Frontiers in Clinical Drug Research (HIV)

Editor:
Atta-ur-Rahman, *FRS*

Bentham Books

Frontiers in Clinical Drug Research-HIV

(*Volume 5*)

Edited by

Atta-ur-Rahman, *FRS*
Kings College,
University of Cambridge,
Cambridge,
UK

Frontiers in Clinical Drug Research-HIV

Volume # 5

Editor: Prof. Atta-ur-Rahman, *FRS*

ISSN (Online): 2352-5916

ISSN (Print): 2468-0397

ISBN (Online): 978-981-14-6445-4

ISBN (Print): 978-981-14-6443-0

ISBN (Paperback): 978-981-14-6444-7

need for a court order if at any point you breach any terms of this License Agreement. In no event will any delay or failure by Bentham Science Publishers in enforcing your compliance with this License Agreement constitute a waiver of any of its rights.

3. You acknowledge that you have read this License Agreement, and agree to be bound by its terms and conditions. To the extent that any other terms and conditions presented on any website of Bentham Science Publishers conflict with, or are inconsistent with, the terms and conditions set out in this License Agreement, you acknowledge that the terms and conditions set out in this License Agreement shall prevail.

Bentham Science Publishers Pte. Ltd.
80 Robinson Road #02-00
Singapore 068898
Singapore
Email: subscriptions@benthamscience.net

CONTENTS

PREFACE i

LIST OF CONTRIBUTORS ii

CHAPTER 1 CLINICAL ERADICATION OF LATENT HIV RESERVOIRS: WHERE ARE WE NOW? 1

Lilly M. Wong and *Guochun Jiang*

HIV PANDEMIC IN THE CURRENT SETTING 2

MOLECULAR MECHANISMS OF HIV LATENCY 3

HIV Latency 4

Early Stage Latency 4

HIV Transcription in the Context of Latency Establishment 5

Late Stage Latency 7

CURE STRATEGIES 8

Shock and Kill 8

Block and Lock 8

Didehydro-cortistatin A 9

Triptolide 10

Gene Editing Technology for Disruption of Latent HIV 11

CRISPR/Cas9 12

DRUG DEVELOPMENT IN PRE-CLINICAL AND CLINICAL TRIALS 12

HDAC Inhibitors 13

Protein Kinase C Agonists 14

PMA, Prostratin and DPP 14

Bryostatin-1 15

Ingenol Compounds and its Derivatives 17

Second Mitochondrial-Derived Activator of Caspases (Smac) Mimetics 19

Smac Mimetics: A Novel Class of LRAs 19

Birinapant 20

Debio 1143 20

LCL161 21

AZD5582 21

Stimulator of Interferon Gene (STING) Agonists 23

TLR Agonists 23

Immune Therapies 24

Broadly Neutralizing Antibodies 24

Therapeutic Vaccines 25

Checkpoint Inhibitors 25

CONCLUDING REMARKS 26

CONSENT FOR PUBLICATION 26

CONFLICT OF INTEREST 26

ACKNOWLEDGEMENTS 26

REFERENCES 27

CHAPTER 2 HIV-1 GENOTYPIC DRUG RESISTANCE TESTING AND NEXT-GENERATION SEQUENCING 39

Binhua Liang, Melanie Murray, Raghavan Sampathkumar and *Ma Luo*

HIV-1 DRUG RESISTANCE TESTING 40

INTRODUCTION 40

Rates of ARV Drug Resistance 41

Resource-Rich Settings 41

Resource Limited Settings 42
Guidelines for HIV Drug Resistance Testing 44
Resource-Rich Settings 44
Resource-Limited Settings 45
Cost-saving Measures 45
Genotype and Virtual Phenotype Interpretation Systems and Databases 45
HIV Sequence Databases and Their Applications 46
Recommendations 47
CURRENT METHODS FOR HIV-1 DR TESTING 47
Phenotypic Testing 48
Genotypic Testing 48
Population-based Sanger Sequencing 49
Allele-specific Assays 50
Next-generation Sequencing 52
Advantages & Disadvantages of the Current HIV-1 DR Testing Methods 53
LOW ABUNDANT HIV-1 DR VARIANTS (LADRVS) 54
LADRVs are Common 54
Mechanism of LADRVs 56
Impact of LADRVs on ART Failure 57
NEXT-GENERATION SEQUENCING-BASED HIV-1 DRUG RESISTANCE TESTING 59
Platforms of Next-Generation Sequencing Technologies 59
Application of NGS in HIV-1 DR Testing 62
Challenges of NGS in HIV-1 DR Testing 63
Future Direction of NGS Application in HIV-1 DR Testing 68
THE INTERPLAY OF ART AND HOST IMMUNE RESPONSES 69
Interaction of HLA Class I - Restricted $CD8^+$ T Cell Responses and ART 70
The Effect of HLA Class I - Restricted $CD8^+$ T Cell Responses on viral Mutations 70
Does Host Immunity have Anything to Do with Pre-treatment Drug-resistant Mutations? 72
Influence of HLA Alleles on ART Treatment 72
How can We Use the Knowledge of the Interactions between Host Immune Response and ARV to Improve Patient Treatment and Care? 73
CONCLUSION AND RECOMMENDATIONS 75
LIST OF ABBREVIATIONS 76
CONSENT FOR PUBLICATION 78
CONFLICT OF INTEREST 78
ACKNOWLEDGEMENTS 78
REFERENCE 78

CHAPTER 3 CURRENT AND PROMISING MULTICLASS DRUG REGIMENS AND LONG-ACTING FORMULATION DRUGS IN HIV THERAPY 95
Murugesan Vanangamudi, Gnana Ruba Priya and *Maida Engels*
INTRODUCTION 96
Key Drug-Targets in Blocking the HIV Replication Cycle 97
Genomic Information 97
Replication Cycle of HIV 97
Drug Targets 98
APPROVED DRUGS 99
ANTIRETROVIRAL COMBINATION THERAPY 105
Abacavir and Lamivudine 115
Dolutegravir and Lamivudine 115

Zidovudine and Lamivudine 116
Lopinavir and Ritonavir 116
Emtricitabine and Tenofovir Alafenamide & Emtricitabine and Tenofovir Disoproxil Fumarate 117
Dolutegravir and Rilpivirine 118
Darunavir and Cobicistat 118
Atazanavir and Cobicistat 119
Lamivudine and Tenofovir Disoproxil Fumarate 119
Abacavir-dolutegravir-lamivudine 120
Abacavir-lamivudine-zidovudine 121
Bictegravir-emtricitabine-tenofovir Alafenamide 121
Darunavir-Cobicistat-Tenofovir Alafenamide-Emtricitabine 122
Doravirine-Tenofovir DF-Lamivudine 122
Efavirenz-Tenofovir DF-Emtricitabine 123
Efavirenz-lamivudine-tenofovir Disoproxil Fumarate 123
Elvitegravir-Cobicistat-Emtricitabine-Tenofovir Alafenamide 124
Elvitegravir-Cobicistat-Tenofovir DF-Emtricitabine 125
Rilpivirine-Tenofovir Alafenamide-Emtricitabine 125
Rilpivirine-Tenofovir DF-Emtricitabine 126
Therapeutic Potency of Maraviroc and its Combination 126
LONG-ACTING ANTIRETROVIRALS 127
CONCLUSION 129
CONSENT FOR PUBLICATION 130
CONFLICT OF INTEREST 130
ACKNOWLEDGEMENTS 130
REFERENCES 130

CHAPTER 4 ROLE OF NANOTECHNOLOGY IN HIV DIAGNOSIS AND PROGNOSIS 140
Sai Akilesh M, Ashish Wadhwani and *Manas Mandal*
INTRODUCTION 141
SECTION – I 143
HIV PATHOGENESIS 143
HIV - CLASSIFICATION AND STRUCTURE 143
HIV Genome, Replication and Lifecycle 144
HIV Pathogenesis and AIDS 147
HIV Diagnosis and Testing 148
HIV Care and HAART Therapy 149
SECTION – II 151
NANOTECHNOLOGY AND INNOVATION 151
Nanometric Scale and Nanomedicine 151
Nanomedicine – Trends and Development 152
Nano-based Pharmaceuticals 153
Pharmacokinetic and Pharmacodynamic Profile 153
Improved Efficacy and Bio-compatibility 154
Reduced Toxicity 155
SECTION – III 156
ROLE OF NANOTECHNOLOGY IN HIV DIAGNOSTICS, ... 156
DRUG DELIVERY AND THERAPY 156
HIV Diagnostics and Nanotechnology 157
$CD4^+$ T Cells and Viral RNA 157
HIV Enzymes 157

HIV Antigens 157
Therapy, Delivery of ART Drugs and Nanotechnology 161
HIV Vaccines and Adjuvants 165
CONCLUSIONS AND FUTURE PERSPECTIVES 166
CONSENT FOR PUBLICATION 168
CONFLICT OF INTEREST 168
ACKNOWLEDGEMENTS 168
REFERENCES: 168

CHAPTER 5 PREVENTIVE AND THERAPEUTIC FEATURES OF COMBINATION THERAPY FOR HIV 175
Sumera Zaib, Nehal Rana, Areeba and *Imtiaz Khan*
INTRODUCTION 176
Replication Cycle of HIV 177
Attachment and Entry 177
Reverse Transcription 178
Integration of Viral dsDNA into Host DNA 178
Transcription and Translation of Viral dsDNA 178
Assembly and Release 179
Anti-Retroviral Drugs for HIV 180
Nucleotide Reverse Transcriptase Inhibitors (NRTIs) 181
Non-Nucleotide Reverse Transcriptase Inhibitors (NNRTIs) 184
Integrase Strand Transfer Inhibitors (INSTIs) 186
Protease Inhibitors (PIs) 189
Entry Inhibitors 192
CONCLUDING REMARKS 195
FUTURE PERSPECTIVE 195
CONSENT FOR PUBLICATION 196
CONFLICT OF INTEREST 196
ACKNOWLEDGEMENTS 196
ABBREVIATIONS 196
REFERENCES 197

SUBJECT INDEX 203

PREFACE

The book series ***Frontiers in Clinical Drug Research-HIV*** presents important recent developments in the form of cutting-edge reviews written by eminent authorities in the field. The chapters in this 5th volume are mainly focused on different therapies, cell reservoirs of HIV-1, the combination of drugs and nanotechnology in the diagnosis and prognosis of HIV infection.

Wong and Jiang in *Chapter 1* review therapeutic interventions for HIV that have entered preclinical and clinical trials. They also highlight their cure potentials and associated limitations. Liang *et al*., in *Chapter 2* review the HIV-1 genotypic DR testing methods and focus on the main NGS platforms which are available for HIV-1 DR diagnosis. *Chapter 3* by Vanangamudi *et al*., presents the currently available information on multiple drug combinations against HIV and the development of long-acting antiretroviral drugs. *Chapter 4* by Wadhwani focuses on HIV pathogenesis and the role of nanotechnology in HIV diagnostics, drug delivery, and therapy. In the final chapter, Zaib *et al*. give an overview of the therapeutic drugs against HIV, their mechanism of action, their side effects as well as their recommended dosage.

I am grateful to all the eminent scientists for their excellent contributions. I also express my gratitude to the editorial staff, particularly Mr. Mahmood Alam (Editorial Director) and Ms. Fariya Zulfiqar (Manager Publications) for their hard work and persistent efforts.

Prof. Atta-ur-Rahman, *FRS*
Kings College
University of Cambridge
Cambridge
UK

List of Contributors

Lilly M. Wong	UNC HIV Cure Center, Institute of Global Health and Infectious Diseases, The University of North Carolina at Chapel Hill, Chapel Hill, NC, USA
Guochun Jiang	Department of Biochemistry and Biophysics, The University of North Carolina at Chapel Hill, Chapel Hill, NC, USA
Binhua Liang	JC Wilt Infectious Disease Research Center, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Canada Department of Biochemistry & Medical Genetics, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada
Melanie Murray	Division of Infectious Diseases, Department of Medicine, University of British Columbia, Vancouver, Canada The Oak Tree Clinic, British Columbia Women's Hospital, Vancouver, Canada
Raghavan Sampathkumar	JC Wilt Infectious Disease Research Center, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Canada ATPC, Regional Centre for Biotechnology, Haryana, India
Ma Luo	JC Wilt Infectious Disease Research Center, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Canada Department of Medical Microbiology & Infectious Diseases, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada
Murugesan Vanangamudi	Amity Institute of Pharmacy (AIP), Amity University Madhya Pradesh (AUMP), Maharajpura, Gwalior, Madhya Pradesh 474005, India
Gnana Ruba Priya	Pharmaceutical Chemistry Department, College of Pharmaceutical sciences, Dayananda Sagar university, CD Sagar building, Shavige Malleshwara Hills, Kumaraswamy Layout, Bangalore-560078, Karnataka, India
Maida Engels	PSG College of Pharmacy, Peelamedu, Coimbatore - 641004, Tamil Nadu, India
Sai Akilesh M	Department of Pharmaceutical Biotechnology, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Ooty - 643001, Nilgiris, Tamil Nadu, India
Ashish Wadhwani	Department of Pharmaceutical Biotechnology, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Ooty - 643001, Nilgiris, Tamil Nadu, India Faculty of Health of Sciences, School of Pharmacy, JSS Academy of Higher Education and Research, Vacoas-Phoenix, Mauritius
Manas Mandal	Department of Pharmaceutical Biotechnology, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Ooty - 643001, Nilgiris, Tamil Nadu, India College of Pharmacy, Roseman University of Health Sciences, Henderson, NV 89014, USA
Sumera Zaib	Department of Biochemistry, Faculty of Life Sciences, University of Central Punjab, Lahore-54590, Pakistan
Nehal Rana	Department of Biochemistry, Faculty of Life Sciences, University of Central Punjab, Lahore-54590, Pakistan

Areeba Department of Biochemistry, Faculty of Life Sciences, University of Central Punjab, Lahore-54590, Pakistan

Imtiaz Khan Manchester Institute of Biotechnology, The University of Manchester, 131 Princess Street, Manchester M1 7DN, United Kingdom

CHAPTER 1

Clinical Eradication of Latent HIV Reservoirs: Where are we Now?

Lilly M. Wong[1] and **Guochun Jiang**[1,2,*]

[1] *UNC HIV Cure Center, Institute of Global Health and Infectious Diseases, The University of North Carolina at Chapel Hill, Chapel Hill, NC, USA*

[2] *Department of Biochemistry and Biophysics, The University of North Carolina at Chapel Hill, Chapel Hill, NC, USA*

Abstract: Antiretroviral therapy (ART) is the leading therapeutic strategy for the suppression of HIV-1 (HIV) replication. However, ART is a life-long treatment with no effective sterilizing or functional cure of HIV. The main challenge with ART is its inability to eradicate HIV residing in the long-lived resting $CD4^+$ T cells, otherwise known as the main latent HIV cellular reservoirs. HIV reservoirs are commonly found in various areas of the body: brain, liver, placenta, skin, GALT, and lymphoid tissues. Withdrawal of ART leads to the rapid rebound of viremia or progress into AIDS without re-treatment. Current clinical approaches such as "shock and kill," "block and lock," and gene editing exploit the molecular pathways of HIV latency for eradication or permanent suppression of the latent reservoirs. Novel pre-clinical or clinical approaches must take several limitations into consideration: dose-limiting toxicity, potency, and specificity. These limitations are the barriers to reservoir clearance. The "shock and kill" method employs latency reversal agents (LRAs), including histone deacetylase inhibitors (vorinostat, romidepsin, and panobinostat), PKC agonists (bryostatin-1, prostratin, ingenol, or Kansui), SMAC mimetics, STImulator of INterferon Gene (STING) agonists, and TLR agonists, for the disruption of HIV latency and subsequent eradication of latently infected cells. This is followed by immune clearance, including broadly neutralizing antibodies (bnAbs), therapeutic vaccines, or the use of immune checkpoint inhibitors (ICPi). LRAs have exhibited the ability to increase transcription. However, of the recognized LRAs, none have single-handedly reduced the reservoir, which underscores a potential need for combinational strategies. While some of these interventions have entered trials, repurposing our efforts towards a functional cure of HIV may also be productive. The "block and lock" method seeks permanent silencing of HIV transcriptional machinery through targets such as HIV protein Tat to possibly achieve remodeling of the epigenetic landscape at HIV LTR. Here, we review therapeutic interventions that have entered preclinical and pilot clinical trials and highlight their cure potentials and associated limitations. Prospective directions will be discussed for the development of these new therapeutics into drugs for the cure of HIV.

* **Correspondence author Guochun Jiang:** UNC HIV Cure Center, Institute of Global Health and Infectious Diseases, The University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; Tel: 919-445-0384; E-mail: Guochun_Jiang@med.unc.edu

Prof. Atta-ur-Rahman (Ed.)

Keywords: Deep latency, Epigenetics, HIV latency, HIV reservoir, Shock and kill.

HIV PANDEMIC IN THE CURRENT SETTING

Human immunodeficiency virus type 1 (HIV-1), a retrovirus discovered in 1983, is the causative agent of acquired immune deficiency syndrome (AIDS). HIV transmission occurs through three routes: sexual intercourse, vertical (mother-to-child) transmission, and intravenous injection. Since the discovery of HIV, there have been 32.7 million deaths worldwide, and it affects more than 38 million individuals [1]. In the past few decades, the course of treatment for HIV infection has greatly improved through the establishment of antiretroviral therapy (ART). In 1985, the development of Retrovir® (zidovudine) by a collaboration between GlaxoSmithKline (GSK) and Samuel Broder demonstrated that HIV infection is manageable and survivable [2]. With the discovery of Retrovir®, seven distinct drug classes emerged with FDA approval: (1) nucleoside reverse transcriptase inhibitors (NRTIs), (2) nonnucleoside reverse transcriptase inhibitors (NNRTis), (3) protease inhibitors, (4) entry inhibitors, (5) integrase inhibitors, (6) coreceptor antagonists and (7) post-attachment inhibitors [3]. Of the seven drug classes, some HIV regimens exploit several drugs as a combinational strategy with the intention of targeting several mechanisms for HIV [4].

In 2019, only 66% (25.4 million out of 38 million individuals) of HIV-positive individuals had access to ART [1]. Individuals often deal with social stigma from the diagnosis of HIV/AIDS along with socioeconomic stress. These factors impact adherence, where pill fatigue and drug resistance can lead individuals to further complications or AIDS. Comorbidities can also introduce pharmacokinetic drug-drug interactions, a consequence often observed in aging populations [5]. ART has the ability to manage HIV infection effectively by suppressing HIV replication to minimal proviral loads (*i.e.*, <50 copies/ml is the standard for the limit of detection) and latent HIV or possibly residual viral replication is responsible for the rebound of HIV when treatment is interrupted. Infected $CD4^+$ T while cells turn to quiescence in the form of memory T cells, which harbor integrated HIV DNA, also called HIV provirus. These quiescent memory T cells have an estimated half-life of 44 months by quantitative viral outgrowth assay (QVOA) [6] or 48 months by intact proviral DNA assay (IPDA) [7]. Even with ART, it would require more than 73 years of treatment to eliminate the latent infection as the provirus remains undetectable by the immune system and continues to evade viral cytopathic effects (CPE); therefore, individuals must remain on treatment indefinitely in order to purge the reservoirs. In addition to the lifelong requirement of ART, treatment interruption poses another problem. Upon interruption of ART, the memory T cells harboring the stable proviral DNA can

generate new replication and infections, which may be therapy-resistant [8]. Most HIV-positive individuals have a viral rebound, typically observed two weeks post-interruption due to the induction of productive HIV replication *via* cytokine induction encounters with latent proviruses [9]. These limitations reveal the need to develop therapeutic strategies that can effectively target the latent reservoirs.

Elite controllers, individuals who maintain silent viral reservoirs without intervention, make up less than 0.5% of HIV-infected individuals [10, 11]. For these HIV controllers, it indicates the potential for an HIV cure. Notably, only two individuals have been cured of HIV: the "Berlin Patient" and the "London Patient." In 2009, the "Berlin Patient" (Timothy Ray Brown) was cured of HIV after receiving a hematopoietic stem cell (HSC) transplant from a donor containing an entry chemokine receptor (CCR5) mutation to prevent HIV infection of healthy cells (CCR5D32) [12]. The "Essen Patient" underwent an allogeneic HSCT, but upon ART interruption, rapid viral rebound occurred through the alternative entry coreceptor, CXCR4, *via* receptor switch [13]. After several unsuccessful attempts over the years with various candidates, the "London Patient" (Adam Castillejo) was announced free of HIV in 2019 after complications with grade 1 graft-*versus*-host disease (GVHD), cytomegalovirus (CMV), and Epstein-Barr virus (EBV) [14]. Unfortunately, HSCTs are limited in their benefits to provide a cure for HIV as HSCTs are hard to scale for populations due to difficulty in finding donor matches. In addition, the procedure has risks such as opportunistic infections and immunosuppression [15]. While the two individuals who are cured of HIV are a cause for celebration, the recurring success of an HIV cure through HSCTs indicates that we should move forward towards a sterilizing or functional cure through other methods for purposes of scalability and efficiency.

MOLECULAR MECHANISMS OF HIV LATENCY

Acute onset of HIV replication in susceptible target cells is followed by the rapid depletion of $CD4^+$ T cells, which indicates the existence of HIV infection [16]. As a result of the rapid replication, high viral plasma loads are apparent [16]. There are three hallmarks for an HIV-infected individual: acute infection, clinical latency (also known as chronic HIV infection), and AIDS diagnosis [17]. Several genes are required for HIV replication and transcription in host immune cells to necessitate its survival; these genes include structural (*gag*, *pol*, and *env*), regulatory (*tat* and *rev*), and accessory (*vif*, *vpr*, and *nef*), which are flanked by two long terminal repeats (LTRs) at the 5' and 3' end. HIV transcription is further promoted through strict control of several mechanisms that target the Tat-TAR complex *via* viral host factors such as P-TEFb or cyclin T1 (CycT1)/cyclin-dependent kinase 9 (CDK9). During infection, clinical latency can be established

CHAPTER 2

HIV-1 Genotypic Drug Resistance Testing and Next-Generation Sequencing

Binhua Liang[1,2,*], Melanie Murray[3,4], Raghavan Sampathkumar[1,5] and **Ma Luo[1,6]**

[1] *JC Wilt Infectious Disease Research Center, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Canada*

[2] *Department of Biochemistry & Medical Genetics, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada*

[3] *Division of Infectious Diseases, Department of Medicine, University of British Columbia, Vancouver, Canada*

[4] *The Oak Tree Clinic, British Columbia Women's Hospital, Vancouver, Canada*

[5] *ATPC, Regional Centre for Biotechnology, Haryana, India*

[6] *Department of Medical Microbiology & Infectious Diseases, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada*

Abstract: The emergence and spread of HIV drug resistance (DR) is threatening the global advances gained from antiretroviral therapy (ART) in suppressing HIV-1 infection and reducing AIDS-related morbidity and mortality over the last decade. Next-generation sequencing (NGS) has fundamentally altered the landscape of HIV-1 DR testing through widely and deep sequencing in a much more cost effective and rapid manner. NGS is improving our ability to understand, diagnose, and prevent HIV DR by accurately identifying low abundant (< 20%) HIV DR variants (LADRVs) relevant to ART outcomes. NGS has been increasingly adopted by research and clinical laboratories for research, surveillance, and clinical monitoring of HIV DR in the last decade. However, NGS faces a number of limitations in its application of HIV DR testing, including sequencing error management, standardization of NGS procedures and instruments, external quality assurance of laboratories, computational and bioinformatics challenges. In this chapter, we will review the HIV-1 genotypic DR testing methods with the focus on the main NGS platforms available for HIV-1 DR diagnosis, their characteristics, applications, and limitations. In addition, we will systematically review LADRV in its distribution, prevalence, mechanism, and impact on ART outcomes. In the end, we will review the host factors, including the human leukocyte antigen (HLA), which effects the efficacy of ART.

* **Corresponding author Binhua Liang:** JC Wilt Infectious Disease Research Center, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Canada; Tel: 1-204-789-2039; E-mail: ben.liang@canada.ca

Prof. Atta-ur-Rahman (Ed.)

Keywords: Allele-specific assays, Antiretroviral therapy, Drug resistance, HIV-1, Low abundant HIV-1 drug-resistant variants, MiSeq, Next-generation Sequencing, Roche 454, Sanger Sequencing.

HIV-1 DRUG RESISTANCE TESTING

INTRODUCTION

With the availability of numerous antiretroviral (ARV) medications from a variety of classes and the standard use of combined antiretroviral therapy (ART) for human immunodeficiency virus (HIV), there are now multiple ARV regimen options available for the treatment of persons living with HIV (PLWH). However, resistance to ARVs remains a barrier to obtaining the 90-90-90 targets set forth by UNAIDS [1]. Understanding why HIV resistance emerges, and how best we can detect and manage it, is an important step forward in ending the HIV epidemic.

Resistance to ARVs emerges as a result of the fact that HIV couples a high replication rate (approximately 10^{10} virions per day) with a low fidelity reverse transcriptase enzyme (2.6×10^{-4} errors/base) [2] to produce an immense variety of viral variants each day [3]. Because of these extraordinary rates, low frequency drug resistant viral variants are already present prior to the start of therapy. In this setting, any situation whereby an antiretroviral drug is present at the same time as replicating HIV may result in the selection and amplification of viral variants resistant to the drug(s) present. Examples of this include the step-wise introduction and use of monotherapy/dual therapy with ARVs as they became available in the late 1980s and 1990s, or for the prevention of mother to child HIV transmission [4, 5], the presence of pretreatment HIV drug resistance [6 - 8], and imperfect adherence to ART [9, 10]. ARV resistance to one or more drugs thus remains fairly common. In addition, the lack of access to drug resistance testing, with reported testing rates of 0-2% in developing nations, has added to the emergence of drug resistance in patients who have started on standard therapies in the setting of pretreatment drug resistance (DR), and subsequently, failed these regimens [7].

Standard HIV drug resistance testing (genotyping) is usually carried out using standard Sanger sequencing techniques capable of detecting virus variants comprising greater than or equal to 20% of the HIV viral population present in the individual [11]. With the exception of the cited study by Taffa *et al* [12] which uses an allele-specific assay, studies presented herein represent results from in-house or kit-based population-based Sanger sequencing methods.

Rates of ARV Drug Resistance

Resistance of the HIV virus to ARV therapy may be classified into two forms. Resistance transmitted from one person to another at the time of infection is known as transmitted drug resistance (TDR), while resistance to ARVs that arises at any point after infection, usually as a result of exposure to ARV drugs is known as acquired drug resistance (ADR). In this chapter, "baseline" resistance testing will refer to any testing done prior to initiation of ARVs.

ARV drug resistance testing is an important part of clinical care. When done during acute infection or when infection is recent, resistance testing may identify cases of TDR and thus allow physicians to start patients on a fully active ARV regimen. When done before therapy initiation, but after infection has been present for many months to years, resistance testing may still be useful in identifying TDR, through predominant virus may by this time have reverted to wild type.

Later, testing identifies ADR in those individuals for whom therapy has failed and guides next line ARV regimen selection. A 2015 study by Baxter *et al*. examined baseline drug resistance testing at several international sites. Testing rates were 0.1% in Africa and 1.8% in South America.

In contrast, testing frequencies were much higher in resource-rich settings, ranging from 81.3% in the United States (US) to 86.7% in Europe and 89.9% in Australia [7].

Resource-Rich Settings

Studies from resource-rich settings examining TDR by Sanger sequencing have demonstrated pre-treatment rates of resistance varying between 6.6% and 19.4% depending on study date and cohort [13 - 18]. Transmitted resistance was mainly toward nucleos(t)ide reverse transcriptase inhibitors (NRTI's) 0.9-9.5% and/or non-nucleoside reverse transcriptase inhibitors (NNRTIs) 1.9-4.5%, while resistance to protease inhibitors (PIs) was less frequent ranging from 0.4-2.7% [13, 15, 16, 19, 20]. Only one of these studies reported resistance to integrase inhibitors (INSTI) with 1/461 patients, exhibiting a major integrase strand transfer inhibitor (INSTI) mutation [17]. In some resource-rich settings, rates of TDR decreased over time, while in others, rates have stayed the same. Specifically, in the large European CASCADE cohort study, the estimated TDR prevalence was 19.4% in 1996, a number that decreased significantly to 8.5% by 2012 [13]. Similarly, in a study from the United Kingdom by Tostevin *et al*. [14], TDR decreased over time from 8.1% in 2010 to 6.6% in 2012 (p=0.02), while a study by D'Costa *et al*. [17] from Australia reported TDR rates of 11.4% from 2005-

CHAPTER 3

Current and Promising Multiclass Drug Regimens and Long-Acting Formulation Drugs in HIV Therapy

Murugesan Vanangamudi[1,*], Gnana Ruba Priya[2] and **Maida Engels[3]**

[1] *Amity Institute of Pharmacy (AIP), Amity University Madhya Pradesh (AUMP), Maharajpura, Gwalior, Madhya Pradesh 474005, India*

[2] *Pharmaceutical Chemistry Department, College of Pharmaceutical sciences, Dayananda Sagar university, CD Sagar building, Shavige Malleshwara Hills, Kumaraswamy Layout, Bangalore-560078, Karnataka, India*

[3] *PSG College of Pharmacy, Peelamedu, Coimbatore - 641004, Tamil Nadu, India*

Abstract: In HIV-1 therapies, dual-drug and triple-drug combinations of antiretroviral therapy (ART) have radically improved the prognosis of HIV-1 infected people. The clinical usage of drug combinations has established high efficacy with constant viral load repression, saving T cells, and low adverse drug reactions compared to mono drug therapy treatment and thus has drawn intensive attention from researchers and pharmaceutical enterprises for HIV treatment and prevention. The switching of antiretroviral regimens from one combination to another is relatively easier for patients experiencing adverse effects or drug toxicities or requesting modification or simplification of their regimen. In addition, the choice of the combination regimen reduces viral resistance drastically when compared to the mono drug regimen. Several two-and three-drug complete regimens like Delstrigo, Complera, Stribild, Dovato, Juluca, *etc.*, were approved by the U.S. Food and Drug Administration (FDA) for the treatment of HIV-1 infection in adults and children. Multiclass combination drug regimens, which include varied classes of antiretroviral agents that work by interrupting the two or more enzymes required for the life cycle of HIV replication, have proved effective in the treatment of HIV infections, resulting in the approval of novel combination regimens for antiretroviral therapy. For complete virologic inhibition, antiretroviral combination regimens should include at least two or preferably three active drugs from two or more classes of nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs or NtRTIs), Protease inhibitors (PIs), and Integrase Strand Transfer Inhibitors (INSTIs). At present, several researchers are focused on developing newer, long-acting formulations of different classes of mono-and dual-antiretroviral drugs. Recently, the first and only complete long-acting regimen of extended-release injectable suspension of cabotegravir and rilpivirine by intramuscular gluteal was approved once monthly for HIV treatment by the FDA.

[*] **Corresponding author Murugesan Vanangamudi:** Amity Institute of Pharmacy (AIP), Amity University Madhya Pradesh (AUMP), Maharajpura, Gwalior, Madhya Pradesh 474005; India; Tel: +91-8555984792, E-mail: vmurugesann@gmail.com

Prof. Atta-ur-Rahman (Ed.)

Several antiretroviral drugs are under investigation in preclinical and clinical studies through various formulations, such as implants, injectables, intravenous, and subcutaneous. This book chapter aims to summarize the currently available multiple drug combinations information and the development of long-acting antiretroviral drugs for HIV treatment and prevention in the last two decades.

Keywords: AIDS, Antiretroviral therapy, HIV, Long-acting therapy, Three-drug combination therapy, Two-drug combination therapy.

INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) is caused by a human retrovirus, Human immunodeficiency virus (HIV), and has been considered a deadly disease since its discovery in 1983 [1]. To date, 79.3 million people have been infected by the virus globally, with around 36.3 million deaths. The demographic data shows about 0.7% of the currently infected patients fall in the age group of 15–49 years, with a varying burden of the epidemic observed in different regions around the globe. According to the UNAIDS report, in 2020, 37.7 million people were living with HIV. Among them, 36 million were adults, and 1.7 million were children, indicating these infections are a major threat to humans all over the world. In 2020, 20.7 million people had HIV (54%) in Eastern and Southern Africa, 4.9 million (13%) in Western and Central Africa, 5.8 million (15%) in Asia and the Pacific, and 2.2 million (6%) in Western and Central Europe and North America. Young adults in the age group of 15-24 and women are at a higher probability of risk. There are approximately 4,500 new infections every day arising among adults and children. The human immune system weakens after 10–15 years of infection due to the entry of HIV into the host cell followed by replication with the help of the host cell mechanism and ultimately killing the T-cells, giving way to opportunistic infections such as tuberculosis, pneumonia, herpes simplex, kaposi’s sarcoma, and coccidioidomycosis. The devastated CD4+ T-cells are the characteristic feature of AIDS that leads to the death of a patient infected with HIV [2]. HIV is effectively transmitted from one individual to another individual by sexual contact that takes place on the mucosal surfaces of the anus, rectum, vagina, and semen secretions containing the HIV. Pneumocystis pneumonia, cachexia in the form of HIV wasting syndrome, and esophageal candidiasis are the most frequent early symptoms occurring in AIDS patients [3, 4]. Sometimes, viral-induced cancers like Kaposi's sarcoma, Burkitt's lymphoma, primary central nervous system lymphoma, and cervical cancer develops among patients with AIDS [5 - 7].

Although there have been intense efforts towards the development of drugs used effectively for the treatment of HIV infections, several drugs fail to function due to acquired mutations in the virus and the development of resistant strains. To

overcome viral mutations and drug resistance, combination treatment regimens called combined antiretroviral combination therapy (cART) are being explored. For effective treatment of resistant strains, several promising clinical trials are currently in progress. One of the rational approaches to effectively combating resistant strains of HIV is by using the structural information of the target protein. Through the knowledge of the binding pocket and the important amino acid residues at the HIV target site, drugs could be designed effectively to treat mutant strains that have resistance to routine drug therapy.

Key Drug-Targets in Blocking the HIV Replication Cycle

Genomic Information

HIV-1 belongs to a *lentivirus* from the family of *Retroviridase*. It consists of approximately 9800 base pairs and is flanked by long terminal repeats (LTR). The genes of HIV are present in the central region and encode nine functional genes, which can be classified into three structural proteins, Gag, Pol, and Env; the two regulatory proteins, Tat and Rev; and four accessory proteins, Vpu, Vpr, Vif, and Nef. Among the structural proteins, the Gag polyprotein is further processed into six protein domains, which include matrix (MA), capsid (CA), nucleocapsid (NC), and p1, p2, and p6 spacer peptides. The genomic information of protease (PR), reverse transcriptase (RT), and integrase (IN) is derived from the proteolytic processing of Gag-Pol. The *Env* gene contains a signal peptide (SP), gp120, and gp41 [8].

Replication Cycle of HIV

The development of mature viral and multiplication are carried out at various stages in the replication cycle of HIV (Fig. **1**). The early replication cycle starts from the outside membrane viral envelope proteins such as glycoproteins, gp120 and transmembrane gp41, protein binding towards the C-C chemokine receptor type 5 (CCR5 or R5) or CXCR4 co-receptor in T-lymphocyte cells. The interface of the viral and host cell proteins fuse and further allows the entry of the viral nucleocapsid into the host cell and liberates the RNA strands along with the key enzymes such as reverse transcriptase (RT), integrase (IN), and protease (PR) for replication. RT is one of the crucial enzymes responsible for the reverse transcription of the retroviral single-stranded RNA genome into viral double-stranded DNA (dsDNA) at the polymerase and ribonuclease H domains. Consequently, dsDNA as proviral DNA integrates into the host genome by the viral integrase enzyme. Once the cell gets activated, it stimulates the transcription of pro-viral DNA into viral messenger RNA, commanding the cell to start producing the new building blocks for long-chain HIV proteins. Assembly occurs at the cell membrane and generates an immature virus that buds into the

CHAPTER 4

Role of Nanotechnology in HIV Diagnosis and Prognosis

Sai Akilesh M[1], Ashish Wadhwani[1,2,*] and **Manas Mandal[3]**

[1] *Department of Pharmaceutical Biotechnology, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Ooty - 643001, Nilgiris, Tamil Nadu, India*

[2] *Faculty of Health of Sciences, School of Pharmacy, JSS Academy of Higher Education and Research, Vacoas-Phoenix, Mauritius*

[3] *College of Pharmacy, Roseman University of Health Sciences, Henderson, NV 89014, USA*

Abstract: The diagnosis and prognosis of HIV (human immunodeficiency virus) infection has been revolutionized through advances and development in the field of nanotechnology. Since its onset in the early 1980s, HIV has gradually attained *'pandemic'* status and increased the need for efficacy in the rapid identification and treatment of AIDS (*Acquired Immunodeficiency Syndrome*). Despite the triple-drug therapy initiated by the next decade in the 1990s, the number of affected individuals increased to 2.8 million, and developing countries faced this crisis on multiple fronts. Today, a range of antiretroviral drugs are available with reduced toxicities and improved pharmacokinetic and pharmacodynamic profiles, along with improvement in diagnostic tools and kits, which have been made possible largely due to the advancement of nanotechnology. We have divided this chapter into the following three sections:

• HIV pathogenesis

• Nanotechnology and the need for innovation

• Role of nanotechnology in HIV diagnostics, drug delivery, and therapy

In section I, the disease characteristics of HIV infection and the viral life cycle are discussed, and the possible target sites for therapeutic intervention are also assessed. The second section delves into the basics of nanoscience and the myriad of possibilities that it offers. The pros and cons of nanotechnology-based therapeutics, along with the need for newer, rapid, and realistic approaches to tackle HIV infection, are explored. Section III examines the various advancements and trends in the diagnosis of the disease condition through nanotechnology-based applications, materials, and tools. This section then progresses to the critical aspects of drug delivery and therapy and

[*] **Corresponding author Ashish Wadhwani:** Faculty of Health of Sciences, School of Pharmacy, JSS Academy of Higher Education and Research, Vacoas-Phoenix, Mauritius, Tel:+230 57130426; E-mails: dradwadhwani@jssuni.edu.in, adwadhwaniad@gmail.com

Prof. Atta-ur-Rahman (Ed.)

concludes by outlining the potential for the development of future nano-based antiretroviral therapies.

Keywords: Antiretroviral Drug Development, HIV, Infectious Diseases, Nanomedicine, Nanotechnology, Viruses.

INTRODUCTION

Infectious diseases predominantly of viral origin have been detrimental for many centuries; HIV (*Human Immunodeficiency Virus*) grew into a pandemic during the 1990s, leading to serious complications on multiple fronts, such as healthcare and socio-economic, ultimately affecting the quality of life of the people. HIV primarily targets white blood cells, lowers the $CD4^+$ T cell count to a critical level, and eventually progresses to the clinical stage of AIDS (*Acquired Immunodeficiency Syndrome*). AIDS impedes effective immune response, thereby leading to the vulnerability of opportunistic infections and cancer. Therefore, without therapeutic interventions, HIV infection rapidly progresses to AIDS and causes death.

Initial descriptions and reports of the HIV-1 (Human Immunodeficiency Virus type I) were provided in 1983 [1 - 3] and HIV-2 in 1986 [4]; these two types of virus have since been identified as the primary cause of AIDS. Approximately, 38 million people across the globe were affected by HIV/AIDS in 2019, and an estimated 1.7 million individuals worldwide acquired HIV infections in 2019, marking a 23% decline in new HIV infections since 2010 (Fig. **1**).

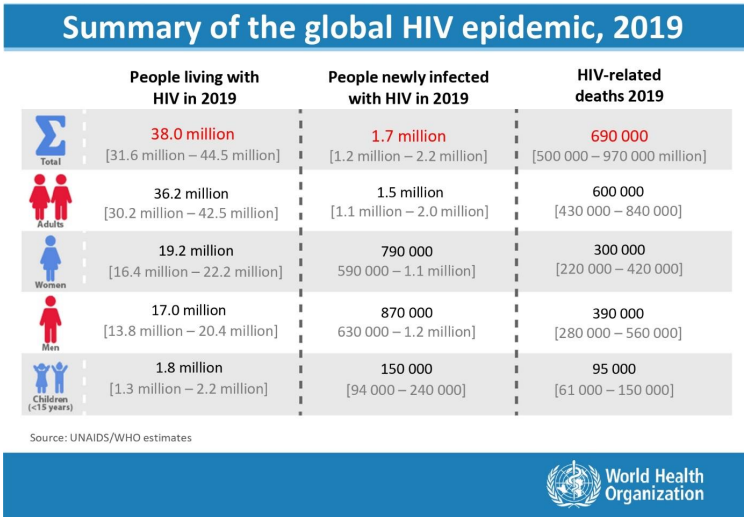

Fig. (1). Global HIV Epidemic (WHO Report - 2020).

The availability and accessibility of HIV testing, diagnostic devices, and medicines amongst the vulnerable population and communities is a key objective to eradicate HIV and AIDS in the long term. Only 81% of people infected with HIV globally knew their HIV status in 2019, and the remaining 19% (about 7.1 million people) still need access to HIV testing services. As of the end of 2019, 25.4 million people with HIV (67%) were accessing antiretroviral therapy (ART) globally, which leaves a whopping 12.6 million people still awaiting any kind of therapeutic interventions and care. Due to the advancements in HIV care and therapy, AIDS-related deaths have been reduced by 60% since their peak in 2004 [5].

Currently, there is no cure for HIV, but treatment with antiretroviral therapy is capable of preventing the rapid advancement of AIDS. HIV medicines significantly prolong the expected lifespan of the infected population. However, studies and clinical observations over the many years have indicated the presence of HIV in other organs of the body, besides cells of the immune system [6]. Also, the emergence of resistant virus strains is a major challenge to the containment of the disease. It is reported that 5%-78% of the patients infected with HIV-1 and receiving antiretroviral therapy are resistant to at least one of the currently available antiretroviral drugs. Thus, there is a need for newer antiretroviral agents to overcome the drug resistance and target different stages of the viral lifecycle [7]. Fig. (**2**) shows the global population of newly infected individuals with a projected decreasing target through the year 2030.

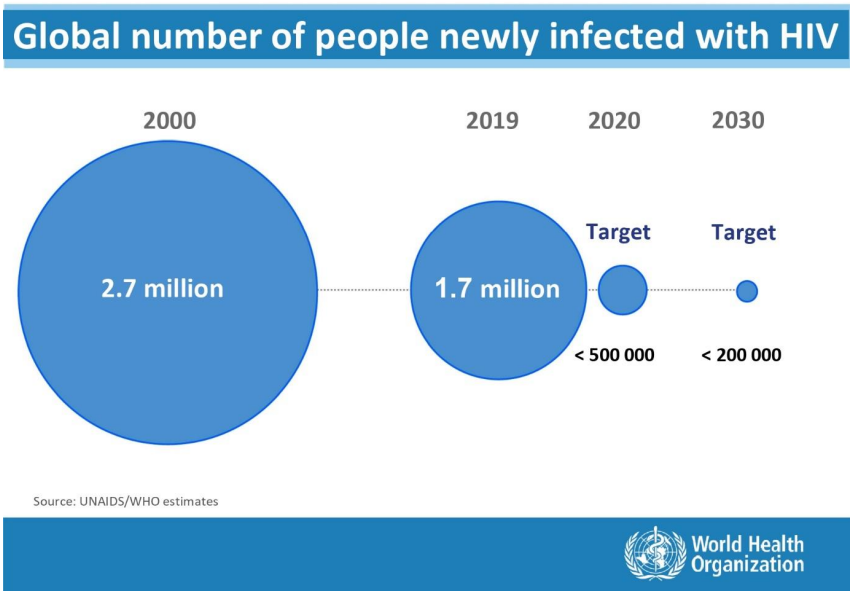

Fig. (2). Number of individuals affected with HIV Globally.

CHAPTER 5

Preventive and Therapeutic Features of Combination Therapy for HIV

Sumera Zaib[1,*], Nehal Rana[1], Areeba[1] and **Imtiaz Khan[2,*]**

[1] *Department of Biochemistry, Faculty of Life Sciences, University of Central Punjab, Lahore-54590, Pakistan*

[2] *Manchester Institute of Biotechnology, The University of Manchester, 131 Princess Street, Manchester M1 7DN, United Kingdom*

Abstract: The human immunodeficiency virus (HIV) is a retrovirus characterized by a reverse transcriptase enzyme and is known for causing acquired immune deficiency syndrome (AIDS), a chronic condition with progressive failure of the immune system. HIV poses a major health issue globally, infecting cells containing $CD4^+$ and CCR5 or CXCR4 receptor sites, *i.e*., T-lymphocytes, macrophages, monocytes, and dendritic cells. HIV infects the T-lymphocytes by suppressing the immune system leading to several pathogenic infections, thus critically demands healthy measures to strengthen the immune system. For this purpose, diverse classes of drugs have been developed that effectively decrease the viral load in the patients, inhibiting the replicative cycle of HIV at a specific point. These specific inhibitors (drugs) may include inhibitors of entry/fusion, protease, nucleotide reverse transcriptase, non-nucleotide reverse transcriptase, and integrase. This chapter provides an overview of the drugs used to treat HIV, their mechanism of action and side effects, as well as their dosage recommended for the treatment of HIV. Notable examples are zidovudine, abacavir, lamivudine, didanosine, tenofovir, stavudine, emtricitabine, nevirapine, delavirdine, efavirenz, etravirine, dolutegravir, bictegravir, raltegravir, cobicistat, indinavir, ritonavir, nelfinavir, saquinavir, darunavir, atazanavir, lopinavir, tipranavir, fusion inhibitors (enfuvirtide) and chemokine CCR5 receptor antagonist. These drugs are administered to HIV patients throughout their life mostly as a combination therapy as HIV can become resistant to these drugs after some period. Additionally, these drugs have several side effects such as nausea, dizziness, liver diseases, kidney disorders, and heart diseases. Many of the above-mentioned side effects are temporary and are resolved spontaneously. However, some of these (hepatic, renal, or cardiac failure) can lead to the death of the patient. Another drawback of antiretroviral drugs is their latency for HIV and its reactivation. By determining and controlling all the factors that regulate the gene expression of HIV, such as HSP90, required for HIV gene expression, reactivation of HIV can be stopped from latency. Moreover, the latency of

* **Corresponding author Sumera Zaib:** Department of Biochemistry, Faculty of Life Sciences, University of Central Punjab, Lahore-54590, Pakistan; Tel: +92 (42) 35880007; E-mail: sumera.zaib@ucp.edu.pk and **Imtiaz Khan:** Manchester Institute of Biotechnology, The University of Manchester, 131 Princess Street, Manchester M1 7DN, United Kingdom; Tel: +44 (0) 161 306 500; E-mail: imtiaz.khan@manchester.ac.uk

Prof. Atta-ur-Rahman (Ed.)

HIV can also be controlled by studying its mechanism, thus enhancing the effectiveness of antiretroviral treatment (ART). The development of plant-based drugs exhibiting improved inhibition of HIV replication compared to available antiretroviral drugs has also been reported.

Keywords: Antiretroviral drugs, Antiretroviral treatment, Cardiac failure, Human immunodeficiency virus, Macrophages, T-lymphocytes.

INTRODUCTION

Human Immunodeficiency Virus (HIV) is an enveloped retrovirus strongly associated with the acquired immunodeficiency syndrome (AIDS). The disease started in Africa and is now present across the globe, with 38.9 million infected people. As HIV is a member of the *Lentivirus* genus that belongs to the *Retroviridae* family, so, like all other retroviruses, it also contains a reverse transcriptase enzyme that was discovered by Baltimore and Temin in 1970 [1 - 4]. HIV contains a diploid single-stranded positive-sense RNA genome that encodes 2 envelopes, 3 structural, 6 accessory proteins, and 3 enzymes inside the host cell [3, 5]. Based on the arrangement of the genome, HIV has two types, *i.e*., HIV-1 (genomic organization represented in Fig. (**1a**) and HIV-2, and the former one is more prevalent and virulent and is transmitted by sexual contact, body fluids, mother to a child when mother viral load is high, and by contaminated syringes or other surgical instruments [3, 6]. On entering the host, the virus mainly infects the cells that express $CD4^+$ receptors on their cell membranes, such as T-lymphocytes, macrophages, and dendritic cells. When HIV infects and replicates inside T-lymphocyte, it causes the death of T-lymphocytes resulting in the decline of immune functions, and consequently, many opportunistic infections can occur to the patient. HIV can also infect brain cells where it can cause encephalopathy [7].

HIV patients can be treated by antiretroviral therapy (ART), which includes drugs that are given in various combinations (two, three, or four). These nucleic acid aptamers target the HIV-1 genome and several proteins like Gag, Tat, Rev, integrase, and reverse transcriptase, *etc* [1] (Fig. **1b**). As ART is given to the patients throughout their life so primary infection due to HIV is reduced to a greater extent, but the threat that develops is the enhanced risk of secondary infections due to the associated side effects caused by the long-term use of antiretroviral drugs [6]. These toxic effects include heart diseases, renal infections, and nervous disorders that are the cause of death among many HIV patients [9].

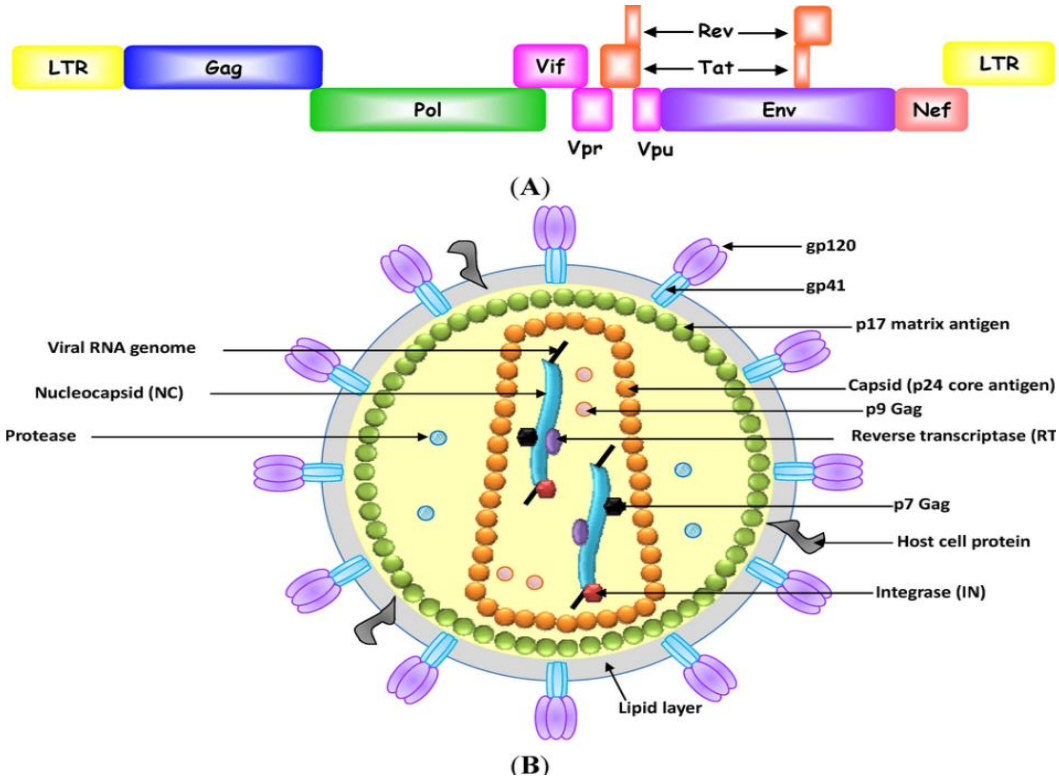

Fig. (1). a) Genomic organization of HIV-1; **b)** virion of HIV-1 and the potential targets. Reproduced from an open-access source [8].

Replication Cycle of HIV

Here is a brief description of the replicative cycle of HIV that will help us to understand the mechanism of action of each drug used to treat HIV patients.

Attachment and Entry

The first step in replication is the binding of HIV with $CD4^+$ receptor that is present on the cell surface of monocytes, macrophages, lymphocytes, microglial cells of CNS, and dendritic cells. Under normal conditions, $CD4^+$ receptors are used for the recognition of any foreign antigen by T-cells, but the same receptors can also serve as a portal of entry into the host cell for HIV. As HIV is an enveloped virus, so its lipoprotein envelope is basically responsible for its attachment to the receptor site on the host cell surface by utilizing envelope proteins like gp (glycoprotein) 120 and gp41 [10]. First of all, gp120 attaches to the $CD4^+$ receptor, and because of this binding, the viral envelope undergoes a change that actually refers to the change in the gp120 resulting in the exposure of a specific binding site allowing gp120 to glue with chemokine receptors. So far,

SUBJECT INDEX

A

Accessing antiretroviral therapy 142
Acetylatransferase 7
Acid, nucleic 45
Acidosis 102
Acquired 1, 2, 4, 5, 9, 96, 41, 42, 43, 73, 140, 141, 142, 147, 148, 149, 150, 167, 175, 176
  drug resistance (ADR) 9, 41, 42, 43, 73
  immune deficiency syndrome (AIDS) 1, 2, 4, 5, 96, 140, 141, 142, 147, 148, 149, 150, 167, 175, 176
AIDS-related 54, 99
  morbidity 54
  mortality 99
Alzheimer's disease 16
Amplicon variant analyzer (AVA) 52
Antigen-presenting cells (APCs) 146
Anti-retroviral therapy (ART) 1, 2, 8, 9, 15, 17, 18, 22, 39, 40, 43, 56, 58, 69, 70, 72, 73, 148, 161, 176
Antiviral therapy 6, 158
Apoptosis 6, 20, 21
ART 16, 161
  drugs and nanotechnology 161
  suppressed HIV-infected individuals 16
  therapy 164, 167
ARV 41, 43, 49, 69
  regimens 43, 49, 69
  therapy 41
Assays, radio-immunoblot 148
Atazanavir-based antiretroviral therapy 188

B

Bioinformatics
  analysis 76
  platforms 67
  skills 67
  strategies 66, 67
Biopolymer-based nanocomposites 152
Blood 52, 104, 141, 150, 164, 187
  brain barrier (BBB) 164
  cells, white 141
  plasma 150
  specimens 52
  sugar, high 187
  triglycerides 104
Bone marrow 22
Bovine immunodeficiency virus (BIV) 143
Burkitt's lymphoma 96

C

Cancer immunotherapy 155
Cancers 19, 96, 141, 147, 148, 155, 156
  cervical 96
  metastatic breast 155, 156
  viral-induced 96
Canonical NF-κB signaling pathway 14
Caprine arthritis encephalitis virus (CAEV) 143
Cardiac disorders 118
Cardiovascular risk factors 115
Charge-coupled device (CCD) 60
Cholelithiasis 104, 107
Combination 9, 10, 12, 14, 16, 18, 20, 24, 26, 97, 114, 116, 175, 180, 185
  antiretroviral therapy 114
  therapy 9, 10, 12, 14, 16, 18, 20, 24, 26, 97, 116, 175, 180, 185
Conduction system disorders 119
COVID-19 pandemic 166
Creatinine 120
Crotonylation, histone lysine 5
CTL-mediated therapy 26
Cyclin-dependent kinase 3, 9
Cytotoxicity 9, 10, 25, 164
Cytotoxic T lymphocytes (CTL) 21, 25, 69, 147

D

Dendritic cells (DCs) 6, 146, 175, 176, 177, 195
Detection 2, 6, 18, 25, 60, 154, 158, 159, 160, 161, 166
  electrochemical 159
  fluorescence-based 160
  fungal 6
Disease 3, 96, 100, 106, 114, 142, 149, 151, 152, 156, 157, 160, 165, 167, 176, 180, 183, 186, 189, 195
  cardiac 195
  cardiovascular 180, 183
  chronic kidney 186
  deadly 96
  graft-versus-host 3
  kidney 114
  neurological 189
  renal 106, 183
Dizziness 100, 101, 103, 105, 106, 110, 111, 113, 175, 182, 185, 190
DNA 4, 9, 11, 47, 48, 49, 51, 68, 160, 161, 165, 178, 182, 186
  amplified 51
  based assay 49
  cleaving nuclease 11
  co-receptor tropism assay 48
  data bank of Japan (DDBJ) 47
  extraction 68
  hybridisation 160
  methylation 9
  non-complementary 51
  repair mechanism 178
  synthesis 182
Drug(s) 70, 71, 72, 73, 99, 118, 161, 180
  antipsychotic 118
  anti-retroviral 70, 71, 161, 180
  combination therapies 99
  hypersensitivity 70, 73
  induced liver injury (DILI) 72, 73
Drug resistance (DR) 2, 39, 40, 43, 45, 46, 49, 50, 55, 63, 126, 129, 150, 156
  genotyping assay 50
  mutations 49

E

Effector-memor--transition (EMT) 7
Electrochemical
  impedance spectroscopy 160, 161
  Impedance Spectroscopy (EIS) 160, 161
ELISA, double-antigen sandwich format 149
Encephalopathy 195
Enzyme(s) 14, 127, 148
  linked immunosorbent assay 148
  metabolic 127
  protein kinase 14
Epstein-Barr virus (EBV) 3
Esophageal candidiasis 96

F

Failure 16, 55, 120, 175, 176
  cardiac 175, 176
  mitochondrial replication 184
  renal 120
Fatal hypersensitivity reactions 106, 107
Fatigue 111, 116, 182, 183, 185, 186, 187, 189, 190
Feline immunodeficiency virus (FIV) 143
Fever 100, 106, 111, 147, 183, 184, 186, 187
Fluorescence imaging 60
Food and drug administration (FDA) 95, 105, 106, 115, 116, 118, 128, 129, 151, 153, 155, 156, 185
Function 6, 46, 96, 103, 117, 145, 150, 151, 161, 163, 164, 166, 176, 181, 187, 188
  abnormal liver 187
  biomedical 166
  immune 176
  immunomodulatory 145
  impaired renal 103
  renal proximal tubule 117
Fusion process 145

G

Glomerular filtration rate (GFR) 188
Graft-versus-host disease (GVHD) 3
Gut-associated lymphoid tissue (GALT) 1, 165

H

HAART Therapy 149
Health and human services (HHS) 99, 120
Heart diseases 115, 175, 176, 183, 191
  coronary 115
  ischemic 115
HeLa-transfected cells 10
Hematopoietic stem cell (HSC) 3
Hemodialysis 119
Hepatic 100, 116, 193
  disorders 193
  dysfunction 116
  transaminases 100
Hepatitis B virus (HBV) 103, 107, 112, 113, 122, 182, 183
Histocompatibility 155
HIV 11, 12, 15, 21, 23, 40, 46, 63, 70, 71, 95, 99, 102, 103, 105, 128, 129, 140, 142, 143, 146, 148, 149, 156, 157, 160, 161, 167, 175, 180, 182, 183, 189, 193
  care and HAART therapy 149
  core protein 157
  Diagnostics and Nanotechnology 157
  disease 156, 157, 167
  drug resistance assays 46
  Enzymes 157
  expression 12, 15
  fusion inhibitor 193
  gene expression 175
  genome sequencing 70
  genotypic assays 63
  infected macrophages 21
  integrase resistance 105
  medicines 142, 146, 149
  multidrug resistance 189
  pathogenesis 23, 140, 143, 161
  protease 71, 160
  replicating 40
  reverse transcriptase enzyme 182
  sequence databases 46
  suppressing 183
  treatment 11, 95, 99, 102, 103, 128, 180, 182
HIV infection 2, 3, 8, 9, 11, 95, 96, 128, 129, 140, 141, 147, 148, 150, 157, 158, 165, 188, 189, 190
  asymptomatic 148
  treatment of 95, 96, 188, 189, 190
HIV-related 24, 148, 190
  cancers 24
  morbidity 190
  symptoms 148
HIV RNA 22, 127, 190
  levels 22
  reduction 190
  suppression 127
HIV-1 1, 2, 45, 46, 48, 49, 72, 99, 102, 103, 104, 142, 143, 145, 160, 177, 191, 193
  and receiving antiretroviral therapy 142
  drug resistance mutations 72
  envelope protein 48
  fusion inhibitors 193
  reverse transcriptase 49, 99
HIV-1 infection 39, 100, 101, 103, 105, 108, 109, 111, 112, 119, 122, 125, 127, 188
  multidrug-resistant 100
  suppressing 39
HLA-ART interaction database 75
HMGCoA reductase inhibitors 108
Human 5, 39, 69, 73, 102, 106
  HeLa cells 5
  leukocyte antigen (HLA) 39, 69, 73, 102, 106
Hybridization techniques 49
Hyperglycemia 105
Hyperhidrosis 185
Hyperlipidaemia 191
Hyperlipidemia 189, 190
Hypersensitivity reactions 72, 101, 102, 112, 113
  life-threatening 101, 102

I

IFN-stimulated genes (ISGs) 6, 23
Immature virus 97
Immune 22, 25, 100, 105, 106, 107, 108, 109, 112, 113, 114, 118, 142, 145, 147, 148, 149, 150, 154, 157, 162, 165, 167, 175, 185
  reconstitution syndrome 100, 105, 106, 107, 108, 109, 112, 113, 114, 118, 185
  system 22, 25, 142, 145, 147, 148, 149, 150, 154, 157, 162, 165, 167, 175
Implementing NGS-based assays 76
Infection 3, 7, 41, 96, 101, 102, 103, 104, 105, 111, 140, 147, 149, 165, 175
  eye 101
  pathogenic 175
Inhibitor(s) 1, 2, 4, 6, 8, 9, 13, 15, 17, 117, 119, 124, 150, 175, 180, 181, 184, 186, 189, 190, 191, 192
  of apoptosis (IAP) 6
  non-nucleotide reverse transcriptase 180, 184, 186
  nucleoside reverse transcriptase 2, 117, 150, 190
  of histone deacetylases 1, 4, 8, 13
  side reverse transcriptase 119, 124
  synthetic protease 191
Initiating abacavir therapy 72
Intact proviral DNA assay (IPDA) 2
Integrase 2, 41, 44, 48, 95, 99, 105, 118, 121, 123, 125, 150, 180, 183, 186, 187, 188
  enzymes 99, 186
  inhibitors 2, 41, 105, 123, 186, 187
  strand transfer inhibitors (INSTIs) 41, 44, 48, 95, 99, 118, 121, 125, 150, 180, 183, 186, 188

K

Kaplan-Meyer survival analysis 71
Kaposi's sarcoma (KS) 24, 96
Kidney disorders 175

L

Label-free electrochemical assay 160
Lactic acidosis 100, 102, 106, 107, 114, 119, 184, 191
LADRV 59, 62
  assays 59
  detection 62
Large unilamellar vesicles (LUVs) 193
Latency reversal agents (LRAs) 1, 4, 8, 12, 13, 19, 20, 21
Lipoatrophy 102, 107, 114
Lipodystrophy 102, 185, 189
Liposomes, pH-sensitive 155
Liver 114, 119, 175, 182, 186
  disease 114, 175
  enzymes 119
  infections 182
  injury 186
  problems 110, 182, 187, 193
Long terminal repeats (LTR) 3, 11, 97, 145
Lymphatic 144, 155
  drainage 155
  tissue 144

M

Machine learning techniques 46
Macrocytosis 184
Mechanism of action 162, 182
  of lamivudine 182
  of Metal Nanoparticles on Viruses 162
Mechanism of LADRVs 56
Medicines, traditional Chinese 17, 18
Membrane fusion processes 157, 163
Methods 49, 50, 64, 65, 159, 161, 164
  bioinformatics 64
  fluorometric 50
  microchip 161
  nano-based diagnostic 159
  nanotechnology-based 164
  restriction 65
  sensitive 49
Methylation, lysine 4

Methylergonovine 109
Mono drug therapy treatment 95
Monotherapy 102, 116, 185
Myocardial infarction (MI) 100, 106, 107

N

Nanoparticles 152, 154, 155, 158, 164, 166
  lactoferrin 164
  metal-based 152
  synthetic 166
Nasal congestion 182
Natural killer (NK) 6
Negative regulatory element (NRE) 5
Nephrolithiasis 104, 107, 191
Nervous disorders 176, 195
Neurological deficits 21
Neuropathy 184
Neutropenia 102, 107, 114, 116, 185
Newest gene-editing technique 12
Next-generation sequencing (NGS) 39, 40, 47, 52, 53, 54, 56, 59, 60, 62, 63, 64, 65, 67, 76
  technologies 59
NGS technologies 49, 55, 62, 63, 71
Non-nucleoside reverse transcriptase inhibitors (NNRTIs) 41, 42, 43, 44, 54, 55, 56, 58, 99, 101, 150, 184, 185, 186, 187
NRTIs in combination therapy 185
Nuclear factor-κB 8
Nucleocapsid 97, 145, 179, 180
Nucleoprotein 144
Nucleoside reverse transcriptase inhibitors (NRTIs) 2, 41, 42, 43, 44, 55, 58, 101, 105, 150, 164, 181, 184, 186, 190
Nucleotide sequence 60, 182

O

Oligonucleotide ligation assay (OLA) 49, 50, 51, 52, 62
Osteoporosis 102, 106, 114, 120

P

Pancreatitis 102, 103, 104, 114
Peptides 5, 10, 15, 18, 23, 147, 164, 165
  brain-targeting 165
  cells display HIV 147
Peripheral blood mononuclear cells (PBMCs) 5, 10, 15, 18, 23, 149
Personal genome machine (PGM) 63
PKC 1, 4, 14, 15, 17, 18, 19
  agonists (PKCa) 1, 4, 14, 15, 17, 18, 19
  pathway through phospholipase C (PLC) 14
Pneumocystis pneumonia 96
Pneumonitis and immune reconstitution syndrome 193
Population-based Sanger sequencing (PBSS) 40, 49, 53
Post-translational modifications (PTMs) 4, 5, 179
Pre-integration complex (PIC) 178
Progenitor cells 12
Properties 10, 16, 17, 155, 157, 158, 159, 163
  anti-cancer 10, 17
  anti-tumor 16
  antiviral 163
  catalytic 157
  electrical 159
Protease 41, 42, 44, 55, 58, 62, 65, 74, 97, 99, 104, 118, 122, 145, 146, 150, 157, 160, 175, 178, 179, 180, 189, 190, 192
  cleaves 98
  enzyme 189, 191
  inhibitors (PIs) 41, 42, 44, 55, 74, 95, 99, 104, 118, 122, 150, 180, 189, 190, 192
  viral 58
Protein(s) 4, 5, 6, 7, 8, 14, 15, 98, 99, 120, 143, 144, 145, 146, 149, 151, 158, 163, 176, 178, 179, 191, 193
  adhesion 144
  expression 5
  kinase 8, 14
  kinase C (PKC) 8, 14, 15
  synthesis 4, 7

urine 120
viral infectivity 145
viral surface 163
Proteosome 7
Proviral DNA 2, 12, 48, 65, 66, 97, 149
latent 48
stable 2

Q

Q-PCR 51, 52
amplification 51
assay 52
detection reaction 51
Quantitative viral outgrowth assay (QVOA) 2
Quiescent memory 2

R

Raltegravir therapy 127
Rash 100, 101, 103, 104, 106, 107, 111, 113, 114, 119, 120, 183, 184, 185, 187
Reactions 104, 105, 108, 109, 110, 120, 121, 182, 185, 187, 193
allergic 110, 120, 121, 182, 185, 187, 193
hypersensitive 185
life-threatening skin 104
severe skin 105, 108, 109
Real-time PCR assay, quantitative 51
Receptors 8, 19, 73, 98, 126, 154, 163, 166, 177, 178, 193
adaptive immune 166
chemokine 177, 178, 193
human cell surface 98
target cell membrane 163
targeting 8
Regimens 40, 45, 101, 103, 106, 114, 117, 120, 121, 122, 123, 124, 126
abacavir-lamivudine-zidovudine 121
efavirenz component 123
rilpivirine-containing 123
tenofovir-alafenamide-containing 124
Regulation, epigenetic 9
Rel homology domain (RHD) 6
Renal 100, 107, 108, 113, 116, 117, 118, 119, 124, 126
impairment 100, 107, 108, 113, 116, 118, 119, 124
proximal tubulopathy 116, 126
tubular cells 117
Reservoirs 1, 2, 26
cellular 1, 26
Resistance 48, 56, 95, 120, 190
genotypic 48, 56
multidrug 190
viral 95, 120
Resources, renewable 15
Retroviridase 97
Retroviruses 8, 167
and opportunistic infections 167
infectious 8
Reverse transcriptase (RT) 62, 64, 65, 66, 97, 98, 99, 144, 145, 146, 157, 175, 176, 178, 181, 183, 184, 185, 189
complex (RTC) 178
enzyme 99, 145, 175, 176, 178
nuclease 189
regions 62
Reverse transcription process 195
Rhesus macaques 10, 17, 22, 25
Ribavirin-based regimens 107
Ribonuclease 97, 146
RNA 11, 149, 158
assay 149
binding protein 158
induced silencing 11
interference (RNAi) 11
RNA polymerase 4, 10
II transcriptional machinery 4
RT 52, 62, 146
enzyme 146
genes 52, 62

S

Salvage 104, 107, 114, 127
antiretroviral therapy 114
therapy 104, 107, 127

Sentosa SQ 63
assay 63
HIV genotyping assay 63
Sequencing 49, 50, 65, 67
based genotypic assay 49
dideoxynucleoside cycle 50
error model 67
methods 65
Side effects 102, 125, 193
cobicistat-induced gastrointestinal 125
gastrointestinal 102
life-threatening 193
Side effects and toxicity of ART 69
Signal peptide (SP) 97
Silver nanoparticles interaction 163
Simian immunodeficiency virus (SIV) 17, 143
Single 102, 120, 121, 124, 125, 126, 160
frequency impedance measurement (SFIM) 160
tablet regimen (STR) 102, 120, 121, 124, 125, 126
Skin 18, 185, 186, 189
biopsies 18
diseases 185
lesions 18
rash 186, 189
Squamous cell carcinoma (SCC) 18
Stanford HIV database system 50
Steatosis 106, 107, 108, 109, 112, 113, 114, 118
Stevens-Johnson syndrome 107, 111, 114, 119, 187, 190, 191
Stimulator of interferon gene (STING) 1, 8, 23
Structure 144, 194
of HIV 144
of maraviroc 194
Suppressing viral replication 129, 151
Suppression 1, 12, 107, 118, 151, 161, 162, 167
hematologic toxicity/bone marrow 107
Surface 158, 159
enhanced raman spectroscopy (SERS) 159
plasmon resonance (SPR) 158, 159
Symptoms, reduced neuropsychiatric 122
Syndrome, acquired immunodeficiency 96, 140, 141, 176
Systems 45, 60, 61, 63, 127, 155, 157, 159, 164, 166, 185
central nervous 164
drug-delivery 127
electrochemical detection 159
nano-based drug delivery 157
nanoparticle delivery 166
nanoparticulate drug delivery 155
nervous 185

T

Tagged pooled pyrosequencing (TPP) 62, 64
Target 26, 50, 51, 97
DNA 50, 51
HIV-1 genes 49
HIV reservoirs 26
protein 97
Tat 3, 5, 6, 9, 10, 11, 97, 145, 158, 176, 178, 179
activating region (TAR) 5
expression 10
GFP in HeLa-transfected cells 10
in preclinical assays 10
proteins 10, 179
transactivation 5
transcription 6, 10
TDR by sanger sequencing 41
Tenofovir disoproxil fumarate (TDF) 58, 104, 105, 110, 111, 112, 113, 114, 117, 119, 120, 124, 126, 183
Therapeutic(s) 1, 11, 24, 25, 74, 75, 140, 143, 155
nanotechnology-based 140
treatment 11
vaccines 1, 24, 25, 74, 75
Therapy 23, 24, 40, 41, 43, 44, 45, 101, 117, 118, 119, 140, 142, 143, 161, 164
anti-cancer 23
immune 24
initiating 45
Three-drug combination therapy 96

Thymic organoid 22
Thymidine analog mutations (TAMs) 55
TNF signaling pathway 19
Toll-Like Receptors (TLRs) 6, 166
Total internal reflection fluorescence (TIRF) 60
Toxic epidermal necrolysis 114
Toxicity 11, 17, 22, 69, 103, 104, 105, 107, 122, 125, 143, 152, 155, 156, 166, 168, 184, 185
  cellular 11
  hematologic 107
  idiosyncratic skin 185
  minimal 105
  mitochondrial 184
  renal 122, 125
  systemic 156
Toxoplasmosis 189
Transcriptional 5, 9
  gene silencing (TGS) 9
  machinery 5
Transcription factors 9, 20
Transmitted drug resistance (TDR) 41, 42, 43, 46, 47, 72
Triple antiretroviral therapy 123
Triptolide's cytotoxicity 10
Tumor necrosis factor (TNF) 6, 19

V

Vaccines 23, 75, 155, 165, 166, 167, 168, 194, 195
  genome-based 166
  high-efficacy 166
  live attenuated viral 165
  prophylactic 75
Viral 2, 9, 57, 68, 69, 70, 98, 100, 122, 123, 124, 129, 143, 144, 145, 146, 147, 148, 157, 158, 163, 164, 165, 179, 186, 189, 190
  cytopathic effects 2
  infectivity factor 158
  like particles 179
  load suppression 190
  pathogenesis 147
  polyprotein precursor 189
  proteins 70, 144, 145, 157, 179
  replication 9, 57, 98, 100, 143, 146, 148, 157, 163, 186
  reservoirs 164
  silencing 9
  stress 129
  suppression 68, 69, 122, 123, 124, 165, 190
Viral DNA 98, 99, 182, 184, 186
  growth 182
Viruses, human immunodeficiency 69
Vomiting 102, 103, 104, 114, 117, 119, 183, 184

W

WHO 47, 167
  data 167
  guidelines 47
Whole-genome sequencing (WGS) 52
World health organization (WHO) 45, 50, 56, 68, 72, 149, 167, 168

X

Xanthomonas bacteria 11

Z

Zidovudine 116, 121
  monotherapy 116
  regimen 121
  therapy 121
Zinc-finger nucleases (ZFNs) 11, 12