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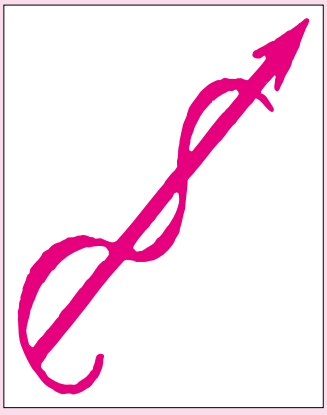

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TAF clinical pharmacology

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INTRODUCTION

Tenofovir alafenamide (TAF) is the 2nd Tenofovir (TFV) prodrug released into the international market [1]. The term prodrug entails the fact that the molecule does not generate any significant therapeutic effect in its original form but requires to be metabolically transformed to became active. TFV is the molecular form undergoing intracellular phosphorylation, the final metabolic step required for the drug in order to compete with natural phosphorylated substrates of viral reverse transcriptase [2]. In the initial phase of development it was soon found that TFV as such was not absorbed at the intestinal level and that suitable pharmaceutical formulations should have been devised to allow the drug to be developed for oral intake [3]. The 1st TFV prodrug to be clinically developed was Tenofovir disoproxil fumarate (TDF), that was released into the market in 2001 (USA) for the treatment of HIV infection [4] and in 2008 its use was also approved for the treatment of chronic HBV infection [5]. The decision to develop TAF inspite of years of worldwide successful TDF use was taken with the purpose of improving several aspects of the long-term safety of the drug [6].

TENOFOVIR METABOLISM, PHARMACOKINETICS AND CLEARANCE

Although, by definition, the final product is the same, the clinical pharmacology of TFV is largely influenced by the prodrug considered. While both prodrugs, TDF and TAF, make the drug absorbable from the intestine, once TFV is in the circulation its distribution shows marked differences depending on which of the two oral formulations is taken. Most of TFV absorbed following TDF oral intake (25% oral bioavailability) dissolves from its link with the disoproxil fumarate salt and is evenly distributed into a wide range of different tissues [7]. The reverse is true when TFV is taken as TAF (40% oral bioavailability estimated), as the link with the alafenamide salt is stronger, and most of the drug circulates bound to it [8]. A major property of TAF is that of driving a rather selective distribution of TFV (Figure 1). Here comes the definition of "magic bullet", as TFV when given as TAF undergoes a rather selective uptake by cells in which most of viral replication occurs. This applies both to the first-pass metabolism, where the carboxy-esterase 1(CES1)-rich hepatocytes are able to internalize the drug, and to the catepsin A (CatA) expressing PBMCs [8]. This selective distribution accounts for the much lower (25 mg) dose of TAF that is required to generate comparable clinical antiviral effects as the standard 245 mg dose of TDF [9]. Such different distribution of TFV when given as TAF or TDF was first described in a pioneer study in 2005 by Lee and coworkers, who compared the pharmacokinetics of TFV when administered by the intravenous route (IV, 1 mg/kg bw) and by the oral route as TDF (245 mg) and TAF (25 mg) [10]. While the highest plasma AUC was measured in decreasing order for IV TFV (4800 ng/h/mL), TDF (1900 ng/h/mL) and TAF (16 ng/h/mL), the PBMC/plasma ratio showed the opposite order, with TAF achieving the highest value, 150, followed by TDF, 5 and IV TFV, 1. These relevant differences in terms of intracellular distribution were mirrored by the EC_{50} for HIV-1 (mM), that was as low as 0.005 for TAF, 0.05 for TDF and as high as 5.0. for IV TFV [10]. The clinical relevance of these different values have been consistently confirmed in clinical stud-

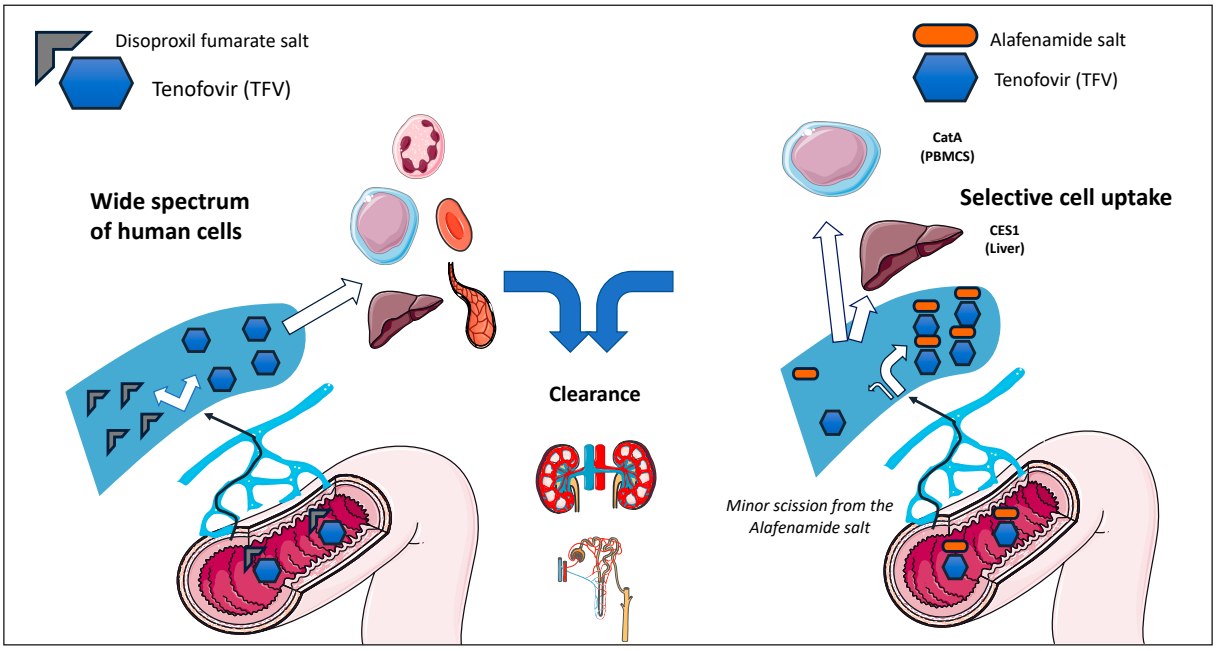

Figure 1 - The different tenofovir (TFV) distribution following intestinal abosrption of Tenofovir disoproxil fumarate (TDF) and Tenofovir alafenamide (TAF) id represented.

ies. Plasma and intracellular pharmacokinetics of TFV was measured in 30 patients who switched from TDF- to TAF containing regimens and it was found that while TFV plasma concentrations decreased by 90% [TDF: 99.98 (2.24) ng/mL vs TAF: 10.2 (1.6) ng/mL, p<0.001] following the switch to TAF, the white cell associated TFV-diphosphate (TFV-DP) increased 2.41 fold [TAF: 834.7 (2.49) vs TDF: 346.85 (3.75) fmol/10^6 cells, p=0.004] [11].

The main reason why TAF was clinically developed following the extensive and successful use of TDF was not however the higher intracellular penetration of TFV achieved by TAF intake but rather its much lower plasma pK exposure. The major clinical relevance of these findings, and specifically the negligible plasma pK exposure of TFV, is thus on the toxicity side as TAF has been consistently found to be associated to a much lower impact in terms of both renal toxicity and bone structural integrity as compared to its ancestor TDF [12-15].

The main difference in terms of clinical impact between TDF and TAF lies thus in the process of clearance of TFV, where the much lower plasma pK exposure of TFV when taken as TAF plays a key role in improving the safety profile of the drug. TFV is cleared by the renal route, with glomerular filtration accounting for approximately 2/3 and secretion by the renal proximal tubule for the rest [16]. The amount of TFV escaping glomerular filtration reaches the epithelial cells of the proximal renal tubule by the efferent arterioles. Uptake of TFV by these cells is efficient, but following internalization of the drug, the subsequent phase of apical secretion into the urine has a lower capacity, so that a variable amount of TFV tends to accumulate into proximal tubule epithelial cells [17]. Although TFV was shown to have minimal mitochondrial toxicity, once the local concentration increases alteration in mitochondrial structure and function follow, with decrease in energy production by mitochondria resulting in lower efficiency of membrane transporters [18]. A vicious circle is thus generated with chronic impairment of proximal tubule function. The latter can be measured by the capacity or reabsorbing low-molecular protein molecules, such as retinol-binding protein (RBP) and b2-microglobulin. These two markers have been extensively used in clinical trials to demonstrate the lower proximal renal tubule reabsorbing efficiency in TDF vs TAF intakers, with unambiguous results consistently

showing a significantly higher preserved proximal renal function in TAF recipients [12-15]. It must be noted that these markers of proximal tubular function are rarely used in clinical practice, where creatininemia and creatinine-based calculation of glomerular filtration are more commonly measured. Creatinine renal clearance mainly occurs by glomerular filtration (85%), with a minor contribution by proximal tubular secretion [16, 19]. This explains why the increases in creatininemia and parallel decreases in the estimated value of glomerular filtration are common occurrences in TDF-treated patients, but these markers actually underestimate the impact of TDF on renal proximal tubule function. In an horizontal clinical study on 289 TDF-treated patients with steady normal creatinine values and a median exposure to TDF of 5.2 years, the measurement of the urinary RBP/creatinine ratio showed that 54% of these patients had a reduced proximal tubule function inspite of normal creatinine values. As expected, these alterations were inversely proportional to the TFV urinary concentration, thus testifying a reduced capacity of clearing the drug by tubular secretion [20].

A further difference between TDF and TAF that is also attributable to the lesser impact of the latter on renal function is the reduced impact of TAF on bone structural integrity. Lower reduction in bone mineral density (BMD) have been constantly detected in TAF vs TDF intakers, possibly reflecting a reduced phosphate loss by the proximal renal tubule [12-15]. In clinical studies evaluating the effects of switching from TDF to TAF-containing regimens an increase in patients BMD has also been regularly measured [21, 22].

All this data points on the benefit associated to the lower plasma pK exposure of TFV when administered as TAF. Before the clinical development of TAF was completed, differences in terms of BMD were already seen in TDF recipients according to the companion drugs. Depending on the companion drugs, the pK plasma exposure of TFV in patients receiving TDF may significantly differ, with both efavirenz (EFV) and raltegravir (RAL) being associated to the lowest TFV concentrations [23]. In an equivalence clinical trial comparing darunavir/ritonavir (DRV/r), atazanavir/ritonavir (ATV/r) and RAL, all associated to emtricitabine/tenofovir (FTC/TDF), the lowest impact on BMD was seen in the RAL treatment arm [24], and this is in full accordance to pK clinical studies measuring the TDF-associated TFV pK exposure according to companion drugs [23, 25].

CONCLUSIONS

It is thus the lower concentrations of TFV in plasma that account for the significantly better safety profile of TFV, and to complete the TAF definition of "magic bullet", the property of being less concentrated where potentially toxic (e.g. the plasma bathing the renal proximal tubule) well fits with its higher concentration into cells where HIV and HBV replicate.

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Virology of Residual HIV Infection

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BACKGROUND

The suppression of HIV-1 replication in treated patients is nowadays one of the biggest achievements of antiretroviral therapy. The combination of different drugs in the so-called cART (combination antiretroviral therapy) has dramatically reduced the overall incidence of mortality of HIV-infected individuals and delayed clinical disease progression [1].
Current cART is very effective in suppressing plasma virus levels to below the limit of detection (LLoD) of clinical commercial assays (20-50 copies HIV-1 RNA/mL), thus allowing the achievement of a nearly full control of virus replication and damage that it causes [2, 3]. Yet, the complete HIV-1 eradication from the infected individual (hence, "biological" cure), is still impossible, and this makes lifelong treatment an absolute necessity for every individual living with HIV [4-6].
With the aging of people living with HIV (PLWH), new sets of HIV-associated complications are emerging and contributing to the excess risk of non-AIDS events, leading to complications and/or death. Among them, we count, beyond traditional risk factors not directly linked to HIV (such as substance abuse, obesity, and hypertension), some HIV-related alterations of homeostasis, such as chronic immune activation, and inflammation [7]. HIV-1 persistence appears to be a critical factor driving immune activation in the context of virological suppression [8], and a possible source of residual viremia in cART-treated patients.

THE ORIGINS OF RESIDUAL HIV-1 VIREMIA

Residual viremia, defined by a measurement <50 copies/mL with positive HIV-RNA PCR signal (so called detectable, not quantifiable viremia), is not a rare phenomenon in HIV-infected patients treated with ART. In real-life cohorts, steady-state VL was shown to be <3 copies/mL, though the proportion of patients with steadily-controlled viremia during follow-up ranges between 40.6% and 53.3% [9, 10].
Residual viremia can depend from 2, non mutually exclusive, functional models. The first suggests that residual viremia represents the product of ongoing viral replication, presumably from specific sites which are poorly reached by global cART penetration [11]. The second proposes that detectable residual viremia depends on the stable periodic release of HIV-1 from latently infected cells, possibly due to antigenic stimulation [12]. In the latter case, HIV-1 persistence would be enhanced by cell-to-cell transmission of HIV-1 [13, 14]. Regardless, these mechanisms are generally nonexclusive in the same individual, and may co-occur to different extents [15], thereby contributing to residual viremia. The same studies suggest that residual viremia can be produced by clonally expanded cell(s) harbouring a resistant variant that escapes the immune clearance [16, 17].
Many studies aim to find how viral reservoirs establish in PLWH, and which mechanisms allow their persistence. Most infected cells harbour defective proviruses that, being unable to replicate, are progressively lost during effective cART treatment [18, 19], as a result of lytic infection or elimination by cytotoxic T-lymphocytes (CTL). With our current knowledge, it is thus reasonable to affirm that these defective proviruses are incapable of triggering viral rebound contributing to residual viremia.
Intact proviruses, on other hand, are not necessarily responsible for the maintenance of an active

reservoir, as often integrated into transcriptionally silent genomic regions that maintains their "deeply" latent persistence [19, 20].
The active HIV reservoir as we know it is thus sustained by a small replication-competent pool of cells, that carry integrated provirus in genomic sites that allow reactivation and production of viral progeny [21].
On the other side of the coin, cART has demonstrated a limited (if any) effect on viral transcription of integrated viral genomes, reactivation of latent cells, and delimitation of the inflammation derived from chronic HIV replication. Chronic inflammation, together with the immune dysfunction, might enhance HIV persistence by generating new target cells and by increasing the proliferation of infected cells; these two phenomena are mutually connected and act like a positive-feedback mechanism [22].

HOW ANTIRETROVIRAL THERAPY CAN IMPACT RESIDUAL VIREMIA

During virological suppression induced by cART, plasma HIV-1 is reduced to levels undetectable by common commercial assays, whose lower limit of detection (LLoD) is usually 20-50 copies/mL.
The presence of this residual viremia (potentially indicating an ongoing, low-level, viral replication), was shown to anticipate viral rebound

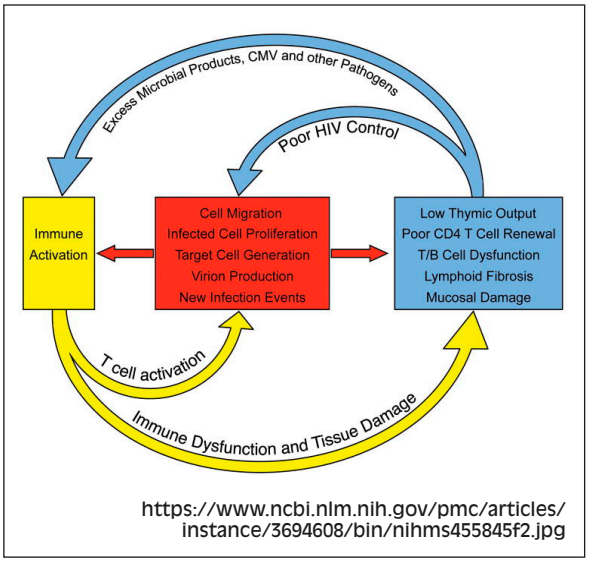
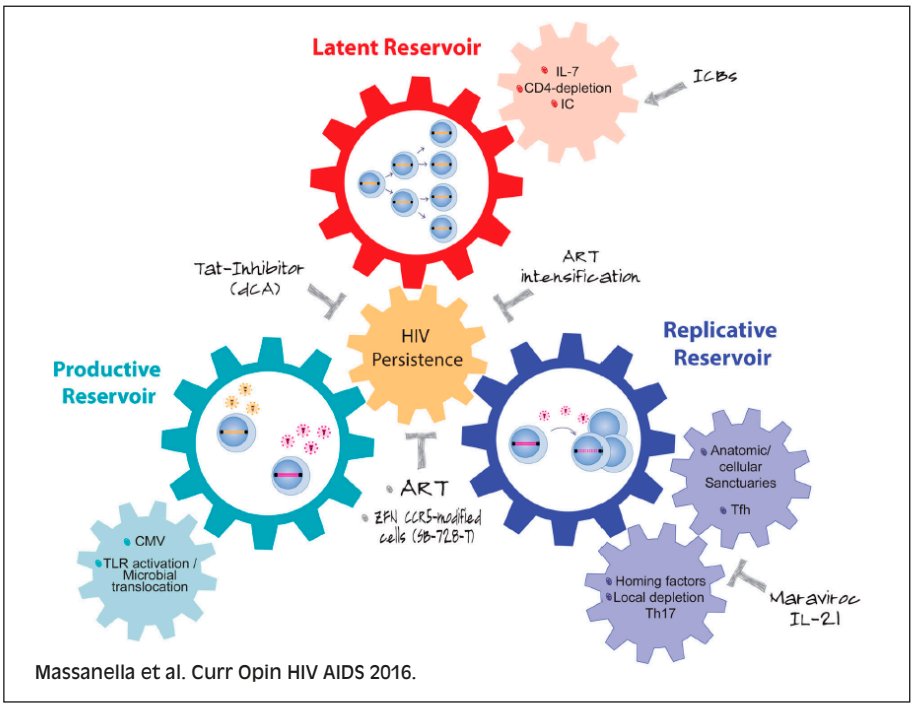


Figure 1

in PLWH on cART [9, 23], and thus represent a critical virological factor to evaluate and monitor.
All currently recommended initial cART regimens have excellent potencies, and a demonstrated outstanding ability of rapidly (and consistently) inhibiting viral replication, with N-years efficacy approaching N% [24-28]. Two treatment-related features contribute to the establishment, and persistence, of residual and/or low-level viremia:

a) the ability to rapidly overcome viral replication once first-line treatment is initiated, thereby reducing HIV-1 reservoir size;
b) the ability to guarantee a full suppression of viral replication on the long run.

HIV-1 RNA suppression is dependent on baseline HIV-RNA levels and the type of regimen used. Past researches have shown that patients with a slower time to achieving <50 copies/mL are more likely to continue to have residual viremia [29], and when <50 copies/mL occurs >6 months after initiating cART, they have an almost 2-fold risk of subsequent virological rebound [30].
Once residual viremia is detected, no univocal treatment strategy has been shown to be more effective in lowering the HIV-RNA load to reach <20-50 copies/mL [31].
Adjunctive therapy capable of increasing $CD4^+$ cell counts beyond levels achievable with cART alone has not been shown to decrease morbidity or mortality. In addition, findings of several studies have shown that intensification of a suppressive cART regimen with either the integrase inhibitor (INI) raltegravir, a boosted PI, efavirenz or maraviroc, does not alter the frequency of latently infected cells or low-level viraemia [33, 34-36]. Morón-López et al. observed a decrease in residual viremia after switching from PI-based to DTG-based regimen in a randomized clinical trial [37], but Rasmussen et al. concluded that the intensification of ART with DTG did not reveal or affect residual viremia in PLWH [38]. In the light of these contrasting results, intensification or modification of cART after viral suppression is not currently recommended as a strategy to reduce immune activation [32], even though further studies are definitively required, to assess this key point in a long-run period.
In HIV-infected individuals the different frequency of detectable residual viremia is surely a combination of several viral, treatment and clinical factors, including VL zenith, CD4 nadir, time un-



Massanella et al. Curr Opin HIV AIDS 2016.

Figure 2

der viral suppression, pharmacokinetic characteristics, adherence to therapy, and tolerability issues [39]. Excellent adherence to treatment is a critical determinant for the maintenance of optimal virological suppression.

LABORATORY STRATEGIES TO EVALUATE RESIDUAL VIREMIA

Both total HIV-1 DNA and plasma HIV-1 RNA can be quantified to assess the success of cART and define HIV-1 persistence in different cellular types. Molecular assays are characterized by high sensitivity and reproducibility, and are thus the most extensively used or such purpose.

A variety of commercial assays are available for quantitation of HIV-RNA in plasma. Of them, the reverse transcription (RT)-PCR assay is the only approved by the Food and Drug Administration (FDA), and the most widely used method for plasma HIV-1 RNA quantification [42], while quantitative PCR (qPCR) is traditionally used to measure the latent HIV-1 reservoir, represented by HIV-1 DNA and cell-associated HIV-1 RNA [43-45].

Despite their sensitivity, versatility and ease of use, PCR-based assays have a main caveat in the unwarranted detection of defective integrated proviruses, and thus overestimation of functional HIV-1 reservoir. Many studies have proposed more accurate methods for the precise assessment of HIV-1 reservoir, able to separately quantify intact and defective proviruses [45].

As PCR- or sequenced-based assays cannot prove replication-competence or inducibility of proviruses, the qVOA still remains the gold standard for measuring replication-competent latent HIV in resting $CD4^+$ T cells [60]. Traditional qVOA methods that include serial dilutions of CD4+ T-cells, stimulation with gamma-irradiated peripheral blood mononuclear cells (PBMCs), and amplification in allogeneic T-cell blasts from healthy donors are costly and time-consuming [47]. In addition, the assay requires weekly addition of HIV-negative allogeneic blasts, which limits the capacity of most laboratories to run qVOAs.

In the setting of cART-treated patients, total HIV-DNA and residual HIV-RNA quantification may support the evaluation of disease progression [47, 48]. For this purpose, digital droplet PCR (ddPCR) is becoming a promising quantification strategy that combines absolute quantification with high sensitivity than real-time PCR [49, 50]. However,

it is a non-standardized testing, not yet included in the recommendations for laboratory monitoring of persons with HIV-1 by international guidelines [26, 32]. One of the potential applications of ddPCR is in the study of correlations among total HIV-DNA, plasma HIV-RNA, CD4+T cells and CD4/CD8 ratio during ART. In particular, in pre-ART phase, HIV-DNA level may predict long-term virological success in patients starting their first-line ART [51-54]. Alteri *et al.* confirmed that pre-ART total HIV-DNA, normalized on CD4+T cells, is an excellent indicator of HIV-1 reservoir burden, residual viremia, and immune status. It also appears to be useful to predict the response to antiretroviral treatment since 6 months after virological success. Moreover, comparing virological and immunological features of patients, the study highlighted a potential correlation between poor immunological reconstitution and residual viremia at success and 6 months after virological success [55].

Deeply understanding the factors responsible for HIV-1 replication despite cART, and identifying the cells harbouring the virus, represent major gaps that need to be filled. Suppressing the background inflammation could decrease the activation of the immune system and the related consequences, whilst identifying reservoirs provide the chance to target them and reduce their burden.

Recent advances in clinical practice and laboratory experience allow us to better examine the complexity of processes associated with this infection. One of the main difficulties is dealing with the fact that we are moving in an extremely small field, since only a small percentage of cells harbour HIV, and an even smaller number of them are transcriptionally active. Researchers are using modern methods to obtain a larger pool of transcriptionally active cells Compared to what achieved until now. These methods include genetically modified viruses, latency-reversing agents and cell lines transfected with the virus, having a larger percentage of transcriptionally active cells. Recently Leon-Rivera *et al.* used single-cell RNA sequencing (scRNAseq) to provide the first extensive characterization of HIV-positive mature monocytes with and without ART, and host-virus interactions directly affecting the monocyte transcriptome, to reveal novel therapeutic targets for blocking formation and reseeding of viral reservoirs [56].

CONCLUSIONS

In conclusion the residual viremia, despite cART, represents one of the major obstacle for HIV-1 eradication, and it is recognized as a potential factor associated with persistent immune activation and inflammation, thus favouring disease progression in cART-treated patients [57]. In particular, viral persistence and residual inflammation are interdependent and fuel each other in a "vicious circle" that seems difficult to interrupt. Under these circumstances, despite the absence of specific recommendations regarding how to deal with this pathological situation, it is reasonable to consider that the maintenance of the maximum possible pressure over the virus during years, including after achieving undetectable viremia, represents an essential tool to decrease virus damage to the lowest possible level. In this frame, the perfect adherence to therapy, together with the choice of drug regimens characterized by optimal efficacy against virus replication, represent still today, and today more than ever, elements of paramount importance to grant to PLWH the longest and most qualitative live possible.

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Test & Treat

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The natural history of HIV infection has been deeply modified by the development and introduction of antiretroviral therapy. In optimal circumstances the life expectancy of persons living with HIV infection is now approaching that of the general population. Following decades of debate concerning the immunological threshold when the treatment should be initiated, the START and Temprano clinical studies eventually demonstrated that significant improvements in terms of decrease of clinical progression and reduced death rate are recognizable even in patients who started treatment with CD4+ T-cell counts higher than 500/μL [1, 2]. This prompted international advisors to recommend the immediate beginning of antiretroviral treatment regardless the CD4+ T-cell count measured at HIV diagnosis. Besides these unambiguous experimental clinical data, the benefit of an earlier start of antiretroviral therapy has been also conceptualized as a measure to reduce HIV transmission according to the definition given by Julio Montaner of "*treatment as prevention*" [3]. The presence of large numbers of HIV-infected persons with suppressed viraemia by antiretroviral therapy has been consistently found to be associated to the reduction of the so called "*community viral load*" and consequently to a diminished viral circulation and decrease of new infections [4]. As soon as in 2007 the protection from new infections among sexual partners resulting from suppressed viraemia was first described by Pietro Vernazza in the Swiss Statement [5]. On this line, the confirming results by three subsequent clinical studies, HPTN052 and PARTNER 1 and 2, led to the formulation of the nowadays popular equation known as *undetectable* = *untransmissible*, whose acronym is U = U [6-8]. The achievement of a steadily suppressed viraemia has also brought about a normalization of another aspect of patients' life, such as the one concerning reproduction and vaginal delivery. The current counselling about the rationale of receiving antiretroviral therapy thus includes all these issues, spanning from individual to public health issues. As a direct consequence of these properties, an immediate start of antiretroviral therapy should be thus offered. The best evidence concerning the benefits of the adoption of the *test & treat* strategy mostly come from developing countries, but similar results are also being generated in the USA [9-11]. It is intuitive that the critical moment to retain in care any new patient is right at the time of diagnosis and such strategy seems to have a significant impact as shown by a Cochrane meta-analysis [12]. The relevance of this strategy may vary according to the geographical and socioeconomic setting. In western countries, where most doctors customarily prefer to carry out an immunovirological evaluation before starting the treatment, the risk of losing a freshly diagnosed patient appears to be negligible. Such assumption is being however challenged by the COVID-19 pandemic and its impact on health care organization. The reduction in hospital-based assistance driven by both newly devised restrictions for hospital access and the associated general perception of hospitals as risky environment, actually upgrade the appeal of a *test & treat* strategy. Beyond these mere organizational reasons, this issue requires to be analyzed under the doctor-patient perspective. From a virological view-

point, as shown by a study of the Italian ICONA cohort, the likelihood of achieving virologic suppression might actually be similar when immediate and delayed (2 weeks) treatment start are compared [13]. However, it is my personal opinion that the most relevant benefit of the *test & treat* strategy resides in the acceptance by the patient and Her/His overall perception of such accelerated procedure linking expeditiously the diagnosis of HIV infection to its treatment. This is likely to be related to the critical emotional aspects generated by the beginning of antiretroviral therapy, with a switch from negative (e.g. fear) to positive insights concerning the patient's expectancies, as described by the Italian Diamante study presented at ICAR [14]. More recently these evolving expectancies and the wish to begin the antiretroviral treatment without any delay have also been reported by an American study [15]. It must be noted, however, that not all therapeutic regimens are equally suitable for such immediate strategy. This is mainly due to the lack of any basic immunovirological data on which basing the individualized choice of the most appropriate regimen. The first point here, in spite of a very low prevalence of transmissible drug resistance in Italy, concerns the risk of baseline mutations in the viral genome coding for reductions or loss of antiviral activity by specific drugs. A second challenge lies in the lack of knowledge of the initial viral load, a variable that might contraindicate the adoption of dual regimens. A third point is the possibility that the patient also harbors an active HBV infection, a rather frequent finding in foreigner patients, whose treatment requires the introduction of an active drug with high genetic barrier like Tenofovir alafenamide (TAF). The last but not least issue here concerns the degree of immune deterioration of the patient; should this variable be critically low (e.g. < 200 CD4+ T-cell/μL), the choice of a dual regimens might be suboptimal, as shown by a negative trend seen in patients with baseline CD4+ T-cell/μL < 200 in the GEMINI studies (16). And it must be considered, especially in this COVID-19 pandemic, that the frequency of such low immune profile is rather frequent in newly diagnosed infections, often exceeding the 50%. These uncertainties, that are all present in a *test & treat* perspective, clearly drive the choice toward an initial triple regimen including both TAF and an integrase inhibitor (INSTI).

The *test & treat* or *same-day ART* is today also included in the WHO treatment recommendations [17], mainly for organizational reasons, although in western countries it is fundamental to deliver any initial treatment by taking also into account the patients' feelings by also offering this strategical opportunity.

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TAF: the ART of Forgiveness

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INTRODUCTION

The term 'forgiveness' has entered the lexicon of HIV management.

Most pharmacological parameters in common usage can be defined with precision.

In contrast, forgiveness currently lacks an established, quantitative measure. Notwithstanding the lack of a specific forgiveness scale, the medical community has embraced the concept that some regimens are more forgiving of nonadherence than others, and this recognition influences therapeutic choices that are made every day clinics [1].

Forgiveness, like many properties related to antimicrobial treatment, depends on pharmacological, viral and host factors [1, 2].

In the following sections we try to answer to some key questions concerning definition, evaluation, and the role of forgiveness in the modern management of HIV-positive patients.

WHAT IS FORGIVENESS?

In the treatment of most chronic diseases, forgiveness increases in therapeutic importance and clinical explanatory power as a patient's dosing history is increasingly interrupted by episodic lapses in dosing of varying lengths, creating pharmacokinetically corresponding gaps of low or undetectable concentrations of drug in plasma [1].

From the pharmacokinetic perspective, most drugs in today's pharmacopoeia have plasma half- life of 12 hours or less. When the dosing of these drugs is interrupted, their concentrations in plasma will, by the second day of lapsed dosing, have fallen far below the range prevailing during continuous dosing [2].

In other words, the circumstance of how sensitive therapeutic success is under imperfect adherence is driven by the property known as forgiveness.

THE RELATIONSHIP BETWEEN ADHERENCE AND FORGIVENESS

Medication adherence rate is typically defined as the proportion of doses taken as prescribed [2]. The adherence rate threshold is the minimum adherence rate needed to maintain a therapeutic effect. Thus, if a drug is taken once daily for 'N' number of days, and 'm' is the number of missed doses, then: $A = (N – m)/N$ [2].

The 'forgiveness', F, of a drug or regimen can be defined as the number of consecutive doses that can be missed while still maintaining the therapeutic drug effect. If D is the duration of the drug's effect, and I is the dosing interval, then the forgiveness of an ARV drug can be expressed as [2]:

$$F = D – I$$

Of note, this generic mathematical description of forgiveness does not describe the likelihood of drug resistance emerging in cases of loss of therapeutic effect – a key consideration when treating HIV and selecting a regimen.

The distribution pattern of missed doses may even be more important than the number of missed doses in deciding whether a therapeutic effect has been maintained (Figure 1). Thus, the therapeutic effect of a once-daily drug will likely be maintained if doses are not missed consecutively (Figure 1A, patterns 2 and 3), but is at increased risk of not being maintained if doses are missed consecutively (Figure 1A, pattern 4) [2].

Figure 1B illustrates the pharmacokinetic consequences of different patterns of adherence for 25%

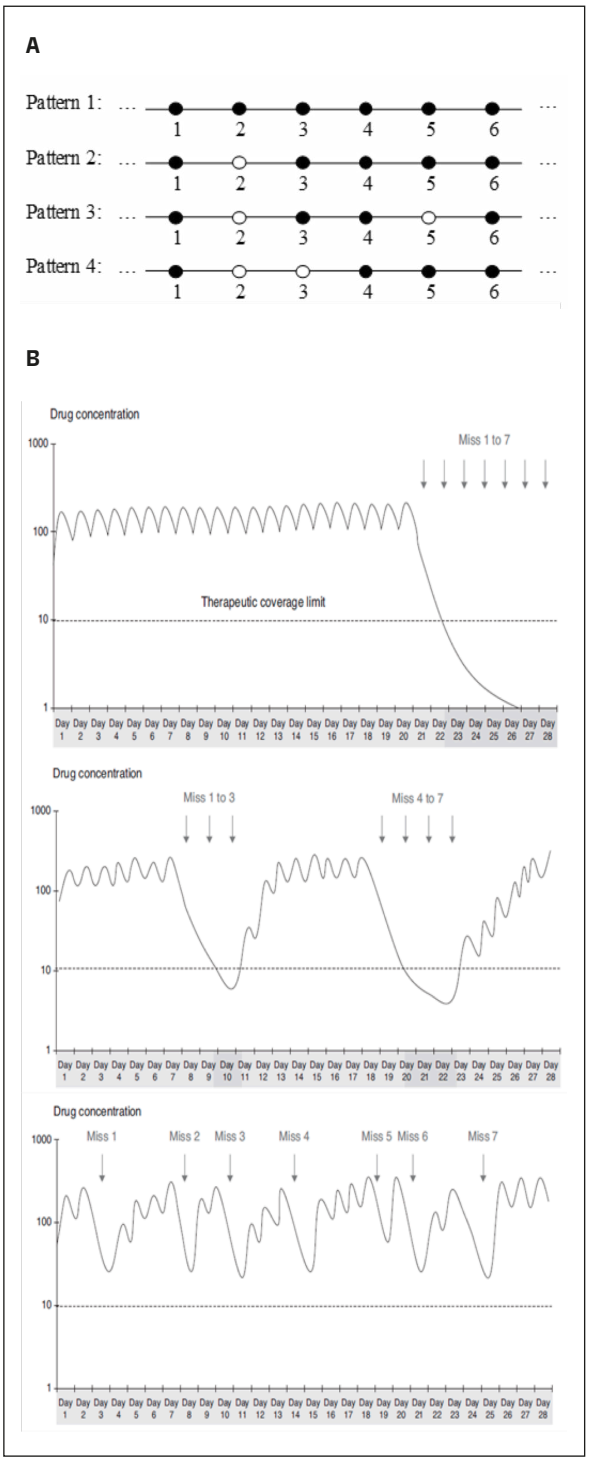

Figure 1 - Different patterns of missing doses (A) and different patterns for 25% average nonadherence and their pharmacokinetic consequences (B) (adapted from ref. 2 and 3).

average non-adherence [3]; thus, an ARV with a long half-life may be necessary to avoid subtherapeutic concentrations resulting from too-widely interspaced missed doses.
Kaufman et al [4] found that runs of >3 and >4 consecutively missed doses occurred more frequently than expected in the clinical setting leading to relatively long period with potential suboptimal expoxure.

WHICH IS THE FORGIVENESS OF ANTIRETROVIRALS?

ARV forgiveness relates to the number of doses that can be missed without causing viral relapse. Several properties of ARV drugs determine their forgiveness, especially pharmacokinetics and barrier to resistance. Forgiveness in the context of missed doses is possible when either the elimination half-life of a drug or its inhibitory effect exceeds the recommended dosing interval [5].
The best data for estimating forgiveness of an antiretrovirals regimen derive from 'tail' studies tracking drug elimination following treatment cessation in healthy volunteers where median time to reaching mimimum effective concentration (MEC) can be calculated. Tail data in healthy volunteers are available for different compounds, including coformulation with TDF/FTC [5-11].
Although MECs for NRTIs are not established, the pharmacokinetic parameters of the active intracellular metabolites of tenofovir (given as TDF) and FTC have been established in one tail study, where both were found to have long terminal half-lives of 164 and 39 h, respectively (Figure 2) [10]. TDF and emtricitabine have been considered the antiretrovirals with longest intracellular half-life [12], suggesting a key role for forgiveness (Figure 3). This gives some reasurance that regimens containing these long-acting NRTIs provide an element of forgiveness for late dosing.

FROM DRUG FORGIVENESS TO REGIMEN FORGIVENESS: A CONCEPTUAL EVOLUTION

TDF availability (along with FTC) can be considered a milestone of HAART history, making possible a new concept of backbone.
It has been postulated [13] if one drug in a regimen has a significantly longer half-life than others, and all agents are missed then the patient will

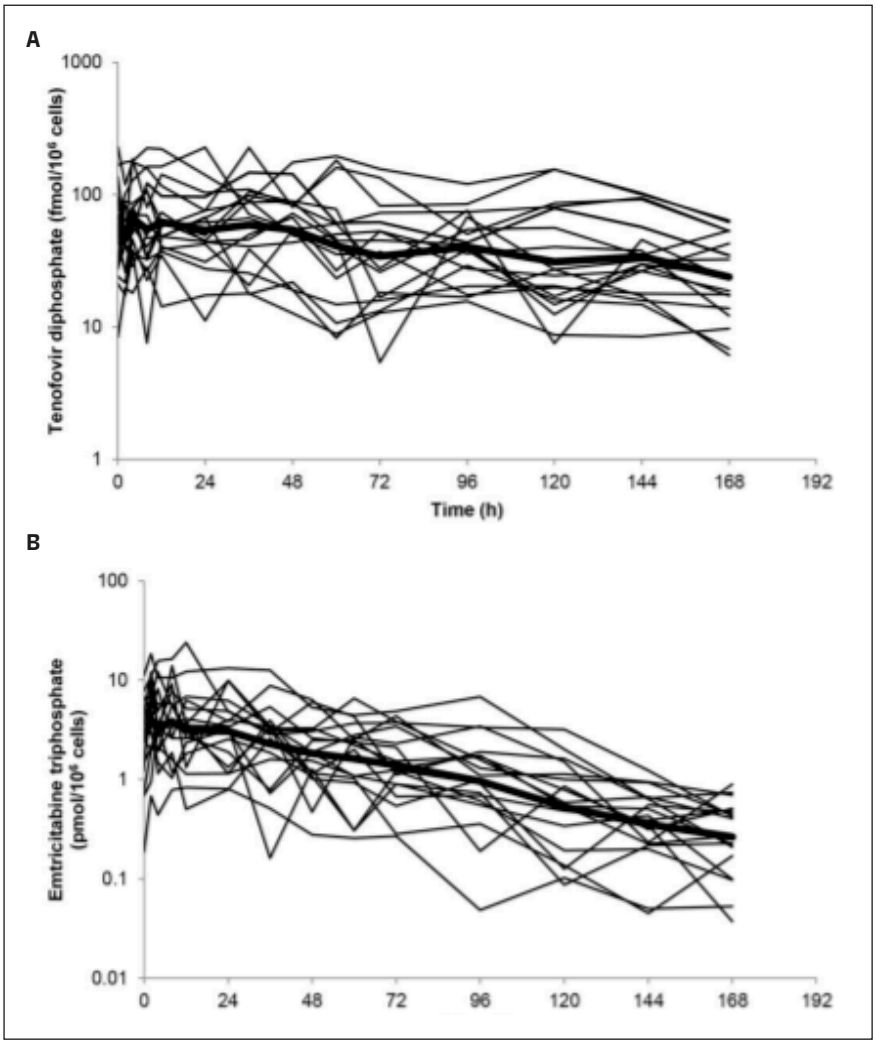

Figure 2 - Individual predicted intracellular tenofovir diphosphate (A) and emtricitabine triphosphate (B) concentrations over 168 h following drug intake cessation in healthy) The bold line represents the geometric mean concentration-time profile. (adapted from ref. 8).

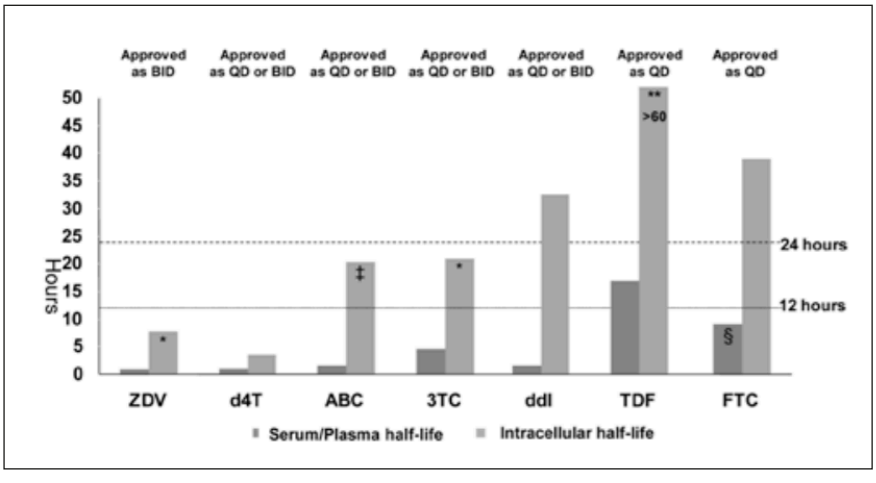

Figure 3 - Comparison of plasma and intracellular half-lives of different N(t)RTIs (ref. 9-11).

be taking essentially 'functional monotherapy' after the shorter half-life drugs are eliminated (Figure 4a). The probability of resistance emerging will depend on:

1. the drug's genetic barrier;
2. the magnitude of viral replication when the drug remains at a concentration capable of inducing resistance;
3. the length of time the drug remains in the zone of resistance selection [13].

In the case of old short half-life NRTIs (AZT, d4T, ddI) associated with a low genetic barrier NNRTI, such as EFV, this could lead to virological failure with high probability of selection of resistance mutations to NNRTI. After adoption of TDF/FTC as a backbone, scenario 4a evolved in scenario 4d: the association of three drugs with relatively 'balanced' long half-lives mantains therapeutic concentrations for a prolonged period of time after stopping these agents simultaneously [13]. This will provide a large degree of 'forgiveness' for missed doses, delaying the risk of virological failure and decrease the probability of selection of resistance mutations. In a clinical trial [14], development of reverse transcriptase resistance was significantly lower among subjects receiving either FTC + TDF + EFV as compared to the ones administred with 3TC + ZDV + EFV.

Simultaneous missing of three drugs A, B and C with 'balanced' short half-lives was depicted in figure 4b (e.g. old short half-life NRTIs + PI) [13]. The degree of 'forgiveness' for missed doses will be low, but on stopping simultaneously the drugs may have cleared from the body before viral rebound occurs. The potential for resistance developing may be less than the scenario depicted in figure 4a. In the case of a similar third drug with a backbone with significantly longer half-life (as TDF/FTC), the degree of pharmacokinetic coverage maintained by the latter should increase the forgiveness of regimen and decrese the risk of virological rebound (figure 4c) [13]. This scenario can be representative of many modern HAART, including association with InSTI [11].

In a clinical study [15], although the gold-standard adherence threshold for older ARV regimens was been previously fixed to 95%, aa 80-90%, or maybe lower, adherence appeared sufficient to maintain virologic suppression in patients treated with TDF/FTC-containing regimen (plus EFV, DRV/r, or RAL).

FROM TDF TO TAF-BASED REGIMENS: IMPLICATIONS FOR FORGIVENESS

In another paper of this Journal issue, pharamacological differences between TDF and TAF are fully elucidated. TFV plasma concentrations has been shown to decrease by 90% and intracellular TFV-DP concentrations to increase 2.41-fold in participants who were switched from TDF to TAF as part of routine clinical care [16]. The augmentation of intracellular persistence of TFV in PBMCs could theoretically further increase the magnitudo of forgiveness when dosed as TAF as compared to TDF. Although no clinical study has so far specifically investigated this issue, also other recent findings could support a potential for such forgiveness increase.

The secondary lymphoid tissues (LT), lymph nodes (LN) and gut-associated lymphoid tissue are the primary sites of HIV replication and where the latent pool of virus is maintained [17].

Penetration of drugs into LTs depends on several physico-chemical characteristics including molecular weight, ioniza-tion, dissociation constant (pKa), lipophilicity (logP), protein binding and particle size [17].

Studies in HIV-infected persons have shown low LN concentrations of some antiretroviral drugs and an association between low antiretroviral concentrations in LN and measures of persistent viral production.

TAF has greater stability than TDF in human plasma and more efficiently delivers TFV to lymphoid cells and tissues that is facilitated also by efficient metabolism of TAF by cathepsin A, which is highly expressed in lymphoid cells [18].

In a recently published [19] comparison of the LT pharmacokinetics of the intracellular pharmacologic-active moiety, tenofovir-diphosphate, in HIV-infected persons following oral administration of tenofovir disoproxil fumarate (TDF) or tenofovir alafenamide (TAF), tenofovir-diphosphate concentrations in PBMCs and LN were 7.3-fold and 6.4-fold higher, respectively, with TAF. The finding that TAF achieved higher LN concentrations of tenofovir-diphosphate provides the first human correlate of the observation in animals that TAF produced higher TFV LN concentrations[19].

The ability to improve pharmacokinetic conditions in the LN allows investigations of whether

ARV regimens with enhanced LN concentrations, elicit a more complete virologic response, although a focus on all lymphoid tissues is warranted, given they are where >98% of the reservoir resides.

2DRS: FORGIVENESS WITHOUT TAF

A 2DR based on the INSTI dolutegravir (DTG) demonstrated noninferiority to a 3DR in a treatment-naive population in the GEMINI-1 and GEMINI-2 studies [21]. DTG has a longer elimination half-life than EVG and RAL, suggesting that it may be more forgiving of missed doses [22]. The terminal elimination half-life of DTG is ~14.30 h compared with 12.9 h for EVG when boosted with cobicistat and 10-12 h for RAL [22] For BIC, the half-life reported in HIV-1-infected, treated, or INSTI-naive participants ranged from 16 to 21 h [22].

Plasma concentrations of DTG were >2-fold higher than the IC90 for 72 h after the last dose, whereas the concentration of EVG when boosted with cobicistat only exceeded the IC95 through 36 h, further supporting higher forgiveness of missed doses for DTG [22]. Combination therapy should include drugs with complementary pharmacokinetic profiles, such as those demonstrated for DTG and the pharmacologically active triphosphate form of 3TC, which have similar half-lives further supporting adequate maintenance of plasma concentrations of DTG and intracellular 3TC-TP for 3 days after the last dose (Figure 5).

However, forgiveness of 2DRs and TAF-based 3DRs has not been so far fully compared.

In a post hoc analysis [23] of GEMINI studies a lower Week 48 virological response was observed in participants with < 90% adherence, but the impact of lower adherence on viral success was similar in the DTG+3TC compared with DTG+TDF/FTC arms. It is noteworthy, however, that a main limitation of the analysis is the small number of participants in the lower adherence subgroup (5% in both arms). In my opinion, definitive conclusion cannot be drawn, considering these trial conditions not fully representative of real life setting, in terms of heterogeneity of patients and high variability of compliance attitudes. In Figure 6, for example, some clinical scenarios of critical forgiveness, still not captured by clinical trials of 2DRs, is reported.

An interesting *in vitro* contribution comes from Mulato et al. [24]. HIV breakthrough experi-

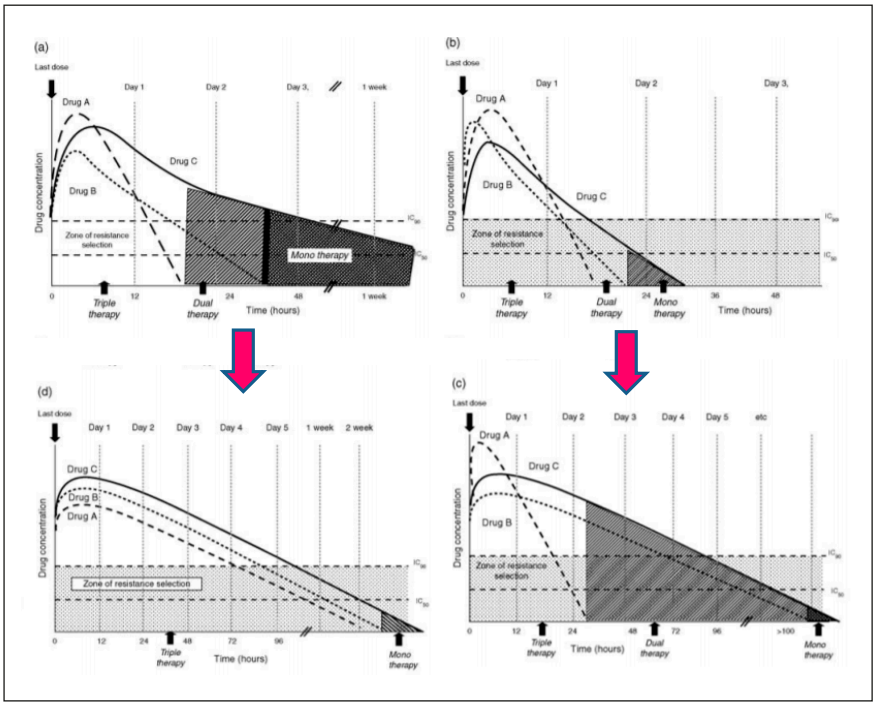

Figure 4 - Pharmacokinetics of missed doses or stopping therapy of different regimens. (a) Representation of three drugs A, B, and C with very disparate half lives, e.g. old NRTIs + EFV or NVP. (b) Simultaneous stopping of three drugs A, B and C with 'balanced' short half-lives, e.g. old NRTIs + PI. (c) Simultaneous stopping of drugs A, B and C when drug A has a significantly shorter half-life than the other two agents, e. g. TDF/FTC + PI or InSTI. (d) Simultaneous stopping of three drugs with relatively 'balanced' long half-lives, e.g. TDF/FTC + EFV of RPV.

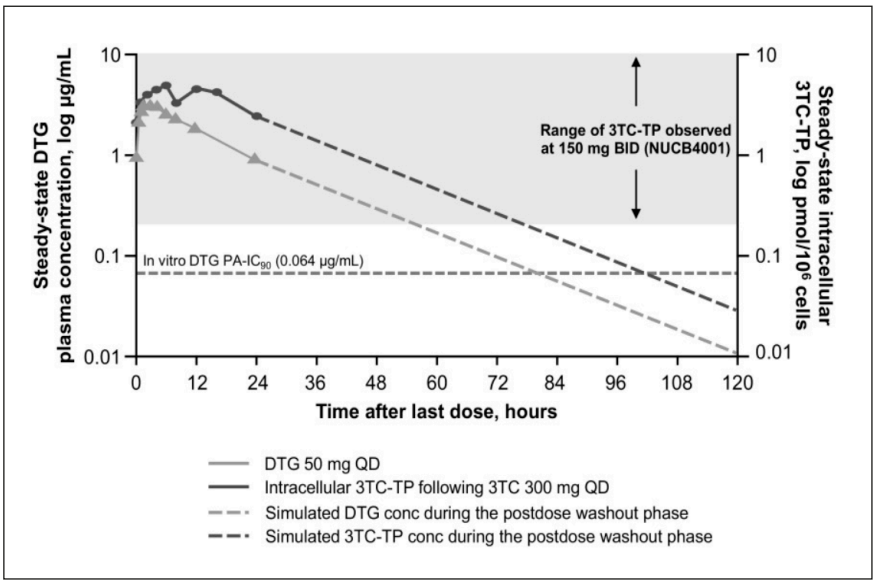

Figure 5 - The PK profiles of DTG and 3TC after interruption.
Steady-state DTG and intracellular 3TC-TP concentration-time profiles after administration of DTG 50 mg or 3TC-TP 300 mg daily. BID, twice daily; conc, concentration; DTG, dolutegravir; PA-IC90, protein-adjusted 90% inhibitory concentration; QD, once daily; 3TC-TP, lamivudine triphosphate (adapted from ref 22).

ments simulated drug exposures at full adherence or suboptimal adherence to BIC+FTC+TAF or DTG+3TC. Drug concentrations were determined using human plasma-free adjusted clinical trough concentrations (C_{min}), at simulated C_{min} after missing 1 to 3 consecutive doses (C_{min} - 1 or C_{min} - 2, and C_{min} 3) based on drug or active metabolite half-lives (Figure 7A). Cultures infected with wild-type HIV-1 showed no viral breaktrough with BIC+FTC+TAF at drug concentration corresponding to C_{min}, C_{min} -1, or C_{min} -2 but breakthrough did occur in 26 of 36 cultures at C_{min} -3, where the M184V variant emerged in one culture. Experiments using DTG + 3TC prevented most breakthrough at Cmin concentrations (9/60 had breakthrough) but showed more breakthroughs as drug concentrations decreased (up to 36/36) (Figure 7B) and variants associated with resistance to both drugs. These results, however, need to be confirmed in the clinical setting.

CONCLUSIONS

Forgiveness is a key element for long term success of HAART.

- Naive pts with very high VLs and low CD4+
- Unavailability of GRT (e.g. rapid HAART)
- Pregnancy and other PK changes
- Confirmed or suspected (lack of GRTs) previous selection of resistance mutations (e.g. M184V , INI-R)
- Subjects at risk of low adherence

Figure 6 - Real life scenarios where comparative forgiveness of 2DRs and 3DRs still needs to be investigated.

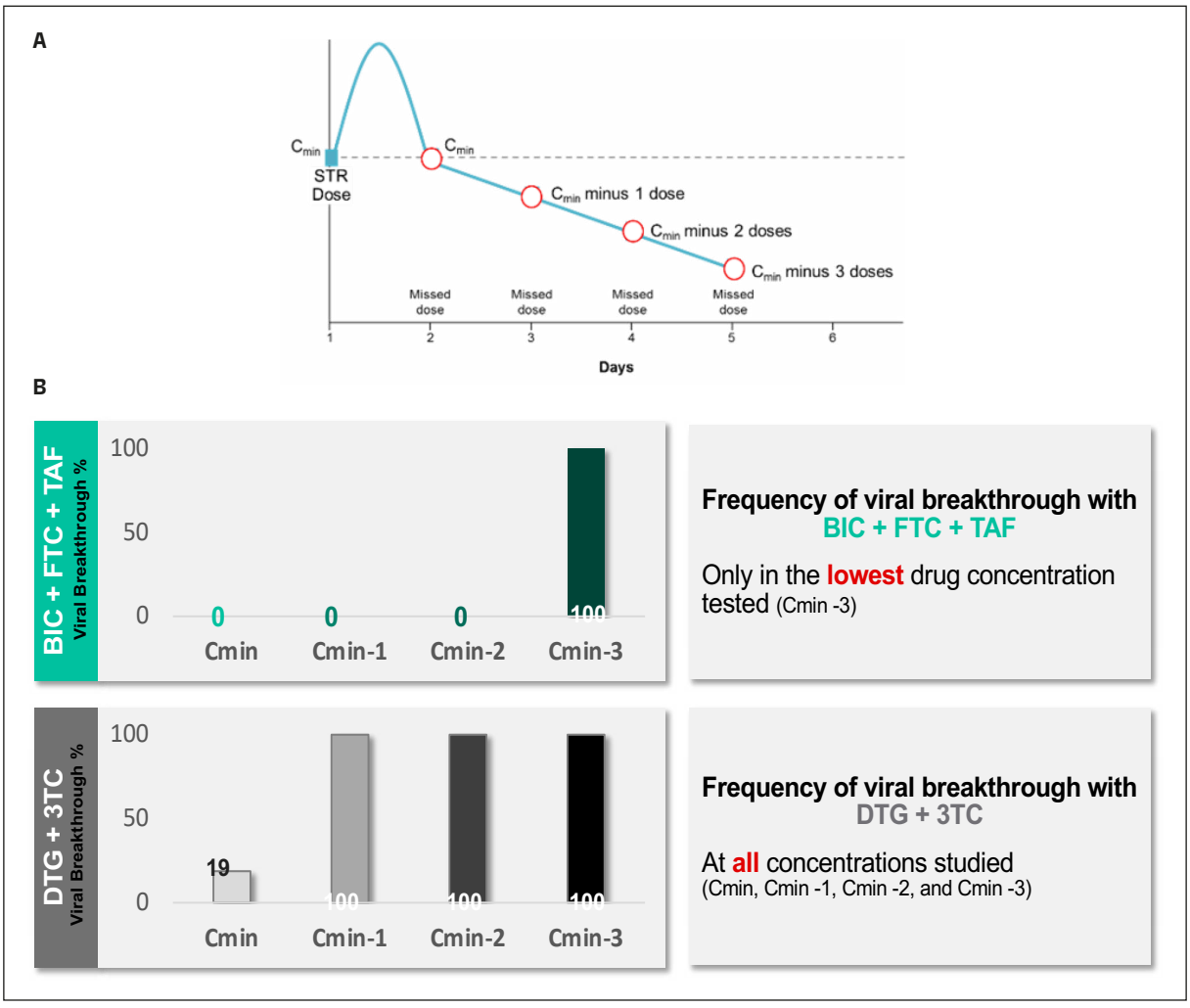

Figure 7 - Viral breakthrough assays using the drug combinations of BIC+FTC+TAF or DTG + 3TC were performed in parallel at fixed drug concentrations simulatingHuman plasma-free adjusted clinical trough concentrations (Cmin), at simulated Cmin after missing 1 to 3 consecutive doses (Cmin - 1 or Cmin - 2, and Cmin - 3).
Time to viral breakthrough in MT-2 cells infected with wild-type HIV IIIB strain. Viral breakthrough selections for each drug combination were tested in replicate infected cultures in the presence of constant drug pressure for up to 32 days or until viral breakthrough was observed. The number of cultures with viral breakthrough based on the observed cytopathic effect was scored at each time point. Selections were performed at Cmin (minimum drug exposures based on in vivo pharmacokinetics), drug concentrations simulating Cmin minus 1 dose, Cmin minus 2 consecutive doses, and Cmin minus 3 consecutive doses (adapted from ref 24).

Evolution of backbone from old NRTIs to modern N(t)RTIs, relying on compounds with long or very long half-life, as FTC and TDF/TAF, was a milestone of HAART history. This allowed not only the optimization of drug forgiveness but also the definition of regimen forgiveness as a new pharmacological perspective. TAF mantains, and probably improves, the key pharmacological characteristics of forgiveness attributed to TDF.
Tha lack of TAF in 2DRs could be associated to a lower regimen forgiveness in several scenarios of low adherence, still not captured by clinical trials. Therefore, more clinical research is needed to characterise patients who should continue to benefit of TAF-based regimens to support forgiveness.

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Inflammation, Immune Activation, Antiretrovirals and HIV Infection

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Current treatment goals of antiretroviral therapy, such as viral suppression (below the most ambitious quantitative cutoff) and recovery and/or maintenance of an effective immune function, are today relatively easy to achieve. The evolution of antiretroviral therapy has made available an increasing number of drugs with higher intrinsic activity and reduced toxicity, with excellent long-term performance. The fact that regimens consisting of two rather than three drugs have been formally approved for selected circumstances, well testifies the current standard of anti-HIV treatment [1]. In optimal therapeutic circumstances, particularly when the treatment is started very early in the course of HIV infection, the efficacy of antiretroviral therapy is such that the life expectancy of patients may approach that of the general population [2].

As different from almost all infectious diseases, however, HIV infection requires the uninterrupted intake of antiretrovirals to prevent the resumption of viral replication and the ensuing deterioration of immune function. While most AIDS-associated disorders are prevented by regular intake of antiretroviral therapy, a number of non-AIDS heterogeneous diseases are nevertheless more common in HIV-infected subjects as compared to the general population, in spite of long-term virologic suppression and full CD4+ T-cell recovery [3]. Neurocognitive impairment, higher cardiovascular risk and several neoplastic diseases have been listed among the AIDS-unrelated events that occur more frequently in successfully treated HIV-infected patients [4, 5]. This area of investigation finds thus its rationale in the fact that antiretroviral therapy might not restore completely the inflammatory/immune balance of patients in spite of persistent viral suppression. Although the magnitude of such residual vulnerability might also depend on the time when antiretrovirals are introduced along the natural course of HIV infection [6], the finding of humoral and cellular signs of persistent inflammation and immune activation may actually remain in most successfully treated individuals and provides a pathophysiological link with disease forms that are less frequent in the general population.

The cascade of discoveries about the role of immune activation in AIDS pathogenesis stems from the early times. In the very first description of patients with AIDS (well before HIV was recognized as the etiologic agent) increased levels of the surface activation marker formerly known as T10 (now called CD38) were reported on peripheral T-lymphocytes of AIDS patients [7].

Further to be repeatedly confirmed, it was subsequently found that the level of immune activation in patients with HIV infection was proportionally associated to disease progression. Quantitative expression of the immune activation marker CD38 was found to be even more accurate than CD4+ T-cell counts in predicting disease progression [8, 9].

The key role of immune activation in the progres-

sion of HIV infection was further demonstrated in the SIV primate infection (Sooty mangabey), in which normal lymphocyte balance was maintained in spite of viral replication in CD4+ T-cells but with low levels of immune activation [10].
The mechanism leading to chronic immune activation in HIV infection was unknown until increased levels of LPS (lipopolysaccharide) were described in patients with HIV infection, possibly resulting from microbial translocation from the gastrointestinal tract [11].
Similar findings were also described in a different primate (Macacus rhesus) SIV infection, in which the degree of immune activation (both innate and adaptive) was associated with increased LPS levels. Disruption of the gastrointestinal barrier function, likely resulting from acute local CD4+ T-cell depletion, was thus hypothesized as a fundamental event in HIV pathogenesis [12]. The role of intestinal mucosal integrity in controlling immune activation has been recently confirmed in a further primate model (African green monkey) infected by SIV [13]. Regardless the hierarchy of the different factors contributing to immune activation, the most accepted hypothesis is that the hyperactive inflammatory state of chronic HIV infection is associated to an increased turnover of activate naïve T-cells resulting in progressive T-cell depletion by apoptopic phenomena [6].
Along this line of research a fundamental change occurred when the key element of effective antiretroviral therapy was introduced, thus raising the question about the effects of the latter on immune activation and chronic inflammation in patients who had no longer HIV-RNA detected in plasma and experienced a sustained immune recovery. Among the many investigations carried in virologically suppressed patients, from a clinical standpoint the most striking evidence supporting a role of antiretroviral therapy in decreasing immune activation was provided by the SMART study in 2006. The trial compared a series of clinical endpoints and immunologic markers in patients receiving continuous vs interrupted therapy and the main result consisted in an higher death rate due to non-AIDS events but secondary to immune activation in the arm receiving interrupted treatment [14]. These findings point on the capacity of antiretroviral treatment to decrease the intensity of immune activation, as shown by significant drop of activation markers following successful introduction of antiretroviral therapy [15, 16]. In general, looking at different studies comparing healthy controls to HIV-infected patients characterized by a rather wide range of different immunovirologic conditions, higher levels of circulating soluble markers (and/or mediators) of immune activation and chronic inflammation have been recorded, such as IL-6 and sCD14, together with increased expression of CD38 and HLA-DR testifying activation of CD8+ T-cells, especially in case of patent viraemia and reduced immune recovery [6]. The overall picture however, is not straightforward as fluctuating levels of these indicators have been reported, although with a tendency to decrease when the immune-virologic balance improves.
Many uncertainties however still remain on the pathogenesis of immune dysfunction in HIV infection and alternative or additional mechanisms are being considered, such as the loss of selective CD4+ T-cell subpopulations or specific patterns of cytokine secretion like IL-6, that may also turn to have better predictive value on disease progression as compared to markers of T-cell activation [17].
The key question does thus remain as to what extent a successful antiretroviral treatment might impact in reducing this persistent background of noxious immune activation and chronic inflammation. Such a question acquires further importance following the approval of dual antiretroviral therapy in patients fulfilling several immunovirological requirements. The two dual regimens so far approved (DTG/RPV and DTC/3TC) were found to determine a degree of both virologic suppression and immune reconstitution comparable to that seen with triple regimens [18-20]. In practical terms the basic doubt concerns the possibility of a weaker effect on the reduction of immune activation by a dual vs a conventional triple antiretroviral regimen.
To have some insights on this issue we should look at the several attempts so far made on antiretroviral monotherapy, which represents the lowest extreme in terms of number of drugs in a regimen [21-28]. Different monotherapies have been analyzed in terms of immune activation through the comparison with conventional triple regimens. It has been the case of boosted atazanavir or darunavir that were compared to triple regimens based on the same protease in-

hibitor but also containing a double N/NtRTI backbone. Although virologic failure rates were generally higher in the monotherapy arms, analyses were also focused on the subsets of monotherapy patients with virologic suppression. While different markers of immune activation were measured in these studies, most of the evidence was in favour of a decreased effect of monotherapy in reducing inflammation and T-cell activation, T-cell apoptosis and monocyte activation markers were found to be increased in patients receiving monotherapy in spite of virologic suppression. As a consequence, further to its lower virologic performance, monotherapy is today contraindicated also due to its reduced impact on reduction of immune activation [1].

Looking at dual regimens, some work has been done with two-drug regimens before INSTIs-based dual regimens were approved [29-32]. No striking findings were recorded over a series of small mono-arm or comparative studies in which dual regimens were based on PIs. In an Italian study virologic suppressed patients who were switched from triple regimens to a wide variety of dual combinations had a significant increase in CD8+ T-cell with proportional decreases in the CD4+/CD8+ T-cell ratio. It is noteworthy that 45 out of a total of 104 patients who switched to dual regimens were taking INSTIs, but no separate analysis was made to look at a possible role of specific drugs included in dual regimens [33].

In the registration trials that actually promoted DTG/RPV as maintenance regimen and DTG/3TC as both initial and maintenance therapeutic options some attention was also paid to several markers of immune activation/hyperinflammation [18]. More data are actually needed to fully clarify this crucial point, particularly considering those patients who might lie in a borderline position as referred to the inclusion criteria for dual therapy as initial regimen (e.g. high baseline viral copies, baseline CD4+ T-cell count <200/μL) [19].

Beyond the still insufficient data available on immune activation, an additional good reason for fully considering this issue relies upon the hypothesis that some low-grade HIV replication persists in certain anatomical sites (central nervous system, lymph nodes, gastrointestinal tract) where no sufficient drug levels are achieved to fully inhibit viral replication [34]. It has been hypothesized that this residual viral production might be responsible for the persistence of immune activation. The choice of administering two instead that three drugs might thus further impact on the degree of such hidden viral replication, with possible consequences in terms of persisting inflammation. Again, also this hypothesis requires more clinical studies in order to be properly challenged.

The problem remains thus unresolved, but the therapeutic index of most options today included in the current therapeutic armamentarium is actually much more favourable as compared to the times when the choice of a regimen implied a much deeper scrutiny of possible short-, mid- and long-term consequences. Choosing a triple instead of a dual regimen does not imply today the same tolerability/toxicity scenarios we faced with several 1st generation drugs in older times and triple fixed combinations might consist in a pill size that is indistinguishable from that of fixed dual regimens. The improvements achieved by most drug classes over the last decades allow today a much smoother choice, as first-line drugs are virtually devoid of any signature toxicity, lower dosages guarantee better antiviral effects, fewer are the drug-drug interactions and no long-term untoward effects are reasonably expected.

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Treatment Switching: the role of TAF-based therapy

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The success of triple therapy in people living with HIV-1 infection (PLWH) has decreased mortality and improved life expectancies near to those of the general population in properly treated patients [1]. Virologically suppressed patients, usually defined by the presence of HIV-RNA <50 copies/ml for at least 6 months, may switch from their ongoing regimen because of several reasons including documented toxicity, prevention of long-term toxicity, ageing and/or comorbidity, avoidance of drug-drug interactions, pregnancy or wishing of pregnancy and protection from HBV replication. Moreover switching to another regimen may be proposed for simplication in order to reduce the number of pills or the number of drugs in the regimen. The primary outcome when switching ARV therapy is to maintain virological suppression, but because PLWH are ageing and thus at increasing risk of chronic diseases and long-term complications, switching goals have to refocus not only on the primary oucome but also on the mitigation of adverse effects and long-term toxicities. Therefore current guidelines clearly recommend to properly evaluate the complete clinical and antiretroviral history, hypersensitivity or tolerability issues and cumulative genotypic resistance history before considering switching options. In this ever-changing scenario, the avaliability of tenofovir-alafenamide (TAF)-based triple therapy, particularly within the single-pill fixed-dose combination (FDC) represents a significant step forward in terms of tailored intervention.

SWITCHING TO TENOFOVIR-ALAFENAMIDE

A considerable amount of retrospective and prospective data support switching to a TAF-based

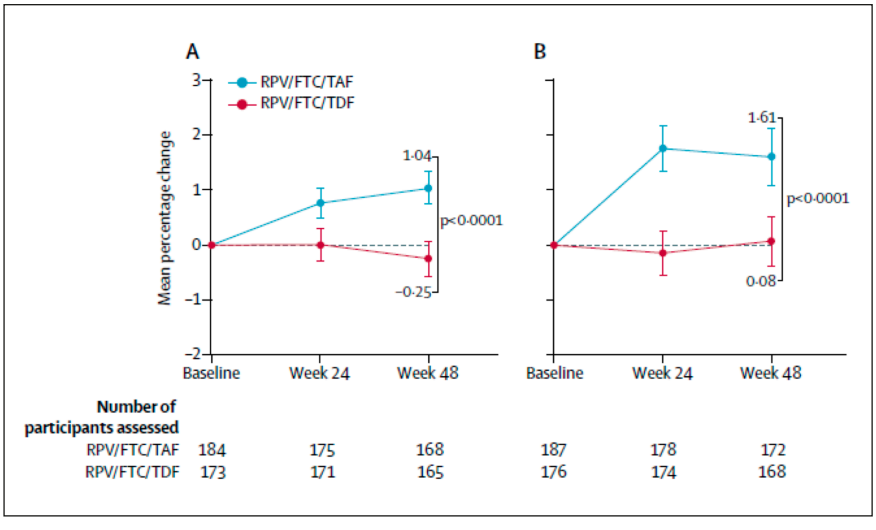

Figure 1 - Mean percentage change from baseline to week 24 and 48 in hip bone mineral density (A) and lumbar spine bone mineral density (B) by dual energy x-ray absorptiometry after switching to RPV/FTC /TAF.

Error bars show 95% CIs. RPV/FTC/TAF=rilpivirine, emtricitabine, and tenofovir alafenamide. RPV/FTC/TDF=rilpivirine, emtricitabine, and tenofovir disoproxil fumarate.

Adapted from C. Orkin, Lancet HIV 2017.

triple therapy in virologically suppressed patients. In a randomized, double-blind study, [2] switching from single tablet rilpivirine, emtricitabine, tenofofovir disoproxil fumarate to *rilpivirine*, emtricitabine and tenofovir alafenamide was associated with a low rate of virological failure (<1%), no evidence of treatment-emergent resistance, a mild increase in cholesterol and tryglicerides and a significant improvement in hip and spine bone mineral density (Figure 1). In the EMERALD study [3] switching to a single tablet regimen of darunavir, cobicistat, emtricitabine/tenofovir alafenamide from *boosted PI* plus emtricitabine and TDF yelded similar 48 weeks efficacy results. Virological suppression was high, no patient discontinued because of virological failure and no drug resistance developed. A similar lipid change and an improved bone and renal biomarkers safety profile in comparison with the control group were observed. Switching to bictegravir, emtricitabine and tenofovir alafenamide [4] maintained high rates of efficacy and was non-inferior to remaining on *dolutegravir, abacavir, and lamivudine*. Both regimens were well tolerated, with fewer participants in the bictegravir group having drug related adverse events (Figure 2). At IAS 2021 the results of the open-label extension phase of this study where all the participants on DTG/ABC/3TC were switched to B/F/TAF were presented. The after a median B/F/TAF duration exposure of 96 weeks 98% of them had an HIV-RNA <50 copies/ml at their last study visit [5]. Viral blips occurred infrequently and did not affect virological outcome or emergence of drug-resistance associated mutation

A large metanalysis including data from 14 clinicals trials (6), including switch trials and enrolling around 15.000 patients confirmed high and comparable levels of safety and efficacy of TAF and TDF overall. Where TAF and TDF were boosted by ritonavir or cobicistat, TAF showed significantly higher rates of HIV-RNA suppression than TDF and lower risks of renal and bone-related adverse events [6-7] (Figure 3). There are still many gaps to be addressed : participants in the trials were relatively young (average 39 years) with few comorbidities and there were also underrepresentation of women and non white population, moreover a follow-up of even 144 weeks in a study may not be enough time to observe major clinical events, therefore long-term follow-up is clearly needed. Nevertheless in order to fill these gaps new data are rapidly accumulating.

Evidence that bictegravir/emtricitabine/tenofovir alafenamide (B/F/TAF) is an effective and durable triple therapy switch option for *black adults* comes from BRAAVE 2020 Study, recently presented ayt IAS 2021 [8]. It is a phase 3 clinical trial designed with community input to evaluate the specific treatment responses of virologically suppressed adults living with HIV who self-identified as Black or African American following a switch to B/F/TAF from a variety of regimens. A total of 495 study participants were randomly

	Bictegravir group (n=282)	Dolutegravir group (n=281)	p value
Any adverse event	225 (80%)	225 (80%)	1·00
Most common adverse events*			
Upper respiratory tract infection	29 (10%)	27 (10%)	0·89
Nasopharyngitis	20 (7%)	22 (8%)	0·75
Headache	19 (7%)	21 (7%)	0·75
Diarrhoea	24 (9%)	14 (5%)	0·13
Arthralgia	19 (7%)	10 (4%)	0·13
Insomnia	8 (3%)	14 (5%)	0·20
Grade 3 or 4 adverse event	16 (6%)	10 (4%)	0·32
Serious adverse event	15 (5%)	22 (8%)	0·24
Treatment-related adverse event	23 (8%)	44 (16%)	0·006
Treatment-related serious adverse event	1 (<1%)†	0	1·00
Adverse event leading to study drug discontinuation‡	6 (2%)	2 (1%)	0·29
Death§	2 (1%)	0	0·50

Figure 2 - Switching to fixed-dose bictegravir, emtricitabine, and tenofovir alafenamide from dolutegravir plus abacavir and lamivudine in virologically suppressed adults with HIV-1. Summary of adverse events.

**Occurring in ≥5% of participants in either group.*

Adapted from JM Molina, LANCET HIV 2018.

Figure 3 - Risk differences and mean differences for efficacy and safety parameters: 10 mg TAF vs boosted TDF.

Adapted from A.Hill, J Virus Erad 2018 .

Measure	TAF/FTC	TDF/FTC	Effect estimate [95% CI]	*P* value
Efficacy				
HIV RNA<50 copies/mL	2411/2679 (90%)	1582/1839 (86%)	+2% [0–4%]	0.05
Primary genotypic resistance	9/1844 (0%)	10/1353 (1%)	0% [0%]	n.s.
Safety				
Grade 1–4 AEs	1123/2047 (55%)	834/1456 (57%)	-8% [-18%, +3%]	n.s.
Grade 3–4 AEs	96/1844 (5%)	87/1353 (6%)	0% [-2%, +2%]	n.s.
Grade 3–4 Lab abnormalities	345/1284 (27%)	316/1078 (29%)	-2% [-15%, +11%]	n.s.
Serious adverse events	165/1999 (8%)	150/1504 (10%)	0% [-2%, +1%]	n.s.
Deaths (any cause)	2/1732 (0%)	3/1295 (0%)	0% [0%]	n.s.
Bone fractures Week 48	3/978 (0%)	8/925 (1%)	-1% [-1%, 0%]	0.04
D/C for bone AEs	0/1081 (0%)	6/975 (1%)	-1% [-1%, 0%]	0.03
D/C for renal AEs	1/2150 (0%)	17/1506 (1%)	-1% [-1%, 0%]	0.002

AE: adverse event; D/C: discontinuation; n.s.: not significant

allocated and treated in a 2:1 ratio to either switch to open-label B/F/TAF for up to 72 weeks (n=330) or to stay on a standard regimen of two nucleoside reverse transcriptase inhibitors (NRTIs) plus a third agent for 24 weeks with a delayed switch to B/F/TAF for up to 48 weeks (n=165). At 72 weeks, 99% of participants (n=246/248, missing=-excluded) who switched to B/F/TAF at the start of the study maintained an undetectable viral load regardless of age or sex at birth. These results provide further evidence that B/F/TAF is an effective and durable treatment option for black adults who are virologically suppressed.

Long-term data from a small Phase 3b open-label trial [9] enrolling PLWH *aged 65 and older* who switched to B/F/TAF (n=86) from either E/C/F/TAF or TDF based triple therapy, showed that 74% of participants (n=64/86) were able to maintain virologic suppression with no virological failures or emergent resistance through 96 weeks and that the rate of drug-related adverse events leading to study drug discontinuation in this fragile population, in the setting of Covid-19 pandemic was low, around 3.5%.

GS-US-380-1961 [10] is a 48 weeks, open label, international randomized study where **women** of 40 years were randomized to receive B/F/TAF or the maintain the ongoing regimen (E/C/F/TAF 53%, E/C/F/TDF 42%, ATV+RTV + FTC/TDF 5%). Non inferiority was confirmed (1.7% vs 1.7% of participants with HIV-RNA ≥50 copies/mL) and none receiving B/F/TAF developed treatment-emergent resistance. Because of neutral effect on bone and kidney, TAF-based triple therapy could represent a proper switching option for use in *pregnant* as well in *menopause* women. Initial data suggest that TAF-based FDCs have high efficacy and low risk of adverse effects during pregnancy [11].

TAF-based triple therapy is a robust switching option for a consistent proportion of PLWH virologically suppressed, including patients with symptomatic advanced disease, those coinfected with HBV.

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