INVESTIGATING THE HIV-1 ANTIVIRAL ACTIVITY OF CRISPR-MEDIATED SLFN11 AND CDKN1A UPREGULATION

KALIDASAN A/L VASODAVAN

UNIVERSITI SAINS MALAYSIA

2025

INVESTIGATING THE HIV-1 ANTIVIRAL ACTIVITY OF CRISPR-MEDIATED SLFN11 AND CDKN1A UPREGULATION

by

KALIDASAN A/L VASODAVAN

Thesis submitted in fulfilment of the requirements
for the degree of
Doctor of Philosophy

July 2025

ACKNOWLEDGEMENT

I started my PhD one year before the onset of the COVID-19 pandemic, a period that presented numerous challenges and uncertainties. Nonetheless, I made it to the finish line! I want to express my deepest gratitude to the exceptional individuals who supported and guided me throughout this transformative journey, enabling me to successfully obtain my doctorate despite the many unforeseen obstacles that arose. First and foremost, I would like to thank my PhD supervisor, Dr. Kumitaa Theva Das (INFORMM, USM), co-supervisors, Dr. Muhammad Amir Yunus (AMDI, USM), Dr. Nor Effa Syazuli Zulkafli (AMDI, USM) and Assoc. Prof. Dr. Reena Rajasuriar (FOM, UM) and ID specialist, Assoc. Prof. Dr. Sharifah Faridah Syed Omar (UMMC). Their constant support, guidance, and encouragement have been invaluable throughout the entire process. From the initial stages of refining my research proposal to the final submission of my thesis, their unwavering presence and wealth of wisdom have been instrumental in shaping my academic growth. In addition to my supervisors, I am indebted to my exceptional lab mates, whose support has been a constant source of motivation. Our collaborative writing sessions and informal chats, whether conducted via screens during lockdowns or in person whenever circumstances allowed, provided a lifeline during the most challenging times. Lastly, I want to express my deepest gratitude to my family, whose belief in my abilities and support. Your encouragement played an integral role in my accomplishments. To my mom, dad, and wife: Thank you for everything. I dedicate this PhD thesis to you.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF SYMBOLS

α	Alpha
’	Apostrophe
~	Approximately equal
*	Asterisk
bp	Base pair
β	Beta
:	Colon
,	Comma
X	Concentration
mm^3	Cubic millimetre
dL	Decilitre
°C	Degree celsius
Δ	Delta
–	En dash
=	Equal
etc	Et cetera
.	Full stop
γ	Gamma
g	Gram
>	Greater than
#	Hash
-	Hyphen
κ	Kappa
kb	Kilobyte
<	Less than

≤	Less than or equal to
L	Litre
RM	Malaysian ringgit
μg	Microgram
μL	Microliter
mL	Milliliter
−	Minus
×	Multiplication
ng	Nanogram
nm	Nanometer
NA	Not available
ND	Not detected
NIL	Nothing or zero
( )	Parentheses
%	Percent
pg	Picogram
+	Plus
Ψ	Psi
?	Question mark
“ ”	Quotation mark
x	Relative centrifugal force
rpm	Revolutions per minute
;	Semicolon
/	Slash
[ ]	Square bracket
cm^2	Square centimetre
U	Unit

LIST OF ABBREVIATIONS

ABC	Abacavir
AIDS	Acquired immunodeficiency syndrome
ATF3	Activating Transcription Factor 3
AP-1	Activator protein 1
AML	Acute myeloid leukaemia
ATP	Adenosine triphosphate
AMDI	Advanced Medical and Dental Institute
AGS	Aicardi-Goutières syndrome
ADCC	Antibody-dependent cell-mediated cytotoxicity
ADCVI	Antibody-dependent cell-mediated viral inhibition
ADCP	Antibody-dependent cellular phagocytosis
ADCD	Antibody-dependent complement deposition
ADNP	Antibody-dependent neutrophil phagocytosis
APC	Antigen-presenting cell
ART	Antiretroviral therapy
APOBEC	Apolipoprotein B mRNA editing enzyme, catalytic polypeptides like
ASK1	Apoptosis signal-regulating kinase 1
ATM	Ataxia telangiectasia mutated
ATR	Ataxia telangiectasia mutated and RAD3-related
ATV	Atazanavir
BST-2	Bone marrow stromal cell antigen 2
bNAb	Broadly neutralizing antibody
BRDi	Bromodomain inhibitors
CA	Capsid protein
T_{CM}	Central memory T cell
CNS	Central nervous system
CRL	Central Research Laboratory
CDC	Centres for Disease Control and Prevention
CCR5	Chemokine receptor type 5
CRF	Circulating recombinant forms
CRISPR	Clustered regularly interspaced short palindromic repeats

COBI/c	Cobicistat
CBV	Combivir
cDNA	Complementary DNA
CBC	Complete blood count
cDC	Conventional dendritic cell
COMMD1	Copper metabolism domain-containing protein 1
CIOMS	Council for International Organizations of Medical Sciences
CBF1	C-promoter binding factor 1
CRP	C-reactive protein
CRISPRa	CRISPR activation
CRISPRi	CRISPR interference
CCTop	CRISPR/Cas9 target online predictor
Cas9	CRISPR-associated protein 9
CTR9	Ctr9, Paf1/RNA polymerase II complex component
CXCR4	C-X-C motif chemokine receptor type 4
cGAS	Cyclic GMP-AMP synthase
CDK	Cyclin-dependent kinase
CDKN1A	Cyclin-dependent kinase inhibitor 1A
CIP1	Cyclin-dependent kinase-interacting protein 1
CMV	Cytomegalovirus
CTL	Cytotoxic T-lymphocyte
CTLA-4	Cytotoxic T-lymphocyte antigen 4
DRV	Darunavir
DBTSS	DataBase of Transcriptional Start Sites
dCas9	Dead Cas9
DC	Dendritic cell
dNTPase	Deoxynucleoside triphosphate phosphohydrolase
dNTPs	Deoxynucleoside triphosphates
DNA	Deoxyribonucleic acid
dCA	Didehydro-corticostatin A
DMSO	Dimethyl sulfoxide
DNMTi	DNA methylation inhibitors
DTG	Dolutegravir
DLI	Donor-lymphocyte-infusions

DSB	Double-stranded break
dsDNA	Double-stranded DNA breaks
DMEM	Dulbecco's Modified Eagle Medium
EGR1	Early Growth Response 1
EFV	Efavirenz
T_{EM}	Effector memory T cell
EC	Elite controller
EVG	Elvitegravir
FTC	Emtricitabine
T20	Enfuvirtide
Env	Envelope
ELISA	Enzyme-linked immunosorbent assay
EBV	Epstein-Barr virus
EDTA	Ethylenediaminetetraacetic acid
EMA	European Medicines Agency
FSW	Female sex workers
FBS	Fetal bovine serum
FACS	Fluorescence-activated cell sorting
FDA	Food and Drug Administration
FSC	Forward scatter
FOS	Fos proto-oncogene, AP-1 transcription factor subunit
FTR	Fostemsavir
GTMP	Gene therapy medicinal product
GMAC	Genetic Modification Advisory Committee
GWAS	Genome-wide association study
gDNA	Genomic DNA
gRNA	Genomic RNA
GCP	Good Clinical Practice
GvHD	Graft versus host disease
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GM-CSFR	Granulocyte-macrophage colony-stimulating factor receptor
GFP	Green fluorescent protein
Gag	Group-specific antigen
GADD45A	Growth arrest and DNA damage-inducible alpha

GALT	Gut-associated lymphoid tissue
HSC	Haematopoietic stem cell
HSP90	Heat shock protein 90
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HSV	Herpes simplex virus
HDACi	Histone deacetylase inhibitors
HMTi	Histone methyltransferase inhibitors
HCP5	HLA complex P5
HDR	Homology-directed repair
HIV-1	Human immunodeficiency virus type 1
HIV-2	Human immunodeficiency virus type 2
HLA	Human leukocyte antigen
HTLV	Human T cell lymphotropic virus
IBA	Ibalizumab
ID	Identification
ITL	Immunotherapeutic Laboratory
IP-10	Inducible protein-10
ID	Infectious disease
ILC	Innate lymphoid cell
IRB	Institutional Review Board
IN	Integrase
INSTI	Integrase strand transfer inhibitors
ICAM-1	Intercellular adhesion molecule 1
IFN	Interferon
IFITM	Interferon-induced transmembrane
IRF3	Interferon-regulatory transcription factor
ISG	Interferon-stimulated gene
IL	Interleukin
IAS	International AIDS Society
IQR	Interquartile range
JAK	Janus-activated kinase
UNAIDS	Joint United Nations Programme on HIV/AIDS
JUN	Jun proto-oncogene, AP-1 transcription factor subunit

KIR	Killer immunoglobulin-like receptor
KWKK	Komplek Wanita dan Kanak-kanak
KRAB	Kruppel associated box
3TC	Lamivudine
LSF	Late SV40 factor
LRA	Latency-reversing agent
LILR	Leukocytes bearing immunoglobulin-like receptors
LFT	Liver function test
LMO	Living modified organism
lnc	Long non-coding
LTR	Long terminal repeat
LTNP	Long-term nonprogressor
LB	Luria-Bertani
LAV	Lymphadenopathy-associated virus
LAG-3	Lymphocyte activation gene-3
MIP	Macrophage inflammatory protein
M-CSF	Macrophage-colony stimulating factor
MHC	Major histocompatibility complex
MVC	Maraviroc
MA	Matrix protein
MFI	Mean fluorescent intensity
MREC	Medical Research Ethics Committee
MDA-6	Melanoma differentiation-associated protein 6
MPER	Membrane proximal MPER external region
March 8	Membrane-associated RING-CH 8 protein
MSM	Men who have sex with men
mRNA	Messenger RNA
miRNA	MicroRNA
MAPK	Mitogen-activated protein kinase
MCP3	Monocyte-chemotactic protein 3
MDM	Monocyte-derived macrophage
Mx2/B	Myxovirus resistance 2
T_N	Naïve T cell
NBB	National Biosafety Board

NCBI	National Center for Biotechnology Information
NK	Natural killer
NLP	Natural language processing
Nef	Negative factor
NVP	Nevirapine
nCas9	Nickase Cas9
NHEJ	Nonhomologous end joining
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NIL	Nothing or zero
NFκB	Nuclear factor kappa B
NFAT	Nuclear factor of activated T cells
NLS	Nuclear localization signal
NC	Nucleocapsid protein
NRTI	Nucleoside reverse transcriptase inhibitor
NLR	Nucleotide-binding oligomerization domain-like receptor
UKKP	Occupational Safety and Health Unit
ORF	Open reading frame
OD	Optical density
p53-RE	p53-responsive elements
PIS	Participant information sheet
POS	Part-of-speech
PAMP	Pathogen-associated molecular pattern
PRR	Pathogen-recognition receptor
PLHIV	People living with HIV
PWID	People who inject drugs
PBM	Peripheral blood monocytes
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate buffered saline
PMT	Photomultiplier tube
pDC	Plasmacytoid dendritic
PCP	*Pneumocystis carinii* pneumonia
Pol	Polymerase
PCR	Polymerase chain reaction
PTB	Polypyrimidine tract-binding protein

P-TEFb	Positive transcription elongation factor b
PEP	Post-exposure prophylaxis
PrEP	Pre-exposure prophylaxis
PD-1	Programmed death 1
PCNA	Proliferating cell nuclear antigen binding site
PR	Protease
PI	Protease inhibitor
PKC	Protein kinase C
PPI	Proton pump inhibitor
PAM	Protospacer adjacent motif
QVOA	Quantitative viral outgrowth assay
RAL	Raltegravir
rDNA	Recombinant DNA
RANTES	Regulated upon activation, normally T-expressed, and presumably secreted
Rev	Regulator of expression of virion proteins
Tregs	Regulatory T cell
RFT	Renal function test
RLR	Retinoid acid-inducible gene-like receptor
RPA	Replication protein A
RRE	Rev response element
RT	Reverse transcriptase
RT-qPCR	Reverse transcription-quantitative PCR
RNA	Ribonucleic acid
RPV	Rilpivirine
RNF39	Ring finger protein 39
RNA Pol II	RNA polymerase II
PAF1	RNA polymerase II-associated factor 1
RNR2	RNA, ribosomal 45s cluster 2
RPMI	Roswell Park Memorial Institute
SAMHD1	SAM domain and HD domain-containing protein 1
SQV	Saquinavir
SLFN11	Schlafen 11
SMAC	Second mitochondria-derived activator of caspase
SDI1	Senescent cell-derived inhibitor 1

SERINC3/5	Serine incorporator
SSC	Side scatter
STAT	Signal transducer and activator of transcription
SIV	Simian immunodeficiency virus
sgRNA	Single guide RNA
ssDNA	Single-stranded DNA
siRNA	Small interfering RNA
Sp1	Specificity protein 1
d4t	Stavudine
SCT	Stem cell transplantation
SAPK	Stress-activated protein kinases
SDF-1	Stromal-derived factor-1
SU	Surface glycoprotein
SAM	Synergistic activation mediator
TCR	T cell receptor
TIA-1	T cell-restricted intracellular antigen-1
TFH	T follicular helper
Tim-3	T-cell immunoglobulin and mucin domain-containing molecule-3
TDF	Tenofovir
T_{EMRA}	Terminally differentiated effector memory T cell
Th1	T-helper type 1
Th2	T-helper type 2
TK1	Thymidine kinase 1
TYMS	Thymidylate synthetase
TLR	Toll-like receptor
TAR	Trans-activation response
Tat	trans-Activator of transcription
TALEN	Transcription activator-like effector nuclease
TGS	Transcriptional gene silencing
TSS	Transcriptional start site
TGF-β	Transforming growth factor
TG	Transgender
T_{TM}	Transitional memory T cell
TM	Transmembrane glycoprotein

TasP	Treatment as prevention
TRIM	Tripartite motif family
TPL	Triptolide
Leu-TAA	tRNA-Leu, anticodon TAA
TNF	Tumour necrosis factor
TRAIL	Tumour necrosis factor-related apoptosis-inducing ligand
URF	Unique recombinant form
USM	Universiti Sains Malaysia
UM	University of Malaya
UMMC	University of Malaya Medical Centre
Vpr	Viral protein R
Vpu	Viral protein U
Vif	Virion infectivity factor
WB	Western immunoblotting
WAF1	Wild-type p53-activated factor 1
WHO	World Health Organization
YY1	Yin and yang 1
ZDV	Zidovudine
ZNRD1	Zinc ribbon domain containing 1
ZFN	Zinc-finger nuclease
moDCs	monocyte-derived dendritic cells

LIST OF APPENDICES

PENYIASATAN AKTIVITI ANTIVIRUS HIV-1 OLEH PENGAWALATURAN NAIK SLFN11 DAN CDKN1A ARUHAN CRISPR

ABSTRAK

Walaupun terdapat kemajuan yang ketara dalam terapi antiretroviral, penawar muktamad bagi jangkitan HIV masih belum dapat dicapai disebabkan oleh beberapa cabaran yang berterusan. Antara cabaran utama termasuk ketoksikan ubat, kemunculan strain virus yang rintang terhadap ubatan, serta kewujudan takungan virus terpendam yang sukar dihapuskan melalui kaedah rawatan sedia ada. Menariknya, terdapat segelintir individu yang dikenali sebagai pengawal elit berupaya menekan replikasi HIV secara semula jadi tanpa memerlukan rawatan, sekaligus menawarkan model penting bagi memahami mekanisme kawalan virus yang dimediasi oleh faktor perumah. Beberapa faktor sekatan perumah seperti SLFN11, CDKN1A, dan SAMHD1 telah dikenal pasti memainkan peranan penting dalam menghalang replikasi virus melalui laluan biologi yang berbeza. Dalam kajian ini, satu pendekatan inovatif pengaktifan gen menggunakan teknologi CRISPRa telah diterokai bagi meningkatkan ekspresi faktor-faktor antivirus perumah ini. Pada peringkat awal, platform Chilibot telah digunakan bagi mengenal pasti SLFN11, CDKN1A serta beberapa gen calon tambahan yang terlibat dalam pengawalan tindak balas imun semula jadi dan adaptif. Kajian eksperimen seterusnya melibatkan pembinaan RNA penunjuk tunggal menggunakan plasmid pSPgRNA, yang kemudiannya ditransfeksi bersama pengaktif transkripsi VP64 ke dalam titisan sel HEK293 dan ACH-2. Strategi penggunaan dwi-sgRNA menunjukkan keberkesanan yang lebih tinggi dalam meningkatkan ekspresi SLFN11 dan CDKN1A berbanding sgRNA tunggal. Peningkatan ekspresi gen-gen ini seterusnya mengaktifkan SAMHD1 dan beberapa sasaran hiliran lain yang terlibat

dalam pengawalan tekanan replikasi, pengaturan kitaran sel, modulasi tindak balas imun, serta penghambatan langsung replikasi HIV-1. Perubahan molekul yang berlaku berkait rapat dengan penurunan ekspresi tRNA-Leu-TAA, peningkatan transkrip RNA HIV-1, serta pengurangan paras protein p24, yang secara keseluruhan mencerminkan keberkesanan penindasan replikasi aktif virus. Analisis sitokin turut menunjukkan perubahan imunologi yang positif, iaitu penurunan paras faktor nekrosis tumor dan peningkatan ekspresi interferon, sekaligus menandakan pengubahsuaian tindak balas imun keradangan dan antivirus. Bagi pengesahan dalam konteks klinikal, sel mononuklear darah periferi (PBMC) daripada lapan individu yang hidup dengan HIV telah dianalisis sebelum dan selepas pemberian ART, diikuti pengaktifan gen secara *ex vivo* menggunakan CRISPRa. Sampel yang menerima ART menunjukkan peningkatan paras protein SLFN11, CDKN1A dan SAMHD1 berbanding sampel yang belum menerima ART. Imunofenotaip menunjukkan pemulihan imun dalam sel T CD4+, dengan peningkatan subset naïf dan memori pusat, serta pengurangan subset memori efektor, efektor terminal, dan penanda keletihan. Bagi sel T CD8+, perubahan pelengkap turut diperhatikan dengan peningkatan populasi memori pusat dan efektor terminal. Selain itu, pengukuhan yang ketara dalam tindak balas sitokin khusus HIV turut dikesan, termasuk peningkatan paras GM-CSF, TNF-α, IL-2, IFN-γ, dan lain-lain. Secara keseluruhannya, dapatan ini menonjolkan potensi pendekatan kombinasi ART dengan pengaktifan gen melalui CRISPRa dalam meningkatkan ekspresi faktor antivirus perumah, sekaligus meniru fenotip pengawal elit. Strategi terapeutik bersepadu ini berpotensi untuk memperkembangkan pendekatan penawar fungsional HIV yang mampu mencapai penindasan virus jangka panjang serta pemulihan imun dalam kalangan individu yang hidup dengan HIV.

INVESTIGATING THE HIV-1 ANTIVIRAL ACTIVITY OF CRISPR-MEDIATED SLFN11 AND CDKN1A UPREGULATION

ABSTRACT

Despite substantial advancements in antiretroviral therapy (ART), a definitive cure for HIV remains elusive due to several persistent challenges, including drug toxicity, the emergence of resistant viral strains, and the existence of latent viral reservoirs that escape therapeutic eradication. Notably, a rare subset of individuals known as elite controllers possess the ability to naturally suppress HIV replication in the absence of therapy, offering a valuable model for uncovering host-mediated mechanisms of viral control. Several host restriction factors, such as SLFN11, CDKN1A, and SAMHD1, have been identified as critical players in inhibiting viral replication through distinct biological pathways. In this study, a novel gene activation approach utilising CRISPRa technology was explored to upregulate these key antiviral host factors. Initially, the Chilibot text-mining platform was employed to identify SLFN11, CDKN1A, and additional candidate genes involved in modulating both innate and adaptive immune responses. Subsequent experimental work involved the construction of single guide RNAs (sgRNAs) using the pSPgRNA plasmid, which were then co-transfected with the transcriptional activator VP64 into HEK293 and ACH-2 cell lines. The dual-sgRNA strategy demonstrated superior efficacy in upregulating SLFN11 and CDKN1A compared to single sgRNAs. The enhanced expression of these genes subsequently induced SAMHD1 and multiple downstream targets implicated in replication stress response, cell cycle regulation, immune modulation, and direct inhibition of HIV-1 replication. These molecular changes corresponded with decreased tRNA-Leu-TAA expression, elevated HIV-1 RNA

transcript levels, and reduced p24 protein levels, reflecting effective suppression of active viral replication. Cytokine analysis further revealed a favourable shift, with reduced tumour necrosis factor (TNF) levels and increased interferon (IFN) expression, indicating modulation of the inflammatory and antiviral immune responses. To validate these findings in a clinical context, peripheral blood mononuclear cells (PBMCs) from eight people living with HIV (PLHIV) were analysed before and after ART administration, followed by *ex vivo* CRISPRa-mediated gene activation. ART-treated samples exhibited increased protein levels of SLFN11, CDKN1A, and SAMHD1 compared to ART-naïve samples. Immunophenotyping demonstrated immune restoration within CD4+ T cells, characterised by elevated naïve and central memory subsets, along with reduced effector memory, terminal effector cells, and exhaustion markers. CD8+ T cells displayed a complementary shift, with increased central memory and terminal effector populations. Furthermore, a robust enhancement of HIV-specific cytokine responses was observed, including elevated levels of GM-CSF, TNF-α, IL-2, IFN-γ, and others. Collectively, these findings underscore the potential of combining ART with CRISPRa-mediated gene activation to upregulate host antiviral factors, thereby mimicking elite controller phenotypes. This integrated therapeutic strategy holds promise for advancing functional cure approaches that may ultimately achieve durable viral suppression and immune restoration in people living with HIV.

.

CHAPTER 1

INTRODUCTION

1.1 Background

The HIV/AIDS pandemic has been around for nearly four decades, with countless stories of loss, activism, rage, resilience, and scientific triumph after years of futility and suffering (Barré-Sinoussi et al., 2013; Beyrer, 2021). The world's attention has moved away from HIV/AIDS as the disease shifted from death to a chronic and manageable condition. According to the Joint United Nations Programme on HIV/AIDS (UNAIDS), in 2022, an estimated 39.0 million people were living with HIV globally (UNAIDS, 2023a, 2023b). Among these, 37.5 million were adults, 1.5 million were children, and 1.3 million had newly acquired HIV infection. About 630,000 people died from AIDS-related illnesses in 2022, having claimed 40.4 million lives since the start of the epidemic.

In such circumstances, UNAIDS proposed targets to end the AIDS epidemic by 2030 (UNAIDS, 2014). The 90-90-90 targets must be achieved by 2020, aiming at 90 % of people living with HIV (PLHIV) know their status, 90 % of people who know their status are on therapy, and 90 % on antiretroviral therapy (ART) are virologically suppressed (Levi et al., 2016). Thus, the annual number of new HIV infections among adults will be reduced to 500,000. If these targets are met, the 95-95-95 targets can be achieved by 2030 toward ending AIDS (Frescura et al., 2022). It is expected that the annual number of new HIV infections among adults will be reduced to 200,000. So far, Botswana, Eswatini, Rwanda, the United Republic of Tanzania, and Zimbabwe are countries that have already achieved the 95-95-95 targets (Musuka et al., 2023; UNAIDS, 2023b).

Advances in treatment and prevention have led some to ask whether the end of AIDS is possible (Sankaranantham, 2019). Nevertheless, numerous approaches and strategies have been put forth to achieve a cure, and breakthrough cases tend to make headlines in recent years (The Foundation for AIDS Research, 2023). Through stem cell transplantation (SCT), the following was reported to be cured: (i) Berlin patient (known as Timothy Ray Brown) (Hütter et al., 2009); (ii) London patient (known as Adam Casteljo) (Gupta et al., 2019); (iii) City of Hope patient (known as Paul Edmonds) (Dickter et al., 2022), (iv) Düsseldorf patient (known as Marc Franke) (B.-E. O. Jensen et al., 2023), (v) New York patient (Hsu et al., 2023); and (vi) Geneva patient (Sáez-Cirión et al., 2023). The other cure approach involves PLHIV, known as elite controllers (ECs), who can spontaneously suppress viraemia to undetectable viral loads without receiving ART (Autran et al., 2011; Cockerham & Hatano, 2015). The San Francisco patient (known as Loreen Willenberg) (Lian et al., 2021) and Esperanza patient (Turk et al., 2022) were ECs to be eliminated from the virus.

Although an HIV cure remains within the uncertain realm of discovery, the global AIDS community must intensify its efforts to diagnose PLHIV and retain them in an effective care continuum (Fauci et al., 2014). Thus, the International AIDS Society (IAS) Global Scientific Strategy 2021 outlined key research goals for an HIV cure, focusing on understanding and measuring HIV reservoirs, mechanisms of virus control, targeting the provirus and the immune system, cell and gene therapy, paediatric remission, as well as social, behavioural and ethical aspects (Deeks et al., 2021). A safe, affordable and scalable cure is much needed to enhance the health and quality of life of PLHIV and reduce the risk of viral transmission to those not infected (Ndung'u et al., 2019).

1.2 Problem statement

The period between the emergence of AIDS in 1981 and the development of ART in 1996 was 15 years of breakthroughs. Nevertheless, limitations to ART regimen still exist, including daily adherence, long-term toxicity of medications, drug-drug interactions, long-term effects of HIV even in viral suppression, high lifetime cost of treatment, and limited options for some patients with multiclass resistance (Desai et al., 2012; Solomon & Sax, 2015). The gold standard of infectious disease cure is eradicating the virus, and SCT has been considered the ideal approach. Nevertheless, it is challenging to scale up, as it cannot be applied to the millions of chronically infected individuals (Hütter, 2016). The main obstacle to an HIV cure is the persistence of transcriptionally silent and immunologically inert latent proviruses in quiescent memory CD4+ T cells (Barouch & Deeks, 2014). These cells serve as viral reservoirs ready to respond to antigenic stimulation and replenish the virus upon ART interruption.

Furthermore, it is very difficult to develop a vaccine candidate against HIV/AIDS because the virus destroys the immune system as soon as it enters the body and inserts its genetic material into human cells (R. Verma et al., 2016). HIV has different subtypes, even within each subtype, HIV is highly variable and constantly changing. Therefore, researchers have pursued other approaches, such as 'shock and kill,' 'block and lock,' and immune-based therapies (Abana et al., 2022; Bonney et al., 2023; Pace & Frater, 2014). Despite the benefits observed in these approaches, some strategies are difficult and extend the period of viral rebound (Matsuda & Maeda, 2024). Also, the side effects of long-term drug administration for the reactivation or inactivation of latently infected cells and their effects on the immune system are critical points that require long-term analysis *in vitro* and clinical trials.

1.3 Study justification

Cell and gene therapy has emerged as a promising avenue in HIV treatment, utilising genetic manipulation to address the complexities of the virus. Particularly, the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) has been emphasised for removing the source of viral rebound and reducing the latent reservoir (Atkins et al., 2021; Bhowmik & Chaubey, 2022). In general, CRISPR/Cas9 strategies can be designed to target viral DNA and RNA encoding for proteins that facilitate various steps of the HIV-1 life cycle or the host DNA, such as the central CD4+ T cells receptor and co-receptors (CCR5 and CXCR4) (Hussein et al., 2023; Sanches-da-Silva et al., 2019). A clinical trial rendering CCR5 deficient in CD4+ T cells using a gene editing approach (NCT02388594) was reported to augment preexisting HIV-specific immune responses (Tebas et al., 2021). The cell and gene therapy product AGT103-T (NCT03215004) was designed to eliminate the CCR5 receptor and target two critical regions of the HIV genome (Vif and Tat), reducing viral load and preventing disease progression (Muvarak et al., 2022). At this juncture, more studies have been conducted on HIV revolving around blocking the viral or the host factors; thus, a promising model for HIV cure is crucial for future research.

The existence of ECs has guided the creation of a plausible model of functional cure, which infers the long-term control of HIV replication without treatment (W. Xu et al., 2017a). ECs follow a heterogeneous pattern of evolution, in which the clinical outcomes are diverse, whereby some patients live more than 30 years (Casado et al., 2020), and some present immune seroreversion (Mendoza et al., 2012). Deciphering the commonality of virological and immunological mechanisms may protect against disease progression and help develop novel anti-HIV treatments. Factors involved in

the suppressive control of HIV-1 infection in ECs include viral, antiviral restriction, host genetics, and the immune system (Tiemessen & Martinson, 2012).

Among these factors, antiviral restriction plays a crucial role in the observed suppression of HIV-1 in ECs. Among 34 anti-HIV-1 restriction genes measured in a study, Schlafen 11 (SLFN11) expression was substantially elevated in CD4+ T cells of ECs in comparison to non-controllers and ART-suppressed individuals (Abdel-Mohsen et al., 2013). Similarly, reduced CD4+ T cell-associated HIV RNA in ECs was strongly associated with an enhanced expression of antiviral factors known as cyclin-dependent kinase inhibitor 1A (CDKN1A/p21) (H. Chen et al., 2011; Yu & Lichterfeld, 2011). Furthermore, CDKN1A/p21 affects HIV replication through its effect on another antiviral factor known as SAM domain and HD domain-containing protein 1 (SAMHD1) (Riveira-Muñoz et al., 2014). Therefore, the interplay between SLFN11, CDKN1A, and SAMHD1 may modulate viral replication and immune homeostasis against HIV-1 infection, rationalising this investigation.

These antiviral restriction factors are often weakly expressed in infected cells of the HIV progressors (Q. Xiao et al., 2019). Since these factors can inhibit HIV infection via different mechanisms, the CRISPR/Cas9 technology could simultaneously activate their expression in infected cells to target different phases of the viral life cycle. In such circumstances, Cas9 can be engineered for expanded use beyond creating targeted double-stranded DNA breaks (dsDNA) (Heidersbach et al., 2023). The fusion of transcriptional activator domains (VP64) to a nuclease-dead form of Cas9 (dCas9) enables CRISPR-mediated transcriptional activation (CRISPRa) (Jinek et al., 2012). For instance, APOBEC3G and APOBEC3B were induced in human cells that typically express neither protein using dual sgRNAs, resulting in the

blockade of HIV-1 replication (Bogerd et al., 2015). Nevertheless, studies on applying CRISPR/Cas9 technology to activate antiviral restriction factors to inhibit HIV-1 infection are minimal.

Using the CRISPRa system, an attempt was made to enhance the endogenous expression of SLFN11 and CDKN1A in HIV-1-infected cells *in vitro.* Also, whether the CRISPR-based upregulation of SLFN11 and CDKN1A would indirectly alter SAMHD1 expression, conferring the cells with inhibitory activity against HIV-1 production, and induce an immune repertoire to combat HIV infection. Moreover, this study deciphers whether a combination of CRISPRa with ART-treated HIV cells confers therapeutic efficacy, in comparison to ART-naïve cells. Such multi-layered combination therapies not only reduce the need for lifelong treatment but also create new avenues for a complete HIV cure. While many hurdles remain, the advancement of gene therapy, alongside ethical guidelines and international cooperation, could represent a major leap forward in HIV treatment, ultimately offering greater benefits.

1.4 Hypothesis

Leveraging on the technology of CRISPRa, the expression of SLFN11 and CDKN1A would be upregulated in HIV-1-infected cells *in vitro*, mimicking the natural suppressive control of ECs.

1.5 General objective

To investigate the HIV-1 antiviral activity of CRISPR-mediated SLFN11 and CDKN1A upregulation.

1.6 Specific objectives

a) To explore the interactive relationship between SLFN11, CDKN1A and SAMHD1 using text-mining and establish sgRNAs using targeted cloning.

b) To study the antiviral activity of SLFN11 and CDKN1A upregulation in a non-specific and specific mammalian cell line model.

c) To recruit and isolate PBMCs of PLHIV and monitor their clinical parameters before and after ART initiation.

d) To study the antiviral activity of SLFN11 and CDKN1A upregulation in PBMCs of PLHIV before and after ART initiation.

CHAPTER 2

LITERATURE REVIEW

2.1 HIV/AIDS: epidemic milestone

The milestone can be characterised by three overlapping phases (Granich et al., 2017, 2018), as shown in **Figure 2.1**: (i) devastation, (ii) discovery and action, and (iii) ending AIDS. The virus began to spread globally soon after being discovered, with Africa bearing the brunt of the pandemic. During the devastation phase, the social response was weak, discrimination was widespread, healthcare workers lost most of their patients, and health centres were filled to maximum capacity without appropriate HIV treatment. The discovery and action phase resulted from the growing collective community and scientific and political responses against the disease. Several developments were achieved, including HIV diagnosis, global surveillance systems, prevention of opportunistic infections, and ART discovery and clinical care.

The focus was on providing care to slow HIV progression and reduce death, calling for human rights and ending stigma and discrimination, building community support for PLHIV, and controlling transmission through prevention methods. On the other hand, the end of the AIDS phase was developed with currently available technology. This phase was built on observational, community-based studies and modelling, suggesting that increasing access to immediate testing and inexpensive treatment would reduce illnesses, end AIDS, prevent deaths, and eliminate HIV transmission.

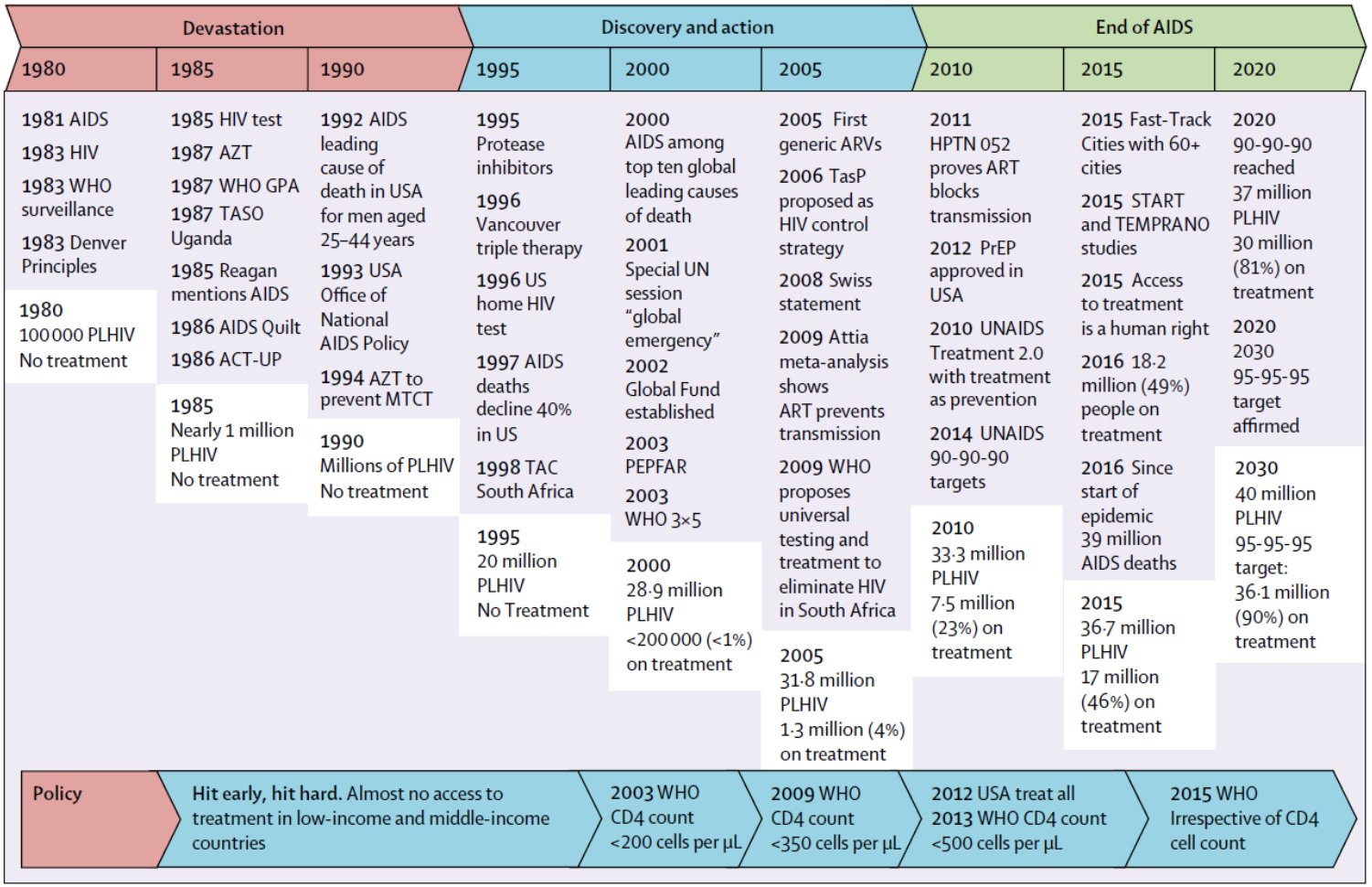


Figure 2.1 Devastation, Discovery and Action, End of AIDS: Key HIV epidemic milestones from 1980 to 2020.
(Adapted from Granich et al., 2017, 2018)

The pandemic was first recognised on 5 June 1981 by the Centres for Disease Control and Prevention (CDC) through the first cases of rare *Pneumocystis carinii* pneumonia (PCP), later Kaposi's sarcoma, among young homosexual men living in Los Angeles (Merson, 1996; Merson et al., 2008; Schmidt, 2018). In 1982, the CDC established the name AIDS and released the first case definition for AIDS. Following that, a French virologist, Luc Montagnier, identified a retrovirus called lymphadenopathy-associated virus (LAV) as the causative agent of AIDS on 20 May 1983, while Robert Gallo announced the human T cell lymphotropic viruses (HTLVs) as the cause of AIDS on 23 April 1984. The Food and Drug Administration (FDA) authorised the first commercial blood test for virus detection on 2 March 1985. Finally, on 1 May 1986, the International Committee on the Taxonomy of Viruses officially announced a virus that causes AIDS, known as HIV. In 1987, the FDA approved the first medication for AIDS, zidovudine (ZDV), and the Western blot blood test kit that specifically tests for HIV antibodies.

On 16 June 1989, the CDC issued the first guidelines for preventing PCP, an AIDS-related opportunistic infection and a significant cause of illness and death for people with AIDS. FDA later approved ZDV for paediatric AIDS cases on 26 October 1990. On 27 May 1992, the FDA licensed a 10-minute diagnostic test kit, which health professionals could use to detect HIV-1. CDC expanded the case definition of AIDS on 18 December 1993, declaring those with CD4+ T cell counts below 200 to have AIDS. On 23 December 1994, the FDA approved an oral HIV test, the first non-blood-based antibody test for HIV. In 1995, the FDA approved the first protease inhibitor called saquinavir (SQV), followed by the first non-nucleoside reverse transcriptase inhibitor (NNRTI), called nevirapine (NVP), in 1996.

More test kits were licensed, including the first HIV home testing and collection kit, a test that measures the level of HIV in blood and the first HIV urine test. In 1997, the FDA approved Combivir (CBV), a combination of two antiretroviral drugs in one tablet, making it easier for PLHIV to take their medications. In 1996, VaxGen, a San Francisco-based biotechnology company, began conducting Thailand's first human AIDSVAX vaccine trials. However, on 24 February 2003, the AIDSVAX vaccine trial was halted as it did not reduce overall HIV infection rates among those vaccinated. In 2002, the FDA approved the OraQuick Rapid HIV-1 Antibody Test for use finger prick, with 99.6% accuracy in as little as 20 minutes, while in 2004, the OraQuick Rapid HIV-1 Antibody Test for use with oral fluid was approved.

Timothy Ray Brown was announced in 2008 as the first person cured of HIV. Cure research gained tremendous attention from researchers in 2013, including two Boston patients and an HIV-infected child, Mississippi Baby. In 2019, the London patient was reported with no detectable virus despite having been off ART for 18 months. In 2012, the FDA approved using Truvada (emtricitabine and tenofovir), the first HIV treatment approved for pre-exposure prophylaxis (PrEP). The World Health Organization (WHO) also recommended that people begin HIV treatment immediately following diagnosis. In 2019, a LATITUDE study evaluated ART for maintaining viral suppression in people who find it challenging to take daily ART in pill form. On 21 January 2021, the FDA approved Cabenuva (cabotegravir and rilpivirine), the first injectable, extended-release, complete HIV regimen administered once a month, the first alternative to daily oral treatment. In December 2021, the FDA approved the first long-acting injectable form of HIV PrEP, Apretude (cabotegravir extended-release injectable suspension). The summary of the 40 years of HIV/AIDS response is shown in **Figure 2.2**.

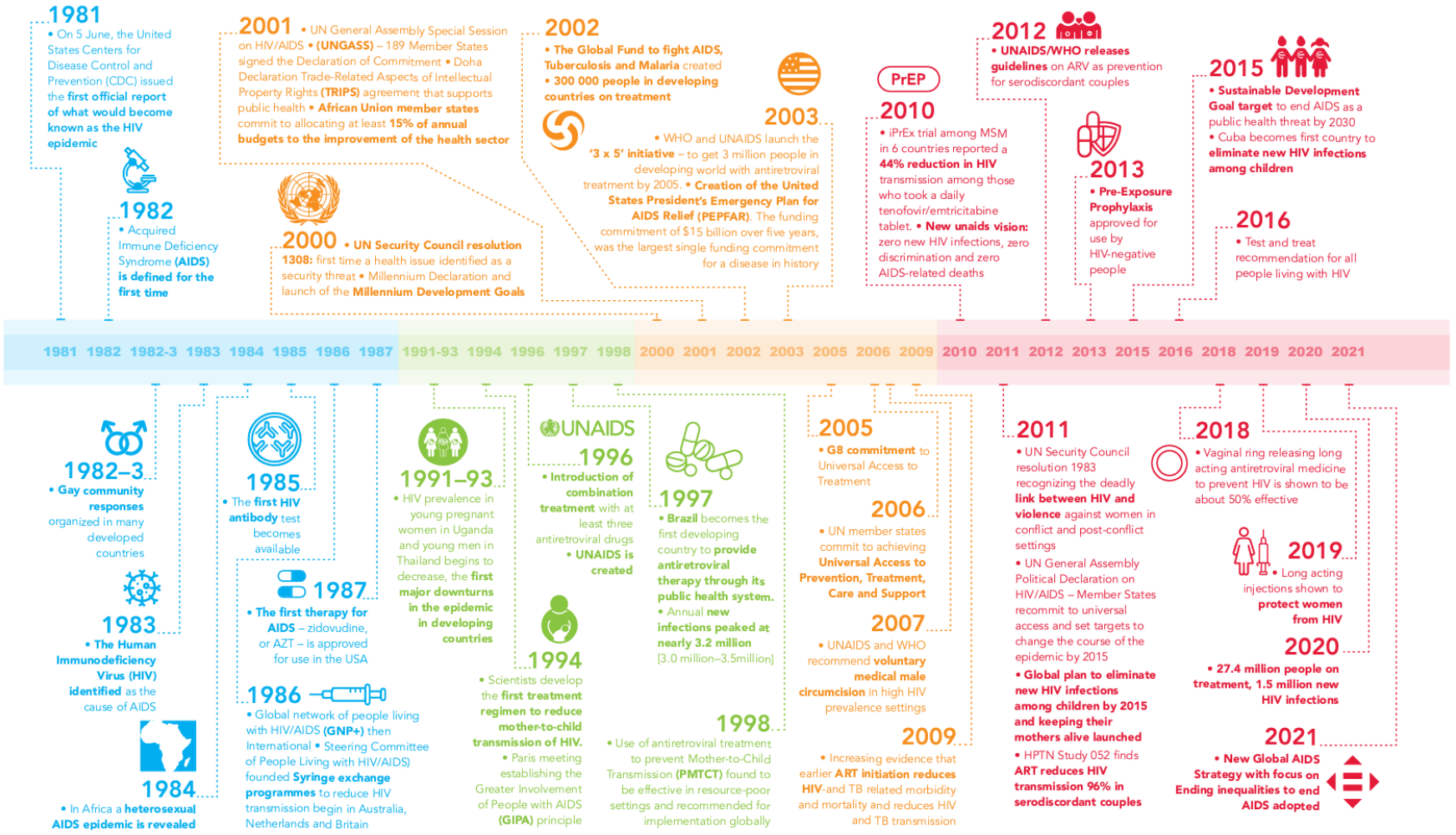


Figure 2.2 40 years of the HIV/AIDS response.
(Adapted from UNAIDS, 2021)

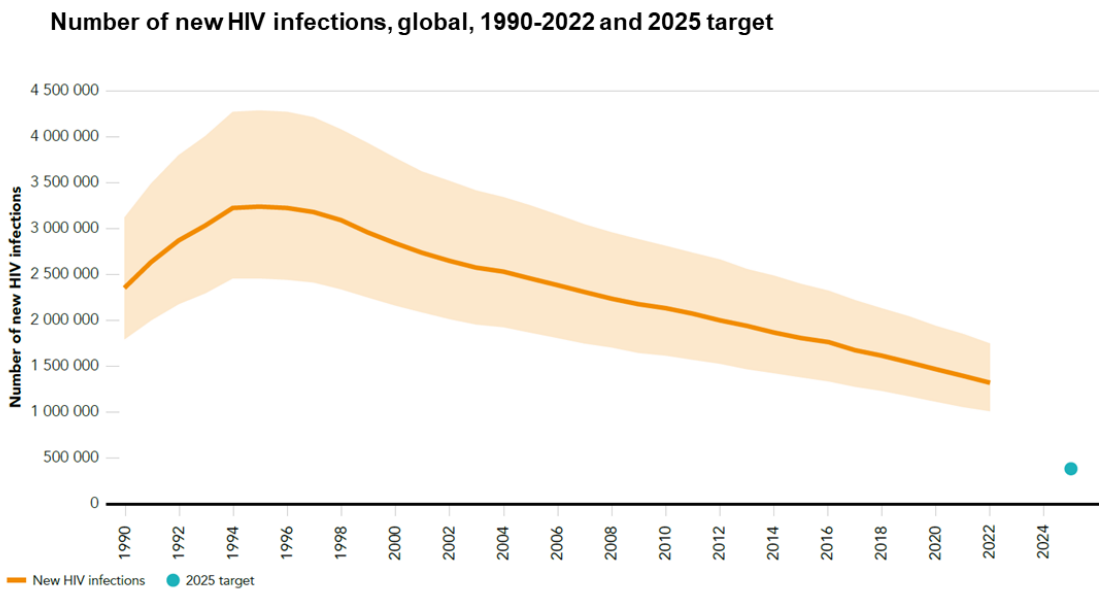
2.2 HIV/AIDS: epidemiology

There was an increase in the number of PLHIV from 2010 to 2022, from 31.5 million to 39.0 million people, as shown in Table 2.1 (UNAIDS, 2023a, 2023b). Since 2010, new HIV infections have declined from 2.1 million to 1.3 million in 2022, particularly among children, from 310,000 to 130,000. AIDS-related deaths have been reduced from 1.3 million people in 2010 to 630,000 in 2022. Therefore, new HIV infections declined by 38 %, and AIDS-related mortality decreased by 51 % since 2010, as shown in Figure 2.3. Access to HIV treatments improved, with around 29.8 million people accessing ART worldwide in 2022, compared to 7.7 million people in 2010, as HIV treatment is vital to the global attempt to end AIDS as a public health threat.

Table 2.1 Global HIV data for the years 2010, 2021 and 2022.

	2010	2021	2022
People living with HIV (PLHIV)	31.5 million	38.7 million	39.0 million
New HIV infections (total)	2.1 million	1.2 million	1.3 million
New HIV infections (aged 15+ years)	1.8 million	1.3 million	1.2 million
New HIV infections (aged 0-14 years)	310,000	140,000	130,000
AIDS-related death	1.3 million	660,000	630,000

Source: UNAIDS, 2023a, 2023b



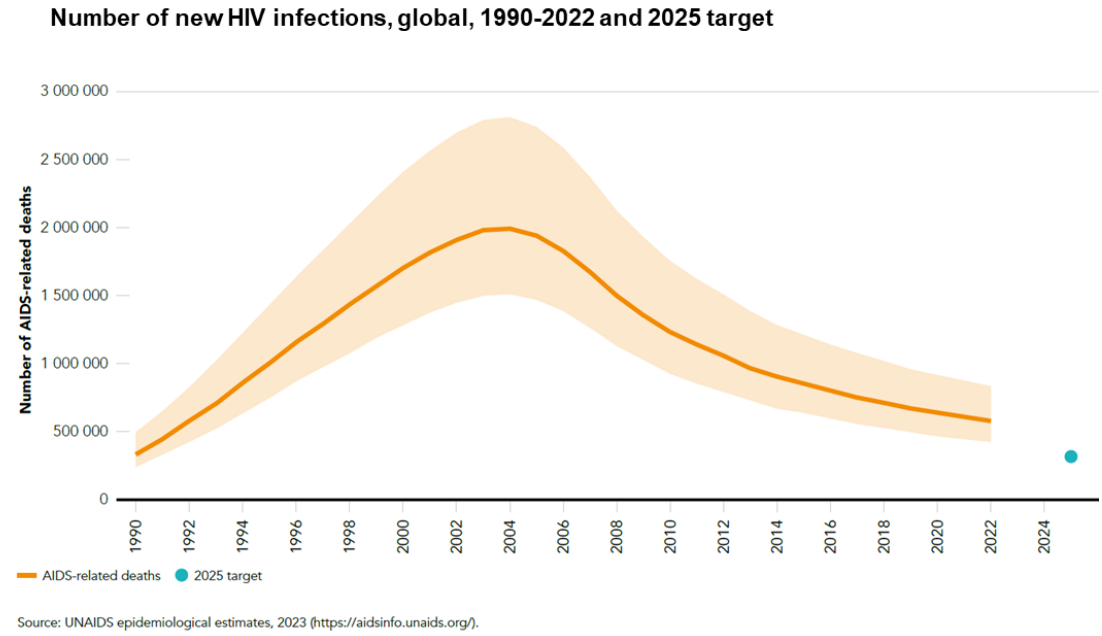


Figure 2.3 Trend of new HIV infections and AIDS-related deaths globally.
The new HIV infections have been reduced by 59 % since the peak in 1995, and AIDS-related deaths have decreased by 69 % since the peak in 2004.
(Adapted from https://aidsinfo.unaids.org)

2.2.1 Prevalence of HIV/AIDS: regional data

Over the past few years, the colliding HIV/AIDS and COVID-19 pandemics placed the global HIV response under increasing threat, with the region of Eastern and Southern Africa reporting the highest morbidity, mortality and new infections, as shown in **Table 2.2** (UNAIDS, 2023a, 2023b). In this region, the key challenges include limited disproportionate resources for healthcare, non-disclosure and lack of timely detection and treatment (Parker et al., 2021). On the other hand, rates of HIV infection in the Middle East and North Africa are among the lowest worldwide due to minimal HIV epidemiological data (Abu-Raddad et al., 2010; Gökengin et al., 2016).

Table 2.2 Regional HIV and AIDS statistics and features for 2022.

Region	PLHIV	Total new infections	AIDS-related deaths
Eastern and Southern Africa	20.8 million	500,000	260,000
Western and Central Africa	4.8 million	160,000	120,000
Middle East and North Africa	190,000	17,000	5,300
Asia and the Pacific	6.5 million	300,000	150,000
Latin America	2.2 million	110,000	27,000
The Caribbean	330,000	16,000	5,600
Eastern Europe and Central Asia	2.0 million	160,000	48,000
Western and Central Europe and North America	2.3 million	58,000	13,000
Global totals	39.0 million	1.3 million	630,000

Source: UNAIDS, 2023a, 2023b

2.2.2 Prevalence of HIV/AIDS: Asia and the Pacific

This region substantially improved in managing HIV epidemics, with 6.5 million PLHIV, 300,000 new HIV infections, and 150,000 AIDS-related deaths. India has the world's third-largest disease burden, as shown in **Table 2.3** (UNAIDS, 2023a, 2023b). The new HIV infections declined by 14 %, and AIDS-related deaths decreased by 51 % from 2010 to 2022. Young people accounted for around a quarter of new HIV infections in this region in 2022.

Table 2.3 2022 HIV/AIDS estimates for Asia and the Pacific (by country).

Country	PLHIV	New HIV infections	AIDS-related deaths
Afghanistan	12,000	1,300	<1,000
Bangladesh	16,000	1,300	<1,000
Bhutan	1,100	<100	<100
Cambodia	76,000	1,400	1,100
Fiji	2,000	<500	<100
India	2,500,000	66,000	40,000
Indonesia	540,000	24,000	26,000
Iran (Islamic Republic of)	46,000	2,900	2,300
Lao People's Democratic Republic	17,000	1,000	<500
Malaysia	86,000	3,100	2,500
Maldives	<100	<100	<100
Mongolia	<1,000	<100	<100
Myanmar	280,000	11,000	6,400
Nepal	30,000	<500	<500
New Zealand	3,600	<100	<100
Pakistan	270,000	NIL	12,000
Papua New Guinea	72,000	6,500	1,100
Philippines	160,000	24,000	1,500
Sri Lanka	4,100	<200	<100
Thailand	560,000	9,200	11,000
Timor-Leste	1,500	<100	<100
Viet Nam	250,000	6,200	4,100

Source: https://aidsinfo.unaids.org; Australia, Brunei, China, Democratic People's Republic of Korea, Japan, Republic of Korea, Singapore: estimates were not available at the time of reporting (accessed on 1st July 2024)

2.2.3 Prevalence of HIV/AIDS: Malaysia data and trends

In Malaysia, there are an estimated 86,000 PLHIV, with 3,100 people who had newly acquired HIV infection and 2,500 people who died from AIDS-related illnesses in 2022, as shown in **Figure 2.4** (HIV and AIDS Data Hub for Asia Pacific, 2023). The new HIV infections declined by 46 %, and AIDS-related deaths decreased by 29 % from 2010 to 2022. 77 % of new HIV infections were reported among people aged 20 to 39, and more than 90 % of them are men (Daim & Harun, 2023; T. Tan et al., 2023).

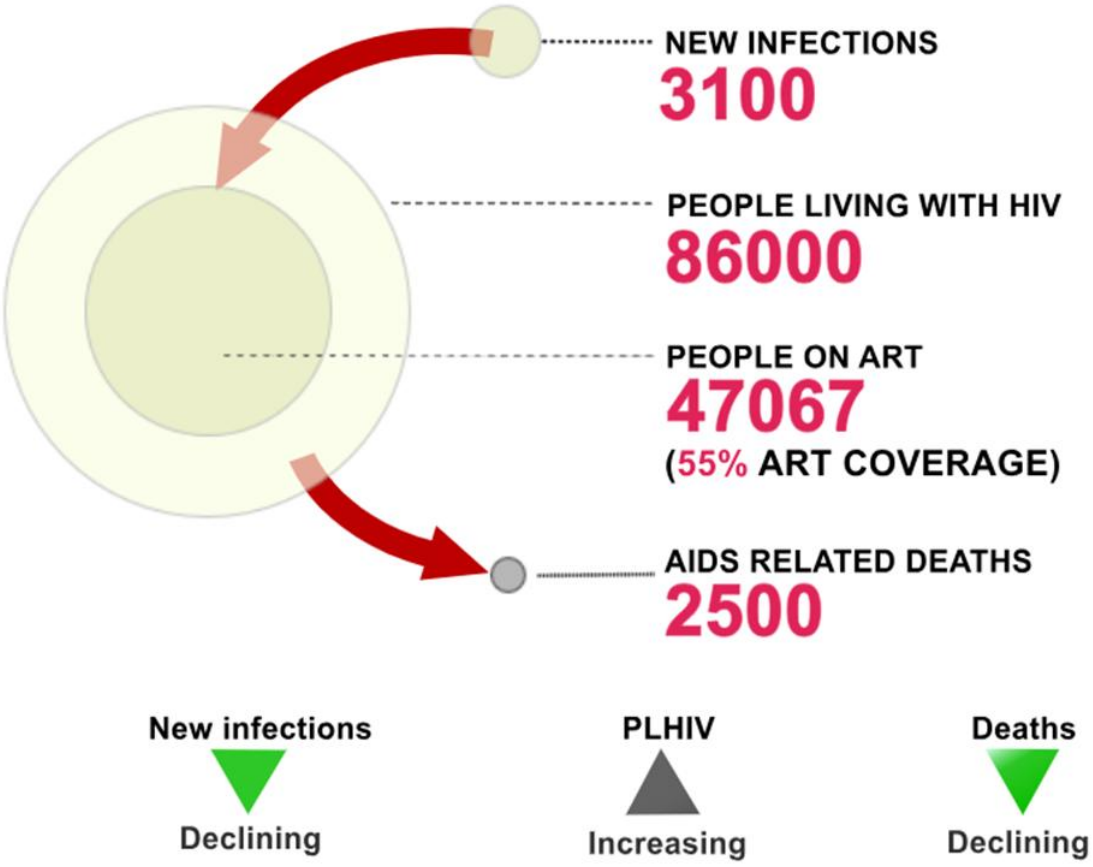


Figure 2.4 Epidemic snapshot and HIV/AIDS profiles for Malaysia for 2022.
(Adapted from https://www.aidsdatahub.org)

Initiating and retaining patients on ART remained the most significant challenge (Ministry of Health of Malaysia, 2023). ART coverage in Malaysia accounted for 55 %, with 47,067 PLHIV on ART, and about 29,515 PLHIV were virally suppressed in 2022, as shown in **Figure 2.5**.

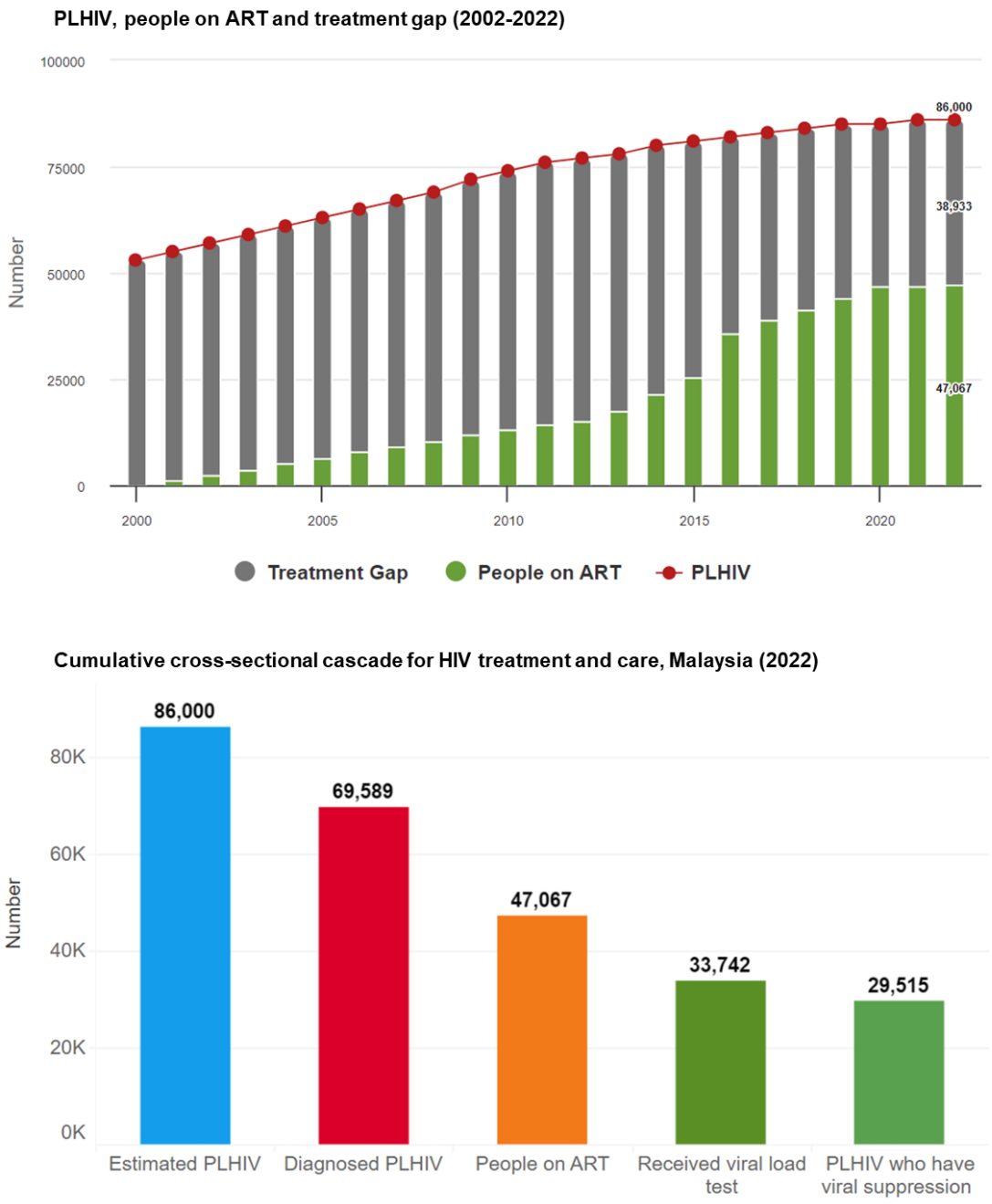


Figure 2.5 Estimated PLHIV receiving ART and treatment cascade for Malaysia.
(Adapted from https://www.aidsdatahub.org)

The initial driving force of the HIV epidemic in Malaysia had been people who inject drugs (PWID), female sex workers (FSW), transgender (TG) people and men who have sex with men (MSM) (R. S. Kim et al., 2023; Ministry of Health of Malaysia, 2023). In the last decade, sexual transmission has become the primary transmission mode, and MSM is expected to become the primary key population in Malaysia, as shown in **Figure 2.6**. In 2022, about 12.9 % of reported HIV cases in Malaysia were linked to MSM, and 20.4 % of cases were reported in Selangor alone. Regarding behaviour and response to HIV prevention among key populations, condom usage was reported to be poorer among PWIDs, while HIV testing coverage is low among FSWs.

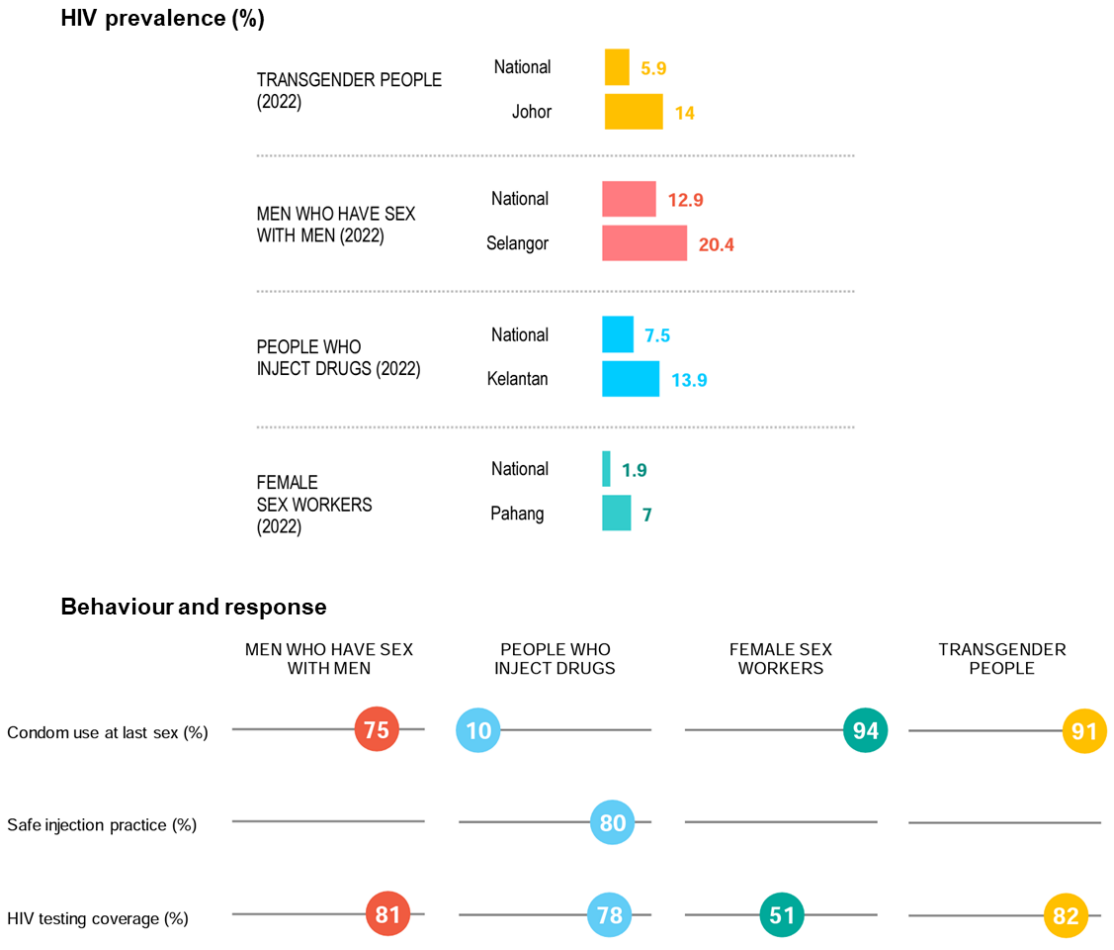


Figure 2.6 HIV prevalence among key populations and their behaviour and responses against HIV prevention strategies.
(Adapted from https://www.aidsdatahub.org)

2.3 HIV/AIDS: origin and virology

HIV is grouped in the genus Lentivirus within the family of Retroviridae, subfamily Orthoretrovirinae (German Advisory Committee Blood, 2016). Epidemiologic and phylogenetic analyses suggested that HIV was introduced into the human population from 1920 to 1940. In 1986, a morphologically similar but antigenically distinct virus was found to cause AIDS in patients in Western Africa (Sharp & Hahn, 2011). This new virus, called human immunodeficiency virus type 2 (HIV-2), was only distantly related to HIV-1 but was closely associated with a simian virus that caused immunodeficiency in captive macaques. Soon after, simian immunodeficiency viruses (SIVs) were found in different primates from sub-Saharan Africa, including African green monkeys, sooty mangabeys, mandrills, and chimpanzees, appeared to be largely non-pathogenic in their natural hosts, as shown in **Figure 2.7**.

The first SIV (SIVmac) was isolated from rhesus macaques *(Macaca mulatta)* at the New England Regional Primate Research Centre (Peeters et al., 2013; Van Heuverswyn & Peeters, 2007). Other rhesus monkeys previously housed at the California National Primate Research Centre were exposed to SIVmac, where they survived a disease outbreak characterised by immune suppression and opportunistic infections. A decade after the first outbreak, stump-nailed macaques *(Macaca arctoides)* developed a similar disease in the same setting, and SIVstm was isolated from the frozen tissue of these monkeys. Due to the geographic coincidence of the natural range of sooty mangabeys and the epicentre of the HIV-2 epidemic in West Africa, as well as being frequently hunted for food or kept as pets, there are suggestions of multiple independent cross-species transmissions of SIVsmm into the human population.

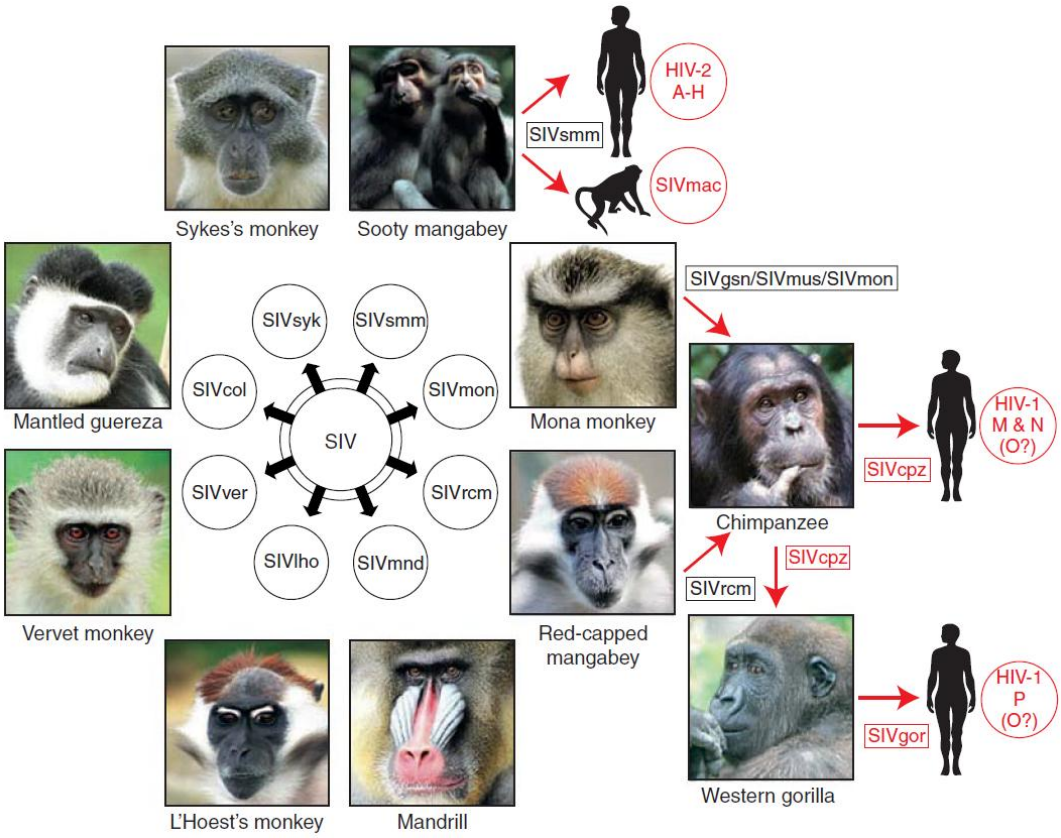


Figure 2.7 Emergences of human AIDS viruses.
The Old-World monkeys were infected with over 40 different lentiviruses, known as SIVs (a suffix indicating their primate species of origin). Different SIVs have crossed the species barrier to humans and great apes, creating new pathogens.
(Adapted from Sharp & Hahn, 2011)

One of the most outstanding examples of cross-species transmission followed by recombination is SIVcpz in chimpanzees (*Pan troglodytes*) (Holmes, 2001). Chimpanzees are known to hunt and kill other animals, such as monkeys, suggesting that they acquired SIV through predation. Furthermore, SIV infection has been described in a second great ape species, western gorillas, termed SIVgor. How gorillas acquired SIVgor remains unknown because they are herbivores and do not hunt other mammals. However, gorillas and chimpanzees are known to feed in the same forest locality, leading to possible transmission. This lineage falls within the branches of SIVcpzPtt (*Pan troglodytes troglodytes*), suggesting that gorillas had acquired SIVgor by cross-species infection, as shown in **Figure 2.8**.

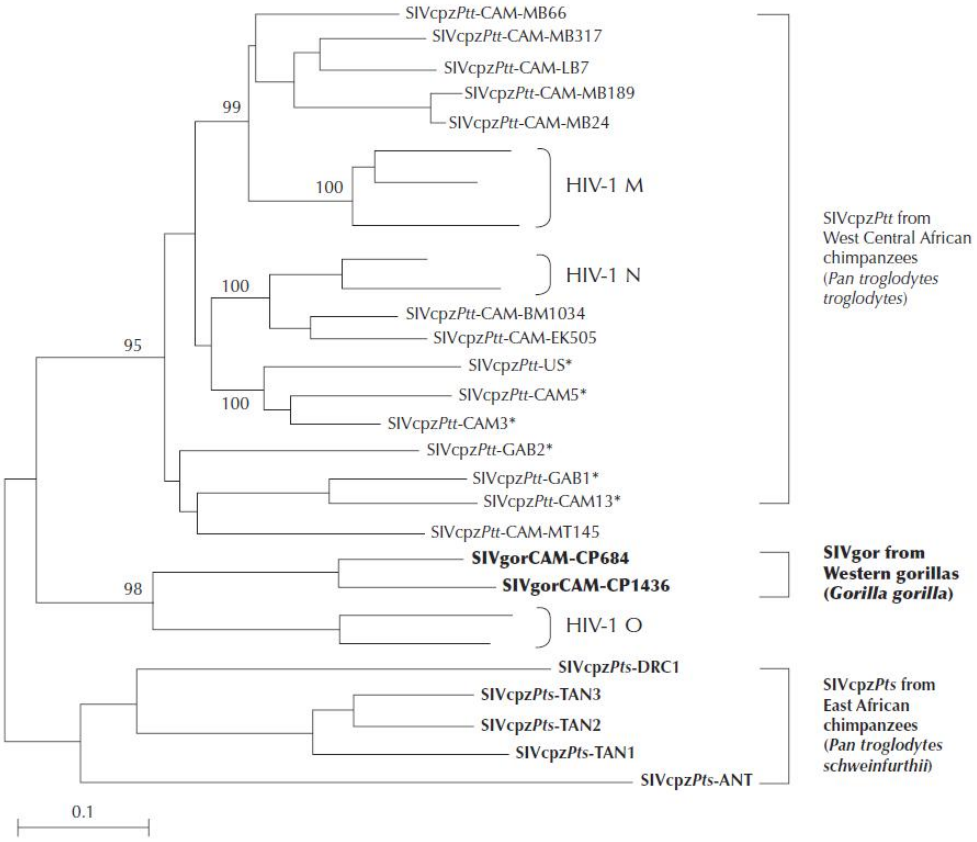


Figure 2.8 Evolutionary relationship of SIVcpz, SIVgor, and HIV-1 strains based on neighbour-joining phylogenetic analysis.
HIV-1 is more closely related to SIVcpzPtt from West Central African chimpanzees. The natural range of western gorillas overlaps with the *Pan troglodytes troglodytes* range in West Central Africa.
(Adapted from Van Heuverswyn & Peeters, 2007)

The origins of HIV-1 groups M and N have been traced to SIVcpzPtt chimpanzees inhabiting the eastern equatorial forests of Cameroon in western central Africa, as shown in **Figure 2.9** (Tebit & Arts, 2011). The exact geographical region was probably also the site of origin for group O HIV-1 SIVgor. Another HIV-1 group-O-like variant, more closely related to SIVgor than group O, has been identified and designated to a tentative new group, P. It is unknown how humans acquired the ape precursors of HIV-1 groups M, N, O, and P; however, the transmission must have taken place through mucous membranes or cutaneous exposure to infected ape blood and fluids during bushmeat hunting.

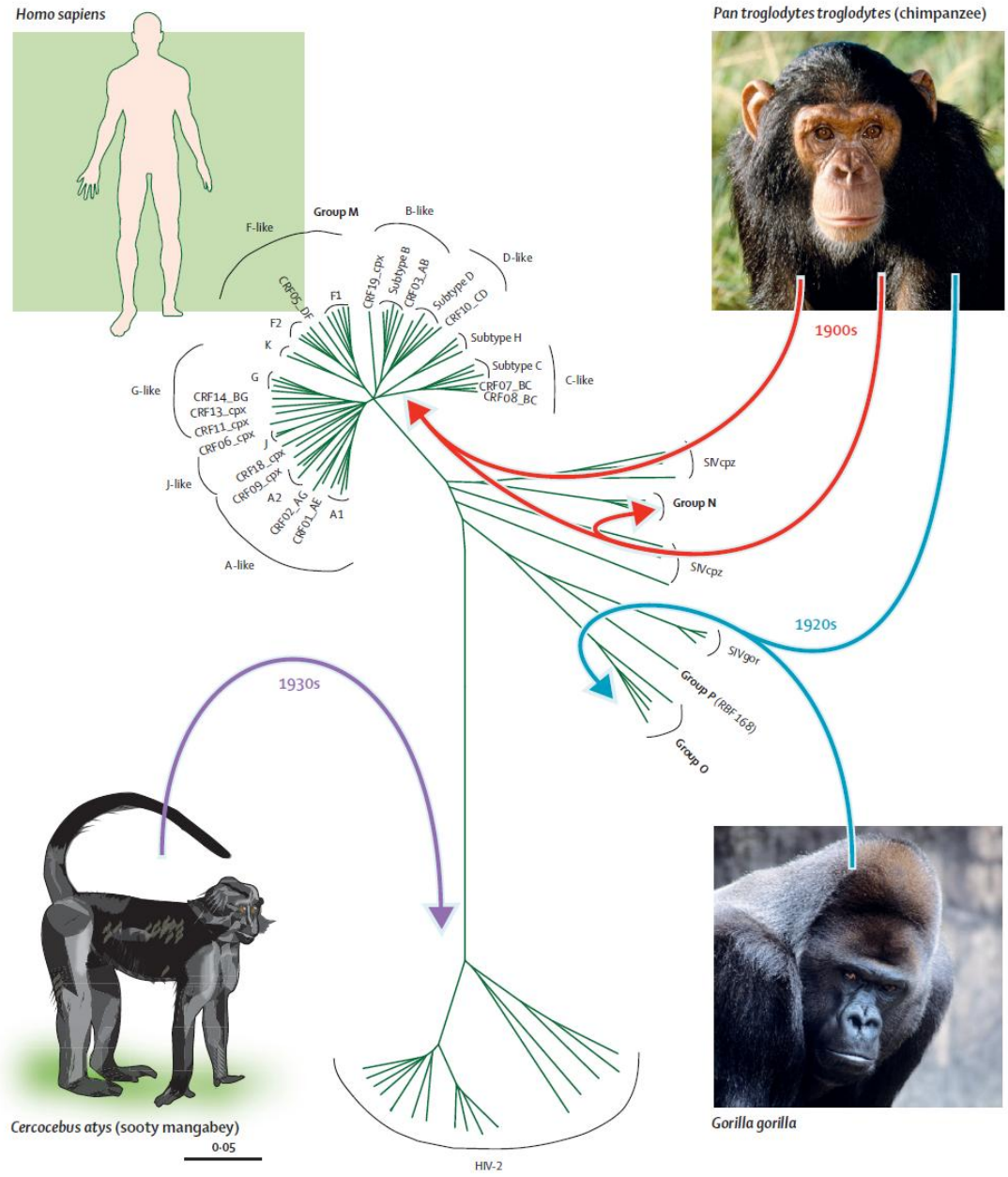


Figure 2.9 Relations between and genetic diversity in HIV-1 groups M, N, O, and P, HIV-2, and SIVs, and patterns of cross-species transmission.
CRF: circulating recombinant form; cpz: chimpanzee; gor: gorilla; cpx: complex; SIV: simian immunodeficiency virus.
(Adapted from Tebit & Arts, 2011)

2.3.1 Diversity and subtypes of HIV-1

HIV genetic diversification is a phylogenetic cluster of viral isolates, referred to as groups or subtypes. To date, HIV-1 has been classified into four groups: M (major), O (outlier), N (non-M, non-O) and the most recent group, P (Bbosa et al., 2019; Hemelaar, 2012; Peeters et al., 2013). HIV-1 group M is reported for the global HIV pandemic. In contrast, group O causes tens of thousands of infections in West–Central Africa, group N has been found in a handful of people in Cameroon, and group P was recently identified in two individuals from Cameroon.

Group M can be subdivided further into minor phylogenetic subtypes (Holmes, 2001; Taylor et al., 2008). Currently, 11 subtypes exist, with contrasting regions of endemicity, including A–D, F–H, and J–L and circulating recombinant forms (CRFs), as shown in **Figure 2.10**. Subtypes A and F are subdivided into sub-subtypes A1 through A4 and F1 and F2, respectively. Meanwhile, the recombinant progeny is classified as CRFs if identified in three or more people with no direct epidemiologic linkage or described as unique recombinant forms (URFs) recovered from only one person.

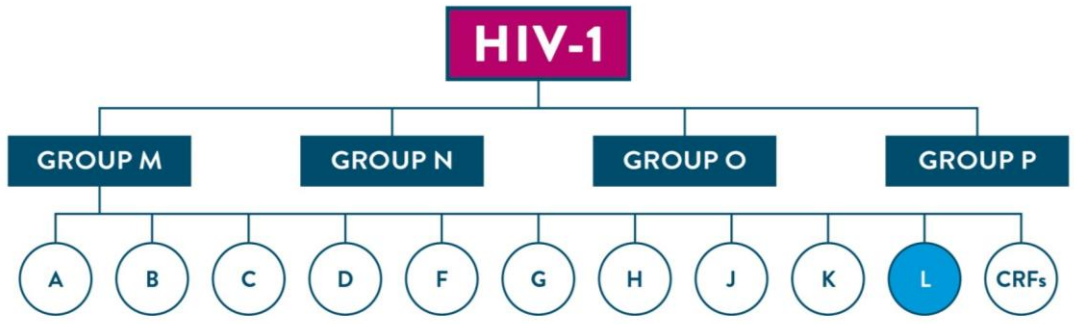


Figure 2.10 Groups and subtypes within HIV-1.
HIV-1 group M is the strain that is responsible for 90 % of the global HIV pandemic. A new subtype of group M has been identified, known as L.
(Adapted from https://www.independent.co.ug/new-strain-of-hiv-discovered)