Universidade de Lisboa

Faculdade de Farmácia

Therapeutic advancements in the management of HIV/AIDS

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Monografia orientada pelo Professor Doutor Bruno Sepodes, Professor Associado com Agregação

Mestrado Integrado em Ciências Farmacêuticas

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Trabalho Final de Mestrado Integrado em Ciências Farmacêuticas apresentado à Universidade de Lisboa através da Faculdade de Farmácia

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Resumo

A infeção pelo vírus da imunodeficiência humana tornou-se numa epidemia global que está a entrar na sua quarta década. Estima-se que, desde a descoberta deste vírus, 79,3 milhões (55,9-110 milhões) de pessoas foram infetadas e que dessas, 36,3 milhões (27,2 milhões-47,8 milhões) perderam as suas vidas. Podemos dividir este retrovírus em dois tipos: tipo 1 e tipo 2. Ambas as espécies pertencem ao grupo dos *Lentivirus*, que por sua vez pertencem à família e subfamília *Retroviridae* e *Orthoretrovirinae*, respetivamente. Ambos possuem a mesma estrutura genética básica, o mesmo mecanismo de replicação intracelular, via de transmissão e progressão da doença; no entanto, eles pertencem a linhagens diferentes, ou seja, representam transmissões entre espécies distintas (zoonose). A síndrome da imunodeficiência humana adquirida é o estadio final da progressão viral e, se não for tratada, acaba com a morte do doente. Com um melhor entendimento da replicação viral e dos mecanismos utilizados para evadir a imunidade do nosso organismo, os alvos para interromper a produção viral também se tornaram mais claros. Atualmente, existe um arsenal de mais de 30 fármacos antirretrovirais, que possuem oito mecanismos de ação distintos. O vírus que antes era visto como uma sentença de morte para todos que o contraíam, é agora é uma doença crónica que pode ser controlada com terapia existente. Esta dissertação está dividida em 4 partes: origem e descoberta do HIV; mecanismo de replicação viral; Terapia antirretroviral; avanços na manutenção da terapêutica do HIV. O objetivo principal do trabalho é entender como uma zoonose com origem em África conseguiu espalhar-se pelo mundo e até hoje permanece sem cura. Será também abordada a progressão da terapêutica disponível e dos medicamentos aprovados ao longo dos anos, desde os primeiros medicamentos que causavam efeitos secundários graves e que eram facilmente suscetíveis à resistência viral devido à ocorrência de mutações no início dos anos 90 até 2021, onde é utilizada uma terapêutica dupla ou tripla, sem que exista toxicidade para o doente e com uma excelente relação benefício-risco. Também serão abordados fármacos e vacinas em ensaios clínicos para o tratamento deste retrovírus, que poderão vir a ser aprovados num futuro a médio-longo prazo.

Palavras-chave: HIV, SIDA, Antirretrovirais, Terapêutica, Replicação

Abstract

The human immunodeficiency virus has become a global epidemic that is now entering his fourth decade. It is estimated that since the discovery of this virus, 79.3 million (55.9 million-110 million) people have become infected and 36.3 million (27.2 million-47.8 million) have lost their lives. We can divide this retrovirus into two main types: 1 and 2. Both species belong to the Lentivirus group, that belongs to the *Retroviridae Orthoretrovirinae* family and subfamily, respectively. Both types share the same basic gene arrangement, equal intracellular replication mechanism, transmission pathway and disease progression however they belong to different lineages and furthermore represent distinctive cross-species transmissions (zoonosis). The acquired human immunodeficiency syndrome is the final stage of viral progression and it ends up in death if not treated. With the better understanding of viral replication and their mechanisms to evade immunity, the targets to stop viral production also became clearer. Nowadays, we are in possession of an armoury of more than 30 antiretroviral agents, acting through eight different mechanisms. The virus that once was a death sentence for everyone who caught it, is now a chronic disease that can be managed by the existing therapy. This dissertation is divided in 4 parts: origin and discovery of HIV; mechanism of viral replication; antiretroviral therapy; advancements in the management of HIV. The main purpose of the work is to understand how a zoonosis originated in Africa was able to spread throughout the world and until this day remains with no cure. It will also approach the progression of the approved drugs throughout the years, from the 1-2 medicines that caused brutal side effects and were easily prone to resistance mutations in the beginning of the 90s to 2021, where 2-3 medicines are simultaneously used in almost non-toxic, with little to no side effects in the treatment of this retrovirus, along with possible drugs that could be approved in a medium-long term.

Keywords: HIV, AIDS, Antiretroviral, Therapy, Replication

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Abbreviations

ABCE1 – ATP-binding cassette sub-family E member 1

AIDS - Acquired Immunodeficiency Syndrome

APOBEC3G – Apolipoprotein B-editing catalytic polypeptide 3G

ART – Antiretroviral therapy

ARV – Antiretroviral

ASF/SF2 – Alternate splicing factor/splicing factor 2

ATP – Adenosine triphosphate

CCR5 – Chemokine receptor 5

cDNA – Complementar DNA

CRF - Circulating Recombinant Forms

CXCR4 – Chemokine receptor 4

CyA – Cyclophilin A

CYP3A4 – Cytochrome P450 3A4

DNA – Deoxyribonucleic acid

DRC – Democratic Republic of Congo

EMA – European Medicines Agency

ESCRT – Endosomal sorting complexes required for transport

FDA – Food and drug administration

GALT – Gut-associated lymphatic tissue

Group M - -Group Major

Group N – Group non-O or New

Group O – Group Outlier

Group P – Group Pending

HIV – Human Immunodeficiency Virus

HR – Heptad repeat

IN – Integrase

INSTI – Integrase strand transfer inhibitors

LEDGF – Lens epithelium-derived growth factor

LTR – Long terminal Repeat

MHC-1 – Major histocompatibility complex 1

mRNA – messenger RNA

NF-kB – Nuclear factor kappa B

NNIBP – NNRTI binding pocket

NNRTI – NonNucleoside Reverse Transcriptase Inhibitors

NRTI – Nucleoside Reverse Transcriptase Inhibitors

NRTTI – Nucleoside reverse transcriptase translocation inhibitor

NtRTI – Nucleotide Reverse Transcriptase Inhibitors

PI – Protease Inhibitors

RNA – Ribonucleic Acid

RRE – Rev response elements

RT – Reverse Transcriptase

RT-PCR – Real Time Polymerase Chain Reaction

SIVcpz - Simian Immunodeficiency Virus on Pan troglodytes troglodytes chimpanzees

SIVgor – Simian Immunodeficiency Virus on Gorillas

SIVsm – Simian immunodeficiency virus of sooty mangabey monkey species

STR – Single tablet regimen

TLR – Toll-like receptors

TSG101 – Tumor susceptibility Gene 101

URF – Unique Recombinant Forms

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1. Origin and Global distribution of HIV

1.1. HIV-1

1.1.1. Discovery and origin of HIV-1

In 1980-1981, five healthy homosexual men that never had presented sexual transmitted diseases, never had contact with the same partners and no common contacts, all began to exhibit the same opportunistic infections (*Pneumocystis carinii* pneumonia, along with cytomegalovirus and candidiasis) (1). Furthermore, they had all presented one thing in common: persistent unexplained lymphadenopathy (1). In 1982, it was suggested that the source of this acquired immunodeficiency syndrome (AIDS) was a retrovirus that had to be transmitted through sexual contact (2,3). It was only by 1983 that HIV (later to be known has type 1) was firstly identified, in a patient with Kaposi's sarcoma, in Paris (3). HIV-1 possesses the same genome organization as the simian immunodeficiency virus on isolated *Pan troglodytes troglodytes* chimpanzees (SIV_{cpz}) populations in Southern Cameroon (4–7). Furthermore, they both share a unique gene, *vpu*, that is absent on all other lentiviruses, suggesting that chimpanzees are SIV_{cpz}'s natural reservoir (4–7).However, unlike HIV-2, SIV_{cpz} is pathogenic in his natural reservoir (8). Studies show that SIV_{cpz} is the result of several recombinations between ancestors of SIV from red-capped mangabeys and greater spot-nosed monkeys (SIV_{rcm} and SIV_{gsn} respectively) (5). The chimpanzees are known to hunt smaller species of monkeys, and by hunting the reservoirs of SIV_{rcm} and SIV_{gsn}, they became the perfect "mixing vessel" of both viruses, ultimately originating SIV_{cpz} (9). The inter-species crossing from chimpanzees to humans occurred in central-west Africa, where being bitten, hunting, butchering and consumption (sometimes uncooked) of chimpanzees was regular (5,10,11). Exposition of cutaneous or mucous membranes to this animal's blood resulted in direct contact with SIV_{cpz} and subsequent inter-species transmission of the HIV-1 species (10,11).

1.1.2. Tracing the origin and history of HIV-1

Phylogeographic analyses show us that the first HIV-1 infection known happened in Leopoldville (now called Kinshasa), in the Democratic Republic of Congo (DRC) around 1920 (12). Leopoldville was the economic centre of central Africa, due to its geographical

localization: it was the terminal station of the entire navigations to Congo and the departure point of the railway to all the Atlantic coast cities (13). The location, along with colonial policies that highly favoured prostitution made the geographic dissemination of the virus possible (14). Public Health interventions by the Belgian Red Cross from 1921 to 1959, even though well intended, have created a massive opportunity for the transmission of HIV, since the needles and syringes used were only rinsed between patients and not sterilised (14,15). Unsterilised injections led to iatrogenic transmission of the virus especially in sex workers, which allowed for further spreading of HIV (16). In 1960, after Congo became an independent country, anarchy followed, alongside an unexpected shift in sex work, with the dramatic appearance of a high rate of prostitution (more than a thousand relations per year), leading to a further spreading of HIV throughout Africa (16). The parenteral transmission in the 1950s, complemented with a sexual transmission in the 1960s, led to an increased prevalence of HIV (0.25 to 3.0%) among mothers in Kinshasa (17). The iatrogenic and sexual transmissions in the 1950s and 1960s respectively, that originated an exponential increase of people with HIV-1, unavoidably got spread throughout Africa - due to bureaucrats, traders, and all kinds of economic migrants, as well as excess of uncircumcised men and a growing sex industry - and throughout the world (16,18).

1.1.3. HIV-1 Diversity

HIV-1 can be separated in 4 different groups that are named M, N, O and P (19). The nucleotide sequences of these groups are approximately 50-60% homologous, which allows us to conclude that each group was the result of unique and independent zoonosis of the SIV_{cpz} (18). Group M (Major) is the most is the only type of virus that surpassed Africa, with worldwide distribution and accounts for 95% of all HIV-1 infections (19,20). Group M is further divided into ten clades (A-D, F-H, J-L) and further sub-subtypes (A1-A4, F1 and F2) (19). The subtypes are 80% similar, which means that individuals with dual or multiple HIV subtypes can recombine the viruses generate new mosaic forms through recombination (21). The recombinants can be unique recombinant forms (URF) – if they were sequenced once from a multi-infected individual and show unique genome breakpoints - or circulating recombinant forms (CRF)- if it was found and sequenced in at least two epidemiologically unlinked patients and fully sequenced (21). As of now, 102 different CRF have been reported throughout the world (22).

1.1.4. Group M

Subtype A is accountable for 12% of all HIV-1 infections and it is principally found in East and central Africa, as well as Russia and other Soviet Union countries (23). Subgroup B is responsible for 11% of all HIV-1 infections and it is the most worldwide spread of all types, in which includes Europe, USA, South America and Oceania (24–26). Subtype C represents more than 50% of all HIV-1 infections on the world, and it is present in Sub-Saharan Africa, India, and Brazil (27–29). Subtypes D, H, J and K circulate mainly in Africa and the middle east (30). Subtype F is mainly reported in south and southeast Asia and Brazil, forming CRFs together with subtype B in this last country (18). Subtype G co-circulates with other subtypes of HIV-1 in eastern and central Africa (31). More recently, a new type of HIV-1 has been identified as group L (18). The virus was isolated, and the genomes were totally sequenced from 3 unlinked transmission events (32). Subtype L is presently restricted to DRC, however, continued molecular surveillance will be the determinant of subtype L's prevalence (32).

1.1.5. Group O

In the 1960s, a Norwegian sailor who was visiting west Africa was infected by group O, along with his family (33). But it was only 30 years later, in 1990, that HIV-1 Group O (for "outlier") was initially detected, in a couple of Cameroonians that were living in Belgium (34,35). The virus first entered the human population around 1920 by cross-species transmission of the simian immunodeficiency virus on gorillas (SIV_{gor}) to humans (36). The SIV_{gor} himself was also the result of an inter-species crossing between chimpanzees and western lowland gorillas (*Gorilla gorilla)* (36–38). Since both apes share the same habitat, physical encounters between them were natural to occur (37). By direct and indirect contact through infected blood or body fluids, the SIV_{cpz} was transmitted to gorillas, resulting in the new SIV_{gor} (37). Ultimately, SIV_{gor} became the precursor for HIV-1 group O, by inter-species transmission to humans by infected blood and body fluids (37,38). Due to their divergent origins, group O is more than 50% phylogenetically homologous to HIV-1, which is not enough to become a different HIV type yet, it is distant enough to not be considered a subtype of group M (39–41). Cases of group O are almost exclusive of Cameroon with a steady 0.6% prevalence over the past two decades (42–44). Sporadic cases have been reported in Europe, United States, and other regions of East,

however all tied to but always patients or partners of patients originating from or linked to western Central Africa (39,40). Co-circulation of groups M and O are also emerging as a public health challenge due to the high genetic diversity of group O (47–49). The consequences of recombination or dual M/O infection, can be antigenic, resulting in false negative serological testing; genetic, leading to wrong quantification of the viral load by using specific kits for group M detection and the need for special antiretroviral (ARV) resistance essays; and therapeutic, since group O possesses a natural resistance to Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) (50,51).

1.1.6. Group N

Group N ("non-M, non-O" or "new") was isolated for the first time in May 1995 from a 40-year-old Cameroonian woman who presented gastrointestinal symptoms (local *Histoplasma capsulatum* infection) (52,53). Interestingly, the patient's serum only reacted with the envelope antigen from SIV_{cpz}, rather than with groups M and O representative antigens (10). The first inter-species crossing occurred between an isolated *Pan troglodytes troglodytes* chimpanzee's community in south centre Cameroon and humans (54,55). Intragroup homogeneity and low prevalence of this group suggests an early introduction of this lineage into the human population that dates around 1963 (56). Group N was an epidemic restricted to Cameroon, with less than 20 reported cases of infection until 2011, when the virus was identified in France, emphasising the need for rigorous HIV epidemiological monitoring (57).

1.1.7. Group P

Group P was the last group to be identified, and it was in 2009, in a 62-year-old woman from Cameroon who was living in France (58). She was seropositive for HIV since 2004 and presented an elevated viral load, however it could not be quantified by HIV-M specific essays (58). With the lack of response to HIV-M specific essays, together with her Cameroonian origins, it was suspected an HIV-1 group O infection but again, specific group O RT-PCR had failed (58). Complete genome sequency unravelled a virus that was evidently distinct from groups M, N and O, yet very similar to SIV_{gor} (58). The entry of this virus into human populations happened around 1960, and the proximity between the phylogenesis of and HIV-1 group P, most likely suggests an independent zoonosis of SIV_{gor} infecting gorillas o humans

(58). Until this day, only two distinct cases have been reported (one in France and one in Cameroon) (58,59), and together with weak neutralization of the human cell restricting factor such as tetherin, suggests an almost null spreading of this virus through human population (60). With strains of this virus being extremely rare, along with less pathogenicity and less adaptation to humans, it is hypothesised that HIV-1/P could correspond or evolve towards a dead-end infection (60,61).

1.2. HIV-2

1.2.1. Discovery and origin of HIV-2

HIV-2 was firstly isolated in 1986, from two patients (from Guinea Bissau and Cape Verde), who were healthy and did not belong to any risk group (62). Both patients began to have fever, diarrhoea, progressive loss of weight and chronic lymphadenopathy (35). Afterwards, they began to exhibit opportunistic infections, associated with a low T4 lymphocyte count, that led to the diagnostic of AIDS and later, discovery of HIV-2 (35). Like HIV-1, HIV-2 is also a zoonosis, however closely related to the simian immunodeficiency virus found in the *Cercocebus atys* (or sooty mangabey) monkey species (SIV_{SM}) (63,64). Both HIV-2 and SIV_{SM} have a similar genome structure, with each virus encoding Vpx, an accessory protein, that has not been encountered in other primate lentiviruses (65). Moreover, SIV_{SM} and HIV-2 cannot be separated into distinct lineages since they are phylogenetically related (it has been reported phylogenetic and geographic connection between HIV-2 and SIV_{SM} at a local level) (65,66). Evidence indicates that human contact with blood of wild sooty mangabeys in Africa (for bushmeat) is the most suitable route for the first interspecies transmission of HIV-2 (65,67,68). The genetic data determined that both HIV-1 and HIV-2 share 30 to 40% of genetic homology, which means that there had to be similar circumstances for the emergence of both HIV-1 and HIV-2 (65). These findings were later the principal factors that unravelled the first ever HIV-1 cross-species transmission to humans (65). Even though the first ever identified case of HV-2 occurred in the late 80s, molecular clock research settles the first interspecies crossing occurring around 1940 and 1945 for subtypes A and B respectively (69,70). Since HIV-2 generally has a lower viral load than HIV-1, it becomes ten times harder to pass through vertical transmission becomes and three times harder to pass through sexual intercourse (due to lower genital shedding in semen and cervical secretions), consequently leading to a slower progression

towards AIDS (25 years average) (71). Given the small life expectancy in the African countries and the opportunistic infections (fatal to AIDS patients) that were common with local environment diseases, HIV-2 was able to remain undetected for almost fifty years (18).

1.2.2. Tracing the origin and history of HIV-2

Even though Guinea-Bissau had the highest prevalence by the time the first blood samples were analysed, the oldest HIV-2 blood samples were collected from Côte d'Ivoire (72). Furthermore, phylogenetic analyses demonstrated strong evidence between groups A, B, C, G, and H and SIV_{SM} from sooty mangabeys in the Tai forest (southwestern Côte d'Ivoire) (68). This is a good example in which the area of greatest clinical impact does not coincide with the original cross-species location (68). Consequently, this data suggests an early migration of HIV-2 A to Guinea-Bissau (68). The high prevalence of HIV-2 A in this country was not caused by inter-species crossing, but due to health, culture and social modifications related to the colonial war with Portugal between 1963-1974 (70,73–76).

1.2.3. HIV-2 Diversity

We can further divide HIV-2 into eight different subspecies (Groups A-H), however there has only been reports of subspecies A and B spreading through humans (67). Subgroup A is exclusively found in The Gambia and Guinea-Bissau whereas subgroup B persists mainly around Côte d'Ivoire (77). Subgroups C-H have been documented in Liberia, Sierra Leone, and Côte d'Ivoire, representing different cross-species transmission events, between humans and sooty mangabey, however, since they have only infected one individual each and there has not been reported any other infection, these exemplify dead-end infections (11,67).

2. Mechanism of viral replication

HIV has the typical genomic structure of retroviruses (78). The mature virion possesses two molecules of single-stranded RNA (the HIV genome) and fundamental enzymes in the process of infection (protease, integrase, reverse transcriptase, and accessory proteins) inside the inner capsid (78). Upon reverse transcription and integration on the human genome by action of the reverse transcriptase and integrase enzymes respectively, the resulting proviral DNA (also known as provirus) is flanked by long terminal repeat (LTR) sequences at both ends (79). The 5' LTR region acts as a promoter of the transcription of the viral genes (78). The genes of HIV are found in the central region of the provirus and encode 15 unique proteins that can be divided into three different classes: structural proteins (group specific antigens (Gag), polymerase (Pol), and envelope (Env)), regulatory proteins (*Tat* and *Rev*), and accessory proteins (*Vpu/Vpx, Vpr, Vif*, and *Nef*) (**Table 1**) (78,80). Accessory proteins *Vpu* and *Vpx* are exclusive of HIV-1 and HIV-2, respectively (18,78). The absence of the *Vpu* protein in the genome of HIV-2 can be one of the reasons for the lower viral load and pathogenicity of this virus compared to HIV-1 (81,82). Due to a biggest infectivity and pathogenesis, the subsequent discussion will focus on the life cycle of HIV-1 (**Figure 1**).

Table 1:Division and function of HIV proteins(adapted from (78))

Class	Gene	Protein	Function
Structural	gag	Capsid protein	Formation of conical capsid
		Matrix protein	Myristilated protein, forming the inner membrane layer
		Nucleoprotein	Formation of the nucleoprotein/RNA complex involved in virus particle release
	pol	Protease	Proteolytic cleavage of Gag (Pr55) and Gag-Pol (Pr160GagPol) precursor protein; release of structural proteins and viral enzymes
		Reverse transcriptase	Transcription of HIV RNA in proviral DNA
		RNase H	Degradation of viral RNA in the viral RNA/DNA replication complex
		Integrase	Integration of proviral DNA into the host genome
	env	Surface glycoprotein (gp120)	Attachment of virus to the target cell
		Transmembrane protein (gp41)	Anchorage of gp120, fusion of viral and cell membrane
Regulatory	tat	Transactivator protein	Activator of transcription of viral genes
	rev	RNA splicing regulator	Allows unspliced and partially spliced viral genomes (mRNA) to leave the nucleus
Acessory	nef	Negative regulating factor	Downregulates CD4 receptor and major histocompatibility complex (MHC) class I, alters T-cell activation, aids viral infectivity
	vif	Viral infectivity protein	Counters the host restriction factor APOBEC3G (critical for infectious virus production in vivo)
	vpr	Virus protein r	Facilitates the nuclear localization of the viral genome, downregulates CD4 receptor, increases viral release
	vpu	Virus protein unique	Efficient virus particle release, control of CD4 degradation, modulates intracellular trafficking
	vpx	Virus protein x	Interaction with p6 in virus particles, involved in early steps of virus replication of HIV-2, component of virus particles
	tev	tat/rev protein	Tat-Env-Rev fusion protein, regulates the activity of Tat and Rev in nucleus

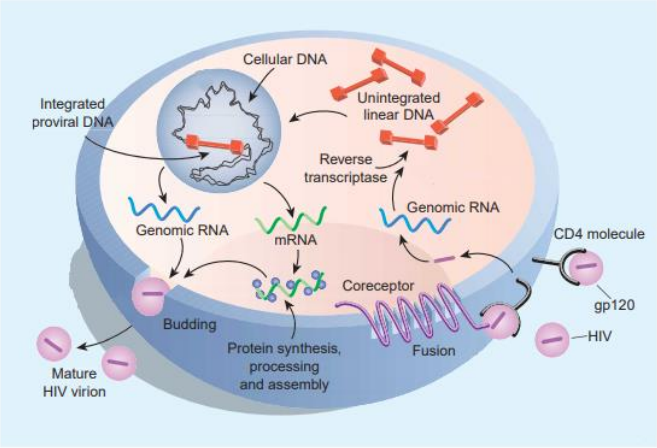

Figure 1: Cycle of viral replication on the human host cell (adapted from (83)**)**

The viral genome of HIV is protected by a capsid (78). The capsid has the function of providing a closed environment to ensure the correct beginning of reverse transcription and elongation of the viral DNA genome as well as maintaining the viral genome undetected from host factors capable of recognizing cytosolic DNA (78,79,84). The capsid is surrounded by a protecting lipidic envelope (84). This envelope displays very high concentrations of cholesterol and sphingomyelin, as well as specific lipids that include ceramide, phosphoinositides, plasmalogen phosphatidylethanolamine, aminophospholipids and dihydrosphingomyelin, and varies depending on the cell line producer (85). In addition, the envelope viral glycoproteins gp41 and gp120 trimers are the only ones projected from the membrane of the virion (86). Both proteins are associated in a conformation structure that is formed by a trimeric functional unit consisting of three molecules of gp120 exposed on the virion surface and associated with three molecules of gp41 inserted into the viral lipid membrane (18,86). The gp120 consists of five conserved domains (C1-C5), responsible for binding to the host cell and five variable domains (V1-V5) that provide constant evolving epitopes to evade humoral immune response and coreceptor binding (84,86,87). The gp41 subunit is composed by an ectodomain and a transmembrane anchor and it is responsible for the anchoring to the host cell and subsequent membrane fusion (88).

2.1. Viral entry process

The entry of HIV on the cell begins with a complex interaction between the viral envelope glycoproteins and the cells receptor (78). The viral glycoprotein gp120 binds to the CD4 receptor of the host cell. Since macrophages, astrocytes, dendritic cells, and T helper cells are all CD4 positive, all of them can be susceptible to HIV (18,89). The conserved domain of gp120, upon binding to the CD4 receptor, goes through conformational changes that end up exposing its binding sites for coreceptors CCR5 (chemokine receptor 5) or CXCR4 (chemokine receptor 4) on the host cell surface (78,79,87). The binding to the coreceptors subsequently triggers another change in the conformation of gp120 that leads to exposure of the fusion domain of gp41 (79). The hydrophobic fusion peptide of gp41 is inserted into the plasma membrane of the target cell and forms a pore through which the viral capsid enters the cell (88). This process is known as fusion (88) (**Figure 2**).

In early HIV transmission and infection, the dominant used coreceptor is CCR5 (18). Mutant alleles in the CCR5 gene have been described to prevent HIV infections (90). Furthermore, drugs that target coreceptor CCR5, blocking his interaction with gp120, as well as deletion of CCR5 gene through stem cell fusion or gene therapy have been reported to achieve HIV remission (91–94).

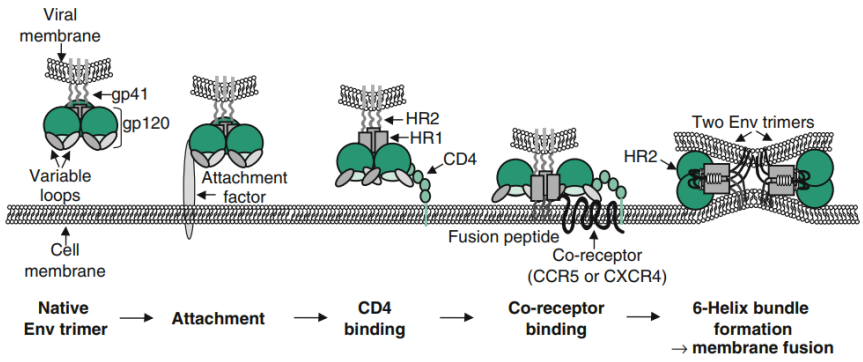

Figure 2: Cycle of viral replication on the human host cell (adapted from (95)**)**

2.2. Uncoating, reverse transcription and integration

After the fusion process, the virus capsid is taken to the cytoplasm by an endosome (96). The process of uncoating is very complex, and firstly involves the binding of capsid protein of the gag domain to host cell protein CyA (97). The binding to this human protein allows the capsid

to pass through the cytoplasm undetected, preventing premature uncoating and facilitating reverse transcription (98). The process of uncoating occurs at or closely to the nucleus, and it is triggered by a matrix phosphorylation mediated by mitogen activated protein kinase, cyclophilin A (CyA), and V-ATPase, a universal proton pump that is mediated by *Nef*, stimulating uncoating by local changes in pH (18,79,96,98–101). The genomic information of HIV is stored as RNA so, in order to be integrated into the host cell chromosome, it requires transcription to DNA. Reverse transcriptase (RT) is a large heterodimer, composed by a smaller subunit (p51) that acts as a structural support and a larger subunit (p66) that executes both polymerase and nuclease functions (89). RT needs to be attached to integrase (IN) to become activated and start the transcription (102). The RT adopts an open conformation when bound to a nucleic acid substrate that binds with the IN (102). This leads to stimulation of the initiation and elongation of the viral DNA by enhancing RT processivity and repressing the formation of pause products, leading to the transcription of the viral RNA into the double stranded viral DNA that will integrate the cell genome (79,102).

The viral reverse transcription activates the natural immunity of the host and the APOBEC3G (Apolipoprotein B-editing catalytic polypeptide 3G) enzyme present in the cytoplasm will provoke hypermutations in the viral cDNA, leading to an early terminus of the elongation (18,103). However, the *vif* protein binds to the APOBEC3G cytidine deaminase, targeting her for proteasome-dependent degradation, overriding the natural immunity of the host, and allowing elongation of viral cDNA (89,104–106). Virions without *vif* or with that protein mutated or supressed cannot perform the reverse transcriptase and consequently, fails to infect the cell (89).

Integration is the final step of the infectious process, with the viral linear, blunt-ended cDNA being incorporated into the host's genome (18,79,89). The cDNA is contained within a pre-integration complex (79). The matrix, the integrase, and the viral protein *vpr* allow for the passage of the cDNA pre-integration complex trough the nucleopores and towards the nucleus (89,107). The interaction between the pre-integration complex and the lens epithelium-derived growth factor (LEDGF), a fundamental host protein co-factor, enables the tethering of the complex to chromatin (108). The already processed viral cDNA 3'-hydroxyl groups forms an integration intermediary linked by covalent bonds by attacking the phosphodiester bounds of host's chromosomal DNA, in an integrase mediated process described as strand transfer (18,108). The integration process is finalized with the cell's normal repairing mechanism, that

fills the single strand gaps to the host chromosome and connects the ends together (79). Interestingly, the integration does not have a specific location to occur on the host cell chromosomes, meaning that the attack can occur in any chromosome and in any part of the genome (89).

2.3. Transcription and replication

The integral viral DNA (or provirus) can remain latent in the cells, even though it is rare, or it can undergo into active expression (18). The expression of this genome is cell-dependent and viral dependent, since coinfection with other agents, cellular activation or production of inflammatory cytokines can all lead to stimulation of HIV (18,109). NF-kB, a protein complex that controls the DNA transcription on the cell has a key role in the stimulation of the provirus, and some subtypes of HIV-1 have even developed more receptors to NF-kB, towards an increasing rate of transcription (79,89,110). The viral protein *tat* (an acronym from Transcription Activator) also plays an essential role in the up regulation of transcription (111,112).

Tat binds to the C-terminal domain of DNA polymerase 2, phosphorylating it and increasing his transcription rate by 100-fold (79,89,113). After fully transcript, the viral pre-mRNA is fully spliced, leading to a partial ~2-kb mRNA sequence that can encode *tat*, *nef* or *rev* (79). This means that in the early stages of virus transcription, large concentrations of the viral proteins *tat*, *nef* and *rev* are present in the host cell (113,114).

The *nef* (Negative Regulating Factor) protein, unlike *tat* or *rev*, does not affect the viral RNA structure directly, but rather modulates the host cell environment to obtain optimal replication (79). This protein is responsible for the impairment of the immune system response to HIV (115). By decreasing the expression of the MHC-1 (major histocompatibility complex 1) on the surface of the host cell, it is less prone to be detected by the $CD8^+$ cytotoxic T cells (115). Moreover, *nef* is unable to lower the expression of HLA-C, allowing for HIV to avoid recognition by the natural killer cells, also escaping the innate immunity of the organism (116). Simultaneously, *nef* also suppresses apoptosis by binding to the apoptosis signal regulating kinase-1 intermediate and by attaching to the p53 protein, inhibiting its function (111). Both mechanisms (in addition to the arrest of the cell cycle at G2/M phase by viral protein *vpr*) prolong the life of the host cell and consequently enhance viral replication (111,117). Adding

to all these functions, *nef* also plays a key role in the decrease of the expression of CD4 receptor in the cell surface, together with *gp120* and *vpu* (117). The envelope protein *gp120* attaches to the CD4 receptor in the endoplasmic reticulum, retarding its arrival to the plasma membrane and *vpu* binds to the CD4 receptor, activating proteins that target the receptor for proteasomal degradation and ubiquitination before the receptor arrives on the cellular membrane (118).

The *rev* (Regulator of Virion) protein interacts with alternate splicing factor/splicing factor 2 (ASF/SF2) and its associated p32 protein, causing an inhibitory effect of splicing (119). Moreover, it also functions as a sequence-specific nuclear export factor, binding to the Rev Response Elements (RRE) present in the env-coding region of the viral mRNA and activating the RNA exportation of unspliced and partial spliced Rev-responsive RNA from the nucleus to the cytoplasm (79,119). Under specific experimentations, it has been reported that the *rev* protein also stabilizes the HIV pre-mRNA, increasing his nuclear half-life from 10 minutes to 6 hours and plays a role in the translation of Rev responsive RNAs (119,120). This ultimately allows for the exiting of fully sequenced ~9kb viral intron-containing mRNA from the nucleus to the cytoplasm and subsequent production of the HIV viral proteins (120).

2.4. Assembly and Maturation

The immature virion starts to assemble at the plasma membrane (79). Each virion is composed by two full-length viral RNA genome, the *vpr* protein and approximately 1500 *gag* proteins and 200 *gag-pol* proteins, in a process that is primarily mediated by the *gag* and *gag-pol* polyproteins themselves and secondarily by *env*, *nef* and ATP- binding protein, HP68 (121,122). Since the proper assembly of the capsid can only be performed in high eukaryotic cells and requires ATP and a sub-cellular fraction, HP68 (or cellular ATP-binding protein ABCE1) is thought to act as a molecular chaperone, promoting conformational shifts in *gag* that are key for the transformation from structural intermediates to immature capsids (79,123).

The process of budding occurs on the plasma membrane, before the full closure of the capsid, pointing towards a competitive process between *gag* assembly and budding (124). Because of lipid post-translation modifications on the *Gag,* protein the lipid rafts, regions on the plasma membrane rich in cholesterol, sphingolipids, and glycosylphosphatidylinositol (GPI)-anchored proteins, are the preferential microdomain for the occurrence of budding (125). Moreover, this lipidic composition provides the virions with a better release, stability, and fusion with the

marked cells (125). On the last step of budding the virus hijacks the host cell ESCRT (endosomal sorting complexes required for transport) pathway (124). The primary late domain sequence PTAP (Pro-Thr -Ala-Pro) existent in the p6 portion of *gag* binds to the TSG101 (tumor susceptibility gene) subunit of the ESCRT-I complex, activating it (124). This process of activation promotes the closure of the viral dome and drives the membrane fission to completion, allowing for the virus to exit the cell (79,89,124).

The viral maturation initiates during or immediately after budding and it is driven by the cleavage of the *gag* and *gag-pol* polyproteins in ten different sites (126). This cleavage is mediated by the protease enzyme protease, and it ultimately leads to the formation of fully processed capsid, matrix, nucleus, p6, integrase, protease, and reverse transcriptase proteins (127). These proteins suffer a huge restructuration, and a non-infectious immature lattice is transformed into an infectious cone shaped particle that is the mature HIV virus (124,126).

The most important step of assembly and maturation is the timing of activation of the protease (18,124). If this enzyme is prematurely activated, or if there is an overexpression, the virion is not able to proceed to the budding process, however, if there is a partial or total inhibition of protease, the virion is unable to maturate and loses its infectivity (124). Since this viral stage requires a lot of timing and a very strict regulation, the use of protease inhibitors can suppress the maturation of HIV and consequently act as antiretroviral agents (18).

2.5. Clinical progression of HIV

As discussed earlier, HIV invades the host's immune system to hijack the host cell machinery that will allow for the virus to replicate (18). The virus can be transmitted through body fluids that are infected (blood, breast milk, rectal fluid, seminal fluid, or vaginal fluid) (128). The epithelial surface is the body's primary barrier against infection as it provides an efficient first line barrier against pathogens, including HIV (128). The epithelium itself works as a strong physical obstacle that prevents the virus from penetrating and enter the bloodstream (128). This means that thicker, mucosal surfaces like the oral cavity and the vaginal lining provide better physical barriers compared to the rectal cavity, which can be easily damaged by sexual intercourse (128,129). Simultaneously, the epithelial cells also play a key role in the immune response against HIV by expressing toll-like receptors (TLR) that will act as a pathogen recognizer (128). The TLR trigger a pathway that promotes the production of proinflammatory

cytokines and type I interferon and the secretion of antimicrobial peptides (128). The defensins, a particular type of antimicrobial peptide has shown direct inhibition of HIV *in vivo* and *in vitro* by inhibition of the viral particles or by stopping viral replication (18). In addition, the production of mucus (in the vaginal and rectal epithelium) lowers the pH of the environment, and together with the innate immune response, restrain the contact between HIV and the epithelium (128). However, a single breach in the epithelium, caused by a microtrauma during sexual intercourse, genital or oral ulcers caused by sexual transmitted diseases (herpes simplex virus 2 or syphilis), or even gingival bleeding or other oral infections, leads to inflammation and can increase the risk of HIV infection (129). The inflammatory process causes an upregulation of CCR5 expression in the dendritic cell, macrophages, and T cells and the HIV infection can start by two main pathways: direct infection of $CD4^+$ T cells macrophages, or indirect infection by entrapment in a non-T cell and subsequent transportation from the inoculation site to the lymph nodes and presentation to the T cells (130–132). The progression of HIV can be divided into 4 distinct stages: The eclipse phase, acute infection (stage 1), chronic HIV infection (stage 2) and AIDS (stage 3), and the progression of the serum concentrations of virus and $CD4^+$ T cells are described in **Figure 3** (133).

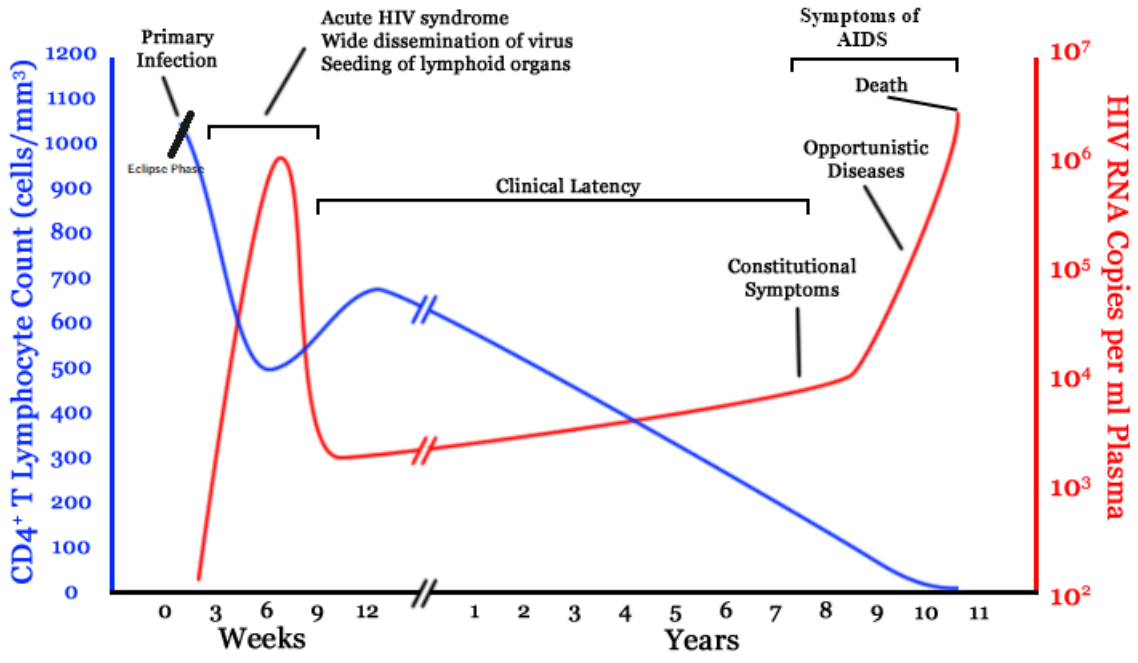

Figure 3: Progression of untreated HIV by stages (adapted from (134)**)**

2.5.1. Eclipse phase

The eclipse phase begins right after the mucosal exposure in which HIV is circulating in the plasma undetectable (18). Dendritic and Langerhans cells start a cell-to-cell signalling upon exposure to HIV, promoting the recruitment of more immune cells, including $CD4^+$ T cells (132). This phase last approximately 10 days and it is characterized by the establishment of HIV on the mucosal breach and subsequent migration and diffusion to the lymph nodes via blood stream (18).

2.5.2. Stage 1 – Acute Infection

The acute phase follows the eclipse phase, and it is characterized by a ramp-up of the viral production and a decline of $CD4^+$ T cells, in a period that can last from weeks to months (18). Once the retrovirus is disseminated to the lymphoid tissues and consequently to the systemic circulation, the viral load starts to increase as fast as a doubling time of 20 hours (135). This sudden increase consequently leads to an immune activation of $CD4^+$ T cells, all susceptible to the HIV, that ends up establishing a positive feedback loop (135). In the beginning of the ramp-up viremia, the viral RNA is the only detectable parameter (18). It is only around 7 days later that the first viral core protein in the stage 1 can be detected in the form of antigen p24, and 12 days after, that the first HIV-1 antibodies attain detectable levels with sensitive enzyme immunoassays(18,135). During this time, the organism enters in an intense inflammatory response to the infection, leading to a massive production of cytokines (18,135). Moreover, during the acute infection, apoptosis activation, redistribution of cells from the peripheral blood to the lymphoid tissue, direct action by HIV and decreased thymic production all lead to a drastic reduction of the circulating $CD4^+$ T cells (136). The gut-associated lymphatic tissue (GALT) particularly affected by this depletion, together with fibrosis on the lymph nodes (associated to HIV replication) provokes a decreasing gastrointestinal mucosal and epithelial immune response (137). This ultimately increases microbial translocation, promoting further immune activation and further stimulation of the viral replication (137). Patients with HIV with increased viraemia in the plasma can develop flu-like symptoms of chills, fatigue, fever, mouth ulcers, muscle aches, night sweats, rash, sore throat, and swollen lymph nodes (133). These symptoms may appear 2-4 weeks after acute HIV infection and last from a few days to several weeks (133).

2.5.3. Stage 2- Chronic HIV infection

The stage 2 or chronic HIV infection starts right after the acute infection, with a drastic reduction of the HIV plasma concentration and an "almost normal" return of the $CD4^+$ T cells count (136). One of the main reasons for the decreased viral load is the clearance of activated $CD4^+$ T cells that, especially with HIV antiretroviral therapy, can lead up to a diminishment in plasma HIV (up to 90 % on the first weeks if the patient is on antiretroviral therapy) (18). During this time, it is established an equilibrium between the elimination and the production of $CD4^+$ T cells (133). This stage is mainly defined by a clinical latency period, where the HIV infection is asymptomatic, and the infected person shows little to no symptoms during this phase (133). Nonetheless, the virus continues to replicate at very low levels, meaning that people who are not receiving treatment for HIV during this phase or haven't achieved yet viral suppression are able to transmit the retrovirus to the community (138). The chronical HIV infection can last from 5 to 15 years without antiretroviral therapy, depending on the $CD4^+$ count and viral replication level post-acute infection (138). During this time, it is observed a slow decline of $CD4^+$ T cells parallel to a viral load increasement in the organism(138). Once the immune system starts to malfunction due to a serious reduction of T cells and the person starts to exhibit symptoms again, the infection has already reached the next state of infection (18).

2.5.4. Stage 3- Acquired Immunodeficiency Syndrome (AIDS)

The final stage of HIV initiates when the $CD4^+$ T cell count is below 200 cells/mm^3 or when the patients start presenting symptoms and later diseases related to AIDS (138). The immune system of this patients cannot defend the organism against opportunistic infections and infection related cancers that were otherwise stopped by a healthy immune system (138). Moreover, due to an exponential increase of the viral load, the patient is susceptible to transmit the infection more easily (133). The continuous drop of $CD4^+$ T cells count, along with the exponential increase if the HIV in plasma ultimately lead to the most severe phase of HIV infection, AIDS (18). Untreated patients have an average lifespan of 3 years, however, with the advancements in antiretroviral therapies and patient's adherence to the medicines, the viral suppression can be achieved, delaying, or even preventing the progression of the infection to the stage of AIDS (133). Moreover, a study in the United States between 2005-2010

demonstrated that more than 80% of the patients that do reach the stage 3 with the correct ARV can surpass the "3-year barrier" of AIDS (139). Current advances in the treatment and management of HIV were able to transform a death sentence infection into a stable chronic condition (18). People living with HIV that are able to reach consistent viral suppression can have a happy and regular life without having to worry about transmitting the virus to their sexual partner (18). On the next point, antiretroviral agents (individual antiretroviral agents (ARV)) and antiretroviral therapy (combination of ARV to stop the HIV infection (ART)) will be discussed.

3. Antiretroviral therapy

3.1. Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (NRTI/NtRTI)

NRTI/NtRTI are nucleoside/nucleotide analogues that lead to a premature ending of the viral DNA elongation (18). Zidovudine was the first substance in this antiretroviral class approved, in 1987 for the treatment of patients with an advanced state of HIV (140,141). NRTI are prodrugs that circulate outside the cell in their non-functional form (142). These prodrugs enter the cell through simple diffusion or diffusion via nucleoside carrier-mediated transporters and it is in the cytoplasm that NRTI/NtRTI are metabolized and activated through three/two phosphorylation steps (142). The active drug phosphorylate accumulates in the cell cytoplasm and competes with the cell nucleosides/nucleotides for substrate binding to the reverse transcriptase (143). Once they get incorporated, the conversion of single stranded RNA to double-stranded is terminated prematurely, preventing DNA elongation and further integration of the virus into the host-cell DNA (142–144). The nucleosides abacavir, emtricitabine, lamivudine and zidovudine and the nucleotides tenofovir alafenamide and tenofovir disoproxil fumarate are currently the only NRTI/NtRTI approved for the treatment of HIV (133,140,141,145–147). These analogues also interfere with the mitochondrial DNA replication (143). By inhibiting mitochondrial DNA polymerase γ, the mitochondria become dysfunctional by inhibiting mitochondrial enzymes, causing oxidative damage, and diminishing ATP synthesis (143). This consequently leads to insufficient energy production, induction cellular apoptosis and in long term, malfunction of tissues and organs (143,144). Kidney and bone toxicity as well as liver failure have been reported as side-effects of chronic exposure to these

antiretroviral agents (140,146,148). Drug interactions, inconvenience, monitoring, multiple toxicities, and viral efficacy were key factors for the abandonment of didanosine, deoxycytidine, and stavudine (all NRTIs) clinical use (149,150). Clinical resistance to NRTI/NtRTI has also been reported (151). Since proofreading is absent in the reverse transcriptase enzyme due to lack of 3' exonuclease activity, the enzyme starts accumulating constant uncorrected errors in its genome (1 base error per cycle) (18). This inaccurate reading causes viral mutations in the reverse transcriptase that result in conformational changes on the enzyme active site or functional areas (143,151–154). This evolutionary selection leads to a partial efficient reverse transcriptase that can function in the presence of the drugs (151). Suboptimal therapy, lack of adhesion, therapeutic cessation or induction are the major factors that increase mutation rates that the development of resistances (155). If not managed carefully, mutations will accumulate and generate a cross-resistance to all NRTI/NtRTIs available on the market, a concerning situation since these analogues are used as a way to control the HIV infection for an extended period of time (155).

3.2. NonNucleoside Reverse Transcriptase Inhibitors (NNRTI)

NNRTI, just like NRTI, have a mechanism of action that is based on reverse transcriptase inactivation however, unlike NRTI, these drugs are non-competitive inhibitors that bind to an allosteric site of the enzyme, altering her structure and stopping the formation of HIV copies (156). The first approved NNRTI was nevirapine, in 1996, and it was used for the treatment of HIV in combination with zidovudine and didanosine in naïve patients (18). The reverse transcriptase possesses two subunits, and it is in the functional subunit (p66) that exists an allosteric binding site NNIBP (NNRTI binding pocket) (157–160). This "pocket" is adjacent to the DNA polymerase active site, and it is responsible for its correct functioning (159). When present in the cytoplasm of the host cell, the NNRTI binds to the NNIBP in the form a "butterfly" or "U" structure, causing a structural change in the p66 subunit of the reverse transcriptase (157–160). This structural change arrests the active catalytic site into an inactive conformation, leading to a reverse transcriptase sterically incapable of growing a DNA chain(157–160). The available NNRTIs can be divided by generations and all of them have been approved by the FDA and EMA (except delavirdine) (140,141,146). Delavirdine, nevirapine and efavirenz belong to the first generation of NNRTIs; etravirine, rilpivirine, and doravirine (2018) were authorized more than a decade later compared to the other generation

and thus belonging to the second generation of NNRTIs (161–163). Delavirdine was approved by the FDA in 1997 however, in 2018, due to business decisions, it was discontinued by their manufacturers (145). Even though well tolerated and safe for long term use, the biggest drawback of the first generation of NNRTIs was the development of resistances (151,164). A single viral mutation (generally because of poor drug adherence) that could alter the structure of the NNIBP or block the entry of the inhibitors in the pocket was enough to diminish the contact between the NNRTI and the NNIBP, leading to a resistance (151,153,162,165). Moreover, the occurrence of cross resistances to this antiretroviral class was also common (151). New discoveries in the mechanism of resistance, along with the better understanding of the different interactions between the NNIBP, contributed for the discovery of the new generation of NNRTIs (162,163). Using the first second-generation NNRTI (etravirine) as an example, its flexible structure, allows it to rotate, leading to multiple interactions within the NNIBP (166). Due to its flexibility and multiple bonding to the binding pocket, etravirine can maintain activity after several HIV mutations and as a potent inhibitor of HIV reverse transcriptase resistant to first generation of NNRTI (157,158,166).

3.3. Protease Inhibitors (PI)

The PIs inhibits HIV protease, a key enzyme in the process of HIV core maturation (167). This blockage stops the cleavage of *gag* and *gag-pol* polyproteins, leading to an interruption of the budding process or, if the budding process does occur, rendering them abnormal and incompetent to infect host cells (18). Saquinavir was the first ever introduced protease inhibitor on the market in 1995 (140). The HIV protease is a 99 amino acid homodimer, and it is composed by two essential aspartyl residues, where the binding to the *gag* and *gag-pol* occurs (167,168). The binding site can avoid total exposure due to the presence of two flexible β-hairpin flaps (glycine rich loops) (one for each monomer) that are able to mask the active site (168,169). However, to occur substrate binding, the β-hairpin flaps need to open, allowing access to the binding site (169,170). The PIs take advantage of this mechanisms and are able to connect to these sites, generating a large conformational change of the protease (170). Structural studies point to a two-step inhibition mechanism, in which the PI binds to the viral enzyme, and quick change of the protease structure, forming a collision complex (170). Shortly after, a new shift in conformation occurs, however at a slower rate, that leads to a tighter complex, shutting down the cleavage of viral genes and preventing maturation of the virions (170). Up to this day,

besides saquinavir, indinavir, ritonavir, nelfinavir, amprenavir, atazanavir, fosamprenavir, tipranavir and darunavir (2006) have been approved by the FDA and EMA for the treatment of HIV, however, according to the most recent guidelines, darunavir and atazanavir are the only ones that are still commonly recommended in the treatment of HIV (18,141,146,148). The most common side effects of this antiretroviral class are nausea and diarrhoea, ischemia heart disease, reduction of estimated glomerular filtration rate and metabolic disorders (especially dyslipidaemia) (142,147,148). Interestingly, since ritonavir presented high pill burden, frequent dosage and high rates of lipid abnormalities and gastrointestinal effects, it started to be used as a pharmacokinetic enhancer (142). Since ritonavir is also a potent CYP3A4 inhibitor, low doses of this PI result in higher half-life and exposure of other PIs, meaning that it is possible to achieve therapeutic effects with lower doses and less frequency of administration of PI, leading to better tolerability, better adhesion, and less side effects (142). Even though resistances have been reported, first line PI, especially with concomitant pharmacokinetic enhancement, have shown very low to no drug resistance rates when virologic failure occurs (151,153).

3.4. Integrase Strand Transfer Inhibitors (INSTI)

The class of INSTI is very recent compared to other classes, being introduced in the market two decades after the first ever approved HIV drug (140,171). The INSTI interferes with the normal function of the viral integrase, blocking its function and preventing the integration of the viral genome on the host cell (140). The first INSTI introduced on the market was raltegravir in 2007 and elvitegravir (as a combination pill), followed by the second generation ISTINs dolutegravir, bictegravir (as a combination pill) and cabotegravir (2021), that have also received approval by the FDA and EMA for the treatment of HIV (140,141,145,146,148). Like the name of the class, all the INSTI target the integrase enzyme during the process of strand transfer, and its inhibition begins when the viral DNA is assembled to the full-length integrase (172,173). The INSTIs are composed by a metal-binding pharmacophore and a hydrophobic group (172,173). The pharmacophore binds to the two coordination Mg^{2+} ions (the cofactors for *in vivo* integration) on the active binding site of the integrase and to the viral DNA, while the hydrophobic group interacts with the hydrophobic pocket of integrase, establishing Van de Waals interactions with the active site of the integrase (173). These interactions disrupt the covalent bonds that were supposed to occur between the viral and the host DNA, blocking the integration of the viral genome onto the host cell DNA and ultimately stopping viral replication (171–175). The most

common adverse effects of this class are nausea, reduction of the estimated glomerular filtration rate, sleep disturbances, headaches, and weight increase (146). It has also been reported resistances to INSTIs, especially the first generation, meaning that every patient should be carefully monitored to understand if the transition to second generation INSTI is required (151,171,175,176).

3.5. Pharmacokinetic enhancer (PKE)

Pharmacokinetic enhancers do not have a direct action against HIV (140). They are able to partially alter the metabolization of other HIV regimens, stopping the metabolization of the antiretroviral drugs, and consequently, increasing their concentration and effectiveness against HIV (177). Up to this date, ritonavir and cobicistat have been the only pharmacokinetic enhancers approved by the FDA and EMA, in 2001 and 2014 respectively (140,145). Unlike cobicistat, who has no antiretroviral activity, ritonavir was design to be a PI (178). However, due to high inhibition of CYP3A4 and unfavourable side effects when compared to other PIs, ritonavir has been used in lower concentrations as a pharmacokinetic enhancer, alongside with another protease inhibitor (178). The mechanism of action of both ritonavir and cobicistat is the inhibition of CYP3A4 (178). By inhibiting this family of cytochromes, the metabolization of drugs that were once metabolized in the CYP3A4 decreases (142,178). These inhibitions generate bigger plasma concentrations of CYP 450 family metabolized drugs and lower pill burden which is reflected on a higher anti-HIV activity (142,178). Cobicistat presents no anti-HIV activity, a higher selectivity for CYP3A4 inhibition and a lower possibility of enzymatic induction, which means that the profile of drug interactions is lower than ritonavir (179). Nonetheless, with a larger clinical experience from the 20 years that has been in the market, ritonavir is still used according to the most recent guidelines as a pharmacokinetic enhancer (146–148). Cobicistat is generally well tolerated and presents less side effects compared to ritonavir; however, it has been reported that the exposure to cobicistat may inhibit the cell transporters of the proximal renal tube, resulting in the increasement of serum creatinine and diminution of the estimated glomerular filtration rate (146,178,179).

3.6. Entry inhibitors

The entry inhibitors are a type of class that affects the viral entry process on the host cell (18). These medicines can block the viral replication by inhibiting the host cell docking, or the process of viral fusion (18). Nowadays, there are four different entry inhibitors approved on the American and European markets (140,141). They all present different inhibition mechanisms to stop HIV, meaning that cross-resistance is not possible, however, individual cases of resistance to single medicines have already been reported (151). The entry inhibitors can be divided into fusion inhibitors, CCR5 Antagonists, attachment inhibitors and post-attachment inhibitors.

3.6.1. Fusion inhibitors

The fusion inhibitors, interfere with the conformation of the gp41 protein, ensuring that it doesn't change conformation and consequently prevents viral fusion with and passage of the virion through the host's membrane cell (140). Enfuvirtide was introduced on the market in 2003 and is currently the only approved fusion inhibitor for the treatment of HIV (146,147). This medicine is a synthetic peptide, that was synthetised to imitate the amino acid sequence of the heptad repeat region, HR2 of gp41 (180). These 36 amino acid fragment binds to the HR1 region of gp41, blocking the essential conformational changes needed to set up the six-helix bundle structure and ultimately inhibiting fusion (180). Changes in the HR2 selection, alongside with polymorphisms in the HR2 region and amino acid modifications in HR1 can be enough to establish resistances to enfuvirtide (180,181). Furthermore, due to a low genetic resistance barrier and a complicated administration route, (subcutaneous injection that needs to be administered twice a day) enfuvirtide is reserved for patients with multiple drug resistances to three orally active agents (140). Hypersensitivity, injection nodules and iatrogenic pneumonia can manifest as side effects of the treatment (147,177).

3.6.2. CCR5 Antagonists

The CCR5 antagonists prevent the entry of HIV in the host cell by binding to the CCR5 co-receptor of the host cell and consequently stopping the attachment of the virus to the host cell (142). Maraviroc has been introduced in the market in 2007 and it is the single FDA and EMA

approved drug in this class for the treatment of HIV in adults and children with more than 2 kg and in combination with other ARV (141,147). Maraviroc binds to the host cell hydrophobic pocket found in the extracellular transmembrane helices (182). This binding ends up altering the conformation of the CCR5 extracellular loops to the point that they become untargetable to the gp120 viral glycoprotein, stopping the attachment to the host cells and preventing viral replication (182). One of the biggest limitations of this class is the CXCR4-tropic HIV, that can interact with other co-receptors and perform viral replication without the need to attach to CCR5 (148). Before using maraviroc, a test of HIV it is necessary to realize a viral tropism test to confirm that the virus only uses the CCR5 receptors and thus, the therapeutic is adequate (177,183). Maraviroc is administered twice a day orally and can have serious side effect like hepatitis, fever, and upper respiratory tract infections, which, altogether with the expensive test of viral tropism, make the use of this medicine in clinical practise uncommon (140).

3.6.3. Post-attachment inhibitors

The post-attachment inhibitors (along with the CCR5 antagonists) are the only medicines that attach to the host cell to stop viral entry (140). However, the post attachment inhibitor is the first ever monoclonal antibody to be approved for treatment of HIV infection (184). Ibalizumab, a recombinant humanized immunoglobulin G, is the only post attachment inhibitor successfully introduced on the market, getting the approval by the FDA and EMA in 2018 (141,184). Ibalizumab binds to the extracellular CD4 domain 2 cells and to two amino acid sites on the intersection between domains 1 and 2, coating $CD4^+$ T cells (184). Moreover, epitope mapping reveals that the attachment site of ibalizumab does not affect the binding sites of MHC-II and *gp120* on domain 1 (184). This means that the medicine has no interference in the MHC mediated immunity cell response, neither with the *gp120* binding to CD4 (185). The binding of ibalizumab provides a steric effect that physically stops the attachment between the viral envelope *gp120* and the CD4 surface cell receptor (185). Consequently, the blockage of the CD4- *gp120* connection stops subsequent interactions with CCR5 and CXCR4 and inhibits viral entry and fusion (184,185). Ibalizumab is a well-tolerated medicine with few to no side effects with diarrhoea, dizziness, nausea, and rash being the most frequent ones (146). The use of this drug in monotherapy or the failure of infusion can lead to resistances, that have already been reported (151,185). Due to recent approval of the drug, there is still no information about its use against HIV-2, HIV-1 N, O and P infections (146).

3.6.4. Attachment inhibitors

The attachment inhibitors are a new class of anti-HIV drugs that binds directly to the *gp120* subunit of the envelope *gp160*, preventing its binding to the CD4 receptor and ultimately stopping viral replication (186). The first-in-class attachment inhibitor is fostemsavir, a prodrug of temsavir, was approved in 2018 by the FDA and EMA (140,141). Even though temsavir had relevant antiviral activity, his pharmacokinetic profile needed to be improved (187). With the phosphonooxymethyl prodrug technology, temsavir was able to increase its dissolution in the gastrointestinal tract and selectively being released, due to an alkaline phosphatase cleavage mechanism found on the brush border membrane (187). Fostemsavir, after being phosphorylated into temsavir, binds to the *gp120* subunit, and stabilizes it (187). The locked-on stable conformation is unable to bind to the CD4 receptor on the host cells, and without this attachment, the virus is unable to perform the viral attachment and fusion, and viral replication is prevented (186,187). Mild headaches, rash were the main side effects on clinical trials, however, It is now known that fostemsavir can cause more serious secondary effects such as prolongation of QTc interval and increase of transaminase and bilirubin levels (146). This medicine is used along with other ARV, it is not clinically recommended as initial therapy, but rather as a last resort, on patients with multi-drug resistant HIV (188).

3.7. Combination therapy

One of the key reasons for virologic failure is the patient lack of adherence to the therapy (18). And in the early years after the discovery and approval of the first antiretroviral, a very large number of pills, along with severe acute and chronic side effects, led to an abandonment of the therapy and consequent virologic failure (18). Combination therapy can be defined by the presence of at least two HIV medicines in a single formulation (generally one pill) (145). With the introduction of combination therapy, the pill burden was reduced, the toxicity minimized, the potency of the treatment was increased, and the drug interactions started to drop, which contributed to a better adhesion to the antiretroviral medicines (18). As a result of the high adhesion, the patients were able to stabilize the overall medication and achieve viral suppression more easily (around 95% of adherence is required for supressing HIV) (18). We can split the different combinations into two groups: the single tablet regimen (STR), where you only need to take 1 tablet a day to get the desired antiretroviral effect; and combinations that need to be

taken along with other HIV medicines to achieve viral suppression (145). **Table 2** shows every combination therapy against HIV that is currently approved by the FDA and EMA (140,141,145).

Table 2: Therapy combinations approved for combating HIV/AIDS (adapted from (140,141))

Drug	Brand Name (FDA/EMA)	Approval year (FDA/EMA)	Class	Administration route
3TC / ZDV	Combivir	1997/1998	NRTI	Oral
LPV / RTV	Kaletra	2000/2001	PI + PKE	Oral
ABC / 3TC / ZDV	Trizivir	2000	NRTI	Oral
ABC / 3TC	Epzicom/Kivexa	2004	NRTI	Oral
FTC/TDF	Truvada	2004/2005	NRTI	Oral
EFV / FTC / TDF	Atripla	2006/2007	NNRTI + 2NRTI	Oral
FTC / RPV / TDF	Complera/Eviplera	2011	2NRTI + NNRTI	Oral
EVG / COBI / FTC / TDF	Stribild	2012/2013	INSTI + PKE + NRTI	Oral
ABC / DTG / 3TC	Triumeq	2014	2NRTI + INSTI	Oral
ATV / COBI	Evotaz	2015	PI + PKE	Oral
EVG / COBI / FTC / TAF	Genvoya	2015	INSTI + PKE + 2NRTI	Oral
DRV / COBI	Prezcobix/Rezolsta	2015/2014	PI + PKE	Oral
FTC / TAF	Descovy	2016	NRTI	Oral
FTC / RPV / TAF	Odefsey	2016	2NRTI + NNRTI	Oral
DTG / RPV	Juluca	2017/2018	INSTI + NNRTI	Oral
BIC / FTC / TAF	Biktarvy	2018	INSTI + 2NRTI	Oral
3TC / TDF	Cimduo (FDA only)	2018	NRTI	Oral
DOR / 3TC / TDF	Delstrigo	2018	NNRTI + 2NRTI	Oral
EFV / 3TC / TDF	Symfi (FDA only)	2018	NNRTI + 2NRTI	Oral
DRV / COBI / FTC / TAF	Symtuza	2018/2017	PI + PKE + 2NRTI	Oral
DTG / 3TC	Dovato	2019	INSTI + NRTI	Oral

Abbreviations: 3TC – lamivudine; ABC – abacavir; ATV – atazanavir; BIC – bictegravir; CAB – cabotegravir; COBI – cobicistat; DOR – doravirine; DRV – darunavir; DTG – dolutegravir; EFV – efavirenz; EVG – elvitegravir; FTC – emtricitabine; IM – Intramuscular; LPV – lopinavir; RPV – rilpivirine; RTV – ritonavir; TAF - tenofovir alafenamide; TDF - tenofovir disoproxil fumarate; ZDV- zidovudine)

4. Advances in the management of HIV/AIDS

The time where HIV was treated by a niche of antiretroviral drugs is long gone. In 2021, more than 30 drugs that work through 8 different mechanisms and 2 pharmacokinetic enhancers, have been introduced on the market to fight HIV(18,141,145). Several medicines that were once used in clinical practise, have given place to more potent, less toxic, and less frequently taken drugs that can fight equally or better HIV/AIDS (18). Moreover, the existence of a large arsenal of antiretrovirals, allows for patient therapy personalisation(18,142,145). The biggest reason for HIV drug resistance mutations is the lack of adherence by the patients, however, with a more individualized therapy, the boundaries of each patient can be overcome, as it is possible to prescribe a treatment that better adapts to the patient's comorbidities and other social, geographical, racial and gender factors (146,147). This is reflected in the adhesion of the patient to the therapy, and on the long-term results of the treatment (146).

4.1. Beginning ARV therapy

The beginning of ARV on a young primary infected patient is still a big debate however, the most recent guidelines recommend the starting of ARV therapy immediately or as quick as possible after diagnostic of HIV (146–148,189–193). This action not only prevents the transmission of HIV to the community, mortality, and AIDS-associated morbidities at long term, but also speed up the linkage care and viral suppression in medium-short term (146,147). However, there are exceptions to these rules, that depend on the patient's physiology, CD4 cell count and/or comorbidities (146).

Patients that are pregnant, with acute symptomatic infection, with a CD4 count<350 cells/μL, with over 50 years old, with neurological disease or with severe/prolonged symptoms are strongly advised to start treatment for HIV immediately after diagnosis (147). On the other hand, patients suffering from psychosocial factors or specific opportunistic infections like active tuberculosis, cryptococcal meningitis and cytomegalovirus end organ disease, might need to delay the treatment of HIV, in order to manage the other comorbidities (146,147).

Randomized control trials in poor countries have demonstrated that people beginning ARV therapy as soon as they are diagnosed, leads to a faster linkage to care and to a faster viral

suppression (191–193). Observational studies in the USA regarding vulnerable population showed very good results, as 90% of the community became viral suppressed in the end of 1,09 years (189,190). This shows that concomitant diagnosis and initiation of the treatment is viable and may even be beneficial, independently of the rich or limited settings available (146). Now, more than ever, the high potency of new treatments, along with low number of pills and few toxicity/secondary effect burdens allow for the use of ART to the point of initiating treatment at the time of diagnosis for many patients (148).

4.2. Initial laboratory assessment

A person that is diagnosed as HIV positive for the first time, needs to perform an extensive laboratory evaluation (18). Along with providing present and past medical history, drug/family/social history, and an initial physical examination, the results of these analysis will serve as baseline parameters for further comparisons after initiating antiretroviral therapy (194,195). General blood tests that can assess general health and safety (eGFR, glucose, cholesterol, liver enzymes and white blood cell count) and comorbidities are important to choose the antiretroviral agents/combination to begin/to switch to and need to be performed, and some of them may never be repeated (194). The HIV specific tests, the resistance, the hypersensitivity tests will be ones highlighted in this point.

For the detection of HIV, it is usual to perform a fourth-generation antibody-antigen test for detection of the p24 antigen in an earlier phase or HIV antibodies on a later stage (195). This test is generally conduced after a first positive self-test performed by the patient. The nucleic acid test can also be performed, however due to its high price and unusual use, it is rarely utilized as a first test. The diagnosis of HIV is mainly important for asymptomatic patients with normal CD4 cell count and low to undetected viral load since it assures the presence of this retrovirus in the organism (133,195).

The CD4 absolute and relative cell count test help the clinician understand the current stage of infection, establish possible stage-related complications and, when below 200 cells/µL, initiate prophylaxis against opportunistic infections (146–148). The relative cell count is relatively more stable than the absolute count and it can be used to evaluate the immunological function (18,195).

The plasma HIV RNA tests should be performed in the beginning of the treatment (177). Viral suppression is defined by any value below the lower threshold of detection of the essays (20-50 copies/ml) and the number of RNA copies needs to be below 200 copies/ml to prevent sexual transmission of HIV (177,195). The clinician needs to have the values of the viral load controlled to understand if the therapeutic is working or if there is any problem on the treatment because of resistances or lack of adhesion from the patient (177,195).

Since drug-resistance can be transmitted between from person to person, all patients beginning treatment should be tested for genotypic resistances to PI, NRTI and NNRTI (195). INSTIs are not included due to low-frequency transmission nevertheless, in case of suspecting resistance, the test should be performed (146,147,195). Patients that present virological failure (>200 copies/ml) also need to realize the resistance testing with the objective of managing the infection (195). If the clinician pretends to begin a therapy with an entry inhibitor that blocks the CCR5 co-receptor, it is mandatory to also perform a chemokine receptor tropism test, to evaluate if HIV only uses the CCR5 pathway to replicate (and the entry inhibitors may be used) or if it uses both CCR5 and CXCR4 pathways (and the clinician needs to take another therapeutic approach) (177).

If ABC is considered as a therapeutic option in a patient, it will be needed to perform an HLA B*5701 haplotype screening (195). In the case of a positive result, ABC should not be administered or, if the patient refuses the test, it should be carefully monitored for signs and symptoms of a possible allergic reaction (195).

4.3. Initial ART regimens

Before the selection of any type of antiretroviral drug, the clinician needs to address any type of comorbidity or physiological state of the person in cause (146). For adult people living with HIV that are receiving treatment for the first time and have no other health problem, it is recommended a combination therapy of 2 NRTIs + INSTI or 1 NRTI + INSTI (196–199). The beginning of treatment with a INSTI combination is preferred over a PI combination since it has showed faster viral suppression (87 vs 81% over 48 weeks), less viral failure and less iatrogenic reactions (19 women performing treatment with PI needed to interrupt therapy due to adverse reactions vs 5 with INSI treatment) (200). The use of the 2-drug therapy with DTG/TC can only be used in patients with viral loads inferior to 500000 copies/ml, and any

value bigger than that needs to be treated with the 3-drug regimen (196) . There are alternative regimens to the ones above mentioned that are still efficient and generally tolerated, however, there are disadvantages or not enough supporting data to surpass the recommended regimens (201–203). 2NRTIs + NNRTI and 2NRTIs + PI/PKE are examples of possible alternative combinations (146–148). INSTIs that are not DTG or BIC don't offer the same genetic barrier that these drugs present, which means that they are less likely to confer protection, before knowing the drug-resistance test (146,147). Emerging resistances to the first line of recommended ART have already been reported, which further addresses the need for a fast drug resistance test and, if possible, faster change to a more suitable treatment for each patient (204–206).

4.4. Optimizing/Simplifying ART

After a first regimen of antiretrovirals, if the patient's adherence is maintained and no resistances to the ARV are detected, viral suppression is naturally achieved (147). The main goal of this process is to maintain the number of viral copies below 50/ml (also known as 'undetectable' level), and to prevent food-drug or drug-drug interactions, diminish toxicity in the short or long term, reduce the ART expenses to the patient and reducing the frequency of dosing and pill burden (146). Patients with no prior history of resistances to any antiretroviral, should be able to be given the choice of the regimen that better fits to their physical, psychological, and social situation (146,147). Nevertheless, patients with reported resistances to some regimens need to have their drug-resistance tests and tropism essays reviewed to correctly select the most fitting therapy (147). Therefore, any modification to the current treatment requires a strategic revision on the patient's complete ARV history, secondary effects, toxicity, and drug relating intolerances (146,147).

Optimization/simplification therapy that maintains a three-drug regimen can be approached by two different manners. The first one is the shift within-class, where the same class of ARV is used, however, with a better profile of safety, inferior pill burden and higher genetic barrier. Examples of this changes are TAF instead of TDF or ABC (207–209) and DOR or RPV instead of EFV or an NNRTI (different from DOR) (210,211). Inter-class swap is also a possibility of therapy optimization/simplification, nonetheless, if there is any suspicion regarding the effect of the other regimens in the treatment (due to resistances to said classes), the switch should be

immediately cancelled (146,147). Examples of shifts between classes are MVC in the place of an enhanced PI (212), an INSTI instead of NNRTI (213) and INSTI to replace an enhanced PI (214–217)

With the therapeutic advancement of the management of HIV, simplified two-drug regimen therapy is becoming more effective in the control of naïve patients who started treatment and attained viral repression (146,147). One exception to the rule are co-infected hepatitis B virus patients, in which the following treatments have an inadequate therapeutic effect against that infection (147). DTG + RPV (218,219), DTG + 3TC or FTC (220), boosted PI +3TC (221–223) and boosted DRV + DTG (224) are the most successful strategies used in clinic to change from a 3 to a 2-drug regimen (146,147).

4.5. Cabotegravir/Rilpivirine – the expansion of long-acting HIV medicines

After the approval of the long-acting monoclonal antibody (ibalizumab) in 2018 for the treatment of experienced HIV patients, Cabotegravir/Rilpivirine was introduced in the American market as the second long-acting antiretroviral agent (147). In Europe, the introduction on the market was different. Even though they are meant to be used together, Cabotegrair (Vocabria) and rilpivirine (Rekambys) were separately approved in 2020 as suspensions of prolonged release (141), Phase 3 clinical studies showed that this medicine was not inferior to a three-drug standard regimen in the management of virally supressed HIV infection (225,226). As of today, long acting cabotegravir/rilpivirine is approved as a simplification of oral therapy for patients with good adherence and committed to the care, that have remained virally suppressed for 3-6 months, without hepatitis B virus, as well as resistance mutations to cabotegravir or rilpivirine, not taking any medication that could interfere with the treatment and not considering getting pregnant (147). Before starting cabotegravir/rilpivirine injections, oral therapy with cabotegravir + rilpivirine should be realized as a preparation therapy before the injections for 4 weeks (141). Afterwards, the injections should be administered by a healthcare professional on the last day of lead-in oral therapy and then every month (141,147). The number of copies/ml should be verified every 4-8 weeks and in case of increasement, resistance testing (INSTI included) should be performed (147). The introduction of injectable treatments increases the quality of life of people that fear that their HIV situation

becomes public, relieves the pill burden, and stops the stigma of daily oral medication for the rest of their life (147).

4.6. Future management of HIV/AIDS

Islatravir is a new prodrug in phase I studies that needs to be phosphorylated to be active like NRTIs, and like this class, it also binds to the viral reverse transcriptase enzyme (227). However, this drug attaches to the active site and does not allow for primer translocation, stopping replication without a direct effect on termination of viral DNA production (227). Early studies reveal safety and toleration in animals (227). In case of approval, this would be the first nucleoside reverse transcriptase translocation inhibitor (NRTTI) ever existing on the market (227).

Another candidate for approval in phase I studies is GS-6207 (227). With a new single mechanism of actuation, GS-6207 prevents the assembly of the viral capsid, stopping any further maturation of HIV in the cell. Its pharmacokinetic properties point to a (subcutaneous) injectable formulation (227). Resistances to this prototype have already been discovered, however, with no other antiretroviral acting through the same mechanism, this could be another drug that wouldn't be subject to cross-resistance mutations (227).

HIV broadly neutralizing antibodies have recently entered into phase I clinical trials (227). They bind to the *gp120* of HIV, ceasing the processes of viral attachment and fusion to the host cell (227). With the development of drug-resistances, especially in monotherapy, if this drug ever gets introduced in the market, it is very probable that will be used in combination with other ARV that have different mechanisms of blocking viral replication (227).

The development of a vaccine that can help in the treatment of HIV or prevent a possible infection is starting to show promise (18). Even though there are phase II and III clinical trials ongoing, there is not any vaccine authorized in the American and European markets for the treatment or prevention of HIV (18,140,141). Vaccines can be divided into two main classes: therapeutic HIV vaccines and preventive HIV vaccines.

4.6.1. Therapeutic HIV vaccines

This kind of vaccine targets the patients that have already been infected with HIV (140). The main objective is to enhance the patient's immune system to the point where it would augment concomitant ART, enable therapy simplification, allow for treatment pauses or even allow for complete stoppage of the ART (18,140,228). With this, the optimal vaccine is the one that retards HIV advancement to AIDS and maintains the viral load undetected without ever needing to take other antiretroviral medication (18,140,228). Early clinical trials in the 90s, failed to demonstrate that a vaccine with inactivated gp120 and 5 others with recombinant gp120 and gp160 could change the advancement of the disease and prevent the drop of CD4 cell count (229–234). A vaccine containing an HIV- gag insert showed a good safety profile and improved viral suppression, nevertheless, these values failed to obtain statistical meaning (235). By 2012 a well-tolerated HIV-1 B recombinant plasmid vaccine with 6 genes incorporated was able to increase the CD4 and CD8 T cell count and diminish the viral RNA with statistical significance on HIV-1 type C patients naïve to any sort of antiretroviral treatment (236). In 2015, a randomized phase II clinical trial, patients that were treated with antibodies that target the protein *tat*, were able to develop antibodies that led to the partial recovery of T, B and Natural Killer cells population and furthermore decrease the circulating viral DNA by week 72 (237).

4.6.2. Preventive HIV vaccines

Preventing vaccines, unlike the first ones, are used in people that haven't been infected with HIV (140). Even though pre-exposure prophylaxis medication and use of sexual protection can block the HIV chain of transmission, a vaccine will be the most efficient way to prevent it (18,140). Furthermore, preventive vaccines could be the key to eradicate HIV and eliminate all the laboratory tests, resistances and side effects that occur during antiretroviral therapy (140). Vaccines with recombinant gp120, recombinant *gag/pol/nef* adenovirus type 5 vector showed no efficacy in clinical trials (238–241) . However, in 2009, a combination of a recombinant canarypox vector vaccine with two booster injections of a recombinant gp120 subunit vaccine was able to prevent HIV infection on volunteers with 31.2% efficiency (242). As of now, there is an ongoing clinical trial with a new chimpanzee recombinant adenovirus Ox1s (243). This trial is funded by the European Aids Vaccine Initiative, and it will take place in the UK (243). Other international studies ongoing internationally are testing the effectiveness of a new bioengineered Adenovirus serotype 26 "mosaic 4"

(optimized env/gag/pol antigens + adjuvant gp140) HIV, that will take place in the United States, south America, Europe and sub-Saharan Africa and are expected to finish in March 2024 (243).

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