

Literature Review

CHAPTER 1

1. 1 Overview of HIV/AIDS

Human Immunodeficiency Virus (HIV) is a human pathogenic virus that cause AIDS (Acquired Immunodeficiency Syndrome). HIV/AIDS has been global pandemic for over the last three decades and is depicted as the modern day plague (1). The United Nations Acquired Immune Deficiency Syndrome (UNAIDS) global report 2012 estimates that by the end of 2011 approximately 34 million people are living with HIV by the end of 2011 (WHO factsheet Number 360 (<http://www.who.int/mediacentre/factsheets/fs360/en/>)); over 95% of them are living in low and middle in come countries (2). There is a significant variation in HIV prevalence among the countries around the globe (Figure 1.1). UNAIDS 2012 report shows that although the global trend of new HIV infections and HIV-related deaths per year is declining, the current number of HIV infections is the highest since 1990 (Figure 1.2). The sub-Saharan region of Africa is the region most aggravated by the virus with 23.5 million people living with HIV (3). UNAIDS estimates that approximately 1 in every 20 adults is HIV infected in this region (3). This is 25 or more times the HIV prevalence in any other region of the world. Countries in Sub-Saharan Africa also have varying HIV prevalence with South Africa at the top followed by Nigeria (2). The next severely affected regions, besides African continent, are Asia (China, Thailand, Indonesia), Caribbean and Eastern Europe, North America, western and central Europe (2).

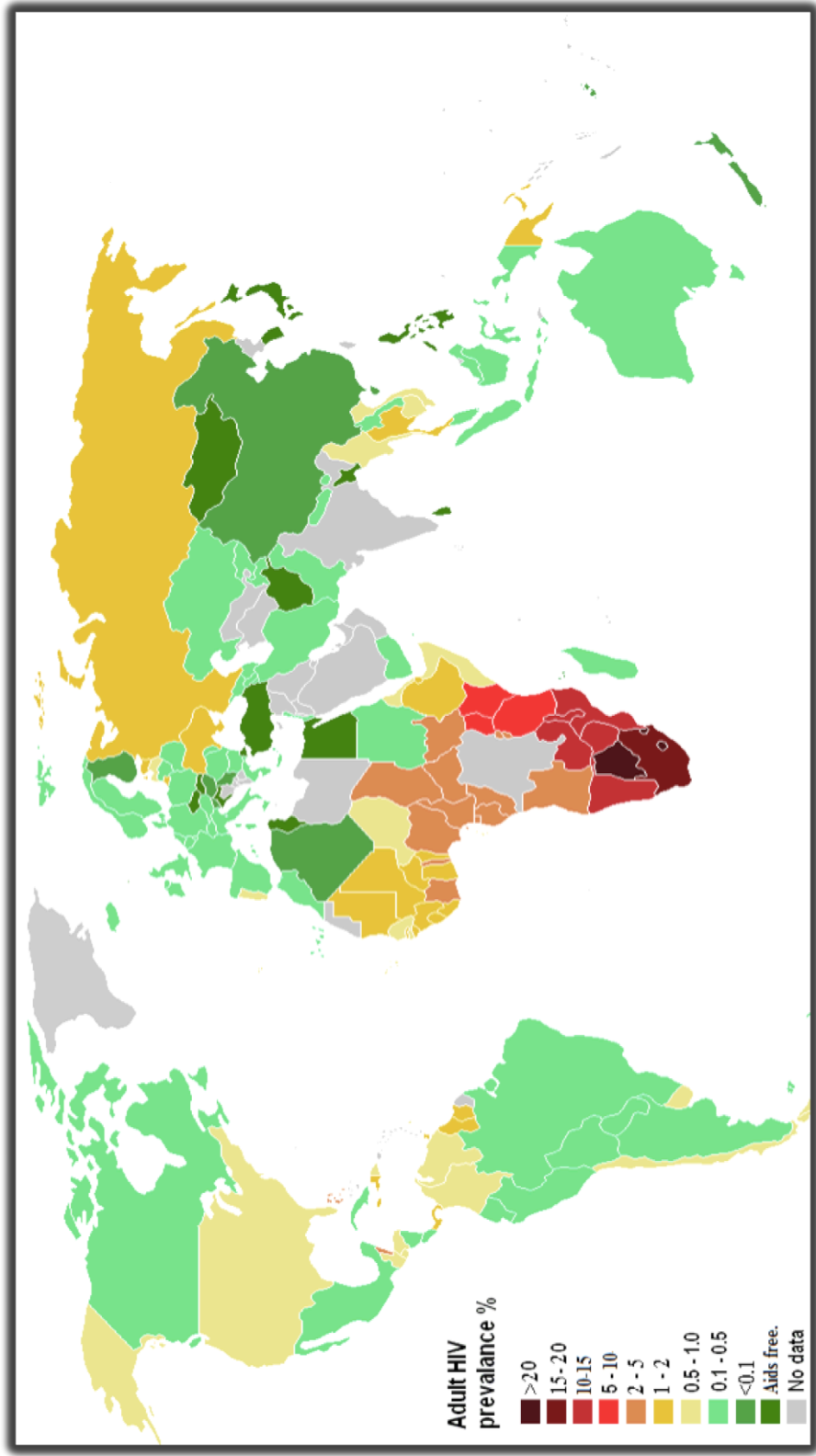


Figure 1.1: Distribution of HIV prevalence in adults around the world.

Source: <http://www.unaids.org/en/dataanalysis/datatools/aidsinfo/>

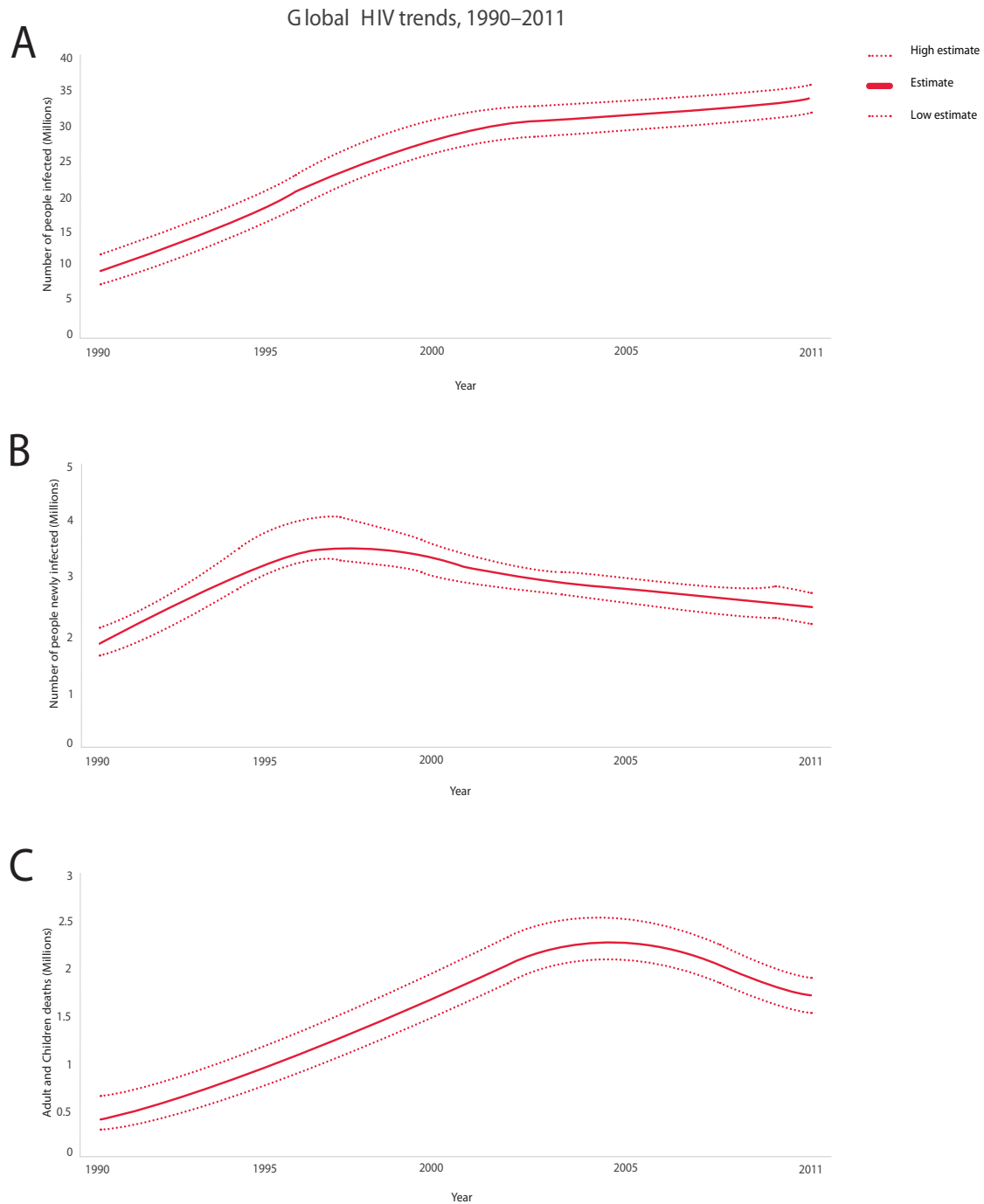


Figure 1.2: Global trend of new HIV infections from 1990 to 2011. The number of people living with HIV globally is increasing (A) while the number of people newly infected with HIV (B) and the number of Adults and child deaths due to HIV are decreasing (C) globally in the time period. This scenario can be attributed to global scale up of drugs while infected people continue transmitting the virus to uninfected people (4). (Source: modified from UNAIDS 2012)

1.2 Discovery and characterization of HIV

As early as 1959, HIV infection cases had been documented but were unreported (5, 6). Curious doctors at that time collected patient blood samples and kept frozen, which were later shown to have HIV antibodies (7). In June 1981, a case of acute immune depletion associated secondary infection was reported in some homosexuals in the USA (8, 9). Their infection was coupled with no lymphocyte proliferation (10). Until 1983, the causative agent responsible for the severe immune depletion, named AIDS (Acquired Immunodeficiency Syndrome), was unknown (11, 12), when Luc Montagnier's group at "Institut Pasteur" in Paris isolated the virus, which was initially named Human T-cell Leucemia Virus (HTLC) and later named as Human Immunodeficiency Virus (HIV) (5). Jay Levy's group in San Francisco, USA also subsequently found the virus confirming the discovery in Paris (13). They found HIV was a lentivirus from Group VI retrovirus with two single strand RNA molecules (14); unique to any other previously isolated viruses and the virus can transmit from infected to healthy people (15, 16), mother to child through umbilical cord (12, 17). Very soon, scientists around the world focused on this transmissible retrovirus. Complete sequencing of HIV genome in 1985 (18) led scientists to know more insights of HIV including its origin, genes/proteins and life cycle (19).

1.3 Origin and evolution of HIV

Exploration of the retrovirus led researchers to identify similarities between HIV and a retrovirus in African non-human primates that were then called as Simian Immunodeficiency Virus (SIV) (20). About 40 different primates, in Africa, are infected with SIV and some are harboring multiple strain of SIVs (21). Phylogenetic analysis of SIV from African non-human primates and HIV in human provided

remarkable understanding of viral transmission as zoonotic (22) and evolution of the virus in human after transmission (23).

HIV is divided into two groups – HIV-1 and HIV-2 (23). Each group is an independent cross species transmission from different African non-human primates to human (24). Discovered in 1986, HIV-2 is transmitted from sooty mangabey monkeys (*Cercocebus atys*) (25) and its prevalence is also high in the geographical location of these monkeys in West Africa (26). Sooty mangabey monkeys are naturally infected by a strain of SIV (25). The phylogenetic analysis of HIV-2 strains shows that they closely group with the SIVsmm strain (25) that are non-pathogenic to its host monkeys (27). SIVsmm evolved in its host to produce multiple strains and subsequent multiple zoonotic transmissions from sooty mangabey monkeys to human (28) gave rise to different subtypes of HIV-2. Although HIV-2 subtypes A to G are identified in human, it is assumed that more subtypes were introduced into human (29) but are lost for low adaptation fitness (30).

HIV-1 is the result of at least three cross species transmission events from chimpanzees (*Pan troglodytes troglodytes* (*Ptt*)) to human (31, 32). Phylogenetic analysis of HIV-1 sequences has shown that three independent cross transmission of the virus in to the human population, each giving rises to three sub groups: group M (Major), group O (Outlier) and group N (Non M or Non O) (28, 33). Recently a new HIV-1 strain, classified as group P, distinct from the previous three groups, has been discovered in a patient in Cameroon (34).

Group M is the most prevalent and accounts for 98% of all infections (reviewed in (24)). Its epicenter is thought to be Kinshasha of present day Democratic Republic of Congo (24, 35). Site stripping for clock detection method shows that group M and its closest simian relative branched out from their common ancestor in 17th century (36)

whereas molecular clock analysis of group M shows that its radiating evolution dates back to late 1920s (37). By 1960, long before human discovered its presence, HIV-1 group M had already diversified substantially (38).

Group O and group N are rare and geographically confined to West African regions such as Cameroon and neighboring countries (23). It is still not understood about the non-pandemic characteristics of group O and N HIV-1 virus after the first zoonotic transmission (39). It has been suggested that reduced replication capacity and transmission fitness are keys to their low prevalence(39). Group O strain has at least 50% genetic identity with group M (29, 40) and the molecular clock model of this group also showed that its origin dates back to 1920s (41). Group N was supposed to be introduced into human population in 1960s (42). Phylogenetic analysis using genetic sequence under evolutionary pressure shows its close grouping with SIV from Chimpanzee (23, 43). This indicates that group N might be a recombinant strain of SIV and HIV-1 group (42).

Group P is transmitted from gorilla as it is closely related to its SIV (34). Population level study of this new group virus, at its discovered region in Cameroon, shows its prevalence at 0.06% in the area but can still be pandemic as it can adapt in human (44).

1.4 HIV-1 Diversity

1.4.1 HIV-1 subtypes

HIV-1 group M is highly diversified and it is classified into nine subtypes: A, B, C, D, F, G, H, J and K (Figure 1.3) (45). The subtype classification is based on the phylogenetic and sequence distance analyses using gene sequence data forming major clades (45). “At least three epidemiologically unlinked sequences are required for defining a subtype” (46).

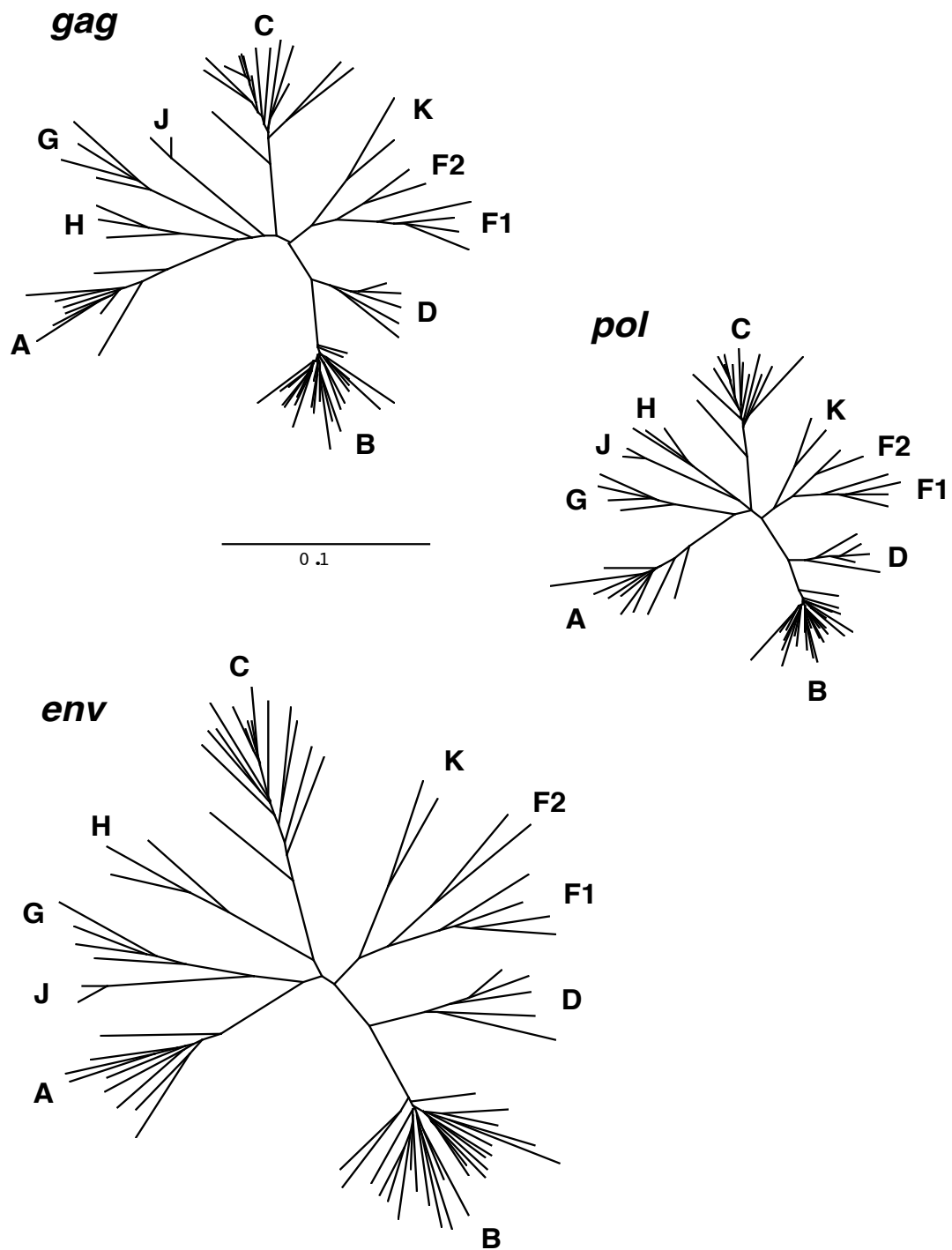


Figure 1.3: Phylogenetic tree showing HIV-1 group M diversification to subtypes A-D, F-H, J and K, inferred from nucleotide sequence alignments of *gag*, *pol* and *env* genes. Source: Robertson et al 2000 (46)

The classification of new subtype should also follow the same rule as “roughly equidistant from all previously characterized subtypes in all regions of the genome with a distinct pre-subtype branch similar to those of other subtypes” (45). The predominating group M subtypes are A, B, C and D (reviewed in (47)). Geographical locations of group M subtypes epidemic are show in Figure 1.4. The analysis from HIV samples collected from 70 countries in 2004 shows that “subtype C accounts for 50% of all infections worldwide” while subtypes A, B, G and D are found in decreasing order 12%, 10%, 6% and 3% respectively (48). Subtypes F, H, J and K are found very scarce and on overall, accounts for only 0.94% infections (48). The range of amino acid variation within a subtype and between subtypes differs from 15%–20% and 25% - 35% respectively (49).

Subtypes are further classified to sub-subtype based on a distinct sister clade formation (50) within a clade with the same rule of phylogenetic and distant analyses but not justifiable to call a subtype due to low genetic distance (45). Only subtypes A and F exhibit distinct sister clades (50). Subtype A has sub-subtypes A1 and A2 (A3 and A4 are mentioned by Taylor et al 2008(51)); Subtype F has sub-subtypes F1 and F2 (Figure 1.3); sub-subtype F3 mentioned by Taylor et al 2008 (51).

The lower diversity observed in Group N (52), O (53) and P (44) correlate to low prevalence and geographical confinement to Western African countries such as Cameroon. It is still unclear if group O can be sub divided into subtypes (54). Group N as well does not show distinct sub clade within itself (Figure 1.5).

1.4.2 HIV-1 recombination

Initially, HIV-1 group M subtypes E (55-57) and I (Figure 1.5) were also classified (reviewed in (47)). With availability of complete HIV genome sequence and phylogenetic analysis from it, the subtypes E and I were reclassified as circular

HIV-1 ^a	Sub-classification ^b	Description	Main geographic concentrations	Global prevalence ^c	Complete genome sequences ^d
Group M					
Subtype					
A		Includes sub-subtypes A1, A2, and A3	East and West Africa, former Soviet Republics	High	62
B		B prime (B') variant in Southeast Asia	America, Western Europe, Australia	High	129
C		50% of all HIV-1 infections worldwide	Southern Africa, East Africa, India	High	209
D		East and West African variants	East and West Africa	High	47
F		F1 and F2 sub-subtypes	West Central Africa	Very low	10
G			West Central Africa	Very low	8
H			West Central Africa	Very low	3
J			West Central Africa	Very low	2
K			West Central Africa	Very low	2
CRF			Southeast Asia	High	52
	CRF01_AE	Former "E"; subtypes A, E	West Africa	High	38
	CRF02_AG	Subtypes A, G	Former Soviet Republics	Very low	3
	CRF03_AB	Subtypes A, B		Very low	3
	CRF04_cpx	Former "I"; subtypes A, G, H, K		Very low	3
	CRF05_DF	Subtypes D, F		Very low	5
	CRF06_cpx	Subtypes A, G, J, K		Very low	3
	CRF07_BC	Subtypes B', C		Very low	4
	CRF08_BC	Subtypes B', C; related to CRF07_BC	Central Africa	Very low	3
	CRF09_cpx	Subtypes A, F, G, related to CRF02_AG	West Africa	Very low	3
	CRF10_CD	Subtypes C, D	China	Very low	3
	CRF11_cpx	Subtypes G, J	China	Very low	3
	CRF12_BF	Subtypes B, F	West Africa	Very low	3
	CRF13_cpx	Subtypes A, G, J, and CRF01_AE	Tanzania	Very low	10
	CRF14_BG	Subtypes B, G	West Central Africa	Very low	7
	CRF15_01E	CRF01_AE, subtype B	Argentina	Very low	3
URF	CRF16_A2D	Sub-subtype A2, subtype D	Spain, Portugal	Very low	6
	AC, AD, CD, ACD	Estimated 30% of strains in East Africa	Thailand	Very low	4
	Complex	Common in West/West Central Africa	Kenya	Very low	3
	BF	Abundant in South America	Nigeria, Rwanda, Tanzania, Uganda	Moderate	44
	CRF01_AE/B	Emerging in Southeast Asia	Nigeria, Cameroon	Moderate	33
Group O		West Central Africa	Brazil, Argentina	Moderate	31
Group N		West Central Africa	Thailand, Myanmar,	Very low	13
HIV-2		West Central Africa	Cameroon, Senegal	Very low	18
			Cameroon	Very low	3
			West Africa	Moderate	22

^aHIV Type 1 and HIV Type 2; HIV-1 strains are classified into groups M, N, and O, respectively. Group M is subdivided into subtypes, circulating recombinant forms (CRF), and unique recombinant forms (URF).

^bSubtypes are assigned letters in order of their discovery; CRF are numbered sequentially in order of discovery with the component subtypes after the underscore. "cpx" (complex) indicates that more than three subtypes are present. Some CRF also contain unclassified regions. URF are grouped according to their geographic origin for this analysis.

^cEstimated from degree of geographic spread, prevalence of HIV in countries and regions, and proportion of total HIV infections in the country or region. Dark grey fill indicates the six most globally prevalent strains; light grey fill indicates strains of moderate abundance.

^dBased on the HIV sequence database at <http://hiv-web.lanl.gov>. Because of recombination, only complete or virtually complete genomes of HIV provide unequivocal classification. Partial genome sequences and multiple sequences from a single individual, were excluded. CRF16_A2D includes two complete genomes from our unpublished data. At least five additional CRF have been described but sequences are not yet publicly available.

Figure 1.4: HIV diversity around the globe, its level of prevalence in the area and number of genome sequenced. Source: McCutchan 2006

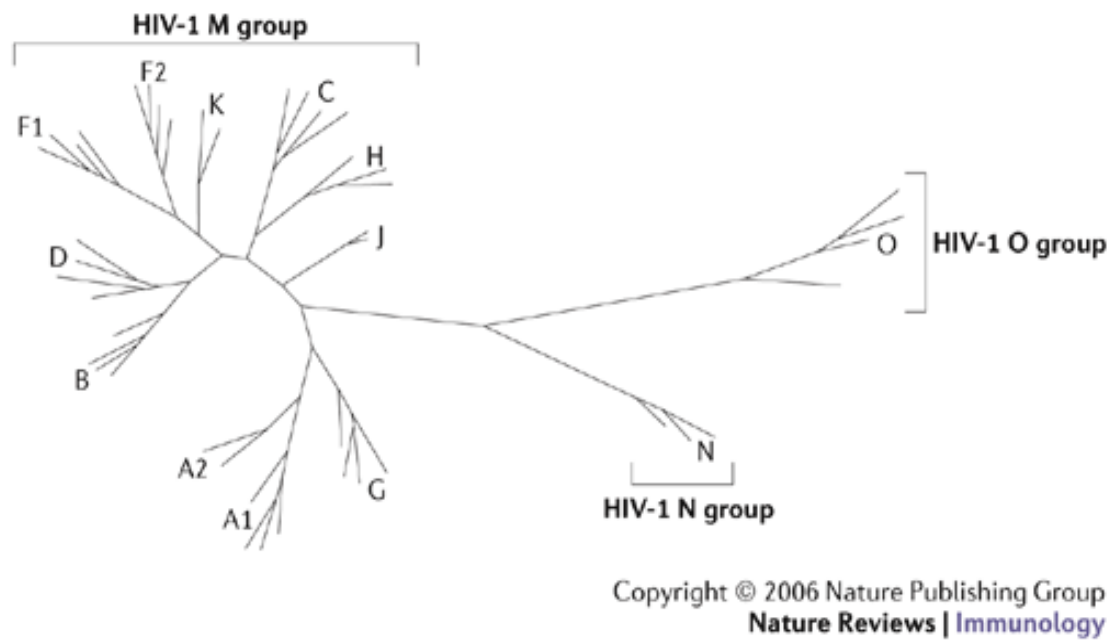


Figure 1.5: Neighboring joining phylogenetic tree showing HIV-1 group M, N and O. Group M shows distinct nine subtypes A-D, F-H, J, K while no specific subtype is observed in group N and O. Source: Letvin 2006 (58)

recombinant forms CRF01_AE (recombinant form of subtype A and E) and CRF04_cpx (recombinant form of more than two subtypes, designated by “cpx”) respectively (reviewed in (47)). The same criterion of epidemiological unlinked isolates from three or more people applies for classification as a circular recombinant form (CRF) (46). A recombinant isolate discovered in single patient is termed as Unique Recombinant Form (URF) (reviewed in (47)). There are 55 CRFs listed in Los Alamos National Laboratory database for HIV sequences (<http://www.hiv.lanl.gov/content/sequence/HIV/CRFs/CRFs.html>) as on July 22, 2013. The recombination breakpoints to shuffle HIV genome (59-62) from different strains of the virus are listed in <http://www.hiv.lanl.gov/content/sequence/HIV/CRFs/breakpoints.html>, each appeared in a publication. CRFs account for at least 20% of all the HIV infections (46, 63, 64). CRF02_AG is the most prevalent circulating recombinant form infecting over 9 million people on the whole (65) and geographically epidemic in the West African region (Figure 1.5). CRF01_AE is the dominant circulating recombinant form in South-East Asia (Figure 1.5) (51).

1.4.3 Intra-patient HIV diversity

HIV infection initiates mostly with a single virion (66, 67). Evidences of multiple HIV variants transmission are also recorded (68, 69). Generally, HIV is genetically homogenous for a short post infection time (70, 71). In the long-term post infection period, virus replicates rapidly to produce genetically heterogeneous population (68). This heterogeneous viral population consisting swarm of highly similar virus but genetically non-identical HIV virus is called HIV quasispecies (reviewed in (47)). It is observed that the diversity at a gene in viral quasispecies can be approximately 30% (72). Factors that contribute to high genetic heterogeneity in viral quasispecies are

higher replication rate and turnover (73), viral genome recombination (74-76), higher mutation rate by erroneous reverse transcriptase (77, 78), and host immune selection (79, 80). On the whole, HIV replication (discussed in section 1.6) is the overall source of genetic heterogeneity in the viral population (reviewed in (81)).

Intra patient HIV genome recombination is a common event (76, 82). Two genomes from different viral strains can be co-packed into single virion during replication (83). In the subsequent HIV replication, the ability of reverse transcriptase to switch between the two template genomes produces a recombined genome (84, 85). Genetic recombination allows rapid and efficient shuffling of advantageous genes and removing deleterious mutations, thus, increasing the viral fitness in the host (reviewed in (81)). Co infection by different HIV subtypes and subsequently co-packed and co-expressed in to a single virion generates Unique Recombinant Forms (URFs) (reviewed in (51)). Successful transmission of URFs with high viral fitness to three or more people and circulates in human population establishes Circulating Recombinant Forms (CRFs) (reviewed in (86)).

1.5 HIV genome and proteins – structures and functions

HIV has nine genes and produces 15 proteins (87). The genes are broadly grouped as accessory (*vif*, *vpr*, *vpu* and *nef*), structural (*pol*, *gag* and *env*) and regulatory (*tat* and *rev*). The higher number of proteins than genes is a result of post - transcriptional proteolysis of the products of structural genes (87).

1.5.1 Accessory genes:

Vif promotes the viral infectivity to the host, but has no role in viral production (88). Vif is produced in the late stage of viral production (89, 90) to suppress the innate antiviral immunity of host (91, 92). It is observed that *vif* is expressed only when the

virus infects immune cells that express cytidine deaminase APOBEC3G (93). The reason is that Vif protein prevents APOBEC3 proteins from hyper mutating HIV reverse transcripts as a mechanism of defense (94-104).

Vpr protein is packed in to nascent virions during budding out (105). Vpr is essential for viral core to enter and localize in the host cell nucleus after infecting the cell (106). Vpr arrests the cell cycle during the transfer from G2 to M phase (107, 108) by preventing the activation of the human p34^{cdc2}/cyclin B complex (109). Vpr is also important for efficient viral replication in monocyte or macrophage cells, but T-cells (105).

Vpu is a protein unique to HIV-1 (110) and is 16 kilo Dalton, 81 amino acids long (111). Biological functions of vpu protein include degradation of CD4 in endoplasmic reticulum (112), interference of host immune cell MHC class II antigen presentation on the cell surface allowing the virus for host immune escape (113, 114) and viral maturation and release from host cell membrane (115).

Nef has no role in viral infectivity but plays a role during the biogenesis of viral particles (116) and virulence (117, 118). Nef down regulates the production of major histocompatibility complex type I (MHC type I) in the host cell (119-121). This impairs the function of cytotoxic T lymphocyte cells to clear the infected cells (122-126). Nef also down regulates CD4 on host cell surface (127, 128) and modulates cellular activation to evade host immune system (123, 126).

1.5.2 Structural genes and proteins

The Gag gene produces a precursor polyprotein (pr55^{gag}) of ~ 500 amino acids long and weighs 55 kilodalton (129). The Gag precursor has all the building blocks to form a fully infectious virion, even in the absence of other viral products (130). A proteolytic cleavage of gag precursor yields the structural proteins – matrix, capsid,

nucleocapsid and p6 (Figure 1.6) (131). The cleavage takes place in the nascent virus after budding out from host cell (132). The matrix protein is at the N-terminal and p6 at the C-terminal of gag precursor (131, 133).

All gag proteins play role at “post assembly and post processing stages in viral infectivity” (130). In the HIV replication cycle, matrix domain of gag plays role in targeting gag precursor to the plasma membrane of the host cell and the viral assembly at the site (130, 134-136). The highly basic region in matrix mediates electrostatic association with phospholipids in plasma membrane during assembly (137, 138). The capsid is a curved and closed shell consisting 250 hexamers and 12 pentamers (reviewed in (139)). Capsid packs viral proteins, nucleocapsid and viral genome during assembly to pass on to new HIV particles (140). Nucleocapsid plays role in efficient viral assembly by making pr55–pr55 inter-protein contacts (135, 136) and localization of viral proteins (141). P6 protein plays role in detaching and releasing the newly formed HIV particles (142).

The *pol* polyprotein is produced by translational frame shift (143) (Figure 1.5), such that, as much as 241 nucleotides at 5’ region of the gene overlaps with 3’ region of *gag* gene (18, 144). The proteolytic cleavage of *pol* precursor produces essential viral replication enzymes – protease (PR), reverse transcriptase (RT) and integrase (IN). The protease enzyme cleaves the *gag* and *pol* polyprotein to form the viral structure proteins and functional enzymes respectively (145-147). The reverse transcriptase enzyme produces a cDNA from the viral RNA after infecting host cell (148, 149). The RNase H domain in RT degrades the viral RNA molecule following cDNA production (150). The integrase enzyme removes two bases from 3’ DNA molecule and functions strand transfer during the process of integrating the proviral DNA into the host genome (151).

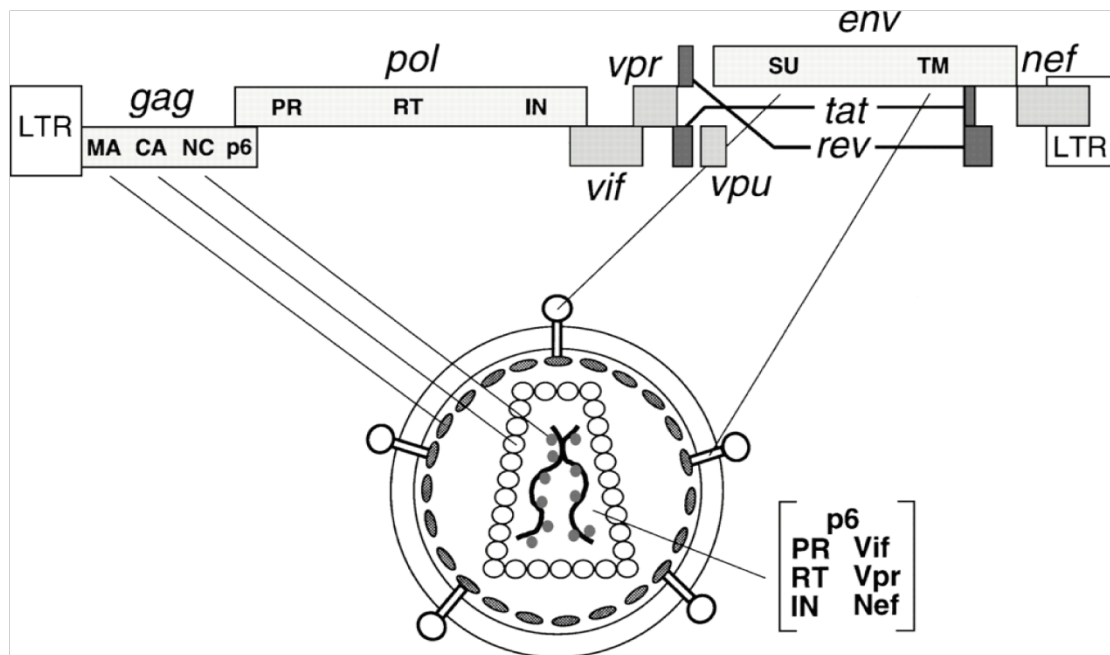


Figure 1.6: HIV genes and proteins positions in the viral genome and their viral parts.

Source: Frankel and Young 1998 (87)

Env gene produces a precursor glycopolyprotein (gp160) that is processed post-translationally by human convertase enzymes - PC1 and furin to produce glycoprotein 120 (gp120, HIV-1 SU) and glycoprotein 41 (gp41, HIV-1 TM) (152). Gp120 is a non-covalent complex of external protein and gp41 is a trans-membrane protein; both play vital role for initial steps in viral infection (153). Three gp120 molecules bound with three gp41 molecules to form envelop spikes (154). They are organized to form trimeric complexes on the surface of HIV and mediate HIV entry into the host cell (155). The exposed external complex gp120 binds to the CD4 receptor on the host immune cell (156). This triggers a conformational shift of trimeric complex that enables a conserved gp120 region binding to a chemokine receptor, either CCR5 or CXCR4, to facilitate fusion of the viral and host membrane (156-159). The gp120-CD4 complex also triggers conformational change in gp41 trans-membrane protein, which plays role in the viral fusion and release of viral contents in to the host cell (160, 161), from native non-fusion state to fusion state (153, 162). The gp41 consists of heptad repeats - HR1 and HR2 that play role in fusion process (160, 163). HR1 is a bundle of three helical motifs and HR2 is trimeric coiled coil structure (164). During fusion process, HR2 makes numerous contacts with HR1 to form stable six helical bundles (165).

1.5.3 Regulator Genes:

Tat is a trans-activating factor localized in the nucleus for HIV gene expression (166, 167). The HIV proviral genome integrated in to the host genome is regulated by cellular as well as the viral transcription regulatory factors (168, 169). Tat is the primary transcriptional regulatory factor (170). An example of Tat action is the control of RNA polymerase II elongation during transcription, which otherwise

disengages from the template DNA strand, terminating the transcription prematurely [206-209]. Sodroski *et al.* (1985) first explained the function of Tat (171).

Rev is a 19 kilo Dalton phosphoprotein (172) trans-activating factor for HIV gene expression (166). Like Tat, it is also mainly localized in the nucleus of host cell (173), but cycles rapidly between the nucleus and cytoplasm as it promotes nuclear export of the transcriptional products (174-177). Rev binds at the Rev Responsive Element (RRE), which is an RNA element encoded within the *env* region of the virus (178, 179).

1.6 HIV replication

There are major 11 events in HIV replication cycle (as numbered in Figure 1.7). The initial step of viral entry (Figure 1.7 step 1) in to a host immune cell is explained in section 1.5.2.

Following the fusion, the viral core enters in to the cytoplasm of host cell. The RT enzyme reverse transcribes the RNA molecule to cDNA (Figure 1.7 step 2) in the intact capsid of the viral core (180). Subsequently the capsid is dissembled, termed as uncoating (Figure 1.7 step 3) (180), assisted by host importin β member protein transportin 3 (TNPO3) (181). The uncoating process can take an hour or less from post infection (182). Uncoating process releases ribonucleoprotein complex in to the cytosol of the host cell (183). The capsid and nucleocapsid proteins dissociate from cDNA but the reverse transcription complex remains intact along with viral matrix, integrase, vpr and human protein high mobility group I (HMG I (Y)) forming preintegration complex (PIC) (184-186). The PIC protects cDNA from endonuclease degradation (186). In an ATP dependent process (187), PIC is transported on host microtubules towards the nuclear membrane (180). Some studies initially suggested that formation of central DNA flap (188), vpr, and matrix mediate nuclear import

(Figure 1.7 step 4) of PIC (189), however, there is no any clear evidence of these factors have been described as such for nuclear import (190). Integrase assists in nuclear import in association with nuclear import machinery like importin (191) and transportin-SR2 (192). It is now established that central polypurine tract-central termination sequence (cPPT-CTS) plays role in kinetics of nuclear import (190).

Post-nuclear entry, integrase processes the viral DNA for integration (193) (Figure 1.7 step 5) into host genome as explained in section 1.5.2. Host transcriptional co-factor LEDGF/p75 and HIV integrase interact to tether to the host chromosome during the integration process (194).

The integrated HIV provirus hijacks the host cell transcriptional machinery for viral genes to transcribe (Figure 1.7 step 6) (195). HIV protein Tat promotes the transcription of the viral DNA (196, 197). The viral transcriptome encodes structural proteins, accessory proteins and viral enzymes necessary for a complete functional HIV (reviewed in (198)).

The viral Rev protein facilitates exporting (Figure 1.7 step 7) of the unprocessed viral transcriptome to cytoplasm for translation (176). HIV has no translation system of its own; the host translational machinery is exploited for translation (Figure 1.7 step 8) of the viral transcriptome to its proteome.

Upon translation of all viral proteins, viral *gag* initiates virion assembly (Figure 1.7 step 9) at the cell membrane (200-202). The complete assembled virion particles bud out and are released (Figure 1.7 step 10) from the plasma membrane by the host ESCRT machinery involving Tsg101 and ALIX regulatory proteins (203-205). The maturation (Figure 1.7 step 11) of the nascent HIV virions begins concomitantly with budding out (115, 206). *Gag* and *Pol* polyproteins are proteolytically cleaved by protease enzyme in the maturation step (207, 208).

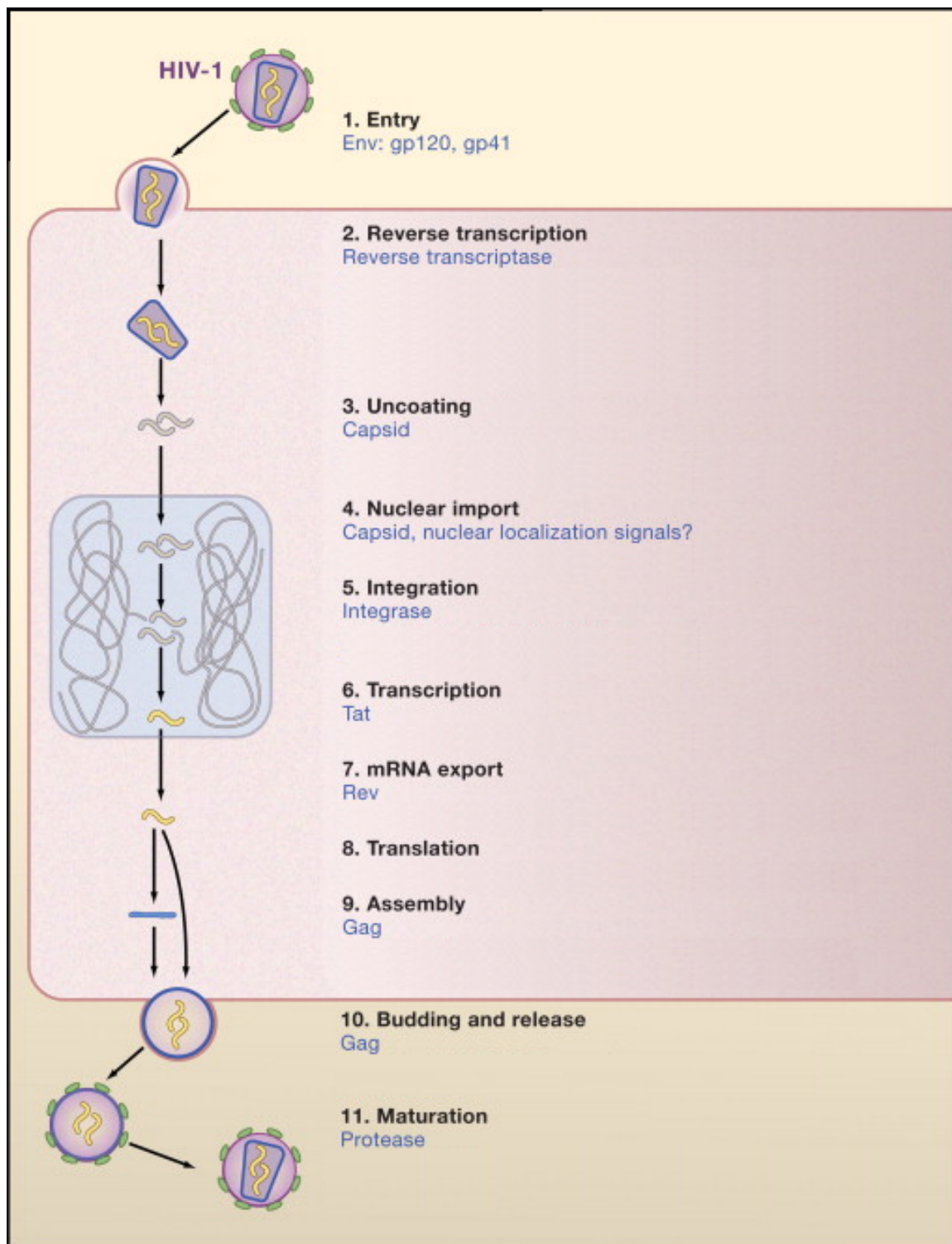


Figure 1.7: The HIV replication cycle showing major stages. Viral proteins that play role in each event are colored blue. (Source: modified from Ho and Bieniasz 2008)

Each HIV replication cycle releases new infectious virions in the order of 10^9 per day (209). The number of new infecting HIV determines the replication rate of the virus (210). With increasing time period of infection, higher turnover rate is observed in both immune cell depletion and viral population expansion (209).

HIV Treatment

Different inhibitory drugs have been designed that interfere with the viral protein normal function at different stages of its replication cycle. Currently available drugs classified on the basis of its target in HIV replication are: Reverse Transcriptase Inhibitors, Protease Inhibitors, Integrase Inhibitors and cell entry Inhibitors (reviewed in (211-213)). The prescribed drug regimen usually consists of drugs from reverse transcriptase inhibitors and protease inhibitors (214). The World Health Organization (WHO) has classified HIV infection to different clinical stages depending upon progression to AIDS (215). The WHO has developed guidelines on usage of anti retro viral drugs to prevent HIV and care infected people (<http://www.who.int/hiv/pub/guidelines/en/>). The WHO guidelines are produced based on the clinical stage of HIV infection, CD4+ count in blood, adult/breast feeding mother and presence of secondary infections (<http://www.who.int/hiv/pub/guidelines/en/>) (216). According to the guideline, patient at clinical stage 3 or 4 having CD4+ count less than 500 per micro liter (μL) should start the treatment whereas CD4+ count less than 350 μL are highly recommended to start the treatment immediately (216). HIV treatment with combination of triple anti retroviral drugs, also called Highly Active Anti Retroviral Therapy (HAART) is done under the guidance of a clinician. The effectiveness of ART treatment and emergence of drug resistant viral variants are monitored.

Reverse Transcriptase Inhibitors

1. NRTIs

NRTIs are analogs of normal nucleotides but without 3' hydroxyl group (reviewed in (217)). The drug is taken in unphosphorylated form, which cytochromes phosphorylates to form 5' triphosphates (reviewed in (211, 218)). It leads to incomplete termination of cDNA synthesis (219). There are eight NRTI drugs currently used in ART drug regimen (Figure 1.8).

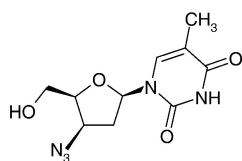
- 2. NNRTIs:** Non-nucleoside and non-nucleotide reverse transcriptase inhibitors (NNRTIs) specifically bind at an allosteric site 10 Å from polymerase active site of the HIV-1 reverse transcriptase (149, 220), close to substrate-binding site. The binding induces conformational change in the enzyme, which distorts the catalytic aspartate triad of active site and inhibits the function of the enzyme (221, 222). NNRTIs drug are administered along with NRTI drugs in ART at non-toxic concentration to human cells (223). NNRTIs drugs from first generation have inherent disadvantageous characteristics like cross-resistance and low genetic barrier (require only one mutation to select) (224). There are six NNRTIs drugs used in ART drug regimen (Figure 1.9)

Protease Inhibitors (PR)

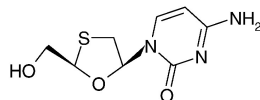
Protease inhibitor is added to second line ART therapy on drug failure to first line therapy (225). Protease Inhibitors interfere with *gag-pol* polypeptide (226) as competitive peptidomimetic inhibitors and have hydroxyethylene core, which prohibits cleavage activity of the HIV-1 protease (227, 228). However, as adverse side effect, patients consuming the inhibitors have developed lipodystrophy and hyperlipidemia (229-235). Besides inhibiting protease enzyme, it also inhibits degradation and secretion of apolipoprotein B; but in the presence of oleic acid,

protease inhibitor increases the secretion of apolipoprotein B (233). There are nine protease inhibitors approved for clinical use shown in Figure 1.10.

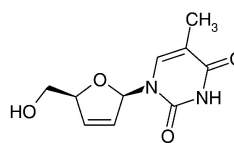
NRTIs



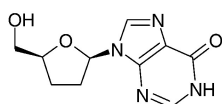
zidovudine



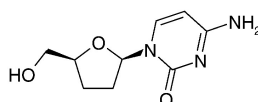
lamivudine



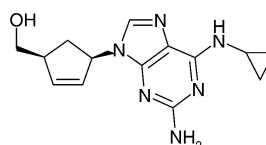
stavudine



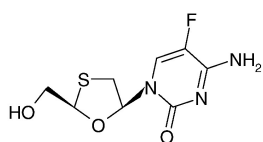
didanosine



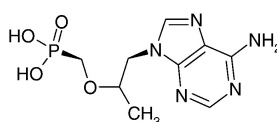
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abacavir



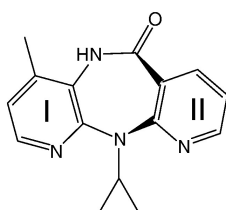
emtricitabine



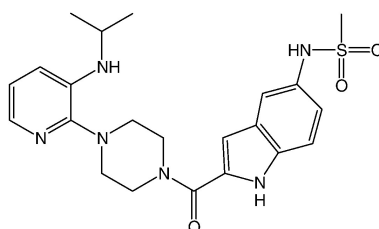
tenofovir

Figure 1.8: Molecular structures of NRTI drugs. Source: Sarafianos et al 2009 (149)

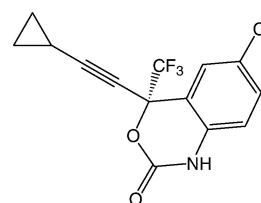
NNRTIs



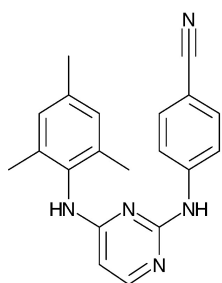
Nevirapine



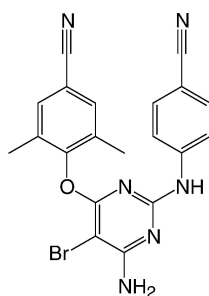
Delavirdine



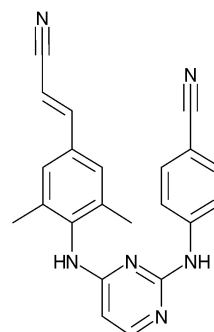
Efavirenz



Dapivirine
(TMC120-R147681)



Etravirine
(TMC125-R165335)



Rilpivirine
(TMC278-R278474)

Figure 1.9: Molecular structures of NNRTI drugs. Source: Sarafianos et al 2009 (149)

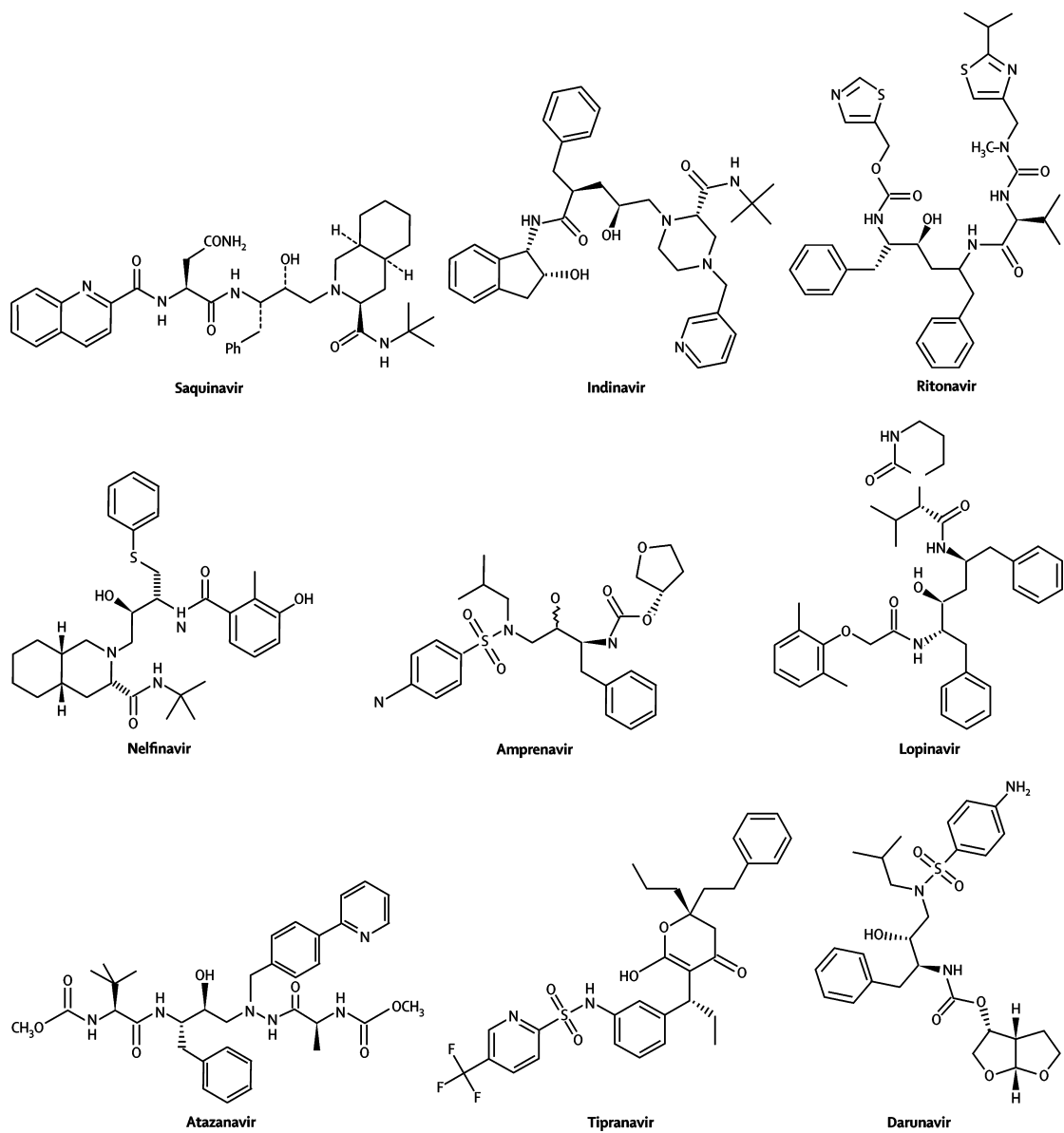


Figure 1.10: Molecular Chemical structures of Protease Inhibitors. Source: Chow et al 2009 (236)

Integrase Inhibitors

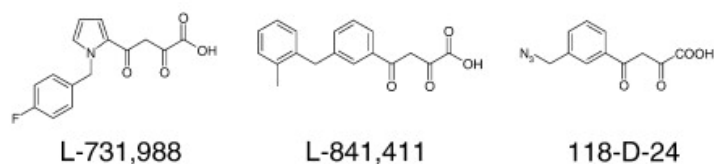
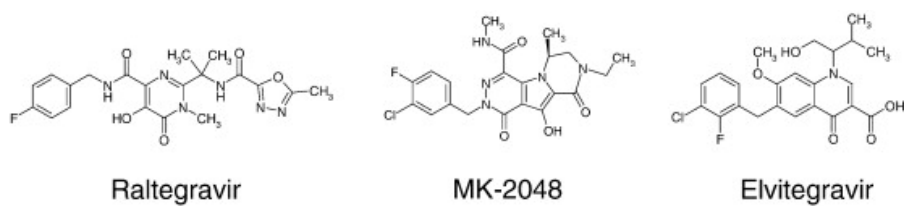
Although the triple anti retroviral drugs therapy could suppress the viral replication, its expensive, leads to multiple drug resistance and requires high adherence (237). This has diverted the search for drugs that target third enzyme, the integrase. Some of the integrase inhibitors are shown in Figure 1.11. The feasibility and efficacy of integrase inhibitors have been tested in Rhesus Macaques (238). Most of the Integrase inhibitors target strand transfer function of the enzyme (239-243). X-ray structure of the integrase enzyme has revealed the active site model of the enzyme complexes with the DNA (244). Diketo acids (DKA) and its derivatives (like L-708,906, S-1360, and V-165 (Fikkert et al 2003)) are the highly potent integrase inhibitors for strand transfer inhibition (241). Low concentration of DKA is sufficient for effective integrase inhibitor (reviewed in (245)).

Cell entry inhibitors

1. Co receptor inhibitors or entry inhibitors:

Early entry inhibitors are designed to target the unglycosylated cavity formed during gp120-CD4 complex ((246, 247), reviewed in (248)). Some of the molecules that inhibit gp120-CD4 binding are: PRP-542 (249), TNX-355(250), CADA (251, 252), BMS-806 (246).

Another target of entry inhibitor is the host immune cell surface co-receptors CCR5 or CXCR4. Co receptor antagonists are designed that binds specifically to targeted co receptors, making it unavailable to HIV gp120 binding (reviewed in (253)).



Diketo Acid

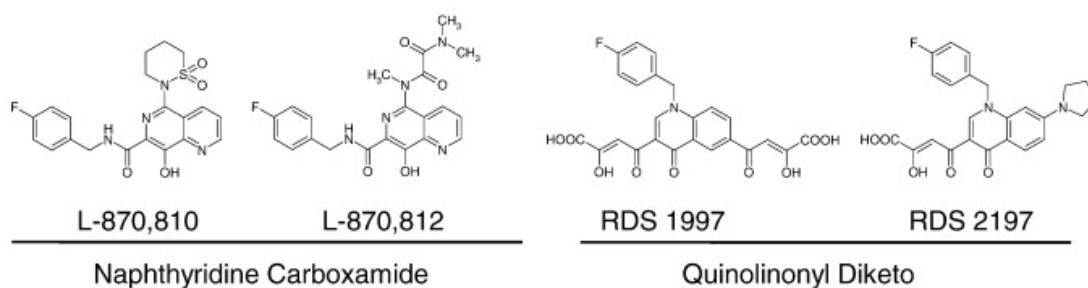


Figure 1.11: Chemical structure of different integrase inhibitors. Structures with similar chemical properties are grouped together. Source: Bera et al 2011 (239)

Some of the CCR5 antagonists developed are TAK-779 (254), TAK-652 (255), vicriviroc (256-258), AD101 (SCH-350581) (259), Maraviroc (UK-427857) (212, 260-264), Aplaviroc (GW-873140) (265, 266), PRO-140 (267). Some of the CXCR4 antagonists are AMD3100 (268), AMD070 (269, 270), KRH-1636 (271) and KRH-2731 (272, 273).

2. Fusion Inhibitors

Fusion inhibitor design is based on targeting the heptad regions HR1 or HR2 of gp41 disabling the virus to make fusion pore (reviewed in (274)). Enfuvirtide (275, 276) is a synthetic peptide, approved for clinical use in 2003 (commercial name-Fuzeon) (277), which can bind to gp41 HR1 region (278). The fusion inhibitor T-1249 also targets HR1 region (279). T-1249 is active against Enfuvirtide resistant viral strains but discontinued in 2004 for clinical use (248, 280). Sifuvirtide is another HIV fusion inhibitor peptide under research (281).

HIV Drug Resistance

Triple anti retroviral drug therapy has been successful at controlling HIV replication to low viral load within a host (282). The therapy has been compromised by the emergence of resistant HIV viral variants through constant evolution (reviewed in (283)). Selective pressure from new environments like presence of anti retro viral drugs and host immune system drive the evolution of resistant virus (reviewed in (284, 285)). HIV drug resistance can occur with accumulation of mutations that increase the viral fitness in the presence of anti retro viral drugs (reviewed in (283, 286)). Mutations that confer evolutionary advantage to virus and escape drug effect

are selected (287). Transmission of resistant virus also confers drug resistance in drug naïve patients (288-290). Although drug resistant mutations escape drugs effect, they confer decrease in viral fitness and replication rate at certain level (291). Other mutations counteract the effect of drug resistant mutations to increase fitness and replication rate (292).

ART drugs are designed based on three dimension structures of the viral proteins to bind at specific active region of the proteins and interfere with its normal function. Mutations at specific positions of HIV genes change the three-dimensional shape of the proteins rendering no drug binding (Figure 1.12). These drug resistant mutations have been well documented with associated drug and resistance level (293). The viral quasispecies sequence information can be used to align against the reference sequence and infer the presence of any drug resistant mutation (294).

Mechanisms of drug resistance

HIV gains drug resistance against nucleoside reverse transcriptase inhibitors with substitution mutations close to the nucleotide-binding site (295, 296). The substituted amino acids either fold over the triphosphate group of the incoming nucleotide analog (297) or exerts steric clash with the oxathiolane ring of the inhibitors and interferes with its binding (298). Another mechanism of gaining resistance to NRTIs is hydrolytic removal of analog inhibitors mediated by nucleotide excision mutations (299). Mutations in RT (M41L, L210W, T215F/Y and K219Q/E) remove NRTIs from the elongating nucleotide chain through phosphorolysis mediated either ATP or pyrophosphate (300-302).

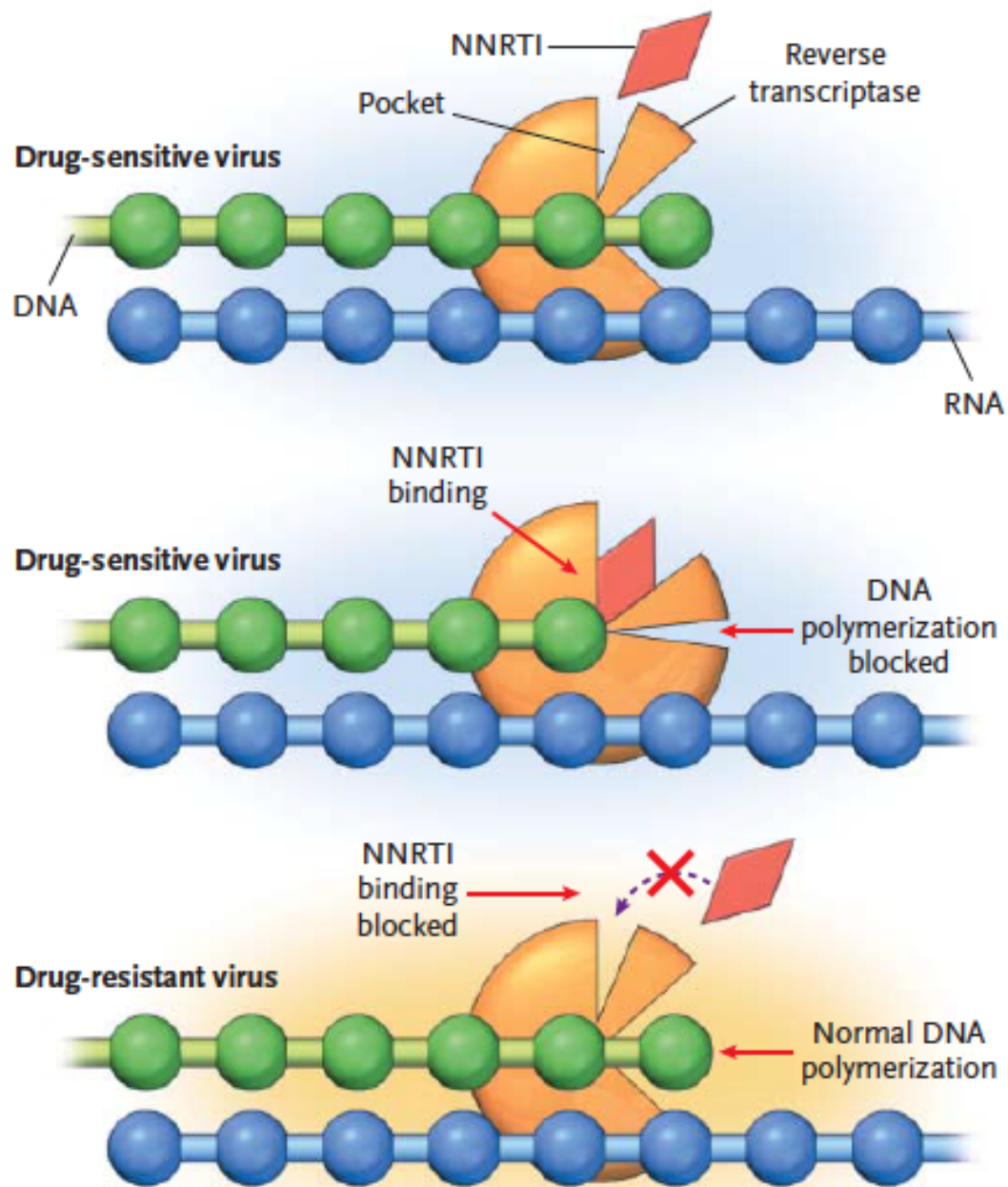


Figure 1.12: Development of HIV drug resistance. Accumulation of Drug resistant mutations changes three-dimensional confirmation of a viral protein, disabling drug binding and carrying out its normal function.

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