSM-C	PhenoSeq	Consensus
50-2178	Mother	CIRPNNNTRET VRI - GPGQTFYRTGEIIGNIRKAYC	35	6	R5	R5/X4 or X4	R5	R5
	Infant	 -	35	6	R5	R5/X4 or X4	R5	R5
60-0048	Mother	CTRPNNNTRKSIRI - GPGQTFFAHNDIIGDIRQAYC	35	4	R5	R5	R5	R5
	Infant	G... - ...R.V...KA.....K...	35	8	R5	R5/X4 or X4	R5/X4 or X4	R5/X4 or X4
60-0902	Mother	CTRPNNNTRKGVGI - GRIQTFYATGEIIGNIRQAHC	35	5	R5	R5	R5/X4 or X4	R5
	Infant	.I.Y.....S.R. -R.....D....Q.	35	5	R5	R5	R5	R5
60-1778	Mother	CARPSNNTRKSIRI - GPGQAFYATNDIIGNIREAHC	35	6	R5	R5	R5	R5
	Infant	 -	35	6	R5	R5	R5	R5
60-5055	Mother	CTRPG- NTRKSIRI - GPGRRVYAI GGIIGDIRQAHC	34	7	R5	R5/X4 or X4	R5/X4 or X4	R5/X4 or X4
	Infant	 -	34	7	R5	R5/X4 or X4	R5/X4 or X4	R5/X4 or X4
60-6027	Mother	CTRHSNNTRRSVRI - GPGQAFYATNDVIGDIRKAHC	35	6	R5	R5	R5/X4 or X4	R5
	Infant	...PG..... -M.....L...	35	3	R5	R5/X4 or X4	R5	R5
60-8171	Mother	CTRPGNNTRRSVRI - GPGQTFYATGDIIGDIRQAHC	35	4	R5	R5	R5	R5
	Infant	 -	35	4	R5	R5	R5	R5

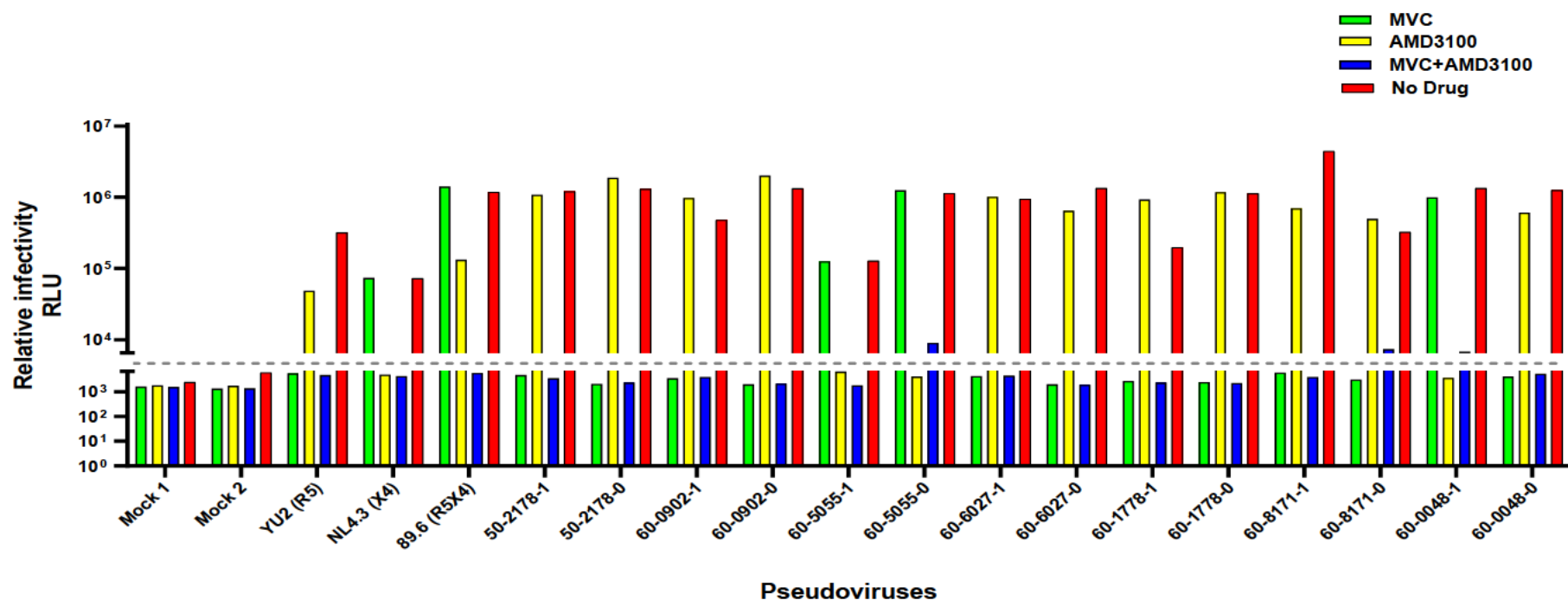


Figure 3.5: Coreceptor tropism of HIV-1 subtype C TF virus using the TZM-bl drug inhibition assay in mother-infant pair. Coreceptor tropism of HIV-1 subtype C TF virus using the TZM-bl drug inhibition assay. TZM-bl cells were infected with patient-derived pseudovirus and virus control YU2 for R5-tropic, NL4-3 for X4-tropic and 89.6 for dual-tropic. Infection was indicated by a mean RLU which was statistically above background (infection of pseudoviral stock generated by HIV-1 backbone; NL4-3delEnv-LUC plasmid with a non-functional env gene) and suppression of 50% or more significant by an X4 or R5 inhibitor, AMD3100 or Maraviroc, respectively. A 3-fold increase in RLU compared to background was considered a successful entry.

3.4 Discussion

Our findings reveal a predominance of CCR5-tropic viruses in vertical transmission of HIV-1 in subtype C, consistent with earlier studies demonstrating the preferential transmission of CCR5-using variants (Ndung'u *et al.*, 2006; Judicate *et al.*, 2021). Among the 21 in utero-infected infants analysed, 71.4% harboured CCR5-tropic viruses, while 29 % were infected with CXCR4-tropic viruses. This represents a notably higher frequency of CXCR4-tropic variants than previous reports in adult and paediatric populations. In our analysis of seven mother-infant pairs, five pairs demonstrated concordant CCR5 usage, one pair showed concordant CXCR4 usage, and one pair exhibited discordant usage, where the mother harboured CCR5-tropic viruses and the infant harboured CXCR4-tropic viruses. This discordance suggests either the transmission of minor maternal viral populations or rapid post-transmission adaptation in the infant (Tscherning *et al.*, 1998; Nakamura *et al.*, 2017). The relatively high frequency of CXCR4-tropic variants observed in our study compared to earlier reports highlights the potential for increasing CXCR4 usage over time (Ndung'u *et al.*, 2006; Nakamura *et al.*, 2017).

The high concordance between computational predictions of coreceptor usage and phenotypic results underscores the reliability of tools such as PhenoSeq and WebPSSM-C charge (Ping *et al.*, 1999; Coetzer *et al.*, 2006). These algorithms demonstrated high sensitivity for predicting X4 or dual-tropic viruses, providing a practical alternative for resource-limited settings where phenotypic assays like the TZM-bl assay may not be accessible. Molecular analyses of the V3 loop revealed key determinants of coreceptor tropism, including a net positive charge $> +7$, the substitution of the crown motif from GPGQ to GPGR, and the loss of N-linked glycosylation at position 6 in one CXCR4-tropic clone (Ping *et al.*, 1999; Jensen *et al.*, 2003).

Previous research has consistently observed a predominance of CCR5-tropic viruses in vertical transmission (Tscherning *et al.*, 1998). Notably, CXCR4-using variants are underrepresented in pregnant women but can occasionally be detected in neonates, supporting the potential transmission of minor viral populations (Tscherning *et al.*, 1998). Additionally, analysis of subtype C envelopes from perinatally infected children has shown that the viral phenotype can evolve from CCR5-tropic to CXCR4-tropic or remain CCR5-tropic despite clinical progression to immune deficiency (Coetzer *et al.*, 2006). The 28.6% CXCR4 usage observed in our infant cohort stands in marked contrast to earlier studies, which reported approximately 5% CXCR4 usage in adult populations from Botswana and South Africa (Ndung'u *et al.*, 2006). This significant difference may be attributed to several factors: first, our study specifically focused

on perinatally infected infants, while previous studies primarily examined adult populations; second, the temporal gap between studies suggests possible evolution in viral tropism patterns over time; and third, regional variations in viral characteristics may play a role. Additionally, analysis of subtype C envelopes from perinatally infected children has shown that the viral phenotype can evolve from CCR5-tropic to CXCR4-tropic or remain CCR5-tropic despite clinical progression to immune deficiency (Coetzer *et al.*, 2006).

The concordance of coreceptor usage between mothers and infants in most pairs and one discordant case underscores the complexity of vertical transmission dynamics. The discordant pair suggests that minor CXCR4-using maternal variants could be transmitted and subsequently selected or adapted in the infant (Tscherning *et al.*, 1998). This observation emphasises the potential role of genetic bottlenecks or immune pressure in shaping transmitted viral populations. Our study demonstrates the value of computational predictions for identifying CXCR4-tropic variants, particularly in resource-constrained settings, by (Ping *et al.*, 1999; Coetzer *et al.*, 2006). Combining these tools with molecular analyses of V3 loop characteristics offers robust approaches for studying HIV-1 tropism and guiding therapeutic interventions.

In conclusion, our findings enhance the understanding of coreceptor usage patterns in HIV-1 subtype C vertical transmission, emphasising the predominance of CCR5-tropic variants while acknowledging the notable presence of CXCR4-tropic viruses. The identification of molecular determinants of coreceptor usage provides valuable insights for developing targeted interventions to prevent vertical transmission and optimise treatment strategies for paediatric HIV-1 infections. Despite its contributions, our study has various limitations such as the small sample size, and use of bulk-PCR amplification instead of SGA may have introduced biases, including potential resampling and the masking of minor variants. While previous studies have shown comparable genetic diversity between bulk-PCR and SGA methods (Jordan *et al.*, 2010), future studies employing next generation sequencing (NGS) in both mothers and infants could provide more granular insights into viral diversity. Additionally, while widely accepted, using the TZM-bl cells for virus tropism assays may not fully recapitulate the complexity of *in vivo* coreceptor usage. Alternative cell lines such as U87 or NP2, which express single coreceptors, or primary CD4⁺ T lymphocytes or macrophages could provide more precise assessments (Koot *et al.*, 1992; Wei *et al.*, 2002). Finally, while informative, our focus on the V3 loop overlooks potential contributions from other regions of the envelope protein to coreceptor usage. Incorporating full-length envelope sequencing in future studies could provide a more comprehensive understanding of the genetic determinants of tropism.

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CHAPTER 4

Variations in neutralisation sensitivity of HIV-1 subtype C isolated from mother-infant pairs

4.1 Introduction

Vertical transmission of HIV-1 remains a significant public health concern, particularly in resource-limited settings where access to comprehensive prevention strategies is constrained (Luzuriaga and Mofenson, 2016). In 2024, approximately 120,000 children were newly infected with HIV globally, predominantly through vertical transmission (UNAIDS, 2024). This transmission occurs in utero, during labour and delivery, or postnatally via breastfeeding (Athena P. Kourtis *et al.*, 2006; Tobin and Aldrovandi, 2013). In utero transmission happens through placental transfer, while intrapartum transmission results from exposure of infants to cervicovaginal secretions and maternal blood during labour (John-Stewart *et al.*, 2004; Milligan and Overbaugh, 2014b). Postnatal transmission occurs through breast milk, which contains both cell-free and cell-associated HIV (Nduati *et al.*, 2000; Bispo *et al.*, 2017). The widespread implementation of ART during pregnancy has significantly reduced vertical transmission rates to below 5% in high-income countries (Fowler *et al.*, 2016). However, despite its success, several challenges limit the effectiveness of ART in preventing vertical transmission, including non-adherence among pregnant women, potential long-term side effects, and the risk of drug resistance development (Nachega *et al.*, 2012a; Hodgson *et al.*, 2014; Wensing *et al.*, 2016; Boerma *et al.*, 2017). These challenges underscore the need for alternative or complementary strategies to enhance the prevention of vertical transmission (Goulder, Lewin and Leitman, 2016; Myer and Phillips, 2017).

BNAbs have emerged as a promising alternative for preventing vertical transmission of HIV-1, given their broad-spectrum neutralisation activity against diverse viral strains, extended half-life, and potential for passive immunisation in infants (Hessell *et al.*, 2009; Ko *et al.*, 2014; Kwong & Mascola, 2018). Several clinical trials have evaluated the efficacy of bNAbs in HIV prevention, including their role in the prevention of vertical transmission. The IMPAACT P1112 trial demonstrated the safety and pharmacokinetics of VRC01 in neonates, highlighting its potential use for passive immunisation (Ajibola, Masheto and Shapiro, 2023). Additionally, the Tatelo study in Botswana explored the use of VRC01LS and 10-1074 in early-treated children, and 44% of the

children maintained HIV-1 RNA levels below 400 copies/ml (Cunningham *et al.*, 2020; McFarland *et al.*, 2021). Recent advances in bNAb development have included the evaluation of VRC07-523LS in infants, demonstrating enhanced potency and durability compared to earlier generation antibodies, with promising safety profiles and sustained neutralization activity in paediatric populations (Cunningham *et al.*, 2025). These findings suggest that bNAbs could provide an effective alternative to ART, or they could be used in combination with ART, particularly in cases where adherence to antiretroviral regimens is challenging. However, further research is needed to determine the optimal combination, dosing, and timing of bNAb administration to prevent vertical transmission effectively.

Variations in the neutralisation sensitivity of HIV-1 variants in mother-infant transmission pairs represent a complex interplay of viral evolution and immune selection pressures that influence transmission dynamics. Studies investigating the characteristics of TF viruses have revealed that infant-derived HIV-1 variants often exhibit distinct neutralisation profiles compared to maternal variants (Goo *et al.*, 2012; Chaillon *et al.*, 2013). TF variants tend to have shorter variable loops, fewer N-linked glycosylation sites, and specific mutations that may enhance their transmission fitness (Wu *et al.*, 2006; Rademeyer *et al.*, 2016; Kumar *et al.*, 2021). These structural differences suggest that neutralization-sensitive variants may be preferentially transmitted during vertical transmission, though the underlying molecular mechanisms remain incompletely understood. Limited data exist on the sensitivity of infant-derived viruses to bNAbs, highlighting a critical gap in knowledge. This study aims to comprehensively compare the neutralisation sensitivity of HIV-1 isolates from transmitting mothers and their infants against a panel of nine well-characterized bNAbs targeting the CD4 binding site, V3 glycan, V2 apex, gp120/gp41 interface, and MPER. Understanding these differences is crucial for informing the development of bNAb-based interventions that could further reduce the burden of paediatric HIV infections.

4.2 Methods

4.2.1 Study Population and Participants

This study focused on a subset of seven mother-infant pairs. This subset was selected based on sample availability and availability of high-quality, full-length *env* sequences and functional clones, ensuring robust comparative analysis. While smaller, this subset is representative of the overall cohort, as it captures diverse genetic and clinical characteristics observed across the population, allowing us to explore key features of vertical transmission dynamics in greater detail.

4.2.2 Characterization of HIV-1 Envelope Variable Regions and Glycosylation Patterns

Variable loop lengths and potential N-linked glycosylation sites (PNGS) were analysed using the Variable Region Characteristic tool available through the Los Alamos HIV Database (https://www.hiv.lanl.gov/content/sequence/VAR_REG_CHAR/) (Foley *et al.*, 2018). Envelope sequences were aligned using the HXB2 reference sequence as a template. The tool calculated the amino acid lengths of V1-V5 variable loops and identified PNGS using the N-X-[S/T] motif (where X can be any amino acid except proline) (Zhang *et al.*, 2004). Total variable loop lengths were determined by counting amino acid residues between conserved cysteine residues that form disulfide bonds at the base of each variable loop, as previously described (Derdeyn *et al.*, 2004; Sok, Doores, *et al.*, 2014b). The number and distribution of PNGS were analysed across V1-V5 regions, with specific focus on conserved glycan sites N156, N160 (V2 glycan), N301, N332 (V3 glycan), and N392 (V4).

4.2.3 Neutralization Assay

The study employed a neutralisation assay to measure the effectiveness of bNAbs against the pseudoviruses. Chapter 2, Section 2.2.11.

4.2.4 Statistics

Statistical analysis was performed using GraphPad Prism software. The study employed the Mann-Whitney test to compare viral loads between infants and mothers, with a significance threshold of $P < 0.05$. We also performed a paired t-test to compare the length of the loops within each pair. These statistical methods helped interpret the relationships and significance of the data collected throughout the study.

4.3 Results

4.3.1 Clinical Characteristics

Our study focused on seven mother-infant pairs, providing insights into the dynamics of mother-to-child HIV-1 transmission. At delivery, all mothers were on a standardised ART regimen, including Efavirenz (EFV), Tenofovir (TDF), and Emtricitabine (FTC), they had a median viral load of approximately 15,000 copies/mL (4.2 Log₁₀) and a median CD4 T cell count of 467 cells/mm³. The infant cohort, consisting of six females and one male, had a median viral load of 63,000 copies/mL (4.8 Log₁₀) and a median CD4 T cell count of 1,748 cells/mm³ at sampling. Notably, CD4 T cell counts for infants ranged from 642 to 3,129 cells/mm³, with a CD4% of 37, suggesting relatively robust immune function despite their HIV status. Following HIV diagnosis, all infants were initiated on a regimen of Zidovudine (AZT), Lamivudine (3TC), and Nevirapine (NVP). While the infants' viral load was higher than their mothers', the difference was not statistically significant (p = 0.48). These findings, alongside the immune profiles of the cohort, offer a robust basis for exploring the complexities of HIV-1 vertical transmission and assessing ART effectiveness.

Table 4.1: Clinical characteristics of Mother-infant pair

Infants							Mothers		
	PID's	Time point	Gender	Viral Load (Log ₁₀)	CD4 %	CD4 count (cell/mm ³)	PID's	Viral Load (Log ₁₀)	CD4 count (cell/mm ³)
1	50-2178-1	1 month	F	4,9	22	642	50-2178-0	6,1	369
2	60-0048-1	Birth	F	6,6	38	831	60-0048-0	5,3	5
3	60-0902-1	Birth	F	4,7	44	3129	60-0902-0	3,5	1201
4	60-1778-1	Birth	M	4,6	37	2397	60-1778-0	3,7	1081
5	60-5055-1	Birth	F	4,8	55	2114	60-5055-0	5,3	588
6	60-6027-1	1 month	F	4,8	24	1748	60-6027-0	4,2	558
7	60-8171-1	1 month	F	3,9	37	1720	60-8171-0	3,7	1736
Median				4,8	37,0	1748	4,2		

*Birth means blood taken within 48 hours

^Mother's VL taken at birth

4.3.2 Env Sequence Analysis

Phylogenetic analysis of full-length *env* sequences amplified from seven mother-infant pairs (14 functional clones) revealed all sequences clustered with HIV-1 subtype C reference strains. Each infant's sequences clustered together with their respective mother's sequences (bootstrap values >90%) while showing distinct separation between pairs. This pattern of high genetic similarity within mother-infant pairs strongly supports vertical transmission. It demonstrates a transmission bottleneck, where only a limited subset of the maternal viral population successfully infects the infant. The restricted genetic diversity during transmission suggests selective pressures may favour specific viral variants that establish infection in infants.

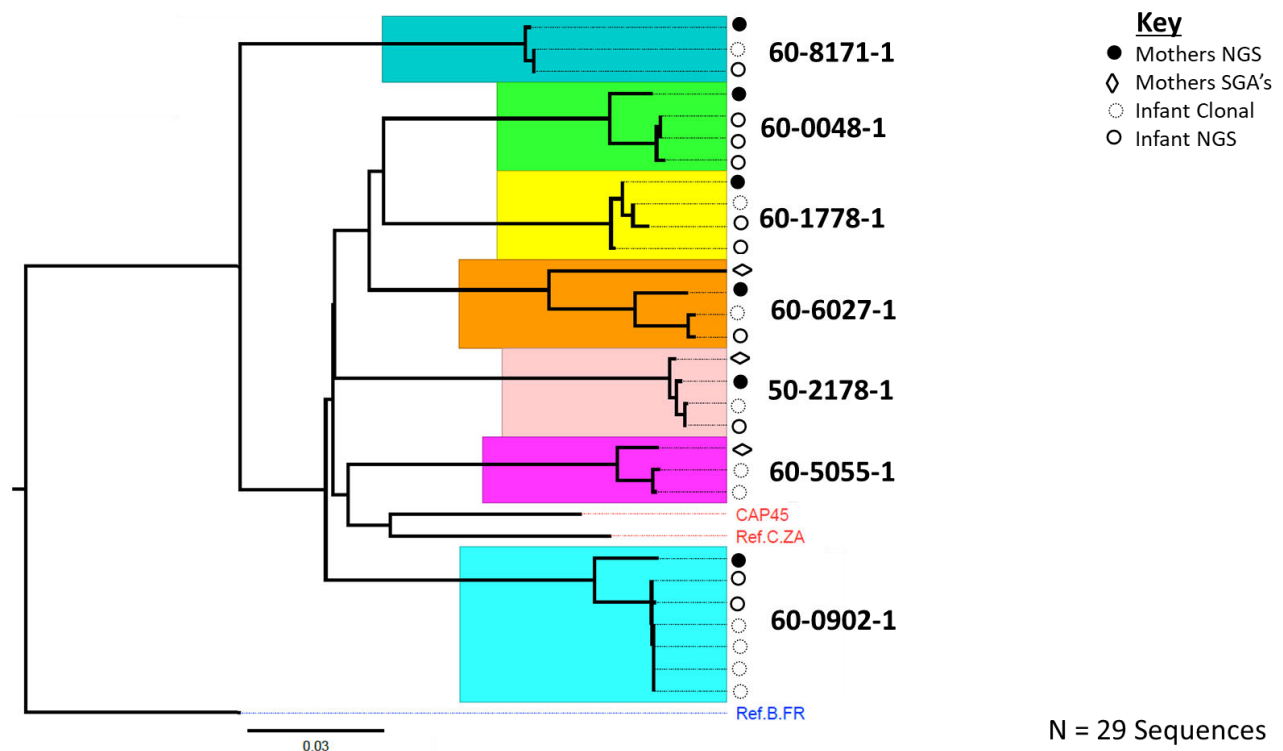


Figure 4.1: Phylogenetic Analysis of T/F HIV-1 Envelope Sequences in Mother-Infant Pairs. The phylogenetic tree illustrates the genetic relationships between HIV-1 *env* sequences derived from 7 mother-infant pairs. Each pair is colour-coded, with open circles representing infant sequences and closed circles and diamonds representing maternal sequences. The types of sequences include NGS sequences (solid and closed circles), clonal sequences (broken circles), and single-genome amplifications (SGAs) (diamonds).

4.3.3 Neutralization Sensitivity Profiles

Comparative analysis of bNAb activity between mother and infant HIV isolates revealed distinct neutralisation patterns across different epitope regions. Among the CD4 binding site antibodies, VRC07-523LS demonstrated the highest neutralisation coverage and potency, achieving 100% neutralisation of both maternal and infant viruses, with infant-derived viruses showing enhanced susceptibility (geometric mean IC_{50} of 0.05 $\mu\text{g/mL}$) compared to maternal viruses (geometric mean IC_{50} of 0.14 $\mu\text{g/mL}$). In contrast, VRC01 and 3BNC117 showed limited activity against maternal viruses, with VRC01 neutralising 43% (geometric mean IC_{50} of 5.49 $\mu\text{g/mL}$) and 3BNC117 neutralising 29% (geometric mean IC_{50} of 7.27 $\mu\text{g/mL}$). Both VRC01 and 3BNC117 demonstrated improved neutralization coverage against infant viruses, VRC01 neutralized 71% of pseudoviruses (geometric mean IC_{50} of 4.06 $\mu\text{g/mL}$) and 3BNC117 neutralised 57% of pseudoviruses (geometric mean IC_{50} of 1.93 $\mu\text{g/mL}$) (Fig. 4.3).

The V2 apex-directed bNAbs showed robust neutralization coverage against maternal pseudoviruses, with both PGDM1400 and CAP256-VRC26.25 achieving 86% neutralisation coverage (geometric mean IC_{50} of 0.06 and 0.04 $\mu\text{g/mL}$, respectively) (Fig. 4.3). However, these antibodies showed reduced coverage against the infant derived pseudoviruses: CAP256-VRC26.25 only neutralised 57% (geometric mean IC_{50} of 0.08 $\mu\text{g/mL}$) while PGDM1400 neutralised 71% of pseudoviruses (geometric mean IC_{50} of 0.12 $\mu\text{g/mL}$). Furthermore, 10-1074 showed higher neutralisation coverage of 71% in pseudoviruses isolated from infants (geometric mean IC_{50} of 0.35 $\mu\text{g/mL}$) compared to 57% coverage of maternal pseudoviruses (geometric mean IC_{50} of 0.45 $\mu\text{g/mL}$). While PGT121 demonstrated a similar neutralisation coverage of 57% for both maternal and infant-derived viruses (geometric mean IC_{50} of 0.73 $\mu\text{g/mL}$) (Walker et al., 2011). The MPER-targeting antibody 10E8 exhibited exceptional neutralization breadth, achieving 100% neutralisation coverage of both maternal and infant viruses. In contrast, PGT151, which targets the gp120/gp41 interface, showed limited activity, neutralising 43% of infant-derived pseudoviruses (geometric mean IC_{50} of 0.34 $\mu\text{g/mL}$) and 57% of maternal viruses (geometric mean IC_{50} of 0.95 $\mu\text{g/mL}$). These findings suggest that mothers may transmit bNAb-resistant variants, especially against the V2 apex and gp120-gp41 interface epitopes. Although the mechanism for this selective resistance is not fully understood.

	PID's	CD4-binding site			V1V2-Loop		V3-Glycan		MPER	Gp120-gp41
		VRC07-523LS	VRC01	3BNC117	PGDM1400	AP256-VRC26.2	10-1074	PGT121	10E8	PGT151
Infants	50-2178	0.04	2.7	0.54	10	0.005	10	10	6.24	10
	60-0048	0.09	4.9	10	0.24	3.3	0.01	0.05	0.07	0.03
	60-0902	0.04	10	10	0.005	0.005	0.06	1.05	3.94	10
	60-1778	0.04	1.79	0.76	0.005	0.005	0.05	0.02	0.26	10
	60-5055	0.01	10	10	10	10	0.35	0.07	0.34	0.04
	60-6027	0.54	9.64	1.26	3.31	10	10	10	0.35	10
	60-8171	0.03	0.81	0.19	0.32	0.24	0.61	10	1.65	0.06
GeoMean		0.051285084	4.069751078	1.925833715	0.349384935	0.192792769	0.349774744	0.68056229	0.710747626	0.934847252
% Breath		100	71	57	71	71	71	57	100	43

	PID's	CD4-binding site			V1V2-Loop		V3-Glycan		MPER	Gp120-gp41
		VRC07-523LS	VRC01	3BNC117	PGDM1400	AP256-VRC26.2	10-1074	PGT121	10E8	PGT151
Mothers	60-0902	0.04	0.33	1.15	0.01	0.005	0.04	0.07	3.74	0.02
	50-2178	0.06	6	10	0.005	0.005	10	10	3.33	0.52
	60-5055	0.06	10	10	10	10	0.12	1.22	0.67	0.01
	60-1778	0.11	10	9.30	0.005	0.005	0.12	0.02	0.36	10
	60-6027	0.35	7.45	10	0.07	0.06	10	10	0.36	10
	60-0048	0.48	10	10	0.02	0.005	0.04	0.008	0.49	10
	60-8171	0.38	10	10	2.01	0.42	10	10	0.89	0.005
GeoMean		0.17131603	8.71969673	9.87977736	0.0938525	0.05619768	0.91467508	0.76163455	0.70813874	0.48490563
% Breath		100	43	28	85	85	57	57	100	57

Figure 4.2: Neutralization heatmap IC₅₀ titers of mother-infant pair viruses against bNAbs. The heatmap illustrates IC₅₀ titers of M-I pair viruses (n = 7) against nine broadly neutralising antibodies (bNAbs) targeting key HIV-1 envelope regions: CD4 binding site, V1/V2 loop, V3 glycan, membrane-proximal external region, and gp120/gp41 interface. Colour-coded tiers represent neutralisation sensitivity: red (<0.1 µg/mL) indicates high sensitivity, orange and light-yellow show moderate sensitivity, light yellow represents minimal sensitivity, and white denotes complete resistance. Neutralisation assays were performed using a TZM-bl-based system, revealing the complex sensitivity patterns within the infant viral population

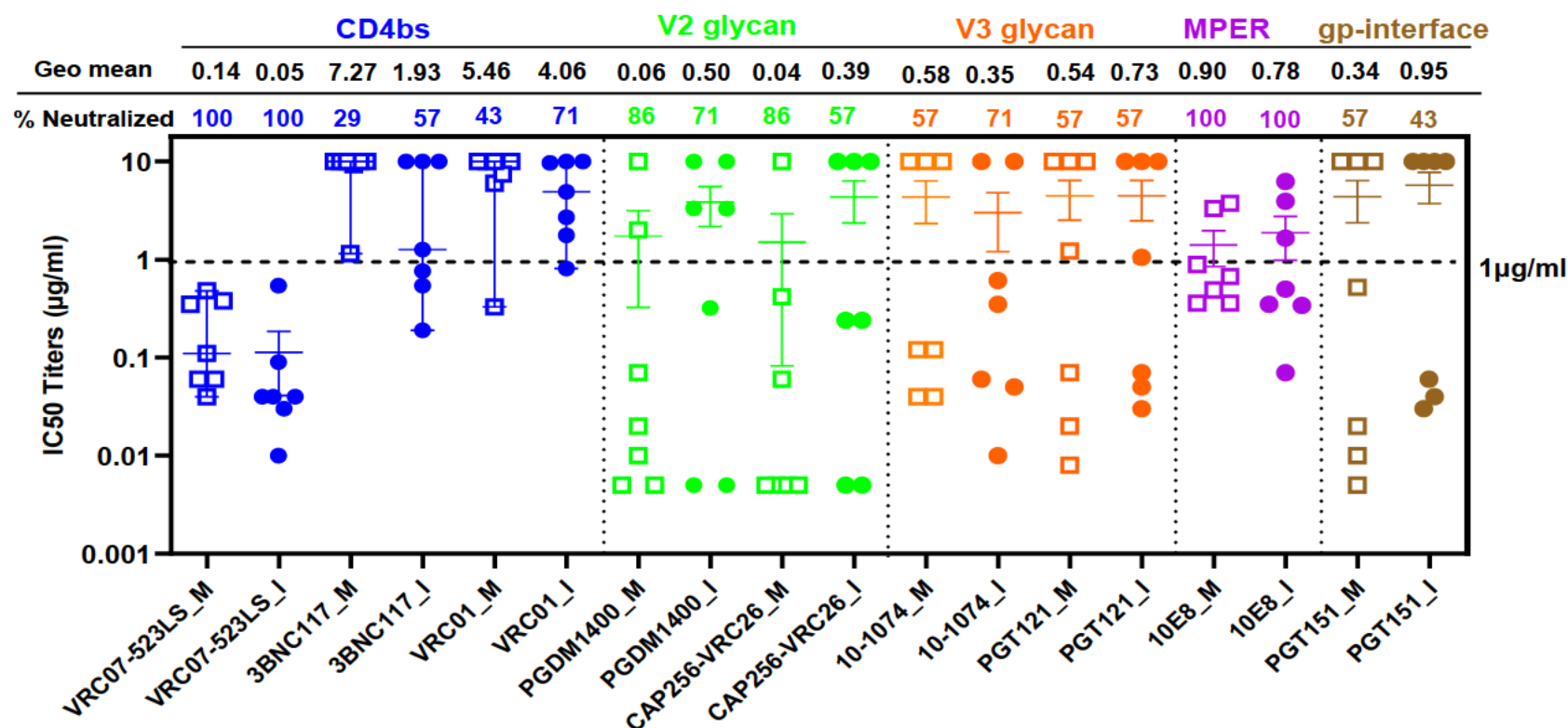


Figure 4.3: Neutralization sensitivity and IC50 titers in mother-infant pairs against nine broadly neutralising antibodies targeting five HIV-1 epitopes. The figure compares IC50 titers ($\mu\text{g/mL}$) and neutralisation sensitivity in mother-infant pairs, with data presented as scatterplots. Each data point represents an individual virus, with circles indicating infant-derived viruses and squares representing maternal viruses. Antibodies are categorised by target epitopes and colour-coded as follows: CD4bs (blue), V2 glycan (lime green), V3 glycan (orange), MPER (purple), and gp120/41 interface (brown). The initial bNAb concentration of 10 $\mu\text{g/mL}$ and the percentage of viruses neutralised ($n = 14$) are indicated. Solid black bars denote median IC50 titers.

4.3.4 Comparison of Mother and Infant Isolates and Genetic Correlates of Neutralization Sensitivity

Analysis of neutralization sensitivity patterns between mother-infant HIV transmission pairs revealed complex epitope-specific variations that provide insights into viral evolution during vertical transmission. In the CD4 binding site epitope region, diverse neutralization profiles emerged among the broadly neutralizing antibodies VRC01, VRC07-523LS, and 3BNC117 across multiple transmission pairs. The 60-5055 mother-infant pair pseudoviruses demonstrated high neutralization sensitivity to VRC07-523LS, while they were resistant to VRC01 and 3BNC117 (Fig. 4.4a). In 60-0902 mother-infant pair, the pseudovirus isolated from the mother exhibited high sensitivity to all three CD4bs bNAbs, while the pseudovirus isolated from the infant was only sensitive to VRC07-523LS. The 60-8171 pair showed discordant neutralization results, the pseudovirus isolated from an infant displayed resistance to all CD4-binding site bNAbs, while the maternal pseudovirus remained sensitive to all tested antibodies. In 50-2178 and 60-6027 mother-infant pairs, maternal pseudoviruses showed high sensitivity to VRC07-523LS, while the infant pseudoviruses demonstrated varied responses. The 60-6027 infant pseudovirus was sensitive to both VRC07-523LS and 3BNC117, but resistant to VRC01 (Fig. 4.4a). Despite these functional variations, the N-linked glycans critical for CD4 binding site integrity were highly conserved across all transmission pairs in the D loop region, including N197, N234, and N276, suggesting that neutralization differences arose from amino acid changes rather than glycosylation pattern alterations.

The V2 apex epitope region demonstrated varied neutralization sensitivity pattern across mother-infant transmission pairs, with most pairs showing conservation but two pairs displaying notable discordant patterns. Mother-infant pairs 50-2178 and 60-6027 both showed high maternal virus sensitivity to PGDM1400 and CAP256-VRC26.25 bNAbs. However, their infant pseudoviruses demonstrated distinct neutralisation patterns: 50-2178 infant pseudovirus was only sensitive to CAP256-VRC26.25, while the 60-6027 infant-derived pseudovirus showed sensitivity exclusively to PGDM1400 (Fig 4.4b). Analysis of the V2 loop revealed that these neutralisation patterns corresponded with specific resistance mutations: in the sequence isolated from the infant 50-2178, we observed lysine residues at position 160 (N160K), this mutation was previously associated with PGDM1400 resistance (Moore *et al.*, 2012). While in the sequence isolated from infant 60-6027, we found a valine (V) mutation at position 164 and a threonine (T) mutation at position 166, these mutations may have contributed to the variations in the neutralisation profiles.

Analysis of the V3 glycan region revealed that pseudoviruses obtained from mother-infant pairs that lacked the N332 glycan were resistant to the V3-glycan bNAbs. In addition, three mother-infant pairs (60-0902, 60-1778, and 60-5055) exhibited differences in neutralisation potency while maintaining sensitivity. Notably, in the 60-8171 mother-infant pair, the pseudovirus obtained from the mother showed complete resistance to both PGT121 and 10-1074, while the infant-derived pseudovirus was only sensitive to 10-1074 while maintaining PGT121 resistance (Fig. 4.3c). In addition, 10E8 mAb showed generally consistent neutralization patterns across mother-infant pairs, key amino acid residues such as tryptophan at positions 672 and 680 (W672, W680), and phenylalanine at position 673 (F673) remained conserved. However, there were variations in neutralization sensitivity in 60-0048 mother-infant pair, the maternal pseudovirus was 3-fold more sensitive to 10E8 ($IC_{50} = 0.07 \mu\text{g/ml}$) compared to the infant virus ($IC_{50} = 0.49 \mu\text{g/ml}$), while the second pair 60-5055 exhibited sequence variations, including serine at position 671 and lysine at position 683 in maternal sequences (Fig 4.4d). The gp120/gp41 interface targeted by PGT151 showed varied neutralisation patterns: mother-infant pairs 60-1778 and 60-6027 demonstrated complete resistance, while pairs 60-8171 and 60-5055 maintained sensitivity. Three mother-infant pairs, 50-2178, 60-0902, and 60-0048, showed different responses; both 50-2178 and 60-0048 maternal viruses were sensitive to 10E8, while their infant pseudoviruses were resistant to 10E8, and 60-0902 displayed the opposite pattern (Fig. 4.4e).

(a)	PID's	99																													CD4-binding site		
																															VRC07-523LS	VRC01	3BNC117
	HXB2	D	N	T	S	N	G	T	V	N	F	T	D	N	A	K	S	S	I	V	N	K	R	G	G	G	D	R					
	Ref.ZA.C	D	N	T	S	N	G	T	E	N	L	T	D	N	A	K	P	A	V	T	N	E	R	G	G	G	N	K					
50-2178	Mother	.	.	.	.	.	.	.	.	E	.	.	.	N	.	.	.	.	S	I	.	.	.	.	.	.	A	.	.	0.06	6	10	
	Infant	.	.	.	S	.	.	.	.	E	.	.	.	N	.	.	.	.	S	I	.	.	.	.	.	.	A	.	.	0.04	2.7	0.54	
60-0048	Mother	.	.	.	S	.	.	.	.	E	.	.	.	.	.	.	.	S	S	I	.	.	G	.	.	.	.	.	.	0.48	10	10	
	Infant	.	.	.	.	.	.	.	.	E	.	.	.	.	.	.	.	S	S	I	.	.	.	.	.	.	.	.	.	0.09	4.9	10	
60-0902	Mother	.	.	.	.	.	.	.	.	E	.	I	.	N	.	.	.	H	S	I	.	R	G	H	.	.	A	D	R	0.04	0.33	1.15	
	Infant	.	.	.	.	.	.	.	.	E	.	I	.	N	.	.	.	H	S	I	.	R	G	H	.	.	A	D	R	0.04	10	10	
60-1778	Mother	.	.	.	.	.	.	.	.	E	.	.	.	.	.	.	.	S	S	I	.	.	G	.	.	.	.	.	.	0.11	10	9.30	
	Infant	.	.	.	.	.	.	.	.	E	.	.	.	.	.	.	.	S	S	I	.	.	G	.	.	.	.	.	.	0.04	1.79	0.76	
60-5055	Mother	.	.	.	.	.	.	.	.	E	.	.	.	.	.	V	.	H	.	I	.	.	.	.	.	.	A	.	.	0.06	10	10	
	Infant	.	.	.	S	.	.	.	.	E	.	.	.	.	.	V	.	H	.	I	.	.	G	.	.	.	A	.	.	0.01	10	10	
60-6027	Mother	.	.	.	S	.	.	.	.	E	.	I	.	.	.	.	.	S	S	I	.	.	G	.	.	.	.	.	.	0.36	7.45	10	
	Infant	.	.	.	.	.	.	.	.	E	.	I	.	.	.	.	.	S	S	I	.	.	G	.	.	.	.	.	.	0.54	9.64	1.26	
60-8171	Mother	.	.	.	.	.	.	.	.	E	.	.	.	N	.	.	.	S	S	I	.	.	G	.	.	.	.	D	R	0.38	10	10	
	Infant	.	.	.	.	.	.	.	.	E	.	.	.	N	.	.	.	S	S	I	.	.	G	.	.	.	.	D	R	0.03	0.81	0.19	

(b)	PID's																V1V2-Loop			
		HXB2	156	157	158	160	161	162	164	165	166	167	168	169	170	171	PGDM1400	CAP256-VRC26.25		
		Ref.ZA.C	N	C	S	N	I	S	S	I	R	G	K	V	Q	K				
50-2178	Mother	.	.	.	.	.	I	.	.	.	.	.	.	.	.	K	K	N	0.005	0.005
	Infant	.	.	.	.	.	K	I	.	.	.	.	.	.	.	K	K	.	10	0.005
60-0048	Mother	.	.	.	.	.	T	.	.	I	.	.	.	.	.	I	R	.	0.02	0.005
	Infant	.	.	.	.	.	T	.	.	I	.	.	.	.	.	R	I	R	3.3	0.24
60-0902	Mother	.	.	.	.	.	M	A	.	I	.	.	.	.	.	K	K	.	0.01	0.005
	Infant	.	.	.	.	.	I	.	.	I	.	.	.	.	.	K	K	.	0.005	0.005
60-1778	Mother	.	.	.	.	.	T	.	.	.	.	.	.	.	.	K	.	.	0.005	0.005
	Infant	.	.	.	.	.	T	.	.	.	.	.	.	.	.	K	.	.	0.005	0.005
60-5055	Mother	.	.	.	T	.	T	.	.	I	G	.	.	.	.	K	.	E	10	10
	Infant	.	.	.	.	.	T	.	.	I	G	.	.	.	.	K	.	E	10	10
60-6027	Mother	.	.	.	.	.	.	.	.	M	K	.	.	.	.	K	.	.	0.07	0.06
	Infant	.	.	.	.	.	.	.	.	V	I	T	.	.	.	K	H	Q	3.31	10
60-8171	Mother	.	.	.	.	.	M	.	.	V	.	.	.	.	.	Q	.	.	2.01	0.42
	Infant	.	.	.	.	.	M	.	.	V	.	.	.	.	.	Q	.	.	0.32	0.24

(c)	PID's																														V3-Glycan	
			HXB2	137	156	157	158	295	296	297	300	301	302	303	304	307	323	324	325	328	330	332	333	334	386	387	388	392	393	394	10-1074	PGT121
			Ref.ZA.C	D	N	C	S	N	C	T	N	N	N	T	R	I	I	G	N	Q	H	N	I	S	N	S	T	N	S	T		
50-2178	Mother	N	.	.	.	E	.	I	.	.	.	.	.	.	V	.	.	.	K	Y	H	.	N	.	.	.	.	S	.	10	10	
	Infant	N	.	.	.	E	.	I	.	.	.	.	.	.	V	.	.	.	K	Y	H	.	D	.	.	.	.	S	.	10	10	
60-0048	Mother	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	D	Y	.	.	.	.	.	.	.	G	.	0.04	0.008		
	Infant	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	D	K	Y	.	.	.	.	.	G	.	0.01	0.05			
60-0902	Mother	T	.	.	.	N	.	.	.	.	.	.	.	.	V	.	.	.	.	.	I	.	N	.	.	T	.	N	.	0.04	0.07	
	Infant	K	.	.	.	N	.	I	.	.	.	.	.	.	V	.	.	.	D	Q	.	.	.	.	T	.	N	.	0.06	1.05		
60-1778	Mother	N	.	.	.	.	A	S	.	.	.	.	.	.	V	.	.	.	E	.	.	.	.	.	.	.	K	A	P	0.12	0.02	
	Infant	N	.	.	.	.	A	S	.	.	.	.	.	.	V	.	.	.	E	.	.	.	.	.	.	.	K	A	P	0.05	0.03	
60-5055	Mother	N	.	.	T	N	.	.	G	.	.	.	.	.	.	.	.	D	.	.	.	.	.	.	.	T	K	L	L	0.12	1.22	
	Infant	N	.	.	.	N	.	.	G	.	.	.	.	.	.	.	.	D	.	.	.	.	.	.	D	.	K	.	0.35	0.07		
60-6027	Mother	T	.	.	.	.	.	S	.	.	.	.	.	.	V	.	.	D	K	.	S	V	G	.	.	.	S	.	10	10		
	Infant	T	.	.	.	.	.	G	.	.	.	.	.	.	V	.	.	D	L	Y	K	V	N	.	.	S	.	10	10			
60-8171	Mother	A	.	.	.	N	.	G	.	.	.	.	.	.	V	.	.	D	.	.	.	.	.	.	.	.	S	.	10	10		
	Infant	T	.	.	.	N	.	G	.	.	.	.	.	.	V	.	.	D	.	.	.	.	.	.	.	S	.	0.61	10			

(d)	PID's		671	672	673	674	675	676	680	683	MPER	(e)	PID's		276	277	278	279	501	503	611	612	613	637	638	639	647	648	Gp120-gp41		
			HXB2	N	W	F	N	I	T	W	K				10E8	HXB2	N	F	T	D	A	R	N	A	S	N	Y	T	E	E	PGT151
			Ref.ZA.C	N	W	F	S	I	T	W	K				10E8	Ref.ZA.C	N	L	T	D	A	R	N	S	S	S	N	Y	T	E	E
50-2178	Mother	.	.	.	D	.	S	.	.	.	3.33	50-2178	Mother	.	.	.	N	.	.	.	A	.	.	.	.	.	.	0.52			
	Infant	.	.	.	D	.	S	.	.	.	6.24		Infant	.	.	.	N	.	.	.	A	.	.	.	.	.	.	10			
60-0048	Mother	S	.	.	N	.	.	.	.	.	0.49	60-0048	Mother	.	.	.	.	.	.	.	.	.	.	.	.	.	K	10			
	Infant	.	.	.	N	.	.	.	.	.	0.07		Infant	.	.	.	.	.	.	.	.	.	.	.	.	.	K	0.03			
60-0902	Mother	.	.	.	N	.	S	.	.	.	3.74	60-0902	Mother	.	I	.	N	.	.	.	.	.	.	.	.	.	D	0.02			
	Infant	.	.	.	N	.	S	.	.	.	3.94		Infant	.	I	.	N	.	.	.	.	.	.	.	.	.	D	10			
60-1778	Mother	.	.	.	N	.	.	.	.	.	0.36	60-1778	Mother	.	.	.	.	.	.	N	.	.	.	.	.	.	K	10			
	Infant	.	.	.	N	.	.	.	.	.	0.50		Infant	.	.	.	.	.	.	.	N	.	.	.	.	.	N	10			
60-5055	Mother	S	.	.	D	.	S	.	R	.	0.67	60-5055	Mother	.	.	.	.	.	.	.	T	.	.	.	.	.	D	0.04			
	Infant	S	.	.	D	.	S	.	.	.	0.34		Infant	.	.	.	.	.	.	.	T	.	.	.	.	.	D	0.01			
60-6027	Mother	S	.	.	.	.	.	.	.	.	0.36	60-6027	Mother	.	I	.	.	.	.	.	.	.	.	S	.	D	10				
	Infant	S	.	.	.	.	.	.	.	.	0.35		Infant	.	I	.	.	.	.	.	.	.	.	S	R	.	10				
60-8171	Mother	S	.	.	D	.	.	.	R	.	0.89	60-8171	Mother	.	.	.	N	.	.	.	V	.	.	.	.	I	0.01				
	Infant	S	.	.	D	.	.	.	R	.	1.65		Infant	.	.	.	N	.	.	.	V	.	.	.	.	I	0.06				

Figure 4.4: Signature mutations and neutralisation activity of antibodies against each epitope. (a) CD4bs antibodies; (b) V3 antibodies; (c) V1/V2 antibodies; (d) MPER antibody; and (e) gp120/41 interface antibody. In each panel, HIV env sequence variation and sensitivity to bNAbs. Grey shading indicates the three amino acids that form the glycan.

Association between HIV *env* Variable Loops and Neutralization Sensitivity

Analysis of HIV *env* variable loops across mother-infant pairs revealed distinct patterns of length variation and glycosylation site distribution that provide insights into transmission bottlenecks and viral adaptation during vertical transmission. The V1/V2 loop lengths exhibited considerable variability, ranging from 63 to 84 amino acids across all analyzed sequences, with transmission pairs displaying two distinct patterns: conservation and expansion (Fig 4.5). Three transmission pairs (50-2178, 60-1778, and 60-8171) maintained similar V1/V2 loop lengths between maternal and infant viruses, with differences of less than 2 amino acids, suggesting that loop length conservation may facilitate successful transmission in these cases. In contrast, the remaining three pairs (60-0902, 60-5055, and 60-6027) showed notable differences in V1/V2 length, with infant viruses consistently displaying longer V1/V2 loops compared to their corresponding maternal viruses, ranging from 3 to 7 additional amino acids (Table 4.2). This expansion pattern suggests potential selection pressure during transmission that favors longer V1/V2 regions in certain transmission contexts, possibly reflecting immune evasion mechanisms or structural requirements for successful establishment of infection in the infant host, although the observed differences did not reach statistical significance across the cohort (Curlin *et al.*, 2010).

The V3 loop demonstrated remarkable conservation in length across all mother-infant transmission pairs, with the majority maintaining 34-35 amino acids in both maternal and infant sequences, consistent with the functional constraints imposed by this region's critical role in coreceptor binding and viral entry. The V4 and V5 loops showed limited variation overall, with notable exceptions observed in specific transmission pairs that highlighted potential selective pressures on these regions. Pair 60-5055 exhibited differences in both V4 and V5 loop lengths, with the maternal V4 loop containing 32 amino acids compared to 28 amino acids in the infant sequence, and the V5 loop showing a reduction from 13 amino acids in the maternal sequence to 10 amino acids in the infant virus. Additionally, two mother-infant pairs (60-1778 and 60-6027) showed specific variations in V5 loop length, with 60-1778 displaying maternal and infant V5 loops of 15 and 12 amino acids respectively, while 60-6027 showed the opposite pattern with maternal V5 loops of 11 amino acids expanding to 14 amino acids in the infant sequence (Table 4.2). These findings align with previous research demonstrating that V3 loops remain relatively conserved in transmitted/founder variants from adult transmission studies, suggesting similar evolutionary constraints operate during vertical transmission.

Analysis of potential N-linked glycosylation sites (PNGSs) revealed generally similar patterns between most mother-infant pairs, with notable exceptions that suggest glycosylation-mediated immune evasion during transmission (Figure 4.6). The most striking variation was observed in pair 60-6027, which demonstrated a significant increase in glycosylation density, with the infant sequence containing four additional PNGSs compared to the maternal sequence (total of 28 versus 24 sites), potentially representing enhanced immune shielding in the transmitted virus. The V3 loop typically maintained one PNGS across most transmission pairs, specifically at position N301 relative to HXB2 numbering, although pair 60-5055 notably lacked this critical glycosylation site in both maternal and infant sequences, which correlated with the observed resistance to V3 glycan-directed broadly neutralizing antibodies. Analysis of the V4 and V5 loop regions revealed contrasting patterns in specific transmission pairs: pair 60-5055 showed maternal viruses with two additional PNGSs in the V4 region compared to infant sequences (4 versus 2 sites), while pair 60-1778 demonstrated maternal sequences with one additional PNGS in the V5 region compared to the transmitted infant virus (2 versus 1 site) (Table 4.2). These glycosylation differences suggest potential selection pressures during vertical transmission that may favour either glycan masking for immune evasion or glycan reduction to optimise viral fitness in the distinct immunological environment of the infant host.

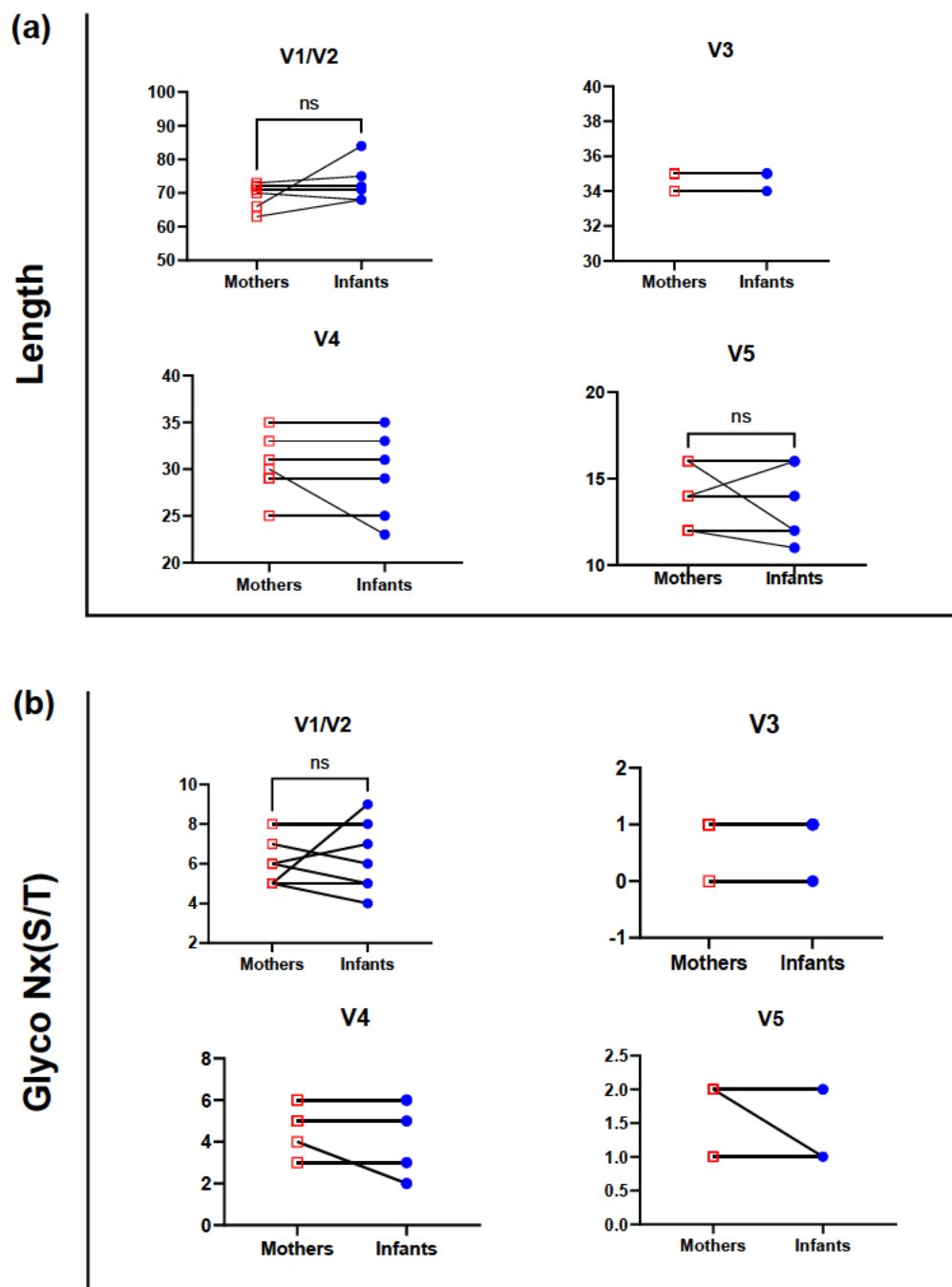


Figure 4.5: Pairwise comparison of mother-infant pairs' V1 – V5 loop characteristics. (a) V1-V2 length, (b) V1-V5 N-linked glycosylation. Each shape represents a different participant, with mothers in open squares and infants in closed circles.

Table 4.2: Pairwise comparison of mother-infant pairs V1 – V5 loop characteristic

PID's	V1 loop			V2 loop			V1/V2 loop			V3 loop			V4 loop			V5 loop		
	Length	Glyco Nx(ST)	Charge	Length	Glyco Nx(ST)	Charge	Length	Glyco Nx(ST)	Charge	Length	Glyco Nx(ST)	Charge	Length	Glyco Nx(ST)	Charge	Length	Glyco Nx(ST)	Charge
50-2178-0	28	3	-1	43	2	1	72	5	0	35	1	0	29	5	1	16	2	0
50-2178-1	28	3	-1	43	1	3	72	4	2	35	1	2	29	5	1	16	2	0
60-0048-0	29	4	0	40	2	3	70	6	3	35	1	3	29	6	1	14	1	-1
60-0048-1	27	5	-1	40	2	0	68	7	-1	35	1	-1	29	6	1	14	1	-1
60-0902-0	29	5	-2	43	1	3	73	6	1	35	1	1	33	5	0	12	2	1
60-0902-1	31	3	0	43	2	3	75	5	3	35	1	3	33	5	0	12	2	1
60-1778-0	23	6	0	47	2	-1	71	8	-1	35	1	-1	25	3	2	16	2	-1
60-1778-1	23	6	0	47	2	-1	71	8	-1	35	1	-1	25	3	2	12	1	2
60-5055-0	21	3	1	41	2	0	63	5	1	34	0	1	30	4	0	14	2	0
60-5055-1	26	3	0	41	2	0	68	5	0	34	0	0	23	2	-1	16	2	0
60-6027-0	26	4	-1	39	1	0	66	5	-1	35	1	-1	35	5	0	12	1	1
60-6027-1	39	7	0	44	2	-1	84	9	-1	35	1	-1	35	5	-1	11	1	1
60-8171-0	30	5	-2	41	2	-2	72	7	-4	35	1	-4	31	5	-1	12	1	0
60-8171-1	30	4	-2	41	2	-2	72	6	-4	35	1	-4	31	5	-1	12	1	0
Median	28	4	-0,5	42	2	0	71,5	6	-0,5	35	1	-0,5	29,5	5	0	13	1,5	0

4.4 Discussion

In a comprehensive analysis of HIV-1 neutralisation patterns in mother-infant transmission pairs, we investigated the neutralisation sensitivity profiles against a panel of bNAbs. Our analysis revealed distinctive trends in sensitivity to various bNAbs. We observed that infant-derived clones showed higher sensitivity to CD4bs and V3 glycan bNAbs than maternal isolates. In contrast, maternal viruses showed high sensitivity to V2 apex-targeting antibodies, although these differences did not reach statistical significance. When examining neutralisation patterns with VRC07-523LS and PGT121, we found comparable neutralization potency between maternal and infant isolates, suggesting that these particular epitopes may be similarly constrained in both populations. Notably, both maternal and infant-derived clones displayed consistent resistance to PGT151, which targets the gp120-gp41 interface. The consistency of this resistance pattern across transmission pairs suggests that PGT151 resistance might be a key feature of successfully transmitted variants. These observations point to specific selective pressures during transmission that influence viral susceptibility to broadly neutralising antibodies, potentially reflecting a balance between immune escape and maintenance of viral fitness.

Sequence analysis revealed minor variations in envelope characteristics between mother-infant pairs, though these differences were not statistically significant. This finding aligns with previous studies showing the conservation of key envelope features during vertical transmission (Goo *et al.*, 2012). Notably, the V3 region showed remarkable conservation between mothers and infants, while the V4 and V5 regions displayed more variability. Maintaining certain envelope features across transmission events suggests a constraint on viral evolution during vertical transmission, possibly reflecting requirements for successful transmission or early viral establishment. The observed neutralisation patterns likely reflect complex interactions between viral evolution and immune pressure during transmission rather than substantial structural changes in the envelope glycoprotein (Behrens *et al.*, 2016a). These findings support that successful vertical transmission may require a delicate balance between maintaining viral fitness and achieving immune evasion.

The subtle variations in glycosylation patterns between maternal and infant isolates are particularly noteworthy, as these modifications can significantly influence viral susceptibility to neutralising antibodies (Doores *et al.*, 2010). Our analysis revealed distinct glycosylation patterns in the V1-V2

regions between mothers and infants, while V3 glycosylation remained broadly consistent. These variations in glycosylation sites might contribute to the observed differences in neutralisation sensitivity between maternal and infant isolates. Previous research has demonstrated that changes in glycosylation patterns can significantly alter epitope accessibility and impact neutralisation sensitivity (Moore *et al.*, 2009). The conservation of specific glycosylation sites, particularly in the V3 region, might reflect their importance in maintaining viral fitness during transmission. In contrast, variations in other regions might represent adaptations to different immune pressures in maternal and infant hosts.

When examining specific amino acid signatures, we identified mutations associated with differential neutralisation sensitivity patterns. In the V2 apex region, we observed N160K mutation in infant-derived clones that displayed resistance to PGDM1400. This mutation, which eliminates a critical N-linked glycosylation site, has been previously associated with resistance to V2 apex-targeting antibodies (Sok *et al.*, 2017). The presence of this mutation in transmitted variants suggests that escape from V2 apex-targeting antibodies might confer an advantage during transmission. Additionally, analysis of the V3 glycan region revealed N332H/S/K mutations in resistant pairs, eliminating a key glycosylation site required for recognition by V3 glycan-specific antibodies (Sok, van Gils, *et al.*, 2014). The co-occurrence of these mutations with specific neutralisation profiles provides strong evidence for their role in modulating antibody sensitivity. These findings highlight the genetic basis of bNAb escape in transmitted variants and suggest that escape mutations might be preferentially selected during vertical transmission.

While our study provides valuable insights, several limitations should be addressed in future research. Our sample size of seven mother-infant pairs necessitates more extensive studies to confirm and expand upon our findings. The cloning approach using bulk PCR products may have missed minority variants, suggesting that single-genome amplification approaches could provide a more comprehensive view of the transmitted viral population. Additionally, our focus on samples collected at birth limits our understanding of viral evolution, highlighting the need for longitudinal studies tracking the evolution of neutralisation sensitivity in infants.

These findings have important implications for developing bNAb-based strategies to prevent vertical transmission. The differential sensitivity to various bNAbs underscores the importance of combination approaches targeting multiple epitopes, which could help overcome the transmission of variants resistant to any single bNAb (Moore *et al.*, 2012). The high resistance to PGT151 observed in maternal and infant isolates suggests that this characteristic may be necessary for successful vertical transmission, though the underlying mechanisms require further investigation.

In conclusion, our study contributes significant insights into HIV-1 transmission dynamics and antibody-mediated protection. The genetic correlates of neutralisation sensitivity identified provide a foundation for understanding the molecular basis of antibody escape. They may inform the design of next-generation broadly neutralising antibodies and vaccines. However, more extensive studies with broader geographical representation are needed to fully elucidate the patterns of neutralisation sensitivity in mother-to-child transmission.

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Chapter 5

5.1 Synthesis

The overarching goal of this thesis was to enhance our understanding of HIV-1 transmission dynamics, mainly focusing on neutralisation sensitivity and coreceptor usage within the context of mother-infant pairs. Through a systematic approach combining virological, immunological, and molecular techniques, each chapter addressed distinct but interrelated aspects of HIV-1 biology in vertically transmitted infections, exploring three interconnected elements: neutralisation sensitivity patterns, coreceptor usage, and the evolutionary dynamics of viral populations during transmission events. This comprehensive investigation has provided novel insights into the mechanisms that underpin HIV-1 evolution and adaptation, uncovering essential insights into the biological mechanisms underlying vertical transmission and identifying potential therapeutic targets for intervention strategies.

5.1.1 Broadly Neutralizing Antibody Sensitivity in Infants

In Chapter 2, we characterised the TF pseudoviruses from infants who acquired HIV-1 in utero in the Baby Cure cohort, neutralisation sensitivity of pseudoviruses was measured against various bNAbs using the TZM-bl neutralization assay. Our findings revealed distinct bNAb sensitivity and resistance patterns in infant TF viruses; VRC07-523LS, 10-1074 and 10E8 achieved the highest neutralisation coverage. Conversely, high resistance was observed for CAP256-VRC26, PGDM1400, and PGT151, suggesting potential limitations in their effectiveness. Analysis of the D loop and V5 loops in the CD4 binding site showed relative conservation of amino acids in important positions, although few mutations were observed at positions 279E (glutamic acid) and 456H (histidine). These mutations were found in pseudoviruses that were resistant to CD4-binding site bNAbs, and they may confer neutralisation escape. Our findings are consistent with previous findings, amino acid mutations in these positions were associated with neutralization escape (Lynch, Boritz, *et al.*, 2015; Bricault *et al.*, 2019).

In the V2-apex region, approximately 48% of infant TF viruses had various mutations at positions 169 and 166, which correlated with resistance to CAP256-VRC26.25. These findings are consistent with previous studies, which demonstrated that position mutations at position 169 are associated with V2 apex bNAb escape in subtype C viruses (Walker *et al.*, 2009; Doria-Rose *et al.*, 2014). In addition, the V3 glycan region showed mutations at position N332, 14% of infants pseudoviruses were resistant to both PGT121 and 10-1074, they lacked the N-linked glycan at position 332, and glycan shifts from 332 to 334 was the most common escape pathway (Pejchal *et al.*, 2011; Sok, van Gils, *et al.*, 2014). In the gp120/41 interface, 43%

of infants displayed resistance to PGT151, potentially linked to polymorphisms that reduce antibody affinity without compromising viral fitness (Cao *et al.*, 2017). The heterogeneity in neutralisation profiles suggests that combinations of bNAbs targeting different epitopes may be necessary for effective treatment. The data can guide the rational selection of bNAb combinations for future clinical trials and inform the development of immunogens tailored for paediatric settings. These findings have significant implications for HIV prevention and treatment strategies.

5.1.2 Coreceptor Usage Patterns

In Chapter 3, we investigated coreceptor usage patterns in HIV-1 TF envelopes isolated from 21 in utero infected infants and their mothers in KwaZulu-Natal, South Africa. We employed computational predictions and TZM-bl cell-based phenotypic assays to determine coreceptor tropism, focusing on CCR5 and CXCR4 usage patterns. Our findings revealed that 71.4% (15/21) of infant isolates utilised the CCR5 coreceptor, while 28.6% (6/21) used CXCR4. This distribution aligns with previous studies in Tanzania and other African regions, showing CCR5 predominance in early HIV-1 subtype C infections (Ndung'u *et al.*, 2006b; Judicate *et al.*, 2021), though our observed CXCR4 usage was higher than previously reported. In mother-infant pairs, most infant viral tropism matched the mother's predominant viral population, with two notable exceptions showing discordant patterns: one pair where the maternal virus was CCR5-tropic, but the infant virus was CXCR4-tropic and another where both were CXCR4-tropic. Analysis of V3 loop characteristics identified key molecular determinants of coreceptor usage. These included specific N-linked glycosylation patterns (Pollakis *et al.*, 2001), overall V3 loop length, and crown motif substitutions, particularly GPGQ to GPGR changes associated with CXCR4 usage (Ping *et al.*, 1999; Coetzer *et al.*, 2006). The distribution of coreceptor usage was consistent across both predictive algorithms and experimental assays, with PhenoSeq and WebPSSM-C charge demonstrating the highest sensitivity for predicting X4/R5X4 viruses. These findings have important implications for HIV prevention and treatment strategies, particularly in the context of mother-to-child transmission. The predominance of CCR5 usage suggests that CCR5 antagonists like Maraviroc might effectively prevent vertical transmission. However, the presence of CXCR4-using viruses in 28.6% of cases indicates that combination approaches might be necessary for comprehensive prevention, especially in regions where HIV-1 subtype C is prevalent.

5.1.3 M-I pairs Neutralization Sensitivity Variation

In this Chapter, we investigated neutralization sensitivity patterns in HIV-1 transmission pairs through comprehensive phenotypic and genotypic analyses of maternal and infant viral isolates. Using a panel of bNAbs, we performed neutralization assays to characterize sensitivity profiles across multiple epitope

targets. This was complemented by genetic sequencing and structural analysis of the viral envelope to identify molecular determinants of neutralisation patterns. Our investigation revealed distinct neutralisation profiles between maternal and infant isolates, suggesting selective transmission of specific viral variants. Infant isolates demonstrated enhanced sensitivity to V2 apex-targeting antibodies like PGDM1400 and CAP256-VRC26.25. (Sok et al., 2017), while showing increased resistance to antibodies targeting the CD4 binding site and the gp120-gp41 interface, particularly PGT151 (Dingens *et al.*, 2019). Certain bNAbs, including VRC07-523LS, 10E8, and PGDM1400, exhibited strong neutralisation potential against maternal and infant viruses. Genetic analysis identified specific mutations associated with neutralisation patterns, including P364H and R456H mutations in infant isolates conferring resistance to CD4 binding site antibodies (Lynch, Wong, et al., 2015), N160K mutations affecting V1/V2 antibody sensitivity, and mutations at position 332 influencing V3 glycan-targeting antibody responses (Sok, van Gils, *et al.*, 2014). The highly conserved MPER region showed consistent sensitivity to antibodies like 10E8, reflecting its essential role in viral fusion (Kong et al., 2019). Structural analysis revealed distinct patterns in variable loop lengths and glycosylation between maternal and infant isolates. These findings align with previous research showing that envelope characteristics modulate bNAb accessibility (Behrens *et al.*, 2016a). Infants showed slightly longer V1-V2 lengths than mothers, while V3 remained stable. Glycosylation patterns varied across regions, particularly in V1-V2 and V5, consistent with studies demonstrating the impact of glycan shields on neutralisation sensitivity (Moore et al., 2009; Doores et al., 2010). These findings suggest complex selective pressures during transmission, where escape from specific antibodies may incur fitness costs. The enhanced sensitivity to V2 apex antibodies indicates these could be particularly effective for preventing vertical transmission through passive immunisation or maternal vaccination. The resistance patterns observed suggest that early bNAb administration might be crucial for maximum effectiveness.

These findings suggest several key considerations for implementing passive immunotherapy strategies. The timing of bNAb administration appears crucial, as the distinct neutralisation profiles of transmitted variants suggest early intervention might be most effective. The heterogeneity in neutralisation sensitivity patterns indicates that combinations of bNAbs targeting different epitopes will be necessary for effective prevention. Additionally, the presence of both CCR5 and CXCR4-using viruses suggests that passive immunotherapy strategies might benefit from broader coverage approaches, potentially combining bNAbs with entry inhibitors. The research emphasises the importance of regional considerations in designing passive immunotherapy protocols, particularly in areas where HIV-1 subtype C predominates. The specific neutralisation resistance patterns and higher frequency of CXCR4-using viruses observed in this cohort

suggest that prevention strategies may need to be tailored to local viral populations. These observations could significantly impact the design of future clinical trials testing passive immunotherapy approaches. This comprehensive analysis provides a foundation for optimising passive immunotherapy strategies using bNAbs. The findings suggest that effective prevention of mother-to-child transmission will likely require carefully selected combinations of bNAbs targeting multiple epitopes, administered early in the transmission window and potentially combined with entry inhibitors. Future clinical trials should consider regional viral characteristics and may benefit from tailored approaches based on local viral populations. The identification of consistently effective bNAbs and understanding of escape patterns provide a clear direction for developing more effective passive immunotherapy protocols for preventing mother-to-child HIV-1 transmission.

5.2 Limitations and Future Directions

The studies presented in this thesis faced several notable limitations that warrant consideration when interpreting the findings. Our cohort size was constrained to 21 infants and seven mother-infant pairs, recruited predominantly from southern Africa with an exclusive focus on HIV-1 subtype C viruses, limiting the generalizability of our findings across different viral subtypes and geographical regions. The temporal scope of our data collection was restricted to baseline time points, preventing the capture of longitudinal dynamics in transmitted/founder virus evolution. Methodologically, bulk PCR amplification may have obscured the detection of minority variants, while our limited antibody panel might have missed other significant neutralisation epitopes. Despite these constraints, our research establishes a robust foundation for future investigations

Future directions should include longitudinal studies essential to track the temporal evolution of neutralisation sensitivity patterns, monitor shifts in coreceptor usage, and document the development of immune responses in mothers and infants. Mechanistic investigations should focus on elucidating the role of maternal antibodies, understanding infant immune system maturation, and characterising viral evolution under selective immune pressures. The development of therapeutic strategies should prioritise novel bNAb combinations and infant-specific interventions that target the unique vulnerabilities of transmitted/founder viruses. Furthermore, expanded population-based studies encompassing diverse HIV-1 subtypes and larger cohort sizes are crucial to enhance the generalizability of findings across different settings and deepen our understanding of vertical transmission dynamics. These research directions will build upon the current knowledge base and advance the development of more effective prevention and treatment strategies for mother-to-child transmission of HIV-1.

5.3 Conclusion

This thesis has comprehensively analysed HIV-1 transmission dynamics in mother-infant pairs, revealing crucial insights into the biological mechanisms governing vertical transmission and identifying potential therapeutic targets. Through a detailed investigation of neutralisation sensitivity patterns, coreceptor usage preferences, and transmission dynamics, our research has illuminated the complex interplay between viral characteristics and successful transmission events. Key findings include enhanced neutralisation sensitivity of infant-derived viruses to specific bNAb classes, a predominance of CCR5-tropic variants in successful transmission events, and distinct envelope characteristics associated with transmitted/founder viruses. The consistent patterns observed across multiple aspects of viral phenotype and genotype during transmission events suggest that mother-to-child transmission represents a unique window of vulnerability that could be exploited for therapeutic intervention. Identifying specific viral signatures associated with successful transmission, including fewer potential N-linked glycosylation sites and altered V1/V2 loop lengths, provides clear molecular targets for therapeutic development and prevention strategies. These findings collectively advance our understanding of HIV-1 vertical transmission and establish a foundation for developing more effective interventions to prevent mother-to-child transmission, bringing us closer to the goal of an AIDS-free generation.

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Chapter 6

Appendices

Table S1: Primers

Amplification of the full-length env gene

	Primer name	Sequence	HXB2 position
1 st round	OMF19	5' GCACTCAAGGCAAGCTTTATTGAGGCTTA 3'	9604 ← 9632
	VIF1	5' GGGTTTATTACAGGGACAGCAGA 3'	4900 → 4922
2 nd round	Env1A	5' CACCGGCTTAGGCATCTCCTATGGCAGGAAGAA 3'	5950 → 5982
	Env1M	5' TAGCCCTTCCAGTCCCCCCTTTTCTTTTA 3'	9068 ← 9096

Sequencing of full-length env gene

Primer name	Sequence	HXB2 position
sq6rc(2)	5' GAATTGGGTCAAAAGAGACCTTTGGA 3'	6839 ← 6864
ef00	5' AAAGAGCAGAAGACAGTGGCAATGA 3'	6204 → 6228
ed31	5' CCTCAGCCATTACACAGGCCTGTCCAAAG 3'	6817 → 6845
sq5.5rc	5' CTAGGAGCTGTTGATCCTTTAGGTAT 3'	7979 ← 8004
sq13f(2)c	5' TATATAAATATAAAGTGGTAGAAATTAAGC 3'	7672 → 7701
sq14fc	5' ACTCACGGTCTGGGGCATTAA 3'	7925 → 7944
sq3r(2)c	5' GCTATGGTATCAAGCAGACTAATAGCACTC 3'	8651 ← 8680
envM	5' TAGCCCTTCCAGTCCCCCCTTTTCTTTTA 3'	9096 ← 9068

Table S2: Full-length HIV env sequence alignment of mother-infant pairs

Figure 1. Multiple sequence alignment of the Hx2 protein from various species. The alignment is shown in a table format with columns representing different regions of the protein: Signal Peptide, Constant Region 1, Variable Loop 1, and Constant Region 2. The rows represent different species, including Hx2, Hx1, Hx3, Hx4, Hx5, Hx6, Hx7, Hx8, Hx9, Hx10, Hx11, Hx12, Hx13, Hx14, Hx15, Hx16, Hx17, Hx18, Hx19, Hx20, Hx21, Hx22, Hx23, Hx24, Hx25, Hx26, Hx27, Hx28, Hx29, Hx30, Hx31, Hx32, Hx33, Hx34, Hx35, Hx36, Hx37, Hx38, Hx39, Hx40, Hx41, Hx42, Hx43, Hx44, Hx45, Hx46, Hx47, Hx48, Hx49, Hx50, Hx51, Hx52, Hx53, Hx54, Hx55, Hx56, Hx57, Hx58, Hx59, Hx60, Hx61, Hx62, Hx63, Hx64, Hx65, Hx66, Hx67, Hx68, Hx69, Hx70, Hx71, Hx72, Hx73, Hx74, Hx75, Hx76, Hx77, Hx78, Hx79, Hx80, Hx81, Hx82, Hx83, Hx84, Hx85, Hx86, Hx87, Hx88, Hx89, Hx90, Hx91, Hx92, Hx93, Hx94, Hx95, Hx96, Hx97, Hx98, Hx99, Hx100. The alignment shows conserved regions and variable loops across the different species.

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HxB2 refer...	E	L	Y	K	Y	K	V	V	K	I	E	P	L	G	V	A	P	T	K	A	K	R	R	V	V	Q	R	E	K	R	A	V		
	Constant Region 5																GP41																	
50.2178-0...	E	L	Y	K	Y	K	V	V	E	I	K	P	L	G	I	A	P	T	K	A	K	R	R	V	V	E	R	E	K	R	A	V		
50.2178-1...	E	L	Y	K	Y	K	V	V	E	I	K	P	L	G	I	A	P	T	K	A	K	R	R	V	V	E	R	E	K	R	A	V		
60.0048-0...	E	L	Y	K	Y	K	V	V	E	I	K	P	L	G	I	A	P	T	K	A	K	R	R	V	V	E	R	E	K	R	A	V		
60.0048-1...	E	L	Y	K	Y	K	V	V	E	I	K	P	L	G	I	A	P	T	K	A	K	R	R	V	V	E	R	E	K	R	A	V		
60.0902-0...	E	L	Y	K	Y	K	V	V	E	I	K	P	L	G	I	A	P	T	K	A	K	R	R	V	V	E	R	E	K	R	A	V		
60.0902-1...	E	L	Y	K	Y	K	V	V	E	I	K	P	L	G	I	A	P	T	K	A	K	R	R	V	V	E	R	E	K	R	A	V		
60.1778-0...	E	L	Y	K	Y	K	V	V	E	I	K	P	L	G	I	A	P	T	K	A	K	R	R	V	V	E	R	E	K	R	A	V		
60.1778-1...	E	L	Y	K	Y	K	V	V	E	I	K	P	L	G	I	A	P	T	K	A	K	R	R	V	V	E	R	E	K	R	A	V		
60.5055-0...	E	L	Y	K	Y	K	V	V	E	I	K	P	L	G	I	A	P	T	K	A	K	R	R	V	V	E	R	E	K	R	A	V		
60.5055-1...	E	L	Y	K	Y	K	V	V	E	I	K	P	L	G	I	A	P	T	K	A	K	R	R	V	V	E	R	E	K	R	A	V		
60.6027-0...	E	L	Y	K	Y	K	V	V	E	I	K	P	L	G	I	A	P	T	K	A	K	R	R	V	V	E	R	E	K	R	A	V		
60.6027-1...	E	L	Y	K	Y	K	V	V	E	I	K	P	L	G	I	A	P	T	K	A	K	R	R	V	V	E	R	E	K	R	A	V		
60.8171-0...	E	L	Y	K	Y	K	V	V	E	I	K	P	L	G	I	A	P	T	K	A	K	R	R	V	V	E	R	E	K	R	A	V		
60.8171-1...	E	L	Y	K	Y	K	V	V	E	I	K	P	L	G	I	A	P	T	K	A	K	R	R	V	V	E	R	E	K	R	A	V		
HxB2 refer...	G	I	G	A	L	F	L	G	F	L	G	A	A	G	S	T	M	G	A	A	S	M	T	L	T	V	Q	A	R	Q	L	L		
	GP41 Fusion Peptide																GP41 NHR																	
50.2178-0...	G	L	G	A	V	L	F	G	F	L	G	A	A	G	S	T	M	G	A	A	S	I	T	L	T	V	Q	A	R	Q	L	L		
50.2178-1...	G	L	G	A	V	L	F	G	F	L	G	A	A	G	S	T	M	G	A	A	S	I	T	L	T	V	Q	A	R	Q	L	L		
60.0048-0...	G	V	G	V	M	I	F	L	G	F	L	G	A	A	G	S	T	M	G	A	A	S	I	M	T	L	T	V	Q	A	R	Q	L	L
60.0048-1...	G	V	G	V	M	I	F	L	G	F	L	G	A	A	G	S	T	M	G	A	A	S	I	M	T	L	T	V	Q	A	R	Q	L	L
60.0902-0...	G	I	G	A	V	F	L	G	F	L	G	A	A	G	S	T	M	G	A	A	S	I	I	T	L	T	V	Q	A	R	Q	L	L	
60.0902-1...	G	I	G	A	V	F	L	G	F	L	G	A	A	G	S	T	M	G	A	A	S	I	I	T	L	T	V	Q	A	R	Q	L	L	
60.1778-0...	G	I	G	A	V	F	L	G	F	L	G	A	A	G	S	T	M	G	A	A	S	I	I	T	L	T	V	Q	A	R	Q	L	L	
60.1778-1...	G	I	G	A	V	F	L	G	F	L	G	A	A	G	S	T	M	G	A	A	S	I	I	T	L	T	V	Q	A	R	Q	L	L	
60.5055-0...	G	L	G	A	V	L	L	L	G	F	F	L	G	A	A	G	S	T	M	G	A	A	S	I	T	L	T	V	Q	A	R	Q	L	L
60.5055-1...	G	L	G	A	V	L	L	L	G	F	F	L	G	A	A	G	S	T	M	G	A	A	S	I	T	L	T	V	Q	A	R	Q	L	L
60.6027-0...	G	I	G	A	V	F	L	G	F	F	L	G	A	A	G	S	T	M	G	A	A	S	I	T	L	T	V	Q	A	R	Q	L	L	
60.6027-1...	G	I	G	A	V	F	L	G	F	F	L	G	A	A	G	S	T	M	G	A	A	S	I	T	L	T	V	Q	A	R	Q	L	L	
60.8171-0...	G	I	G	A	V	F	L	G	F	F	L	G	A	A	G	S	T	M	G	A	A	S	I	T	L	T	V	Q	A	R	Q	L	L	
60.8171-1...	G	I	G	A	V	F	L	G	F	F	L	G	A	A	G	S	T	M	G	A	A	S	I	T	L	T	V	Q	A	R	Q	L	L	
HxB2 refer...	S	G	I	V	Q	Q	Q	N	N	L	L	R	A	I	E	A	Q	Q	H	L	L	Q	L	T	V	W	G	I	K	Q	L	Q		
	GP41 NHR																GP41 Loop																	
50.2178-0...	S	G	I	V	Q	Q	Q	N	N	L	L	R	A	I	E	A	Q	Q	H	M	L	Q	L	T	V	W	G	I	K	Q	L	Q		
50.2178-1...	S	G	I	V	Q	Q	Q	N	N	L	L	R	A	I	E	A	Q	Q	H	M	L	Q	L	T	V	W	G	I	K	Q	L	Q		
60.0048-0...	S	G	I	V	Q	Q	Q	N	N	L	L	R	A	I	E	A	Q	Q	H	M	L	Q	L	T	V	W	G	I	K	Q	L	Q		
60.0048-1...	S	G	I	V	Q	Q	Q	N	N	L	L	R	A	I	E	A	Q	Q	H	M	L	Q	L	T	V	W	G	I	K	Q	L	Q		
60.0902-0...	S	G	I	V	Q	Q	Q	N	N	L	L	R	A	I	E	A	Q	Q	H	M	L	Q	L	T	V	W	G	I	K	Q	L	Q		
60.0902-1...	S	G	I	V	Q	Q	Q	N	N	L	L	R	A	I	E	A	Q	Q	H	M	L	Q	L	T	V	W	G	I	K	Q	L	Q		
60.1778-0...	S	G	I	V	Q	Q	Q	N	N	L	L	R	A	I	E	A	Q	Q	H	M	L	Q	L	T	V	W	G	I	K	Q	L	Q		
60.1778-1...	S	G	I	V	Q	Q	Q	N	N	L	L	R	A	I	E	A	Q	Q	H	M	L	Q	L	T	V	W	G	I	K	Q	L	Q		
60.5055-0...	S	G	I	V	Q	Q	Q	N	N	L	L	R	A	I	E	A	Q	Q	H	M	L	Q	L	T	V	W	G	I	K	Q	L	Q		
60.5055-1...	S	G	I	V	Q	Q	Q	N	N	L	L	R	A	I	E	A	Q	Q	H	M	L	Q	L	T	V	W	G	I	K	Q	L	Q		
60.6027-0...	S	G	I	V	Q	Q	Q	N	N	L	L	R	A	I	E	A	Q	Q	H	M	L	Q	L	T	V	W	G	I	K	Q	L	Q		
60.6027-1...	S	G	I	V	Q	Q	Q	N	N	L	L	R	A	I	E	A	Q	Q	H	M	L	Q	L	T	V	W	G	I	K	Q	L	Q		
60.8171-0...	S	G	I	V	Q	Q	Q	N	N	L	L	R	A	I	E	A	Q	Q	H	M	L	Q	L	T	V	W	G	I	K	Q	L	Q		
60.8171-1...	S	G	I	V	Q	Q	Q	N	N	L	L	R	A	I	E	A	Q	Q	H	M	L	Q	L	T	V	W	G	I	K	Q	L	Q		
HxB2 refer...	A	R	I	L	A	V	E	R	Y	L	K	D	Q	Q	L	L	G	I	W	G	C	S	G	K	L	I	C	T	T	A	V	P		
	GP41 NHR																GP41 Loop																	
50.2178-0...	T	R	V	L	A	I	E	R	Y	L	K	D	Q	Q	L	L	G	I	W	G	C	S	G	K	L	I	C	T	T	N	V	P		
50.2178-1...	T	R	V	L	A	I	E	R	Y	L	K	D	Q	Q	L	L	G	I	W	G	C	S	G	K	L	I	C	T	T	N	V	P		
60.0048-0...	A	R	V	L	A	L	E	R	R	Y	L	K	D	Q	Q	L	L	G	I	W	G	C	S	G	K	L	I	C	T	T	D	V	P	
60.0048-1...	A	R	V	L	A	L	E	R	R	Y	L	K	D	Q	Q	L	L	G	I	W	G	C	S	G	K	L	I	C	T	T	D	V	P	
60.0902-0...	A	R	V	L	A	L	E	R	R	Y	L	K	D	Q	Q	L	L	G	I	W	G	C	S	G	K	L	I	C	T	T	A	V	P	
60.0902-1...	A	R	V	L	A	L	E	R	R	Y	L	K	D	Q	Q	L	L	G	I	W	G	C	S	G	K	L	I	C	T	T	A	V	P	
60.1778-0...	A	R	V	L	A	L	E	R	R	Y	L	K	D	Q	Q	L	L	G	I	W	G	C	S	G	K	L	I	C	T	T	N	V	P	
60.1778-1...	A	R	V	L	A	L	E	R	R	Y	L	K	D	Q	Q	L	L	G	I	W	G	C	S	G	K	L	I	C	T	T	N	V	P	
60.5055-0...	A	R	V	L	A	L	E	R	R	Y	L	K	D	Q	Q	L	L	G	I	W	G	C	S	G	K	L	I	C	T	T	N	V	P	
60.5055-1...	A	R	V	L	A	L	E	R	R	Y	L	K	D	Q	Q	L	L	G	I	W	G	C	S	G	K	L	I	C	T	T	N	V	P	
60.6027-0...	A	R	V	L	A	L	E	R	R	Y	L	K	D	Q	Q	L	L	G	I	W	G	C	S	G	K	L	I	C	T	T	N	V	P	
60.6027-1...	A	R	V	L	A	L	E	R	R	Y	L	K	D	Q	Q	L	L	G	I	W	G	C	S	G	K	L	I	C	T	T	N	V	P	
60.8171-0...	A	R	V	L	A	L	E	R	R	Y	L	K	D	Q	Q	L	L	G	I	W	G	C	S	G	K	L	I	C	T	T	N	V	P	
60.8171-1...	A	R	V	L	A	L	E	R	R	Y	L	K	D	Q	Q	L	L	G	I	W	G	C	S	G	K	L	I	C	T	T	N	V	P	
HxB2 refer...	W	N	A	S	W	S	N	K	S	L	E	Q	I	W	N	H	T	T	W	M	E													

Table S3: Neutralization sensitivity and epitope analysis

(a) CD4-binding site				99	197	234	275	276	278	279	280	281	282	364	365	371	372	425	429	456	458	459	471	474	476
VRC07-523LS	VRC01	3BNC117	HXB2	D	N	N	V	N	T	D	N	A	K	S	S	I	V	N	K	R	G	V	G	D	R
0,005	0,08	0,08	60-6041-1	.	.	.	E	.	.	.	.	V	.	H	.	.	T	.	E	.	.	.	.	.	.
0,005	0,06	0,01	80-0204-1	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
0,005	0,26	0,49	60-6082-1	.	.	.	E	.	.	.	.	T	.	P	.	.	T	.	G	.	.	.	.	N	K
0,005	0,08	0,03	60-4082-1	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	E	.	.	.	.	.	.
0,005	0,11	0,01	60-8195-1	.	.	.	E	.	.	.	.	V	.	P	.	.	T	.	E	.	.	.	.	N	.
0,06	0,15	0,04	80-0215-1	G	.	.	E	.	.	N	.	.	A	.	V	T	.	R	.	.	.	.	.	N	K
0,06	0,22	0,11	70-0778-1	.	.	.	A	.	S	.	.	L	.	H	A	V	E	.	R	.	.	.	.	.	.
0,03	0,81	0,19	60-8171-1	.	.	.	E	.	.	N	.	.	.	.	.	.	T	.	G	.	.	.	.	.	.
0,03	1,05	0,26	60-8277-1	.	.	.	E	.	.	.	.	.	.	A	.	T	.	E	.	.	.	.	.	N	K
0,04	1,4	0,89	70-0239-1	.	.	.	E	.	.	.	.	S	.	P	A	.	T	.	G	.	.	.	.	N	K
0,04	2,7	0,54	50-2178-1	.	.	.	E	.	.	N	.	.	.	P	.	.	T	.	E	.	.	.	A	.	.
0,04	1,79	0,76	60-1778-1	.	.	.	E	.	.	.	.	.	.	.	.	.	T	.	G	.	.	.	.	N	K
0,34	7,14	1,32	60-4063-1	.	.	.	E	.	.	.	.	T	.	P	.	V	T	.	G	.	.	.	.	N	K
0,54	9,64	1,26	60-6027-1	.	.	.	E	.	.	.	.	.	.	.	.	.	T	.	G	.	.	.	.	N	K
0,09	4,9	>10	60-0048-1	.	.	.	E	.	.	.	.	.	.	.	.	.	T	.	E	.	.	.	.	N	K
0,15	5,66	>10	60-8084-1	.	.	.	E	.	.	.	S	V	.	H	A	.	S	.	G	.	.	.	.	N	K
0,19	>10	0,71	80-0180-1	.	.	.	K	.	.	N	.	.	.	.	.	.	T	.	E	.	.	.	T	N	K
0,01	>10	>10	60-5055-1	.	.	.	E	.	.	.	.	V	.	H	A	.	T	.	G	.	.	.	A	.	.
0,04	>10	>10	60-0902-1	.	.	.	E	.	.	N	.	.	.	H	.	.	T	R	G	H	.	.	A	N	K
0,36	>10	>10	70-0758-1	.	.	.	E	.	.	N	.	.	.	H	.	.	T	.	E	.	.	.	E	N	.
>10	>10	>10	50-2285-1	.	.	.	E	.	.	E	.	T	.	H	.	.	T	.	E	.	.	.	E	.	.

(b) V1V2-Loop				156	160	161	162	164	165	166	167	168	169	170	171
PGDM1400	CAP256-VRC26.25	HXB2		N	N	I	S	S	I	R	G	K	V	Q	K
0,005	0,005	60-0902-1	.	.	.	T	E	.	.	D	.	K	K	.	.
0,005	0,005	60-1778-1	.	.	.	T	E	L	.	D	.	K	.	.	.
0,005	0,005	80-0215-1	.	.	.	T	E	L	.	D	R	K	R	N	.
0,005	0,005	80-0180-1	.	.	.	A	T	E	.	D	.	K	R	.	.
0,01	0,005	60-6041-1	.	.	.	V	T	E	L	.	D	.	K	N	.
0,02	0,005	60-6082-1	.	.	.	T	T	E	.	D	.	K	.	.	.
0,01	0,01	60-8084-1	.	.	.	T	T	E	.	D	.	I	R	.	.
0,22	0,01	70-0778-1	.	.	.	M	T	E	L	.	D	.	K	K	Q
0,32	0,24	60-8171-1	.	.	.	M	T	E	V	.	D	.	Q	.	.
3,3	0,24	60-0048-1	.	.	.	T	T	E	.	D	R	I	R	.	.
>10	0,005	50-2178-1	.	K	.	T	E	L	.	D	.	K	K	.	.
0,04	>10	60-8277-1	.	.	.	A	T	E	R	.	D	.	N	.	.
>10	0,01	70-0239-1	.	.	.	T	T	E	.	D	.	K	.	.	.
5,21	>10	60-4063-1	.	.	.	T	T	E	.	D	R	R	.	.	.
3,31	>10	60-6027-1	.	.	.	A	T	V	.	T	D	.	K	H	Q
5,46	>10	80-0204-1	.	.	.	.	.	.	.	D	.	.	.	.	.
>10	>10	60-4082-1	.	.	.	.	.	.	.	D	.	.	.	.	.
>10	>10	60-8195-1	.	S	.	T	T	E	L	.	D	R	.	K	.
>10	>10	60-5055-1	.	.	.	T	T	E	.	G	D	.	K	.	E
>10	>10	70-0758-1	.	Y	V	P	E	T	T	D	R	T	K	.	.
>10	>10	50-2285-1	R	.	.	T	E	V	.	D	.	E	K	N	.

(c) V3 Glycan				156	295	300	301	304	307	323	324	325	328	330	332	334	386	392
10-1074	PGT121	HXB2		N	N	N	N	R	I	I	G	N	Q	H	N	S	N	N
0,01	0,005	60-8277-1	.	E	G	.	.	.	V	.	.	D	.	.	.	.	.	.
0,01	0,05	60-0048-1	.	V	.	.	.	.	.	.	.	D	K	Y	.	.	.	.
0,05	0,02	60-1778-1	.	V	S	.	.	.	V	.	.	E	.	.	.	.	.	K
0,05	0,04	50-2285-1	R	.	.	.	.	.	V	.	.	D	.	.	.	.	.	.
0,05	0,05	80-0180-1	.	V	G	.	.	.	V	.	.	D	S	.	.	.	.	.
0,01	0,01	60-6041-1	.	V	.	.	.	.	V	.	.	.	.	.	.	.	.	.
0,005	0,60	60-8195-1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
0,06	1,05	60-0902-1	.	.	.	.	.	.	V	.	.	D	.	Q	.	.	.	.
0,35	0,07	60-5055-1	.	.	G	.	.	.	.	.	.	D	.	.	.	.	D	.
0,07	0,60	70-0778-1	.	V	G	.	.	.	.	.	.	D	.	.	.	.	.	.
0,04	5,50	60-6082-1	.	.	G	.	.	.	.	.	.	D	.	.	.	.	D	.
0,02	1,36	60-8084-1	.	V	.	.	.	.	.	.	.	D	K	Y	.	.	.	.
0,13	0,12	70-0239-1	.	A	S	.	.	.	.	.	.	D	.	.	.	.	.	.
1,95	2,94	80-0215-1	.	.	.	.	.	.	T	.	.	.	.	Y	.	.	.	.
4,56	0,34	70-0758-1	.	.	.	.	.	.	.	.	.	D	.	.	.	.	.	.
0,61	>10	60-8171-1	.	.	G	.	.	.	V	.	.	D	.	.	.	.	.	.
>10	>10	60-4063-1	.	V	G	D	.	.	.	.	.	D	K	N	T	N	.	.
>10	>10	60-6027-1	.	V	G	.	.	.	V	.	.	D	L	Y	K	N	.	.
>10	>10	80-0204-1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
>10	>10	50-2178-1	.	E	.	.	.	.	V	.	.	.	K	Y	H	D	.	.
>10	>10	60-4082-1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

(d) MPER				671	672	673	674	676	680	683
10E8	HXB2			N	W	F	N	T	W	K
0,07	60-0048-1	.	.	.	.	.	.	.	.	.
0,02	60-4082-1	.	.	.	.	.	.	.	.	.
0,01	80-0204-1	.	.	.	.	.	.	S	.	.
0,02	80-0215-1	S	.	.	.	S	.	.	.	.
0,13	80-0180-1	.	.	.	.	S	.	.	.	.
0,26	60-1778-1	.	.	.	.	.	.	.	.	.
0,28	60-6082-1	S	.	.	S	S	.	R	.	.
0,34	60-5055-1	S	.	.	D	S	.	.	.	.
0,34	60-4063-1	S	.	.	D	S	.	.	.	.
0,35	60-6027-1	S	.	.	S	.	.	.	.	.
0,47	70-0239-1	S	.	.	D	S	.	R	.	.
0,52	60-6041-1	S	.	.	S	.	.	.	.	.
0,67	60-8084-1	.	.	.	O	.	.	.	.	.
1,56	70-0778-1	S	.	.	D	.	.	.	.	.
1,56	60-8195-1	.	.	.	S	.	.	.	.	.
1,65	60-8171-1	S	.	.	D	.	.	R	.	.
2,28	60-8277-1	.	.	.	D	.	.	.	.	.
2,52	50-2285-1	.	.	.	O	S	.	.	.	.
3,94	60-0902-1	.	.	.	O	S	.	.	.	.
6,24	50-2178-1	.	.	.	D	S	.	.	.	.
7,52	70-0758-1	.	.	.	D	S	.	R	.	.

(e) gp120/41				276	279	501	503	611	613	637	639	647	648
PGT151	HXB2			N	D	A	R	N	S	N	T	E	E
0,06	60-8171-1	.	N	.	.	.	.	.	.	.	.	R	I
0,04	60-5055-1	.	.	.	.	.	.	.	.	.	.	.	D
0,03	60-0048-1	.	.	.	.	.	.	.	.	.	.	.	K
0,03	80-0215-1	.	N	.	.	.	.	.	.	.	.	.	D
0,01	70-0778-1	.	.	.	.	.	.	.	.	.	.	.	.
0,01	60-8195-1	.	.	.	.	.	.	.	.	.	.	.	.
0,01	60-8084-1	.	.	.	.	.	.	.	.	.	.	.	K
0,12	50-2285-1	.	E	.	.	.	.	.	.	.	.	.	.
0,21	70-0758-1	.	N	.	.	.	.	.	.	.	.	.	.
0,24	80-0204-1	.	.	.	.	.	.	.	.	.	.	.	D
0,43	60-4082-1	.	.	.	.	.	.	.	.	.	.	.	.
1,18	80-0180-1	.	N	.	.	.	.	.	.	.	.	.	.
>10	60-6041-1	.	.	.	.	.	.	.	.	.	.	.	.
>10	60-6082-1	.	.	.	.	.	.	.	.	.	.	.	D
>10	60-8277-1	.	.	.	.	.	.	.	.	.	.	.	.
>10	70-0239-1	.	.	.	.	.	.	.	.	.	.	.	A
>10	50-2178-1	.	N	.	.	.	.	.	.	.	.	.	.
>10	60-0902-1	.	N	.	.	.	.	.	.	.	.	.	D
>10	60-1778-1	.	.	.	.	.	.	.	.	.	.	.	N
>10	60-4063-1	.	.	.	.	.	.	.	.	.	.	.	D
>10	60-6027-1	.	.	.	.	.	.	.	.	S	.	.	.

Table S4: Summary of all results

PID's	CD4-binding site			V1V2-Loop		V3 Glycan		MPER	Gp120-gp41	Coreceptor prediction softwares				Net Charge	Pos 11/25	PNGS	Length	TZM-bl tropism
	VRC07-523LS	VRC01	3BNC117	PGDM1400	AP256-VRC26.2	10-1074	PGT121	10E8	PGT151	Geno2pheno	PhenoSeq	Web-PSSM	Consensus					
60-6041-1	0,005	0,08	0,08	0,01	0,005	0,01	0,01	0,52	>10	R5	R5	R5	R5	5	SD	1	35	R5
80-0204-1	0,005	0,06	0,01	5,46	>10	>10	>10	0,01	0,24	R5X4/X4	R5X4/X4	R5X4/X4	R5X4/X4	8	SK	1	37	R5X4/X4
60-6082-1	0,005	0,26	0,49	0,02	0,005	0,04	5,50	0,28	>10	R5	R5	R5X4/X4	R5	4	SN	1	34	R5
60-4082-1	0,005	0,08	0,03	>10	>10	>10	>10	0,02	0,43	R5X4/X4	R5X4/X4	R5X4/X4	R5X4/X4	8	SK	1	36	R5X4/X4
60-8195-1	0,005	0,11	0,01	>10	>10	0,005	0,60	1,56	0,01	R5	R5	R5	R5	3	SE	1	35	R5
80-0215-1	0,06	0,15	0,04	0,005	0,005	1,95	2,94	0,02	0,03	R5X4/X4	R5	R5	R5	4	SE	1	34	R5
70-0778-1	0,06	0,22	0,11	0,22	0,01	0,07	0,60	1,56	0,01	R5	R5	R5X4/X4	R5	4	SD	1	35	R5
60-8171-1	0,03	0,81	0,19	0,32	0,24	0,61	>10	1,65	0,06	R5	R5	R5	R5	4	SD	1	35	R5
60-8277-1	0,03	1,05	0,26	0,04	>10	0,01	0,005	2,28	>10	R5	R5	R5	R5	4	SD	1	35	R5
70-0239-1	0,04	1,4	0,89	>10	0,01	0,13	0,12	0,47	>10	R5	R5	R5	R5	4	SE	1	35	R5
50-2178-1	0,04	2,7	0,54	>10	0,005	>10	>10	6,24	>10	R5	R5	R5X4/X4	R5	4	TE	1	35	R5
60-0902-1	0,04	>10	>10	0,005	0,005	0,06	1,05	3,94	>10	R5	R5	R5	R5	3	SE	1	35	R5
60-1778-1	0,04	1,79	0,76	0,005	0,005	0,05	0,02	0,26	>10	R5	R5	R5	R5	4	SE	1	35	R5
60-0048-1	0,09	4,9	>10	3,3	0,24	0,01	0,05	0,07	0,03	R5	R5X4/X4	R5X4/X4	R5X4/X4	8	GA	1	35	R5X4/X4
60-5055-1	0,01	>10	>10	>10	>10	0,35	0,07	0,34	0,04	R5	R5X4/X4	R5X4/X4	R5X4/X4	7	SG	0	34	R5X4/X4
80-0180-1	0,19	>10	0,71	0,005	0,005	0,05	0,05	0,13	1,18	R5	R5	R5	R5	4	SD	1	35	R5
60-4063-1	0,34	7,14	1,32	5,21	>10	>10	>10	0,34	>10	R5	R5	R5	R5	4	SD	1	35	R5
60-6027-1	0,54	9,64	1,26	3,31	>10	>10	>10	0,35	>10	R5	R5	R5	R5	3	SD	1	35	R5
70-0758-1	0,36	>10	>10	>10	>10	4,56	0,34	7,52	0,21	R5X4/X4	R5X4/X4	R5X4/X4	R5X4/X4	6	SK	1	34	R5X4/X4
60-8084-1	0,15	5,66	>10	0,01	0,01	0,02	1,36	0,67	0,01	R5X4/X4	R5X4/X4	R5X4/X4	R5X4/X4	8	GA	1	35	R5X4/X4
50-2285-1	>10	>10	>10	>10	>10	0,05	0,04	2,52	0,12	R5	R5	R5	R5	5	SE	1	35	R5
Geo mean	0,04	0,71	0,19	0,08	0,01	0,07	0,18	0,42	0,06									
IC ₅₀ Breath	95	76	71	67	57	76	71	100	57									

IC ₅₀ titers µg/ml	
	<0,1
	0,1-0,9
	1,0-3,0
	3,1-9,9
	>10

PID's		CD4-binding site			V1V2-Loop		V3 Glycan		MPER	Gp120-gp41	Coreceptor prediction softwares				Net Charge	Pos 11/25	PNGS	Length	TZM-bl tropism
		VRC07-523LS	VRC01	3BNC117	PGDM1400	AP256-VRC26.2	10-1074	PGT121	10E8	PGT151	Geno2pheno	PhenoSeq	Web-PSSM	Consensus					
50-2178	Mother	0,06	6	>10	0,005	0,005	>10	>10	3,33	0,52	R5	R5X4/X4	R5	R5	6	TE	1	35	R5
	Infant	0,04	2,7	0,54	>10	0,005	>10	>10	6,24	>10	R5	R5X4/X4	R5	R5	6	TE	1	35	R5
60-0048	Mother	0,48	>10	>10	0,02	0,005	0,04	0,008	0,49	>10	R5	R5	R5	R5	4	SD	1	35	R5
	Infant	0,09	4,9	>10	3,3	0,24	0,01	0,05	0,07	0,03	R5	R5X4/X4	R5X4/X4	R5X4/X4	8	GA	1	35	R5X4/X4
60-0902	Mother	0,04	0,33	1,15	0,01	0,005	0,04	0,07	3,74	0,02	R5	R5	R5X4/X4	R5	5	GE	1	35	R5
	Infant	0,04	10	>10	0,005	0,005	0,06	1,05	3,94	>10	R5	R5	R5	R5	5	SE	1	35	R5
60-1778	Mother	0,11	>10	9,30	0,005	0,005	0,12	0,02	0,36	>10	R5	R5	R5	R5	6	SD	1	35	R5
	Infant	0,04	1,79	0,76	0,005	0,005	0,05	0,03	0,50	>10	R5	R5	R5	R5	6	SD	1	35	R5
60-5055	Mother	0,06	>10	>10	>10	>10	0,12	1,22	0,67	0,04	R5	R5X4/X4	R5X4/X4	R5X4/X4	7	SG	0	34	R5X4/X4
	Infant	0,01	>10	>10	>10	>10	0,35	0,07	0,34	0,01	R5	R5X4/X4	R5X4/X4	R5X4/X4	7	SG	0	34	R5X4/X4
60-6027	Mother	0,35	7,45	>10	0,07	0,08	>10	>10	0,36	>10	R5	R5	R5X4/X4	5	6	SD	1	35	R5
	Infant	0,54	9,64	1,26	3,31	>10	>10	>10	0,35	>10	R5	R5X4/X4	R5	R5	3	SD	1	35	R5
60-8171	Mother	0,38	>10	>10	2,01	0,42	>10	>10	0,89	0,01	R5	R5	R5	R5	4	SD	1	35	R5
	Infant	0,03	0,81	0,19	0,32	0,24	0,61	>10	1,65	0,06	R5	R5	R5	R5	4	SD	1	35	R5

Supplementary Sheet

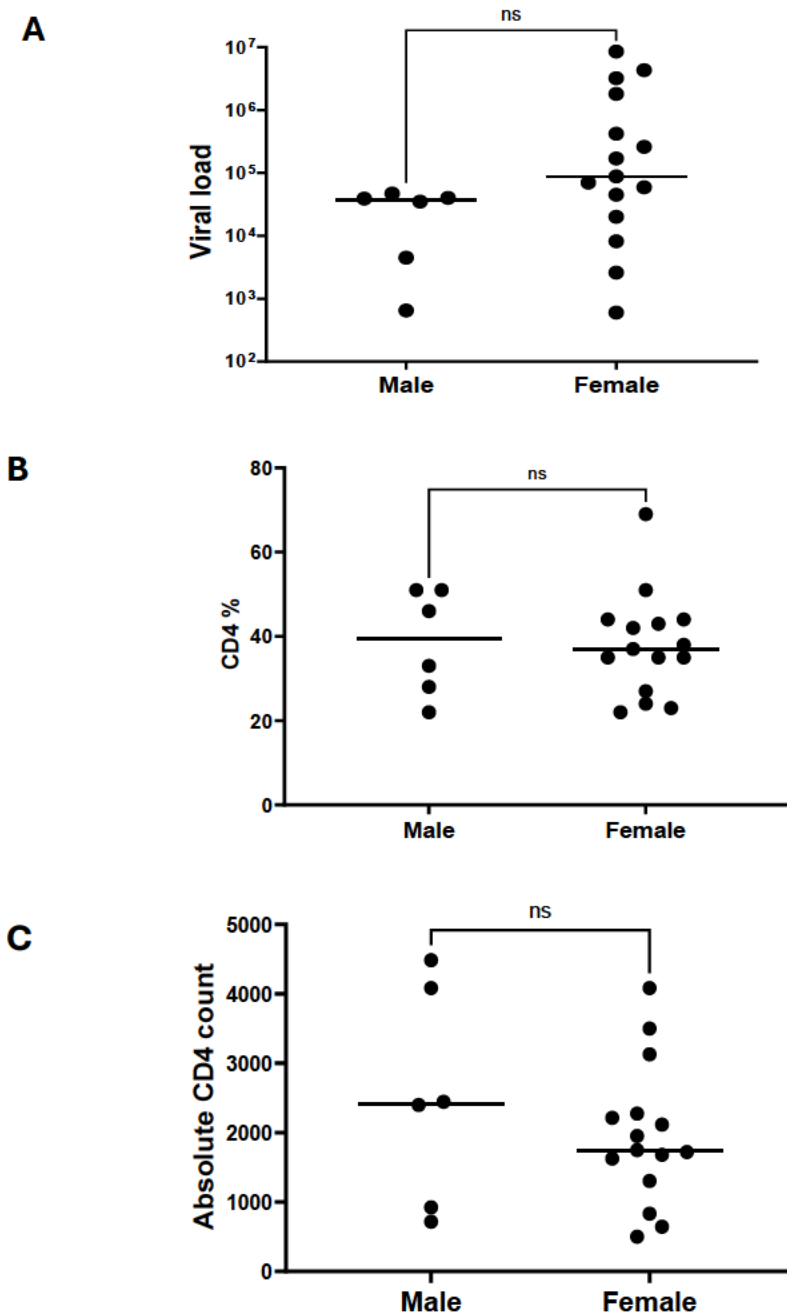


Figure S1: HIV-1 RNA viral load and CD4 count between male and female infants.

There was no significant difference in the viral load ($P = 0.05$), CD4 count ($P = 0.36$) and CD4% ($P = 0.80$), between male and female infants (Mann-Whitney test).

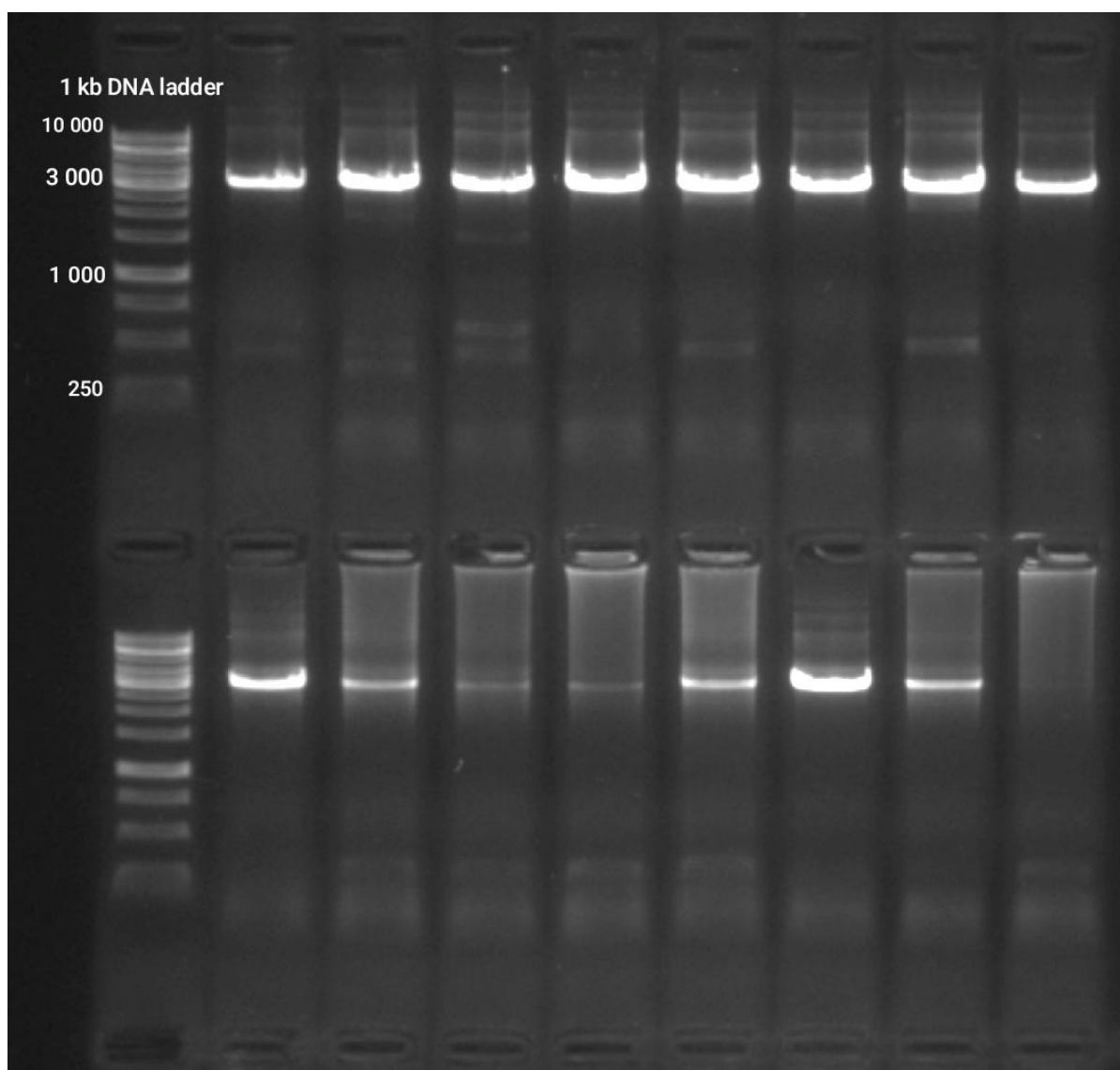


Figure S2: PCR amplification of HIV-1 *env* gene.

Agarose gel electrophoresis showing PCR products of the HIV-1 *env* gene. Lane 1: 1 kb DNA ladder (marker). Lanes 2 – 9: amplified *env* fragments from representative samples. The expected product size is approximately [3000 bp size]

0048-1

	cell control	virus control	1		2		3		4		5
A	1540	52720	44740	47870	46450	46710	42970	40930	41870	44070	
B	1930	52390	41340	43230	41970	42660	33290	29050	29360	33200	
C	1760	50840	31630	33860	33040	26390	22030	21650	23380	25430	
D	1660	52340	17600	19620	6953	5882	7513	7863	21250	20270	
E	1630	53090	6392	10730	3411	3511	2410	2310	20300	22460	
F	1540	55540	2740	3030	3181	3591	1990	1680	21230	19400	
G	1930	51660	1740	1930	3351	3351	1730	1710	17950	17150	
H	1740	51460	1930	2070	3811	3641	1910	1810	17090	18200	

cell control= 1716 +/- 9% star dilution: 1 ug
virus control= 52505 +/- 3% dil factor: 3

samples	dilution	% of inhibition	% std dev	titer ID= 50
10-1074	0,00457	12%	+/- 4%	0,06376
	0,01372	20%	+/- 3%	
	0,04115	39%	+/- 3%	
	0,12346	67%	+/- 3%	
	0,37037	87%	+/- 6%	
	1,11111	98%	+/- 0%	
	3,33333	100%	+/- 0%	
	10,00000	99%	+/- 0%	
PGT 121	0,00457	12%	+/- 0%	0,04653
	0,01372	20%	+/- 1%	
	0,04115	45%	+/- 9%	
	0,12346	91%	+/- 1%	
	0,37037	97%	+/- 0%	
	1,11111	97%	+/- 1%	
	3,33333	97%	+/- 0%	
	10,00000	96%	+/- 0%	
10E8.	0,00457	21%	+/- 3%	0,02212
	0,01372	42%	+/- 6%	
	0,04115	60%	+/- 1%	
	0,12346	88%	+/- 0%	
	0,37037	99%	+/- 0%	
	1,11111	100%	+/- 0%	
	3,33333	100%	+/- 0%	
	10,00000	100%	+/- 0%	
PGT 151	0,00457	19%	+/- 3%	0,02671
	0,01372	42%	+/- 5%	
	0,04115	55%	+/- 3%	
	0,12346	63%	+/- 1%	
	0,37037	61%	+/- 3%	
	1,11111	63%	+/- 3%	
	3,33333	69%	+/- 1%	
	10,00000	69%	+/- 2%	
	0,00457	#DIV/0!	+/- #DIV/0!	#DIV/0!
	0,01372	#DIV/0!	+/- #DIV/0!	#DIV/0!
	0,04115	#DIV/0!	+/- #DIV/0!	#DIV/0!
	0,12346	#DIV/0!	+/- #DIV/0!	#DIV/0!
	0,37037	#DIV/0!	+/- #DIV/0!	#DIV/0!
	1,11111	#DIV/0!	+/- #DIV/0!	#DIV/0!
	3,33333	#DIV/0!	+/- #DIV/0!	#DIV/0!
	10,00000	#DIV/0!	+/- #DIV/0!	#DIV/0!

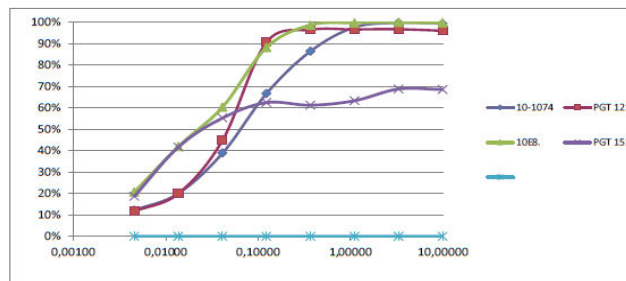


Figure S3: Neutralization sensitivity of cloned *env* pseudovirus (0048-1).

Functional pseudoviruses were tested for sensitivity to a panel of broadly neutralizing antibodies using the TZM-bl assay. Data from the cloned Env exhibit expected neutralization profiles consistent with functional envelope glycoproteins.

14 January 2021

Prof Philip Goulder
The Peter Medawar Building for Pathogen Research
South Parks Road
Oxford OX1 3SY, UK
philip.goulder@paediatrics.ox.ac.uk

Dear Prof Goulder

PROTOCOL: Impact of early diagnosis and anti-retroviral therapy (ART) initiation at birth in children with *in utero* HIV infection: Non-Degree. College of Health Sciences (HIV Pathogenesis Programme).
BREC REF: BF450/14

RECERTIFICATION APPLICATION APPROVAL NOTICE

Approved: 19 March 2021
Expiration of Ethical Approval: 18 March 2022

I wish to advise you that your application for recertification received on 23 December 2020 has been noted and approved by a subcommittee of the Biomedical Research Ethics Committee (BREC). The start and end dates of this period are indicated above.

NOTE TO PI:

- 1) Possible amendment mentioned in item 1.1.2 must be submitted for prior approval on relevant BREC amendment form.
- 2) PI must in future please use the current BREC recertification form available on the BREC website.

If any modifications or adverse events occur in the project before your next scheduled review, you must submit them to BREC for review. Except in emergency situations, no change to the protocol may be implemented until you have received written BREC approval for the change.

The committee will be notified of the above approval at its next meeting to be held on 09 February 2021.

Yours sincerely



Ms A Marimuthu
(for) Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

Biomedical Research Ethics Committee

Chair: Professor D R Wassenaar

UKZN Research Ethics Office Westville Campus, Govan Mbeki Building

Postal Address: Private Bag X54001, Durban 4000

Email: BREC@ukzn.ac.za

Website: <http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx>

Founding Campuses:  Edgewood  Howard College  Medical School  Pietermaritzburg  Westville

INSPIRING GREATNESS

11 April 2024

Mrs Nelisiwe Veronica Zikhali (209528542)
School of Lab Med & Medical Sc
Medical School

Dear Mrs Zikhali,

Protocol reference number: BREC/00005630/2023

Project title: Broadly neutralizing monoclonal antibodies display heterogeneous neutralization activity against HIV-1 subtype C transmitter/founder viruses from infants

Degree: PhD

EXPEDITED APPLICATION: APPROVAL LETTER

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application.

The conditions have been met and the study is given full ethics approval and may begin as from 11 April 2024. Please ensure that any outstanding site permissions are obtained and forwarded to BREC for approval before commencing research at a site.

This approval is valid for one year from 11 April 2024. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on RIG on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2020) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at <http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx>.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be noted by a full Committee at its next meeting taking place on 14 May 2024.

Yours sincerely,



Prof S Singh
Chair: Biomedical Research Ethics Committee