From AZT to treatment as prevention. The evolution of antiretroviral therapy for HIV/AIDS

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ABSTRACT

The antiretroviral therapy (ART) for the treatment of HIV/AIDS has been extremely successful in prolonging the lives of people living with HIV. Since the approval of AZT up to the present, over 26 individual compounds have been added to the arsenal available for physicians. The combination of three of these drugs, directed against more than one target was the key to achieve a prolonged suppression of viral load with the consequent gain in the life expectancy of the patients. The development of drugs with improved virological and pharmacological properties, less adverse effects and a better resistance profile, together with the application of pharmacological boosters such as ritonavir and cobicistat, the implementation of single pill formulations of drugs to reduce pill burden, and a reduction in the production costs associated to the introduction of generics, have allowed a considerable expansion of the ART coverage in low and middle resource countries. Based on the results from the START clinical trial in 2015, which demonstrated the advantages of the early application of ART in patients with CD4+ T cell counts over 500 cells/mm³, the main regulatory agencies has modified the recommendations about when to start ART. Additionally, the demonstration of the protective effect of ART among discordant couples has open new horizons for the implementation of ART as a preventive intervention. Recently, UNAIDS has launched its new campaign aimed at the expansion of the ART coverage to reach a 90% reduction in HIV infections by 2030.

Keywords: HIV, AIDS, antiretroviral therapy, prevention

RESUMEN

Del AZT al tratamiento como prevención. La evolución de la terapia antirretroviral contra el VIH/sida. La terapia antiretroviral (TAR) ha sido muy exitosa para la prolongación de la vida y la salud de los personas infectadas con el VIH/sida. Desde la aprobación del primero de ellos, el AZT, se han sumado más de 26 compuestos al arsenal terapéutico. La combinación de tres de estos compuestos dirigidos contra más de un blanco fue la clave para lograr una supresión prolongada de la carga viral. El desarrollo reciente de drogas con mejores propiedades antivirales y farmacológicas, efectos adversos menos frecuentes y severos, y un perfil de resistencia más favorable, ha permitido ampliar considerablemente la cobertura entre los pacientes tratados en países de mediano y bajos ingresos, junto a la aplicación de potenciadores farmacológicos como el ritonavir y el cobicistat, la obtención de formulaciones combinadas de drogas para reducir el número de tabletas diarias, así como la disminución de los costos de producción para las variantes genéricas. El ensayo clínico START en 2015 demostró las ventajas de la aplicación inmediata de la TAR en pacientes con conteos de células T CD4+ por encima de 500 céluas/mm³, y permitió que las agencias reguladoras modificaran las recomendaciones sobre el estadio clínico para el inicio de la TAR. Adicionalmente, la demostración del efecto protector sobre la transmisión del virus en parejas discordantes, abrió un nuevo horizonte para la implementación de la TAR como instrumento preventivo. La meta lanzada por ONUSIDA para ampliar la cobertura terapéutica hasta el 90 % de los pacientes que la necesitan, persigue disminuir hasta en un 90 % las infecciones por VIH para el 2030.

Palabras clave: VIH, sida, terapia antirretroviral, prevención

Introduction

Antiretroviral therapy (ART) has been very successful for the treatment of HIV/AIDS. Since the former application in 1996 of the so-called highly active antiretroviral therapy (HAART), HIV/AIDS turned from a fatal into a chronic condition [1-4]. Its treatment efficacy and efficient preventive effect on the virus vertical transmission have been proven [5]. In this review, an overview is presented on the ART therapeutic stages for HIV/AIDS treatment, starting from the early days of the pandemic, when ART was not available and life expectancy was very short since the manifestation of the first symptoms, until current preventive strategies with a better outcome for pandemic eradication.

The pre-ART period

The first cases of an unknown syndrome were reported in US in 1981, characterized by a profound drop in CD4+ T lymphocyte counts and subsequent immune depression of patients. In those days, the disease was called “Gay pest”, “Gay cancer” or Gay-related immune deficiency (GRID), due to its major incidence...
among men having sex with men (MSM) [6, 7]. The further demonstration that heterosexual patients were equally susceptible to infection led to its official definition as Acquired Immunodeficiency Syndrome (AIDS) [8].

Discovery of the Human Immunodeficiency Syndrome

The race for discovering the etiological agent of AIDS brought about the first results in 1983, when a team directed by the French virologist Luc Montagnier published on the identification of retroviral particles and reverse-transcriptase activity in cultures of lymphocytes isolated from AIDS patients [9]. This was the first report associating a retrovirus with AIDS, but not conclusive on their causal relationship. Less than a year later, the group led by Robert C. Gallo at the National Cancer Institute provided solid evidences in four reports, supporting the hypothesis of a new retrovirus as the causal agent of AIDS [10-13]. The corner stone in Gallo’s work was to replicate the new virus in a tumor cell line of lymphoid origin (H9), providing enough viral material to characterize its proteins and to develop serologic methods to detect antibodies specific for the virus in patients’ sera [14]. Consequently, the nucleotide sequences of two different but similar viruses were elucidated, markedly different from any previously identified human retrovirus [15, 16]. This was the basis for denoting the new entity as the Human Immunodeficiency Virus (HIV) [14].

The knowledge of the HIV replicative cycle as the basis for the design of viral inhibitors

Shortly after the discovery of HIV, an intense research effort was conducted in several laboratories worldwide to unravel its viral structure and to characterize the proteins coded in the viral genome. Next, we will summarize the main events of HIV replication cycle. For further details the reader may consult one of the following reviews [17-29].

HIV is an enveloped RNA virus, as every known retrovirus. Its genome encompassing 9.6 kb codes for three types of proteins: structural, enzymatic and regulatory (also called auxiliary).

Structural proteins comprise those located in the viral envelope membrane: gp120 or external glycoprotein, and the gp41 or transmembrane glycoprotein. Other four proteins derived from a common precursor protein of 55 kDa form the viral capsid: the matrix (p17), capsid (p24), nucleocapsid (p9) and p7 proteins.

Three proteins display key viral enzyme functions during the viral replication cycle: reverse transcriptase (RT), protease (P) and integrase (I). And five other proteins display regulatory or auxiliary functions, all of them required for an efficient viral replication: Tat [30], Rev [31], Nef [32], Vif [33] and Vpr [11].

The viral particle encapsidates two copies of the RNA viral genome, together with a lysine transfer RNA molecule which functions as primer for the reverse transcription of the viral RNA genome into DNA. HIV mainly infects CD4+ T lymphocytes, those cells with a key function in the orchestration of the adaptive immune response; also capable of infecting efficiently other cell types such as dendritic cells, macrophages, microglia and Langerhans’ cells, which play an essential role in the immune response [34, 35].

The HIV replicative cycle begins with the recognition of the high affinity viral receptor, the CD4 molecule, on the target cell surface. This first contact induces conformational changes in the gp120, these changes expose a second binding site within the gp120 structure known as the viral co-receptor binding domain. This site attaches to several molecules of the chemokine receptors family, mainly CCR5 and CXCR4 [36-38]. Once completed the interaction of the virus with receptor and co-receptor, another major structural change occurs, releasing the hydrophobic N-terminal domain of the gp41. This domain inserts into the cell membrane, taking both the viral envelope and cell membranes into close proximity [39]. Following the internal retro-traction of the gp41 trimers’ alpha helices which wrap around each other to put both membranes closer enough, both membranes fuse together, releasing the viral capsid within the cell [40].

Subsequently, the capsid disassembles in the cytoplasm releasing the viral RNA genome, which is copied by the RT to generate a double-stranded DNA molecule to be transported into the nucleus and inserted into the cell DNA by the action of the viral integrase [41, 42]. HIV RT is characterized by its low-fidelity processing during the copy of RNA into DNA.

It has been estimated that this enzyme introduces an error every 1000 to 10 000 nucleotides [43-45]. This generates virus particles having at least 1 to 10 mutations per viral genome. Once the viral DNA genome is produced, named provirus and indistinguishable but for its sequence from the human DNA genome, it can be inactive and remain untranscribed for long, undefined periods, with no viral protein production [46-48]. When the infected host cell become activated to certain extent, some transcriptional factors such as NFκB acts on the viral promoter and initiate the transcription of viral genes [49, 51].

During the first early stage of transcription, the process is inefficient and the mRNA molecules are subjected to multiple splicing, only producing the low molecular weight proteins. One of those proteins is Tat, a potent transcriptional transactivator which accumulates into the nucleus and increases over 1000 times the levels of mRNA produced [52]. Another key protein in this process is Rev, also located in the nucleus. It binds to the viral mRNA at a site known as RRE and protects the molecule from multiple splicing, aiding to the formation of single or twice spliced, long transcripts, which reach the cytoplasm and are successfully transcribed to produce the viral RNA proteins and used as viral RNA genome. Then, the capsid proteins assemble to encapsulate two viral genomic RNA molecules within a particle that is directed to the cell membrane and released through a process known as budding. The viral particle becomes enveloped by the cell membrane at the site of budding, coated by the viral glycoprotein spikes previously anchored in the cell membrane at the budding point. Outside the cell, the viral particle undergoes a maturation process, finally structure the inner shell of the capsid and making the viral particle infective, ready to initiate another infection cycle [53-55]. There has been 4. Deeks SG, Lewin SR, Hawari DY. The end of AIDS: HIV infection as a chronic disease. Lancet. 2013;382(9903):1525-33.
Estimated that a person living with AIDS generates around 10^2-10^3 viral particles daily [56-58].

**Evolution of ART against HIV/AIDS**

In general, the historical evolution of ART against HIV/AIDS can be divided in three discrete periods:

*First period (1986-1996):* mono- and bitherapy with inhibitors against RT.

*Second period (1997-2014):* highly active antiretroviral therapy (HAART), also known as triple therapy.

*Third period (2015 and beyond):* starting by the extension of ART treatment to every person living with HIV, not only for the benefit of patients but also as a weapon to actively prevent viral transmission.

Let's analyze these three periods in further detail.

**First period: Failure of mono- and bitherapy with RT inhibitors**

ART against HIV started as early as in 1986, after the approval of the first medicine against AIDS by the FDA: azidothymidine or AZT [59]. Between 1981 and 1986, AIDS patients had no medicine available to fight the infection. This caused a dramatic decline in life expectancy to just one year in half the patients developing AIDS, defined as having CD4+ T cell counts below 200 cells/mm³ or characterized by the appearance of well defined opportunistic infections associated to the disease [60].

The first period in the evolution of ART, when one or two combined drugs were applied, did not change much that setting. In spite of attaining certain level of efficacy [61, 62], these drugs did not have a marked impact neither in the intermediate nor the long term of disease progression.

The first viral protein targeted by ART was RT. A series of compounds similar to AZT followed in the years to come, belonging to the family of nucleoside reverse transcriptase inhibitors (NRTI) [63-66] (Table 1). These molecules were called DNA strand terminators, their effects based on the similarity of their structures to some of the nucleotides. RT shows affinity to recognize the nucleoside analogue [67, 68]. Curiously, such a cavity only appears in the presence of the inhibitors. NVP was followed by delavirdine (DLV, ViiV Healthcare) [76, 77]; efavirenz (RPV, Bristol-Myers Squibb) [79] and more recently etravirine (ETR) [80] and rilpivirine (RPV) [81, 82], these last manufactured by Janssen Pharmaceuticals.

The combination of these two types of inhibitors while superior to monotherapy did not significantly improve the clinical outcome of patients, nor steadily reduce viral load [83, 84]. Bitherapy was also a failure for the very same reasons as monotherapy: the emergence of viral resistance as an unbeatable obstacle.

**Second period: Implementing HAART was a huge step forward in AIDS therapeutics**

**HAART**

The second evolutionary period of ART was closely related to the development of a third type of drug: protease inhibitors (PI). These small chemical compounds were capable of inserting into the HIV protease active site, further blocking its catalytic activity [85]. The HIV protease belongs to the family of aspartic proteases [86]. The active form is a homodimer composed by two 11 kDa monomers. Its role in the viral replication cycle is the proteolytic processing of the Gag and Gag-Pol polyproteins to generate the different proteins forming the mature virion [87]. All this proteolytic processing is interrupted by the insertion of PIs into the active site of protease, thereby blocking the formation of mature virions.

**Third period (2015 and beyond):** This period started by the extension of ART treatment to every person living with HIV, not only for the benefit of patients but also as a weapon to actively prevent viral transmission. The combination of those two types of inhibitors while superior to monotherapy did not significantly improve the clinical outcome of patients, nor steadily reduce viral load [83, 84]. Bitherapy was also a failure for the very same reasons as monotherapy: the emergence of viral resistance as an unbeatable obstacle.

**Evolution of ART against HIV/AIDS**

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<table>
<thead>
<tr>
<th>Year</th>
<th>Inhibitor</th>
<th>Acronym</th>
<th>Analogue of</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1996</td>
<td>Indinavir</td>
<td>IDV</td>
<td>–</td>
<td>Merck</td>
</tr>
<tr>
<td>1997</td>
<td>Nelfinavir</td>
<td>NFV</td>
<td>–</td>
<td>Agouron</td>
</tr>
<tr>
<td>1999</td>
<td>Amprenavir</td>
<td>APV</td>
<td>Discontinued in 2004</td>
<td>GlaxoSmithKline</td>
</tr>
<tr>
<td>1999</td>
<td>Lopinavir</td>
<td>LPV</td>
<td>2nd line of treatment in combination with RT</td>
<td>Abbots</td>
</tr>
<tr>
<td>2003</td>
<td>Aftanavir</td>
<td>ATV</td>
<td>–</td>
<td>Bristol-Myers Squibb</td>
</tr>
<tr>
<td>2003</td>
<td>Fosamprenavir</td>
<td>FPV</td>
<td>Amprenavir produg</td>
<td>ViIV Healthcare</td>
</tr>
<tr>
<td>2005</td>
<td>Tipranavir</td>
<td>TPV</td>
<td>–</td>
<td>Boehringer Ingelheim</td>
</tr>
<tr>
<td>2006</td>
<td>Darunavir</td>
<td>DRV</td>
<td>–</td>
<td>Janssen Pharmaceuticals</td>
</tr>
</tbody>
</table>

* Discontinued due to toxicity.
The first PI approved by regulatory agencies was saquinavir [88, 89]. Other nine molecules of this type have been subsequently generated [90-93] (Table 2).

At the same time, the use of these inhibitors as monotherapy did not qualitatively fostered ART efficacy. Resistance was also present, although starting with the gradual emergence of viruses carrying primary mutations which impeded the binding of inhibitors to the protease active site, followed by other mutations at least partially compensating the fitness lost with the primary mutations [94].

Hopefully, the results of clinical trials presented at the HIV World Congress held in Vancouver, in 1996, were about to radically transform the ART perspective. The combination of three anti-HIV drugs (two NRTI plus a PI) successfully reduced viral loads down to undetectable levels and kept it under control for more than a year under treatment [95-99].

Two years after implementing HAART, the first data appeared supporting a decrease in mortality and morbidity due to opportunistic infections in treated patients [100, 101]. Although premature, these results revived the optimism not only on the possible control of HIV/AIDS through therapy, but even its eradication. Dr. David Ho at the Diamond Research Center, one of the most renowned virologists at that time on HIV studies, raised the slogan “to hit early and hard” [102]. This necessarily implied to start what was called HAART in asymptomatic patients as early as possible after infection. It was strongly considered the possibility that, if the virus would be silenced for several years, it would be possible to eradicate itself by the natural immune response of the organism [102, 103]. Unfortunately, such strategy did not stand long enough, due to the abundant and serious adverse effects caused by all these drugs, particularly PIs, in most patients [104-106]. Some of the adverse effects associated to the use of these drugs are listed in table 3.

Therefore, the use of HAART was not properly justified in first instance, due to its cumulative adverse effects and the risk for emergence of resistant viruses, more detrimental than the possible benefits coming from the control of viremia since the very early stages of infection. This led to the decision to postpone its application until the decline of CD4+ T lymphocyte counts below 250 cells/mm³ or until the patient suffering from opportunistic infections defining the AIDS stage. Throughout the years, the tendency has emphasized on their recommendations to start HAART during the AIDS stage. FEBS J. 2012;276(11):2926-46.

Several years after the discovery of T20, another inhibitor capable of blocking viral entry was approved: maraviroc. It was design to interfere the binding of the virus to its co-receptors [111, 112].

Maraviroc as blocker of the HIV binding to the CCR5 co-receptor Once established the binding mechanisms of the virus to its co-receptors, several pharmaceutical laboratories seek for molecules able to block their interaction. The first of those compounds, maraviroc, was approved in 2007, a small chemical molecule specifically binding to CCR5 and blocking its interaction with gp120 [115]. Maraviroc was developed by Pfizer upon optimization of UK-107,543, an imidazoylidine selected by massive screening through a CCR5-binding assay. More than 1000 analogues were evaluated during the optimization process, in order to improve potency and reduce the compound toxicity [116].

Subsequently, the inhibitor demonstrated to be safe and efficacious in clinical trials, both in patients previously treated with other drugs and in those naïve to treatment [117, 118]. Nevertheless, maraviroc had no commercial success, its weakness residing on its efficacy limited to monocyte tropic strains which use CCR5 as co-receptor. This implies that prior to the administration of maraviroc to a patient, it is necessary to investigate on the co-receptor tropism of its resident viral strains [119], an economic cost and a time loss imposed by the complexity of such tests.

Table 3. Main adverse effects of HIV protease inhibitors

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Acronym</th>
<th>Main adverse effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saquinavir</td>
<td>SQV</td>
<td>Gastrointestinal disorders; lipodistrophy (5.4 %); diabetes mellitus; hyperglycemia (2.7 %); fatigue (6.1 %); fever (3.4 %)</td>
</tr>
<tr>
<td>Indinavir</td>
<td>IDV</td>
<td>Renal disorders (nephrotoxicity/uricosuric); asymptomatic hyperbilirubinemia; gastrointestinal disorders; rash; pruritus; cephalexin (5.4 %); dizziness; sleepiness</td>
</tr>
<tr>
<td>Ritonavir</td>
<td>RTV</td>
<td>Hepatic disorders; pancreatitis (pancreas inflammation); severe allergic reactions; heart rhythm disorder</td>
</tr>
<tr>
<td>Amprenavir</td>
<td>APV</td>
<td>Gastrointestinal disorders, such as: nausea (74 %); vomiting (34 %); diarrhea (39 %); hyperglycemia (37 %); hypertriglyceridemia (36 %); hypercholesterolemia (4 %); hepatic disorders; oral/perioral parasthesia and headache; depression (15 %); neutropenia and hemolytic anemia; body fat redistribution and accumulation</td>
</tr>
</tbody>
</table>

Integration inhibitors

The HIV inhibitor is a 32 kDa protein, the major component of the viral integration complex. Its enzyme activity on proviral DNA resides on the elimination of two nucleotides from the 3' terminus and forming a phosphodiester bond between the terminus and the host chromosomal DNA [120].

In 2007 and after two decades of research, raltegravir (RAL; Merck) appeared as the first integration inhibitor, being approved for clinical application [121]. Two other inhibitors of this type have also been approved so far: dolutegravir (DTG; GlaxoSmithKline) in 2013 [122] and elvitegravir (EVG; Gilead Sciences) in 2014 [123]. Three of them are small molecules for oral administration.

Opposed to the experience with entry inhibitors, raltegravir have had a sound commercial success with significant sales. Such results have being determined by its small size and accessibility, as well as its mechanism of action was not interfered by the emergence of resistant mutant viruses. Moreover, the adverse effects it provokes are milder than those caused by precedent ART drugs (Table 4).

This example illustrates that, in spite of the existence of about 26 drugs available against HIV, the arsenal continuously grows, to fight the continuous emergence of resistant viruses. Even when ART has substantially impacted on the AIDS epidemics, with a radical decrease in mortality and improving the patients’ quality of life [124], the complete eradication of HIV from the body remains a goal to be achieved.

Undoubtedly, ART efficacy has improved over time. Particularly since 2010, the number of therapeutic options available in the clinical setting has increased, reducing viral load down to undetectable levels and progressively lowering it. Additionally, the first choice regimes suppress viral load in more than 90% of the cases, even after 8 years on therapy [3, 125].

Auxiliary and capsid proteins as targets for ART

A plausible source for targeting HIV is its regulatory proteins, in spite of having been relegated as targets for ART in clinical trials. But, undoubtedly, interfering with their functions would have an immediate impact on HIV replication as experienced with the enzyme viral proteins. For instance, the levels of viral transcription would be reduced as much as 1000 times upon blocking the activity of Tat. In fact, various molecules have been described capable of inhibiting the action of Tat and thereby viral replication [126-129], but just one of them has been evaluated in the clinical setting and unfortunately with negative results [130, 131].

Likewise, neutralizing Rev would limit the synthesis of structural proteins and, therefore, the formation of new virions [132, 133].

In the case of Vif, this protein is essential for virus formation and infectivity, the proof-of-concept of its inhibition validated by testing a small chemical molecule as inhibitory for viral replication [44]. It is highly probable that in forthcoming years some of these strategies would prove efficacious in clinical studies.

Additionally, there are evidences on the possible impact that inhibition of Nef would have on HIV replication, although it is known that Nef-deficient viruses does not lose completely their capacity to replicate, making of this protein a less attractive target [134].

Other interesting targets are the capsid proteins [135]. It has been demonstrated in in vitro experiments that small molecules interfering with the morphogenesis of new virions and their maturation process could inhibit the viral replication [136, 137]. One of these compounds was evaluated in phase I and II clinical trials but its potency was insufficient [138].

New host targets for ART

It is known that HIV receptors use other cellular proteins as auxiliary factors during its replication cycle. Among them, the nuclear proteins Emerin and BAF are involved in the penetration of the integration complex into the nucleus [139-142]. Other proteins take part in the formation of the integration complex and capsid assembly processes. Any of these cellular factors and others remaining to be identified could become an effective target for ART.

Recommended ART treatment regimes

A large number of combinations have been tested with more or less success for antivirals approved for clinical use. Based on cumulative clinical evidences, a panel of experts consulted by the National Institutes of Health of US established the combinations recommended as optimal for clinical use, either as first choice of treatment for patients naïve to treatment or as second line for those whose ART therapy has been unsuccessful or as rescue treatment in patients with changes more than twice in the ART regime [107].

In the following we summarize the updated recommendations of that panel:

### First line of treatment for patients starting ART

**NRTIs**
- Dolutegravir (DTG)/abacavir (ABC)/lamivudine (3TC).
- Dolutegravir DTG /tenofovir (TDF) /emtricitabine (FTC).
- Elvitegravir (EVG)/bicistcist (c) /tenofovir (TDF) /emtricitabine (FTC).

**NRTIs**
- Raltegravir DTG /tenofovir (TDF) /emtricitabine (FTC).

**Table 4. Common adverse effects of the HIV inhibitor Raltegravir**

<table>
<thead>
<tr>
<th>Type of effect</th>
<th>Most frequent effects (grade, frequency)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic</td>
<td>Increase transaminase levels (ALAT; grade 2, until 11 %; grade 3, until 4 %; grade 4, until 2 %)</td>
</tr>
<tr>
<td>Metabolic</td>
<td>Hyperglycemia (grade 2, until 10 %; grade 3: until 3 %); high alkaline phosphatase (grade 2, until 2 %)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>High lipase (grade 2, until 5 %; grade 3, until 2 %); nausea (moderate to severe, 3 %); high pancreatic amylase (grade 2, 2 %; grade 3, 4 %); abdominal pain, gastritis, dyspepsia and vomits (less than 2 % each)</td>
</tr>
<tr>
<td>Nervous</td>
<td>Cephalalgia (moderate to severe, until 4 %); dizziness (moderate to severe, 2 %)</td>
</tr>
<tr>
<td>Psychiatric</td>
<td>Insomnia (moderate to severe, 4 %); depression (suicidal ideas and conduct, less than 2 %)</td>
</tr>
<tr>
<td>Hematologic</td>
<td>Low neutrophils (grade 2, until 4 %; grade 3, 3 %; grade 4, 1 %); low platelets (grade 2, until 3 %); low hemoglobin (grade 2 and 3, 1 % each)</td>
</tr>
<tr>
<td>Renal</td>
<td>Nephrolithiasis (less than 2 %), renal failure (less than 2 %)</td>
</tr>
<tr>
<td>Hypersensitivity</td>
<td>Fatigue (moderate to severe, 2 %); asthenia, genital herpes and herpes zoster (less than 2 % each)</td>
</tr>
<tr>
<td>Oral</td>
<td>Less than 2 %</td>
</tr>
</tbody>
</table>

Antiretroviral therapy against HIV/AIDS

Combinations including protease inhibitors (PI)
- Darunavir (DRV)/ritonavir (r) /tenofovir (TDF) /emtricitabine (FTC).

Cobicistat and ritonavir are used in these regimes as enhancers of other inhibitors’ effects. These two compounds inhibit liver enzymes belonging to the sub-family of Cytochrome P450 3A4, which metabolize other inhibitors and thereby increase their pharmacokinetic properties, supporting a possible reduction of the dosage and the number of applications [143]. They differ in that ritonavir is simultaneously an HIV PI although ineffective at the concentrations administered in these regimes, while cobicistat is a pharmacokinetic booster with no direct action on HIV replication.

There are a series of alternative regimes recommended solely for those situations in which the previously mentioned combinations cannot be used. All of them are triple combinations, except for the darunavir (LPV/r) /lamivudine (3TC) and darunavir (DRV)/r/raltegravir (RAL) dual regimen, only recommended for patients intolerant to tenofovir and abacavir.

**Cases of virologic failure. Second line of ART treatment**

Virologic failure is defined as the impossibility for maintaining viral RNA levels below 200 copies/mm3 under ART. The causes are varied and its analysis is beyond the scope of this review. But it is worth to mention that, in those cases, there have to be checked if deficient adherence to treatment occurred together with the characterization of the resistance pattern of the viruses infecting the patient. Depending on the results, another ART regime must be proposed, with drugs effective against the virus variants present, the drugs either aimed towards a different target or able to neutralize the viruses resistant to drugs of the same type.

**Repeated virologic failure**

Considering the current drug diversity, the number of people presenting virologic failure against two or more ART regimes has decreased. Nevertheless, such hard to treat cases could occur, commonly associated to viral strains showing resistance against various types of inhibitors. Therefore, the inclusion of inhibitors very different from those recommended, such as T20 or maraviroc, could be very helpful. Ultimately, if the viral load could not be completely controlled below the detection limits of available tests, ART could be applied to maintain a partial control on viral replication levels. This will always provide a more favorable prognosis for patients than leaving them with no therapy.

**The rise back of bitherapy?**

There are renewed attempts to reduce to just two the number of inhibitors included in ART regimes, fueled up by the availability of ‘integrase inhibitors, second and third generations’ drugs against RT and protease, with improved potency, a more favorable viral resistance profile and less adverse effects than previous drugs. In this sense, various clinical studies have demonstrated that biotherapy has similar effects than the recommended tritherapy.

The first of such studies, denominated GAREDEL (Global AntiRetroviral Design Encompassing Lopinavir/r and lamivudine vs LPV/r based standard therapy), demonstrated that the lopinavir (LPV)/r/ lamivudine combination was not inferior to the triple combinations of lopinavir (LPV)/r/ lamivudine (3TC) or emtricitabine (FTC)/other NRTI [144]. It is worth to mention that, in this trial, the effect was measured only for 48 weeks, that is, less than a year of treatment and the regimes used for comparing biotherapy were alternative, second line regimes, not those recommended as optimal by the experts.

A second trial, named PROGRESS, was a pilot, randomized, open trial, run to compare safety and efficacy after 96 weeks of a regime administering lopinavir (LPV)/r/raltegravir (RAL) twice-a-day against the regime with three inhibitors Tenofovir (TDF)/emtricitabine (FTC)/LPV/r once-a-day. The results indicated that bitherapy was not inferior, this time for a longer period, but the limited proportion of patients having a viral load of more than 100 000 copies at the start of the study made difficult to assess the efficacy of this strategy [145].

In the third study, NEAT001/ANRS143, the efficacy was evaluated for a regime based on darunavir (DRV)/ r/raltegravir (RAL) administered twice a day for 96 weeks as compared to a triple regime administering tenofovir (TDF)/emtricitabine (FTC)/darunavir (DRV)/r. This trial concluded with no superiority of the standard treatment over bitherapy to control viral load [146].

These results show a favorable tendency supporting the long term evaluation of such optimized bitherapy regimes, aimed to reduce ART costs and their associated adverse effects. Nevertheless, more clinical testing is required to validate the long term effect of these formulations prior to recommending them as first line of treatment for patients naïve to ART.

**Combo administration: strategies to reduce the number of pills**

One of the difficulties to achieve the adequate adherence of patients to ART is the high pill burden they have to take daily. To solve this problem, the industry has developed combined formulations which integrate more than one inhibitor in the same pill. Some of those formulations are:
- Atripla®, the first once-a-day pill, a turning point for ART combined administration. It was developed by Bristol-Myers Squibb and is recommended for patients intolerant to tenofovir and abacavir.
- Strucerica® recommended tritherapy.
- Triumeq®, combining dolutegravir (DTV) plus abacavir (ABC) and lamivudine (3TC), is manufactured by ViV Healthcare [147].
In the same line, two other formulations of three ART drugs and a pharmacokinetic boosting agent (with no action on HIV-cobicistat) are available from Gilead Sciences, both as single pills: Stri bil® and Genvoya®. Each combines an integrase inhibitor (efavirenz or elvitegravir) with two other inhibitors (emtricitabine/tenofovir) [148]. Moreover, Stri bil® uses 300 mg of tenofovir fumarate, while Genvoya® uses just 10 mg of tenofovir alafenamide which is more specific for the target and induces less frequent adverse events.

There are also tens of combinations of two inhibitors available, but the predominant tendency is to achieve the highest simplification of treatments by administering a single pill a day due to the positive impact this has on the adherence of patients to ART treatment.

**The Test and Treat strategy or back to start**

The improvement of ART treatment in terms of efficacy (i.e., the number of options available to fight viral resistance or more tolerable new inhibitors) has led to a reappraisal on when would be appropriate to start treatment.

On this aspect, a panel of experts from the NIH recommended in 2014 to administer ART to all the HIV+ patients to reduce the risks of progression to AIDS [107].

The strength and evidence of this recommendation changes attending to CD4+ T cell counts:
- Below 350 cells/mm³: Strong, validated in randomized control trials;
- Between 350 and 500 cells/mm³: Strong, based on cohort studies or non-randomized studies;
- Above 500 cells/mm³: Moderate, based on experts’ opinions.

With the aim to objectively settle down the debate for the possible benefits on patients to start ART with CD4+ T cell counts above 500 cells/mm³, a study coded START was conducted from 2009 to 2015, enrolling 1500 patients fulfilling such criteria. Half the patients started ART immediately, and the other half when reaching 350 cells/mm³ as recommended by current guidelines. An unquestionable benefit was demonstrated in patients starting therapy with counts above 500 cells/mm³ in terms of preventing or delaying the progression to AIDS, decreasing the incidence of other severe diseases not associated to AIDS and mortality [149].

**Third period: 2015 and beyond**

A new age in ART treatment against HIV is about to begin, transcending the ART therapeutic function and providing it a preventive and key role in pandemic control.

For years, ART has been strongly recommended to seropositive mothers to prevent the mother to child infection during fetal development [150]. Its usefulness has also been demonstrated to prevent infection in the case of accidental exposure to the virus and in the prophylaxis prior to getting into contact with it [151].

It has been repeatedly proposed that the expansion of ART among seropositive patients could significantly impact on controlling viral transmission [1]. Mathematical modeling of data from populations treated with ART for long periods supports such possibility [125].

On the other hand, ART has proven efficacious to reduce the risk of transmission either by intravenous route [153] or heterosexual contact [154]. A clinical trial named HPTN 052, initiated in 2005 and concluded in 2011 unequivocally demonstrated the preventive potentialities of ART [5]. It enrolled 1763 discordant sexual couples in 13 sites of 9 countries from Africa, Asia and the US. All the seropositive patients showed CD4+ T cell counts in the range 350-500 cells/mm³. A first group started receiving ART when reaching 250 cells/mm³ or the occurrence of an opportunistic infection, as indicated by regulations enforced at that time. In this group, 27 new infections were demonstrated among the 877 couples.

In the second group, ART was immediately administered. At the end of the study, just one out of the 877 seropositive individuals on this group became infected. These numbers indicated that the treatment with ART in patients with T CD4+ cell counts was 96.6 % effective in the prevention of HIV transmission among sexual couples.

These results together with condom use are the best preventive option and eradication strategy available to control the expansion of the pandemic. In fact, they are by far superior to the 50 % of efficacy demonstrated for male circumcision and the 31.2 % reported for the vaccine candidate combining the Canarypox vector from Pasteur Merieux and the gp120 formulated in Alum from Merck [155]. This last results were at the very limit of statistical significance and insufficient to support its further administration as experimental vaccine.

Additionally, UNAIDS has launched the 90 × 90 × 90 campaign to expand ART to all seropositive people, with the slogan Test and Treat [156]. Its main purpose is to make the treatment available to the more than 1.17 million of people infected in low and medium income countries (according to reports in 2013), until reaching the 28 million of people eligible for ART attending to their respective national regulations. In this scenario, a probable, long term control of the epidemic is proposed by mathematical models [157]. Results expected from this campaign look too much optimistic, with a 90 % reduction in HIV infections being predicted for 2030, also with a 90 % reduction in AIDS deaths.

**Current limitations of ART**

Despite the unquestionable success of ART, significant aspects remain to be solved. Let’s take a look at current limitations:

**ART does not eliminate completely HIV** [124]. Viral persistence relates to the silent infection of long live cells that the virus uses as reservoirs, such as memory T cells [158-161]. There are other organs regarded as “immunological sanctuaries”, such as testicles and the central nervous system, where only a very weak immune response can be entangled. All these makes ART a treatment for life [162, 163].

**Significant adverse reactions persist in patients under treatment**, in spite of the new generation ARTs being tolerated better. Decreased adherence of pa-
tients to treatment, at least temporarily, is the direct consequence of their associated adverse effects, with viral replication levels increasing as consequence together with the probability of the selection of new mutants that could emerge over time [164]. Viral resistance to ART is a long term issue. Although of delayed appearance, multiresistant viruses finally emerge after several years under treatment, further requiring a change in the therapeutic combination [164, 165].

The high costs of ART are still a problem for developing and underdeveloped countries. It is worth to mention the significant reduction in the costs of ART derived from the increasing market of generics [166], particularly in India [167], which has markedly lowered the costs for low income countries, in addition to the patent expiry of earlier drugs. Nevertheless, the most recent drugs still are economically unaffordable, such as integrase inhibitors and the last generation of NRTIs. The UNAIDS Program has developed a massive campaign to provide therapeutic coverage of the most deficient regions. According to recent reports, the therapeutic coverage in Africa reached up to 37% among the people living with HIV [168], this resulting from such international efforts in coordination with national programs.

For all these reasons, new ARTs are required, aiming at new therapeutic targets and active against strains resistant to the existing drugs, with improved toxicity profiles, better penetration into the so-called immunological sanctuaries and at lower costs.

**Conclusions**

As extensively discussed herein, the goal of turning HIV infection into a chronic treatable disease has been achieved thanks to the tremendous improvements in ART. Such therapeutic means have been achieved after serious research and a long way of trial-and-error still to go, together with sound advances in the comprehension of basic human immunology, virus-host interactions and an extensive insight into the molecular biology of the HIV virus. This has required a huge amount of economic resources and the concerted will to coordinate efforts enough in achieving such tremendous goals, only rivaled by those successfully eradicating smallpox in the XX century. Hence, when facing current difficulties to obtain a preventive vaccine, ART seems to be the most promising choice offhand to control or even eradicate the AIDS pandemic.

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