

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

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CDKN1A UPREGULATION**

**by**

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## LIST OF SYMBOLS

|                 |                     |
|-----------------|---------------------|
| $\alpha$        | Alpha               |
| ,               | Apostrophe          |
| $\sim$          | Approximately equal |
| *               | Asterisk            |
| bp              | Base pair           |
| $\beta$         | Beta                |
| :               | Colon               |
| ,               | Comma               |
| X               | Concentration       |
| mm <sup>3</sup> | Cubic millimetre    |
| dL              | Decilitre           |
| °C              | Degree celsius      |
| $\Delta$        | Delta               |
| –               | En dash             |
| =               | Equal               |
| etc             | Et cetera           |
| .               | Full stop           |
| $\gamma$        | Gamma               |
| g               | Gram                |
| >               | Greater than        |
| #               | Hash                |
| -               | Hyphen              |
| $\kappa$        | Kappa               |
| kb              | Kilobyte            |
| <               | Less than           |

|               |                            |
|---------------|----------------------------|
| $\leq$        | Less than or equal to      |
| L             | Litre                      |
| RM            | Malaysian ringgit          |
| $\mu\text{g}$ | Microgram                  |
| $\mu\text{L}$ | Microliter                 |
| mL            | Milliliter                 |
| –             | Minus                      |
| $\times$      | Multiplication             |
| ng            | Nanogram                   |
| nm            | Nanometer                  |
| NA            | Not available              |
| ND            | Not detected               |
| NIL           | Nothing or zero            |
| ( )           | Parentheses                |
| %             | Percent                    |
| pg            | Picogram                   |
| +             | Plus                       |
| $\Psi$        | Psi                        |
| ?             | Question mark              |
| “ ”           | Quotation mark             |
| x             | Relative centrifugal force |
| rpm           | Revolutions per minute     |
| ;             | Semicolon                  |
| /             | Slash                      |
| [ ]           | Square bracket             |
| $\text{cm}^2$ | 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                                |
| bNAbs           | Broadly neutralizing antibody                                     |
| BRDi            | Bromodomain inhibitors                                            |
| CA              | Capsid protein                                                    |
| T <sub>CM</sub> | 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 <sub>EM</sub> | 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                         |
| Inc            | 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 <sub>N</sub> | 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                                         |
| NF $\kappa$ B | 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           | <i>Pneumocystis carinii</i> 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 <sub>EMRA</sub> | 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- $\beta$      | Transforming growth factor                                   |
| TG                | Transgender                                                  |
| T <sub>TM</sub>   | 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                         |

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# **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 Chilobot 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. Imunofenotip 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- $\alpha$ , IL-2, IFN- $\gamma$ , 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<sup>+</sup> T cells, characterised by elevated naïve and central memory subsets, along with reduced effector memory, terminal effector cells, and exhaustion markers. CD8<sup>+</sup> 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- $\alpha$ , IL-2, IFN- $\gamma$ , 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 Castelfjo) (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-Ciri3n 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<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> T cells of ECs in comparison to non-controllers and ART-suppressed individuals (Abdel-Mohsen et al., 2013). Similarly, reduced CD4<sup>+</sup> 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.

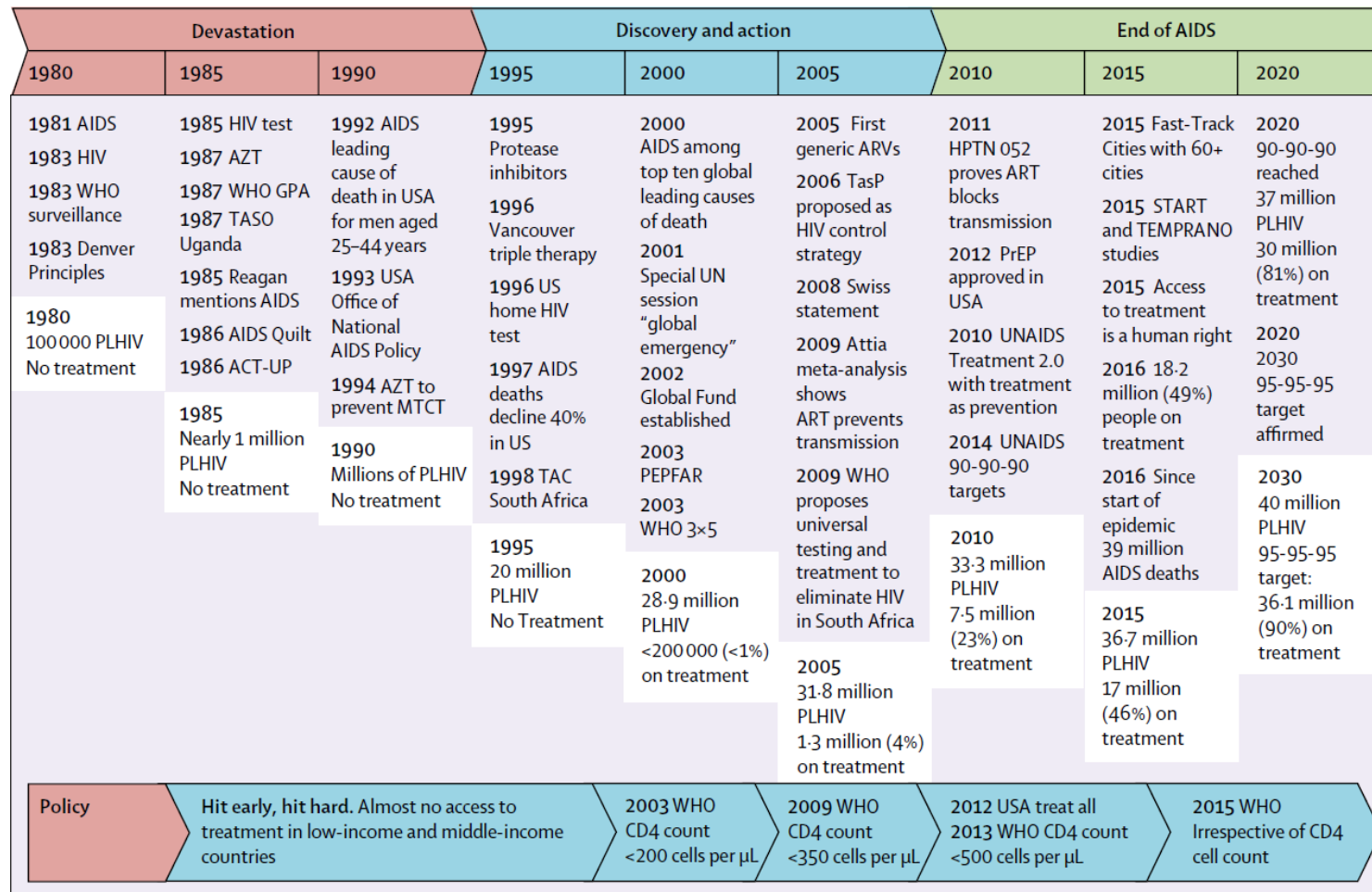
## CHAPTER 2

### 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<sup>+</sup> 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**.

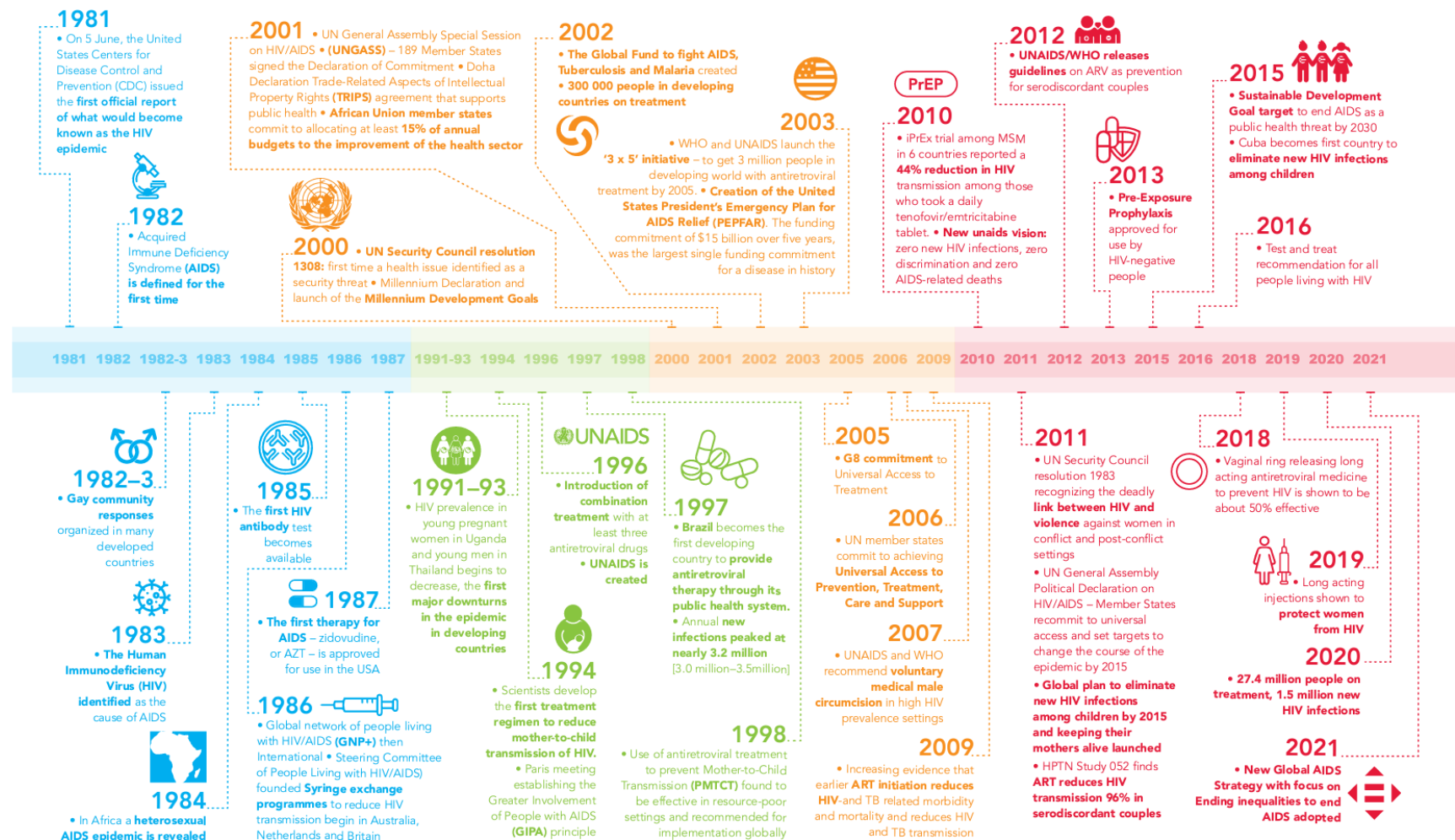


Figure 2.2 40 years of the HIV/AIDS response.  
(Adapted from UNAIDS, 2021)

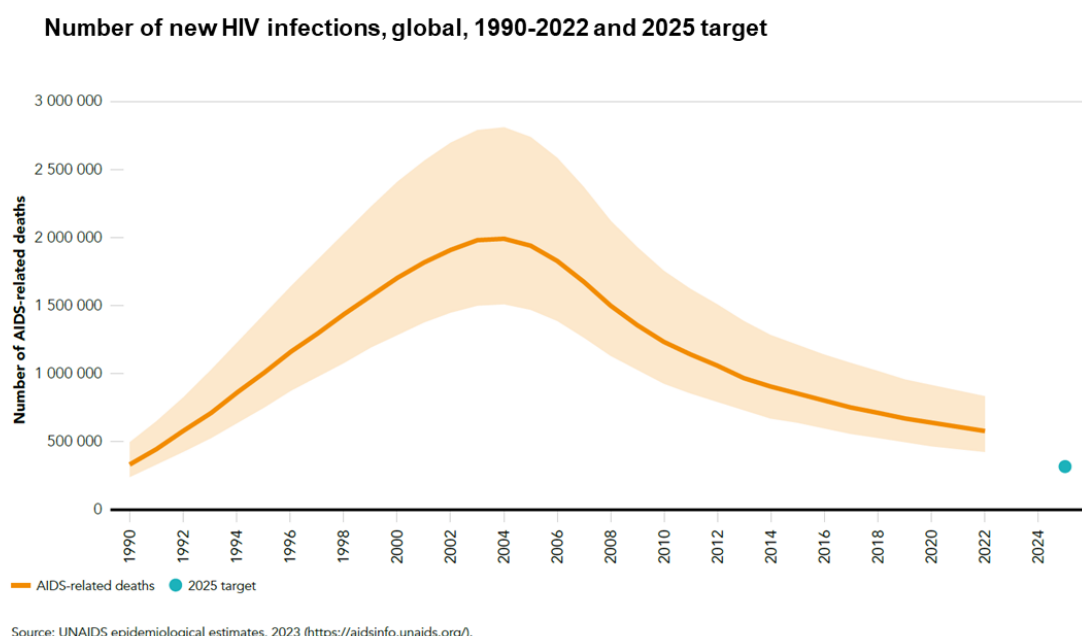
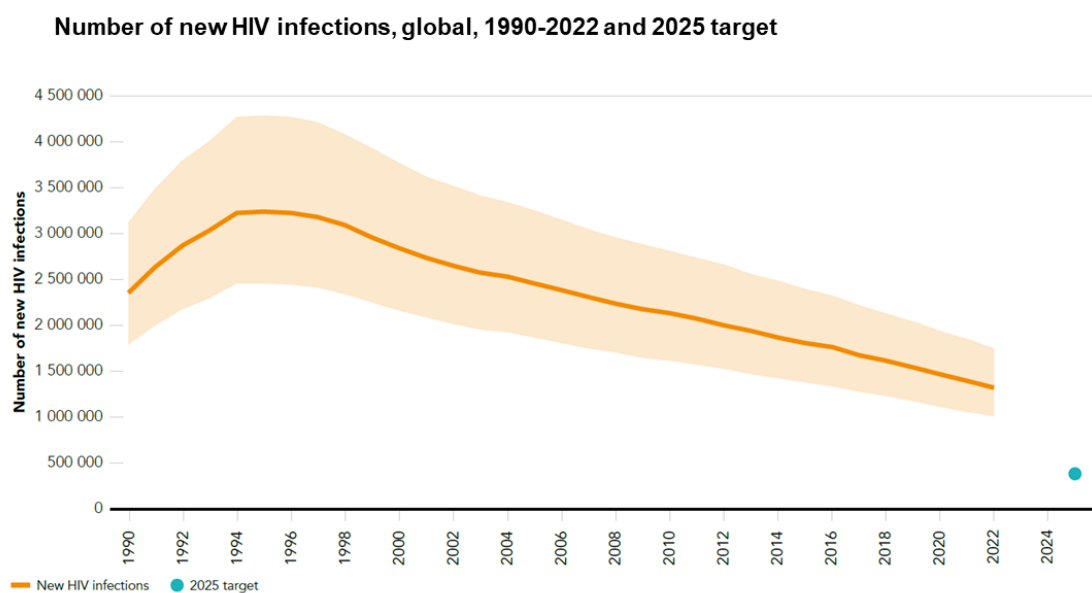
## 2.2 HIV/AIDS: epidemiology

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.**

|                                      | <b>2010</b>  | <b>2021</b>  | <b>2022</b>  |
|--------------------------------------|--------------|--------------|--------------|
| 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.**

| <b>Region</b>                                | <b>PLHIV</b> | <b>Total new infections</b> | <b>AIDS-related deaths</b> |
|----------------------------------------------|--------------|-----------------------------|----------------------------|
| 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.

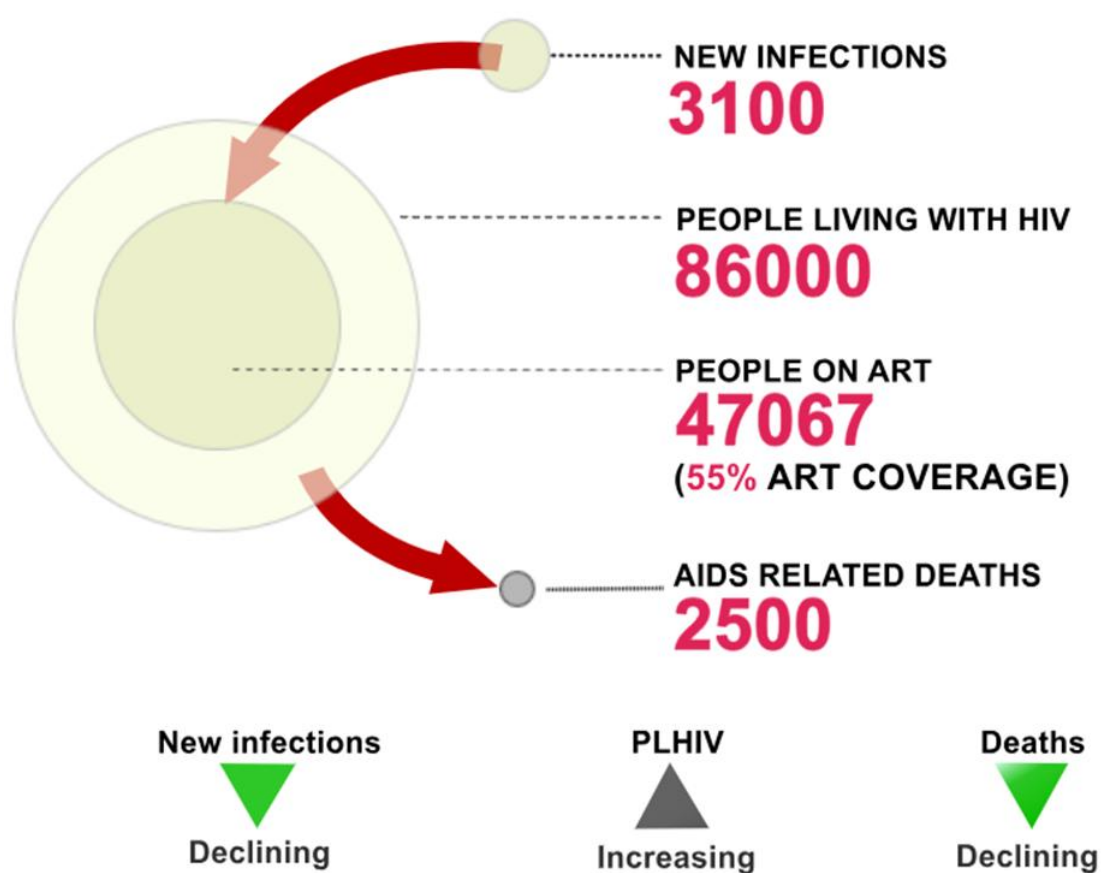
**Table 2.3 2022 HIV/AIDS estimates for Asia and the Pacific (by country).**

| 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 1<sup>st</sup> July 2024)

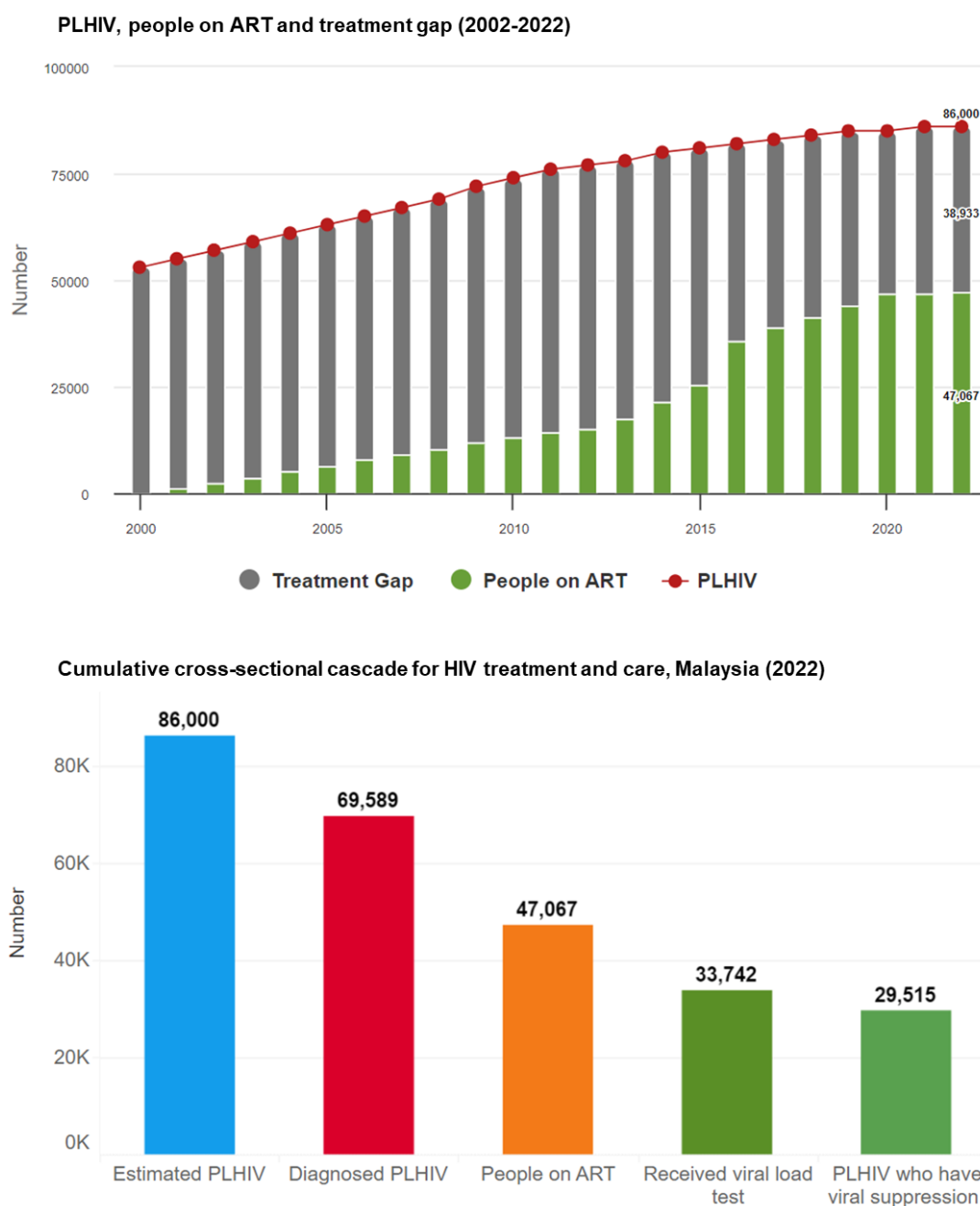
### 2.2.3 Prevalence of HIV/AIDS: Malaysia data and trends

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>)

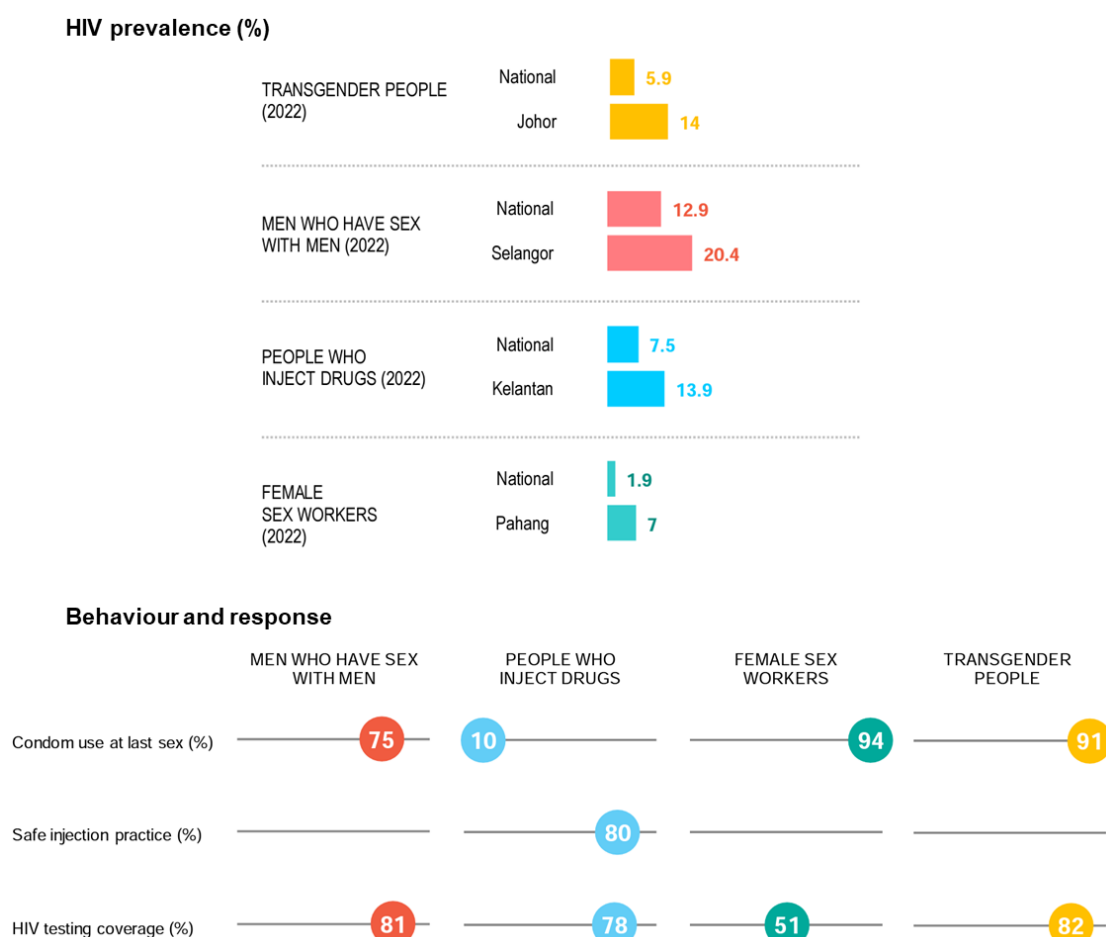
Initiating and retaining patients on ART remained the most significant challenge (Ministry of Health of Malaysia, 2023). ART coverage in Malaysia accounted for 55 %, with 47,067 PLHIV on ART, and about 29,515 PLHIV were virally suppressed in 2022, as shown in **Figure 2.5**.



**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.

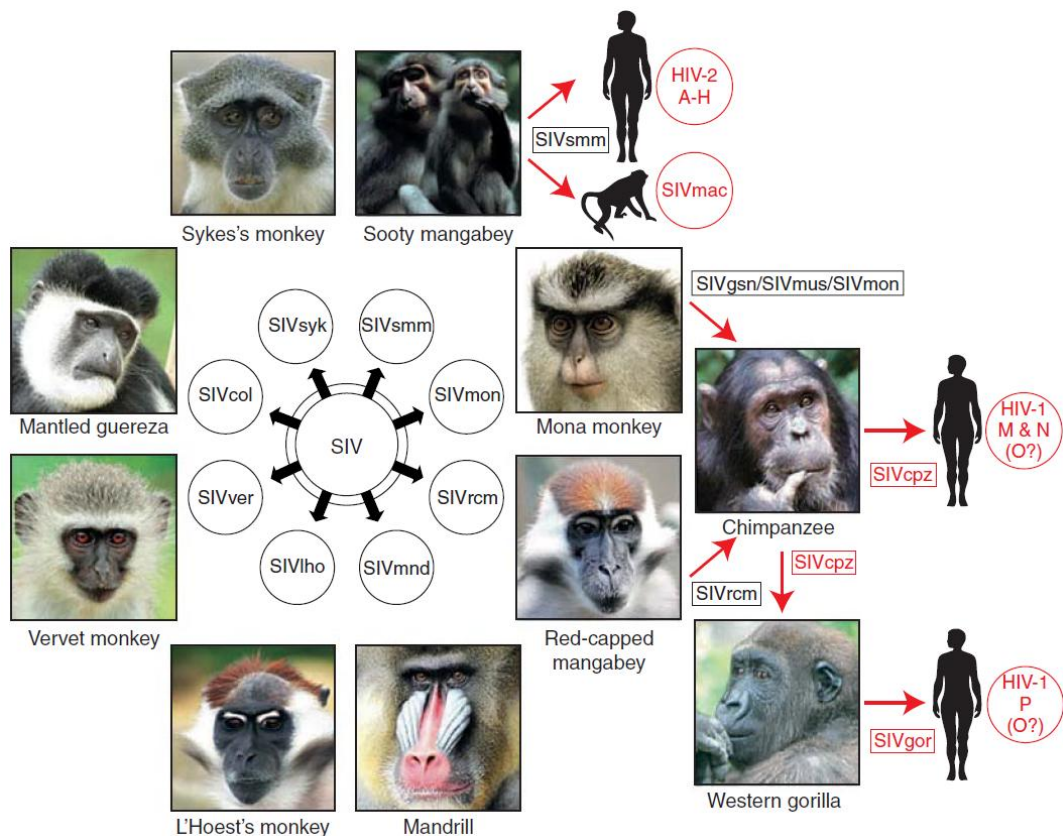


**Figure 2.6 HIV prevalence among key populations and their behaviour and responses against HIV prevention strategies.**  
(Adapted from <https://www.aidsdatahub.org>)

### 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**.

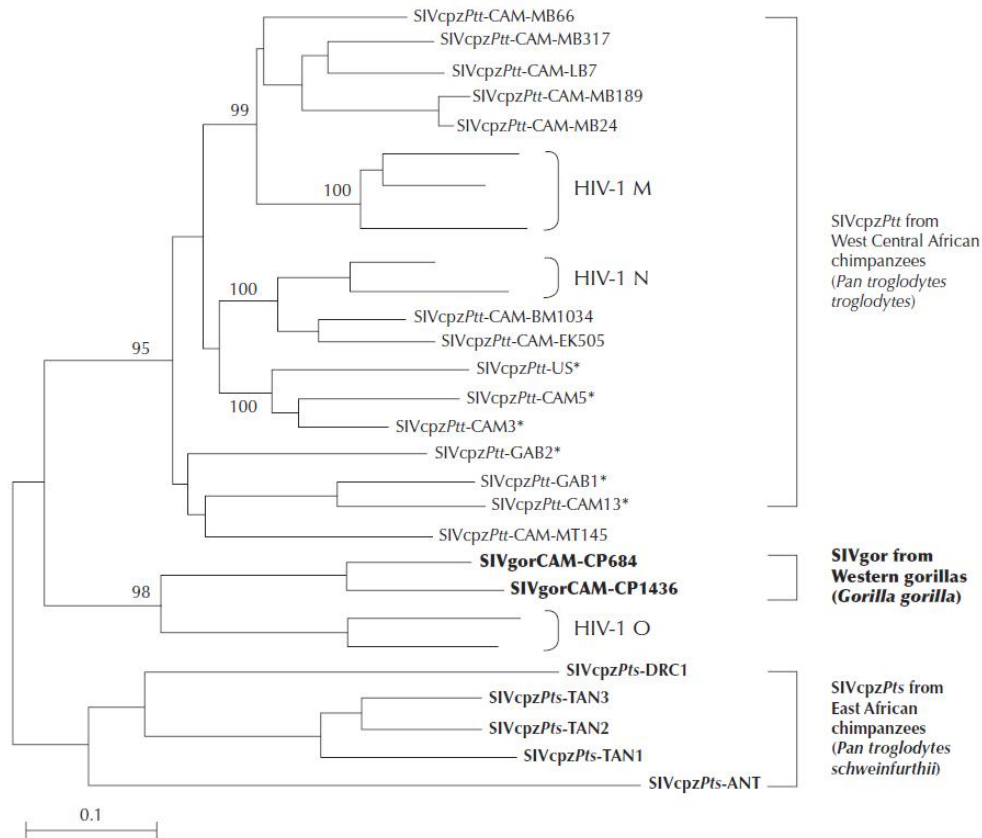
The first SIV (SIVmac) was isolated from rhesus macaques (*Macaca mulatta*) at the New England Regional Primate Research Centre (Peeters et al., 2013; Van Heuverswyn & Peeters, 2007). Other rhesus monkeys previously housed at the California National Primate Research Centre were exposed to SIVmac, where 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**.

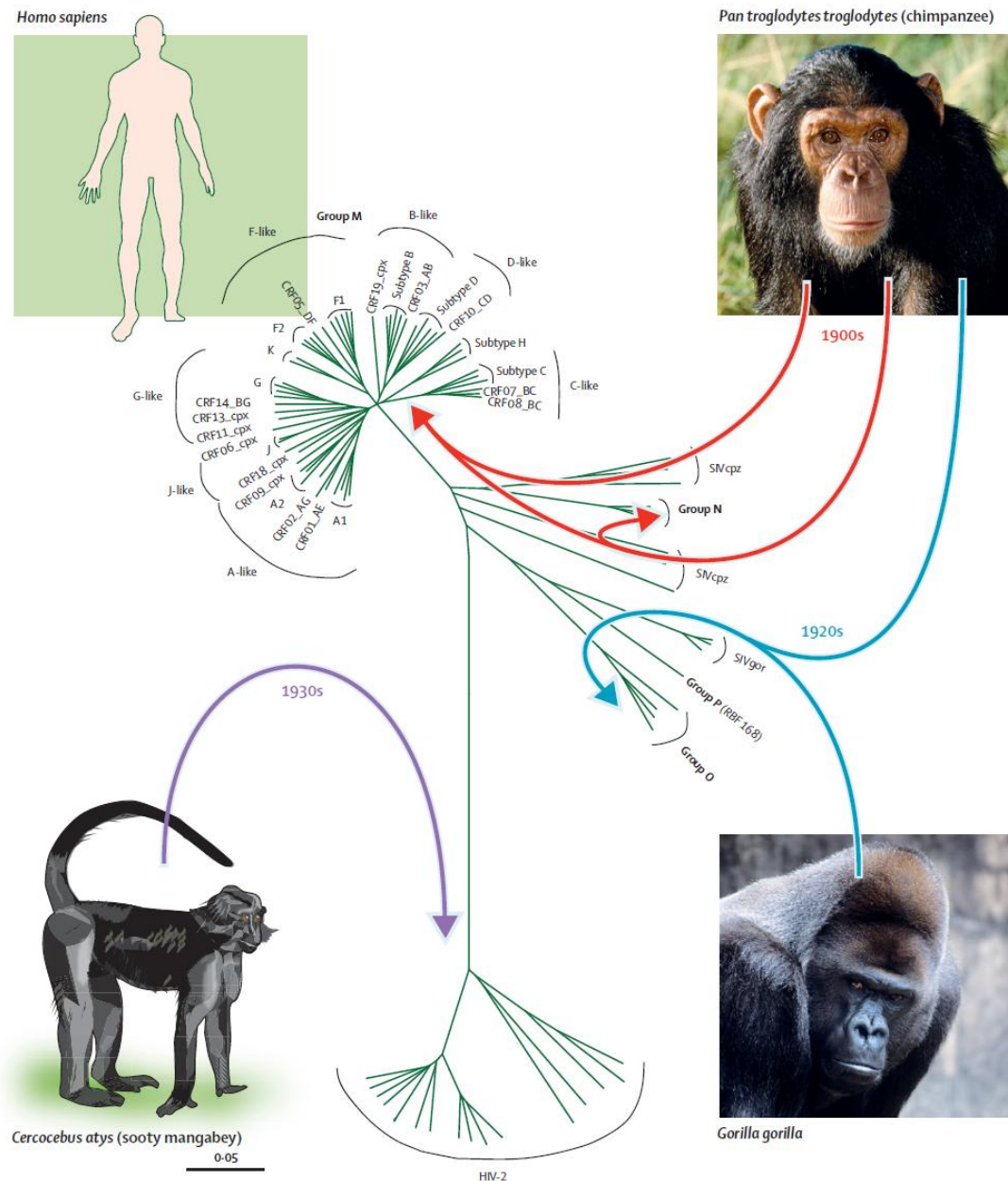


**Figure 2.8 Evolutionary relationship of SIVcpz, SIVgor, and HIV-1 strains based on neighbour-joining phylogenetic analysis.**

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.

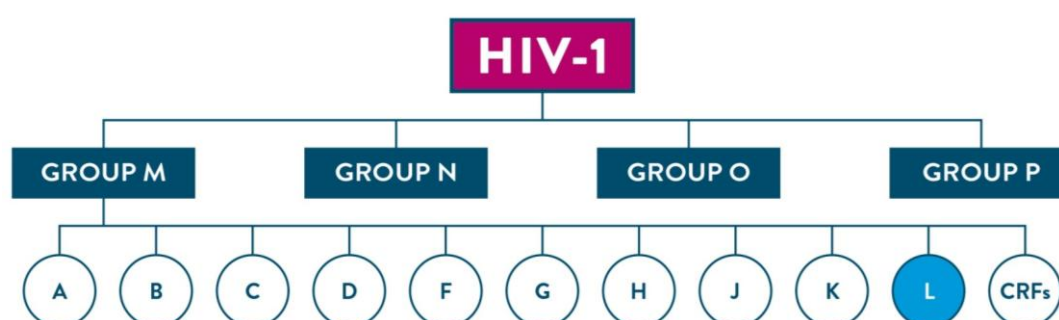


**Figure 2.9 Relations between and genetic diversity in HIV-1 groups M, N, O, and P, HIV-2, and SIVs, and patterns of cross-species transmission.**  
 CRF: circulating recombinant form; cpz: chimpanzee; gor: gorilla; cpx: complex; SIV: simian immunodeficiency virus.  
 (Adapted from Tebit & Arts, 2011)

### 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>)