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Clinical pharmacology of the SingleTablet Regimen (STR) Bictegravir/Emtricitabine/Tenofovir Alafenamide (BIC/FTC/TAF)

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SUMMARY

In Italy a proportion of HIV patients exceeding 50% are diagnosed at advanced stages of disease.
A sizeable proportion of patients under chronic HIV treatment has a story of poor adherence with archived resistance associated mutations, a condition implying some risks in case of treatment with dual regimens.
Conventional three-drug regimens will remain necessary in the short-mid term, in order to avoid treatment failure and selection of drug resistance.
Efficacy, tolerability, safety, genetic barrier, forgiveness and a good compatibility with concurrent medications are all features that describe the overall quality of BIC/FTC/TAF, a combination whose robustness will remain a point of reference for the next years.

Keywords: Bictegravir, Emtricitabine, Tenofovir, single tablet regimen.

INTRODUCTION

In the new era of antiretroviral therapy no regimens consisting of three drugs are any longer in the pipeline of the pharmaceutical industry. Dual regimens (2DR), mostly based on long-acting injectable drugs represent the focus of current and future developments for the treatment of HIV infection [1]. While in their clinical development these lighter regimens were found to provide efficacy rates non inferior to conventional three drug combinations, both in case of treatment-naïve and experienced patients [2-5], it must be recognized that the patients recruited in these registration clinical trials are not representative of the entire HIV+ population. Selection of patients with suitable low-risk conditions for therapeutic failure was a prerequisite in the study designs in order to find the right position for dual therapies in the treatment of HIV infection. This implies that a sizeable proportion of patients not fulfilling study entry criteria were excluded and considered at risk of therapeutic failure with two-drug combinations [6]. As a consequence, for these patients a three-drug regimen (3DR) seems to be still advisable to reduce the likelihood of treatment failure and it appears nowadays unlikely that the currently available dual regimens or newer solutions under development will make 3DR no longer required in the near future. Thus, inspite of the lack of any 3DR in the pharmaceutical pipeline, these conventional regimens will keep on playing a relevant role in the next years. High-risk naïve patients (e.g. AIDS presenters) and patients with prior relevant treatment failures are likely to keep on representing a critical proportion of HIV-infected patients for whom a dual regimen is unlikely to be the best permanent solution. Furthermore, suboptimal adherence is rather common in pa-

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Table 1 - Pharmacologic parameters of the three drugs co-formulated in the single tablet BIC/FTC/TAF. T/2: elimination half-life; IC: intracellular, AUC: area under the curve.

	Bictegravir	*Emtricitabine*	*Tenofovir Alafenamide*
T/2 (h)	17.3	10.0 plasma, 54.5 IC	32.37 plasma, 69.6 IC
VD (L)	15.56	1.4+/-0.3 L/Kg	>100
Absorption	Fat meal increases AUC and Cmax by 24 and 13%	Food irrelevant	Fat meal increases AUC by 65%
Protein binding	>99%	4%	TAF 80%, tenofovir 0.7%
Clearance	35% renal (glucuronide)	86% renal (13% as metabolites - (excreted by glomerular filtration and tubular secretion)	Cleared by glomerular filtration (2/3) and tubular secretion (1/3)
Metabolism	CYP3A and UGT1A1 Inhibitor of OCT2 and MATE	Limited	Carboxylesterase-1, cathepsin A, CYP3A (minimal), substrate of P-gp, BCRP, OATP1B1, OATP1B3

tients receiving antiretroviral therapy, an attitude that emphasizes the lower intrinsic antiretroviral potency of 2DR as compared to 3DR [6].
The last 3DR that underwent successful development consists of the last generation integrase strand-transfer inhibitor (INSTI) bictegravir in co-formulation with the two reverse transcriptase inhibitors (NtRTIs) emtricitabine (FTC, a cytidine analogue) and tenofovir alafenamide (TAF, an adenosine analogue). This single pill combination was approved by FDA in 2018 and represents the combined result of the successful evolution of two drug classes, the INSTIs and the NtRTIs [7, 8].

Basic Clinical Pharmacology of the individual components of the single tablet combination Bictegravir/Emtricitabine/Tenofovir Alafenamide (BIC/FTC/TAF)

The main pharmacologic characteristics of clinical relevance of the three antiretrovirals are represented in Table 1. While more numerous are the parameters describing the pharmacologic features of a drug, here only those of closest relevance to the clinical field are shown and discussed.

Bictegravir (BIC)

Pharmacodynamics – BIC is a last generation strand-transfer integrase inhibitor (INSTI) that binds to the integrase active site and blocks the strand transfer step of retroviral deoxyribonucleic acid (DNA) integration. BIC, which is available only as component of a single tablet regimen also including FTC and TAF, is active against both HIV-1 and HIV-2. The 50% effective concentration (EC50) values for bictegravir [as assessed in lymphoblastoid cell lines, PBMCs, primary monocyte/macrophage cells, and CD4+ T-lymphocytes) was found to be in the range of <0.05 to 6.6 nM. The protein-adjusted EC95 of bictegravir was 361 nM (0.162 µg/mL) for wild type HIV-1 virus [9].
As in case of other INSTIs, BIC was found to determine a rapid viral fall in a 10-day monotherapy study, with a $\log_{10}$ reduction of viral copies exceeding 2.0 [10]. It must be noted that this is a specific property of 2nd generation INSTIs and that beyond the appraisal of a remarkable intrinsic antiviral potency of the drug, such an effect contributes to increasing its genetic barrier by reducing the viral biomass in a significantly shorter time as compared to all other existing antiretroviral drug classes [11].

Pharmacokinetics – Peak plasma concentration following absorption occurs 2.0-4.0 hours after oral intake, with a limited impact on Pk parameters given by intake with a fat meal (AUC and C_{max} increase of 24% and 13%, respectively). The plasma elimination half-life (T/2) is 17.3 hours, a value well allowing once daily administration [9]. However, beyond the plasma concentration of BIC, what is likely to also impact in terms of duration of antiviral suppression is the long dissociation time from the target shown by BIC, which is by far the longest recorded among INSTIs. In *in vitro* studies on cells, in which the antiviral activity was measured following wash-out of the drug from the supernatant, BIC was found to have a dissociation T/2 from wild-type integrase-DNA

Table 2 - Dissociation half-lives of INSTIs from integrase-DNA complex (from White KL, et al. AAC 2021; 65, e02406-20).

INSTI	*Dissociation Time (h) WT*	*Dissociation Time (h) G140S + Q148H*
Bictegravir	163 ± 31	5.7 ± 0.4
Dolutegravir	96 ± 29	1.9 ± 0.2
Raltegravir	10 ± 2	nd
Elvitegravir	3.3 ± 0.9	nd

complex of 163 hours [12]. The dissociation times (half-lives) of INSTIs are shown in Table 2. Such INSTI-specific property suggests that these drugs retain their inhibitory activity against HIV replication for a longer time than otherwise predicted by simply referring to plasma T/2 values. Protein binding of BIC is rather similar to all INSTIs, with values around 99%, and likewise similar is its volume of distribution (15.56 L) [9].

Metabolism – BIC is primarily a substrate of CYP3A and UGT1A1, and 60% of the drug is recovered in faeces as unchanged molecule, desfluoro-hydroxy-BIC-cysteine-conjugate and other minor oxidative metabolites, while 35% is recovered in urine as glucuronide metabolite [9].

Emtricitabine (FTC)
Pharmacodynamics – FTC acts as a nucleoside reverse transcriptase inhibitor (NRTI) with a mimetic mechanism as analogue of 2' deoxycytidine. Following triple intracellular phosphorylation it undergoes incorporation into proviral DNA by the catalyst action of HIV reverse transcriptase, which results in DNA chain termination [13]. FTC at its approved daily dose of 200 mg was found to determine a 1.7 $\log_{10}$ viral fall in a 10-day monotherapy study [14]. In the same study the standard dose of the drug was compared to lower doses of FTC itself and to the standard lamivudine (3TC) dose, and it was shown to achieve a viral load decrease to ≤400 copies/mL or a ≥2 $\log_{10}$ decrease in 53% of cases while with 3TC at the standard 150 mg bid dose the same results were obtained in 29% of patients [14].

Pharmacokinetics – With a 93% of oral bioavailabilty FTC achieves peak plasma concentration 1.5-2.0 hours following oral intake, and absorption is unaffected by food. The plasma T/2 of FTC is around 10.0 hours while its active tri-phosphorylated moiety has an intracellular T/2 of 54.5 hours [13]. For comparison we should take into account that 3TC has a plasma T/2 of 5 hours and an intracellular T/2 of the active triphosphorylated active moiety of 15-16 hours [15]. Protein binding of FTC is of 4% and its volume of distribution is 1.4+/-0.3 L/kg.

Metabolism – FTC undergoes intracellular phosphorylation to be transformed into its active moiety. Following de-phosphorylation FTC is recovered from urine (86%) and faeces (14%) mainly as unchanged molecule, with minor metabolism (13% as 3'sulfoxide diastereomers and 2'O-glucuronide) [13].

Tenofovir Alafenamide (TAF)
Pharmacodynamics – TAF is a phosphonamidate prodrug of tenofovir that was developed after the successful introduction of the former prodrug tenofovir disoproxil fumarate (TDF). Once inside the cells tenofovir undergoes phosphorylation to be transformed into its pharmacologically active metabolite tenofovir diphosphate. The latter acts as mimetic alternative to the natural substrate adenosine triphosphate and gets incorporated into proviral DNA by HIV reverse transcriptase, which results into DNA chain termination [16].

Pharmacokinetics – As compared to TDF, whose bioavailability is 25%, TAF absorption is around 40% and its AUC can be increased by 65% following a fat meal. A strategic difference from TDF is its greater plasma stability as alafenamide salt. This property is associated to a much more selective distribution of tenofovir into cells. The latter is driven by cell richness in carboxy-esterase 1 (CES1), as it occurs in case of first-pass metabolism by hepatocytes, and catepsin A (CatA) expressing PBMCs [17]. The plasma half-life of TAF is 0.51 hours, while tenofovir free of the alafenamide salt has a T/2 of >32 hours. The intracellular T/2 of the active phosphorylated moiety is 69 hours and the volume of distribution exceeds 100 L [18].

Metabolism – TAF is metabolized by CES1 and CatA, then undergoes phosphorylation intracellularly and once de-phosphorylated is cleared by

glomerular filtration (2/3) and proximal tubular secretion (1/3). Tenofovir is transported by P-gp, BCRP, OATP1B1 and OATP1B3 [16].

Clinical/Pharmacologic considerations about the three components together

The single tablet regimen consisting of BIC/FTC/TAF is the last three-drug regimen developed for the treatment of HIV infection. As such it is not surprising that the single components of this regimen display the best antiretroviral properties ever seen in each drug class here considered [19].

Bictegravir is the last oral INSTI that appeared in the antiretroviral market and alike dolutegravir, in the 10-day monotherapy study is associated with a $\log_{10}$ drop in viral copies that exceeds 2.0 [10]. This remarkable intrinsic antiretroviral potency is associated to a faster reduction or disappearance of detectable HIV-RNA from plasma as compared to any other antiretroviral drug class. Since both the size of the microbial biomass and the duration of the exposure to the selecting agent are among the factors driving the selection of resistant mutants, such faster viral decay is by all means a property that contributes to the robust genetic barrier of the drug [11]. In the clinical scenario this is testified by the only anecdotal emergence of drug resistance when a 2nd generation INSTI is part of the regimen [7, 8, 20].

Similar considerations apply to FTC, that is the last cytidine analogue developed for the treatment of HIV infection. When compared to the older NRTI cytidine analogue available in the market (3TC), in a 10-day monotherapy study FTC gave overt evidence of a higher intrinsic antiretroviral potency [14]. Further to its greater potency FTC has a longer intracellular T/2 allowing once daily administration [13].

Tenofovir alafenamide represents the evolution of tenofovir prodrugs, whose development was deemed necessary in the light of the increasing evidence of proximal renal tubular toxicity associated to TDF intake [21]. We must consider that when TDF was made available into the market the average age of the HIV-infected population was well younger than now and that progressive aging was prospectively seen as a further critical factor for renal tubular toxicity [22]. Being TAF and TDF prodrugs of tenofovir, for any comparison we should remember that the final intracellular active moiety is the same. What does indeed change is its distribution, since when tenofovir is taken as TAF much lower plasma concentration of tenofovir are measured as compared to TDF, while the opposite happens at the intracellular level, where the pharmacokinetic exposure of tenofovir may approach a value that is 6, 7 fold the one measured in case of TDF intake [18]. Thus the improvement of TAF over TDF took place in two directions, as more drug is made available in target cells and less drug is present in the systemic circulation where its higher concentration may be harmful. Since approximately a third of tenofovir clearance takes place through proximal tubular secretion (the rest is freely filtered by the glomerulus) in case of TDF intake the capacity of extruding the drug by the tubular epithelium may be insufficient thus leading to some degree of tenofovir accumulation into epithelial cells. It has been demonstrated both in animal models and humans that such intracellular excess of tenofovir may functionally damage mitochondria, which reduces the energy available for the membrane transporters mediating the efflux of the drug into urine [23]. While this functional tubular impairment is not usually followed by reduced glomerular filtration, a downstream consequence of this abnormality is the impaired tubular reabsorption capacity. The reduced reabsorption of phosphate is the likely pathogenetic link with a common feature associated to TDF intake, such as the decrease of bone mineral density, as testified by many clinical studies [24]. Among the variables associated to TDF tubular toxicity, the plasma concentration of tenofovir plays a major role and as a consequence its lower pharmacokinetic plasma exposure achieved when administered as TAF is the reason why this second prodrug is virtually harmless for proximal tubular physiology [21, 25, 26]. Thus, the task of making available a less toxic pharmaceutical form of the same virologically effective molecule was fully accomplished.

On the side of antiretroviral potency no differences between TDF and TAF were recorded, with a partial exception being represented by their use in PrEP; in a randomized, comparative, double-blind clinical trial (both drugs were combined with FTC), although no statistically significant difference was seen, a clear tendency to a greater protective effect was associated to TAF [27]. Recipients of TAF had an incidence of HIV infection

of 0.16 per 100 person-years [7 infections per 4370 persons-year), while for those who were taking TDF the incidence was 0.34 per 100 person-years (15 infections per 4386 person-years), a >50% difference actually just short of statistical significance. In clinical trials comparing TDF and TAF in the treatment of HIV infection the common virologic result was that of non-inferiority, thus suggesting that the increased pharmacokinetic exposure of tenofovir inside the target cells achieved by TAF intake does not provide any additional advantage when compared to TDF in terms of virologic suppression [7, 8]. However such higher intracellular concentration of tenofovir cannot be dismissed as an irrelevant feature, as there are individual circumstances in which having on board more drug might compensate for weaknesses resulting from some degree of drug resistance concerning tenofovir itself or the other components of the regimen [20]. In such a view we should also consider possible advantages from a higher pharmacokinetic exposure into compartments [28]. While the possible virologic advantages of higher intracellular concentrations of tenofovir when given as TAF vs TDF remain hypothetical in the treatment of HIV infection, in the setting of HIV-HBV co-infection some clues about additional benefits coming from TAF intake are emerging. In a small phase-III clinical trial comparing BIC/FTC/TAF and DTG/FTC/TDF, a significantly higher proportion of patients receiving TAF achieved the target of HBV-DNA suppression <29 IU/mL after 48 weeks of treatment as compared to TDF intakers (63.0 vs 43.4%, p=0.023) [29]. Furthermore, TAF recipients also had a higher incidence of HBsAg clearance (12.6 vs 5.8%), anti-HBsAg seroconversion [8.4 vs 3.3%), clearance of HBeAg (25.6 vs 14.4%), anti-HBeAg seroconversion (23.3 vs 11.3%) and ALT normalization through week 48 (73.3 vs 55.3%). These results are unprecedented in the setting of anti-HBV treatment in HIV/HBV co-infected patients, and clearly suggest that the increased intracellular tenofovir concentrations achieved by treatment with TAF are of therapeutic relevance in this setting. It is noteworthy, as a simple matter of fact, that in such clinical therapeutic comparison between TAF and TDF, whose active moiety is by definition the same, the patients who were treated with the pharmaceutical form delivering more drug into the cells had a better virologic outcome. After years of immunomodulant treatment of chronic HBV infection, followed by the successful introduction of antivirals inhibiting the viral polymerase, these results finally suggest that a relevant factor here may be represented by the amount of drug we are able to deliver into infected cells. Such a progress might pave the way to novel strategies to further improve the therapeutic outcome of chronic HBV infection.

CONCLUSIONS

The new orientation of HIV pharmaceutics points on dual regimens, mainly with the characteristics of injectables, long-acting molecules. However many patients keep on having less an ideal conditions for the use of a regimen consisting of less than three drugs. In Italy a proportion of patients exceeding 50% are diagnosed at advanced stages of disease, usually with high viral loads and a deteriorated immunity, and the average perception of the risk of acquiring HIV infection remains rather low, thus contributing to perpetuate the high numbers of AIDS presenters [30, 31]. Furthermore, a sizeable proportion of patients under chronic HIV treatment has a story of poor adherence with archived resistance-associated mutations, a condition implying some risks in case of treatment with dual regimens. As a consequence it can easily predictable that conventional three-drug regimens will remain necessary in the short-mid term, in order to avoid treatment failure and selection of drug resistance. Among physicians working in the HIV field, the responsibility of preserving the efficacy of key components of antiretroviral regimens (e.g. INSTIs) is felt as serious, with the aim of avoiding the use of older drugs, often characterized by lower tolerability and higher toxicity. To this prioritary issue we must add the consideration that the choice between a dual and a triple therapy is no longer justified by the reduced toxicity of a regimen consisting of less drugs. This was the case when the NtRTIs available were characterized by specific signature toxicities, and withdrawal of a drug, when deemed immunovirologically feasible, was the way to improve the long-term tolerability and safety of the regimen [32]. Today is no longer the case, as drugs like 2nd generation INSTIs and TAF, the new tenofovir prodrug, are not actually associated to additional relevant toxicity as compared to the currently available dual regimens. Beyond

the fascinating perspective of treating HIV infection with injectable long-acting options, allowing for long intervals between sequential drug administrations, one reason why triple regimens are no longer in the pipeline of the pharmaceutical industry might also lie in the fact that single-tablet regimens of a standard higher than BIC/FTC/TAF are currently out of reach for most manufacturers. Efficacy, tolerability, safety, genetic barrier, forgiveness and a good compatibility with concurrent medications are all features that describe the overall quality of BIC/FTC/TAF, a combination whose robustness will remain a point of reference for the next years.

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Conflict of interest
None to declare.

REFERENCES

[1] Thoueille P, Choong E, CavassiniM, et al. Long-acting antiretrovirals: a new era for the management and prevention of HIV infection. *J Antimicob Chemother.* 2022; 77 (2), 290-302.
[2] Llibre JM, Hung CC, Brinson C, et al. Efficacy, safety, and tolerability of dolutegravir-rilpivirine for the maintenance of virological suppression in adults with HIV-1: phase 3, randomised, non-inferiority SWORD-1 and SWORD-2 studies. *Lancet.* 2018; 391 (10123), 839-849.
[3] van Wyk J, Ajana F, Bisshop F, et al. Efficacy and Safety of Switching to Dolutegravir/Lamivudine Fixed-Dose 2-Drug Regimen vs Continuing a Tenofovir Alafenamide - Based 3- or 4- Drug Regimen for Maintenance of Virologic Suppression in Adults Living With Human Immunodeficiency Virus Type 1: Phase 3, Randomized, Noninferiority TANGO Study, *Clin Infect Dis* . 2020; 71 (8), 1920-1929.
[4] Murray M, Pulido F, Mills A, et al. Patient-reported tolerability and acceptability of cabotegravir + rilpivirine long-acting injections for the treatment of HIV-1 infection: 96-week results from the randomized LATTE-2 study. *HIV Res Clin Pract.* 2019; 20 (4-5), 111-122.
[5] Cahn P, Madero JS, Arribas JR, et al. Durable Efficacy of Dolutegravir Plus Lamivudine in Antiretroviral Treatment-Naive Adults With HIV-1 Infection: 96-Week Results From the GEMINI-1 and GEMINI-2 Randomized Clinical Trials. *J Acquir Immune Defic Syndr.* 2020; 83 (3), 310-318.
[6] Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents with HIV. Department of Health and Human Services. Available at https://clinicalinfo.hiv.gov/en/guidelines/adult-and-adolescent-arv. Accessed May 2023.
[7] Wohl D, Pozniak A, Workowski K, et al. B/F/TAF Five-year outcomes in treatment-naive adults. CROI 2022. 12-16 February 2022, virtual. Poster abstract 494.
[8] Sax PE, Rockstroh JK, Luetkemeyer, et al. Switching to Bictegravir, Emtricitabine, and Tenofovir Alafenamide in Virologically Suppressed Adults With Human Immunodeficiency Virus. *Clin Infect Dis.* 2021; 73, e485-e493.
[9] Podany AT, Scarsi KK, Pham MM, Fletcher CV. Comparative Clinical Pharmacokinetics and Pharmacodynamics of HIV-1 Integrase Strand Transfer Inhibitors: An Updated Review. *Clin Pharmacokinet.* 2020; 59 (9), 1085-1107.
[10] Gallant JE, Thompson M, DeJesus E, et al. Antiviral Activity, Safety, and Pharmacokinetics of Bictegravir as 10-Day Monotherapy in HIV-1-Infected Adults. *J Acquir Immune Defic Syndr.* 2017; 75 (1), 61-66.
[11] Di Perri G, Calcagno A, Trentalange A, Bonora S. The clinical pharmacology of integrase inhibitors. *Expert Rev Clin Pharmacol.* 2019; 12 (1), 31-44.
[12] White KL, Osman N, Cuadra-Foy E, et al. Long dissociation of bictegravir from HIV-1 integrase-DNA complexes. *Antimicrob Agents Chemother.* 2021; 65 (5), 2406-2420.
[13] Molina JM, Cox SL. Emtricitabine: a novel nucleoside reverse transcriptase inhibitor. *Drugs Today* (Barc). 2005; 41 (4), 241-252.
[14] Rousseau FS, Wakeford C, Mommeja-Marin H, et al. Prospective Randomized Trial of Emtricitabine versus Lamivudine Short-Term Monotherapy in Human Immunodeficiency Virus-Infected Patients. *J. Infect Dis.* 2003; 188, 1652-1658.
[15] Yuen GJ, Lou Y, Bumgarner NF, et al. Equivalent steady-state pharmacokinetics of lamivudine in plasma and lamivudine triphosphate within cells following administration of lamivudine at 300 milligrams once daily and 150 milligrams twice daily. *Antimicrob Agents Chemother.* 2004; 48 (1). 176-182.
[16] Kearney B, Flaherty J, Shah J. Tenofovir disoproxil fumarate clinical pharmacology and pharmacokinetics. *Clin Pharmacokinet.* 2004; 43 (9), 595-612.
[17] Lee W, He G, Einsenberg E, et al. Selective intracellular activation of a novel prodrug of the HIV reverse transcriptase inhibitor tenofovir leads to preferential distribution and accumulation in lymphatic tissue. *Antimicrob Agents Chemother.* 2005; 49 (5), 1898-1906.
[18] Yager JL, Brooks KM, Castillo-Mancilla JR, et al. Tenofovir-diphosphate in peripheral blood mononuclear cells during low, medium and high adherence to emtricitabine/tenofovir alafenamide vs. emtricitabine/tenofovir disoproxil fumarate. *AIDS.* 2021; 35 (15), 2481-2487.
[19] Deeks, E.D. Bictegravir/Emtricitabine/Tenofovir Alafenamide: A Review in HIV-1 Infection. *Drugs.* 2018; 78, 1817-1828.

[20] Andreatta K, D'Antoni ML, Chang S, et al. High efficacy of bictegravir/ emtricitabine/ tenofovir alafenamide (B/F/TAF) in African American adults with HIV including those with preexisting resistance, viral blips, and suboptimal adherence. IDWeek, September 29-October 3, 2021. Abstract 629.
[21] Scherzer R, Estrella M, Li Y, Choi AI, et al. Association of tenofovir exposure with kidney disease risk in HIV infection. *AIDS.* 2012; 26 (7), 867-875.
[22] WING EJ. The Aging Population with HIV Infection. Trans Am Clin Climatol Assoc. 2017; 128, 131-144.
[23] Samuels R, Bayerri CR, Sayer JA, et al. Tenofovir disoproxil fumarate-associated renal tubular dysfunction: noninvasive assessment of mito- chondrial injury. *AIDS.* 2017; 31 (9), 1297-1301.
[24] Brown TT, Moser C, Currier JS, et al. Changes in Bone Mineral Density After Initiation of Antiretroviral Treatment With Tenofovir Disoproxil Fumarate/ Emtricitabine Plus Atazanavir/Ritonavir, Darunavir/ Ritonavir, or Raltegravir. *J Infect Dis.* 2015; 212 (8), 1241-1249.
[25] Calcagno A, Gonzalez de Requena D, Simiele M, et al. Tenofovir plasma concentrations according to companion drugs: a cross-sectional study of HIV-positive patients with normal renal function. *Antimicrob Agents Chemother.* 2013; 57 (4), 1840-1843.
[26] Calcagno A, Cusato J, Marinaro L, et al. Clinical pharmacology of tenofovir clearance: a pharmacokinet- ic/pharmacogenetic study on plasma and urines. *Pharmacogenomics J.* 2016; 16 (6), 514-518.
[27] Mayer KH, Molina JM, Thompson MA, et al. Emtricitabine and tenofovir alafenamide vs emtricitabine and tenofovir disoproxil fumarate for HIV pre-exposure prophylaxis (DISCOVER): primary results from a randomised, double-blind, multicentre, active-controlled, phase 3, non-inferiority trial. *Lancet.* 2020; 396, 239-254.
[28] Nicol MR, Pastick KA, Taylor J, et al. Cerebrospinal Fluid and Brain Tissue Penetration of Tenofovir, Lamivudine, and Efavirenz in Postmortem Tissues with Cryptococcal Meningitis. *Clin Transl Sci*. 2019; 12 (5), 445-449.
[29] Avihingsanon A, Lu H, Leong CL, et al. Week 48 of a phase 3 randomized controlled trial of bictegravir/emtricitabine/tenofovir alafenamide (B/F/TAF) vs dolutegravir + emtricitabine/ tenofovir disoproxil fumarate (DTG+F/TDF) as initial treatment in HIV/ HBV-coinfected adults [ALLIANCE). *AIDS.* 2022, July 29-August 2, Montreal. Abstract OALBX0105.
[30] European Centre for Disease Prevention and Control/WHO Regional Office for Europe. HIV/AIDS surveillance in Europe 2021-2020 data. Stockholm: ECDC; 2021.
[31] Dorrucci M, Regine V, Pezzotti P, et al. Demographic and socio-economic determinants of poor HIV-risk perception at first HIV diagnosis: analysis of the HIV Surveillance data, Italy 2010-2016. *Ann Ist Super Sanita.* 2020; 56 (3), 267-276.
[32] Baril JG, Angel JB, Gill MJ, et al. Dual Therapy Treatment Strategies for the Management of Patients Infected with HIV: A Systematic Review of Current Evidence in ARV-Naive or ARV-Experienced, Virologically Suppressed Patients. *PLoS One.* 2016; 11 (2), e0148231.