Lack of a metabolic and antiviral drug interaction between tenofovir, abacavir and lamivudine

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Objective: An anti-HIV regimen composed of the nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) tenofovir (TFV) disoproxil fumarate (TDF), abacavir (ABC) and lamivudine (3TC) has performed poorly in patients. This study evaluated the combination of TFV, ABC and 3TC for metabolic or antiviral antagonism in vitro.

Design: Procedures were developed to evaluate the in vitro metabolism and antiviral activity of drug combinations of TFV, ABC and 3TC in cell types relevant for HIV infection.

Methods: Anabolism of combinations of TFV and ABC were studied over a 24 h period in the human T leukaemic CEM lymphoblast cell line and human primary peripheral blood mononuclear cells (PBMCs) stimulated with human interleukin-2 and phytohaemagglutinin. The anti-HIV activity of combinations of TFV and ABC in the presence or absence of 3TC was studied in stimulated PBMCs infected with the HXB2 strain of HIV-1.

Results: Levels of the active metabolites produced from TFV and ABC after incubation with CEM or PBMCs showed no significant change upon introduction of the other NRTI. Moreover, the pool sizes for the natural substrates of 2′-deoxyadenosine triphosphate and 2′-deoxyguanosine triphosphate were also unchanged. In anti-HIV assays in PBMCs, the combination of TFV and ABC was found to be additive with respect to inhibition of HIV replication. Addition of 3TC to the combination did not result in synergistic or antagonistic effects.

Conclusions: The poor efficacy of the triple NRTI regimen of TDF, ABC and 3TC is probably not due to a metabolic drug interaction resulting in antagonism of antiviral activity.

Introduction

High rates of virological non-response and virological failure have been observed in several clinical trials assessing the ability of triple nucleoside reverse transcriptase inhibitor (NRTI) therapies to suppress HIV infection. Study ESS30009 was conducted in 194 treatment-naive patients and compared the efficacy of tenofovir (TFV) disoproxil fumarate (TDF)/abacavir (ABC)/lamivudine (3TC) to efavirenz/ABC/3TC, all in once-daily regimens. In an unplanned interim analysis, a 49% virological failure rate was observed at 12 weeks in the triple NRTI arm versus 5% in the efavirenz-containing arm [1]. Other studies with the same combination of TDF/ABC/3TC also found poor response and a high incidence of viral resistance mutations [2,3]. Since TFV and ABC have each been used successfully in regimens with 3TC, a problem between TDF and ABC has been surmised. These results have led to the proposal of different mechanisms for the poor efficacy of triple NRTI regimens containing TDF and ABC, including (i) intrinsic poor potency of all triple NRTI regimens, (ii) a low barrier for viral resistance to the combination and (iii) a pharmacological drug interaction between TDF and ABC. The lack of a systemic pharmacokinetic drug interaction between TDF and ABC in plasma has already been established [4].

The oral prodrug TDF is converted to TFV upon absorption. Within cells, both ABC and TFV require intracellular phosphorylation to their nucleoside triphosphate analogue forms in order to have antiviral activity. The intracellular anabolism of ABC to its active 2′-deoxyadenosine triphosphate (dATP) analogue, carbovir triphosphate (CBV-TP) is complex. ABC is first phosphorylated by adenosine monophosphate phosphotransferase to ABC monophosphate, which is then deaminated to carbovir monophosphate (CBV-MP) and then phosphorylated to CBV-TP via two cellular kinases (Figure 1A) [5]. TFV follows a different anabolic pathway to the active 2′-deoxyadenosine triphosphate (dATP) analogue TFV diphosphate (TFV-DP). The activation pathway involves the formation of a single key intermediate, TFV-MP, by the activity of adenylyl kinase 2 followed by further phosphorylation...
to the active metabolite TFV-DP (Figure 1B) [6]. TFV-DP and CBV-TP compete with the intracellular dATP and dGTP pools, respectively, for incorporation by the viral RT. Due to their lack of 3'-hydroxyl groups, incorporation of these nucleotide analogues causes chain termination of viral reverse transcripts and inhibition of viral replication.

In this article, we further examine the potential for a drug interaction between TFV and ABC by studying the in vitro intracellular pharmacology and anti-HIV activity of the two agents incubated either alone or in combination in cell types relevant for HIV infection.

Materials and methods

Chemicals

Cell culture supplies were purchased from Irvine Scientific (Santa Ana, CA, USA). TFV and TFV-DP as well as adefovir-DP (used as an internal standard) were synthesized by Gilead Sciences, Inc (Foster City, CA, USA). Stable isotope-labelled 13C15N dATP and dGTP were purchased from Spectra Stable Isotopes (Columbia, MD, USA). TFV-MP was synthesized by Dr Ivan Rosenberg of the Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic. ABC, 3TC and CBV-TP were provided by GlaxoSmithKline (Research Triangle Park, NC, USA). All other chemicals used were the highest grade available from Sigma-Aldrich, Inc (St Louis, MO, USA).

Cell culture

Human T leukaemic CCRF-CEM cells were obtained from the American Type Culture Collection (Manassas, VA, USA) and were maintained in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 units/ml penicillin G and 100 µg/ml streptomycin sulphate. Stanford Blood Center (Palo Alto, CA, USA) and the American Red Cross (Durham, NC, USA) supplied buffy coats prepared from the blood of healthy donors. Peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats by density gradient centrifugation on Ficoll-Paque Plus using standard techniques as recommended by the manufacturer (Amersham Pharmacia Biotech, Inc, Piscataway, NJ, USA). Two additional washes in RBC lysis buffer (eBioscience, San Diego, CA, USA) were performed for PBMC preparations used in assays for triphosphate evaluations. PBMC pellets were resuspended in 1640 RPMI medium containing 10% heat-inactivated FBS, 2 mM l-glutamine and penicillin/streptomycin. Cells were stimulated with 20 U/ml of human interleukin-2 (IL-2; Roche Diagnostics Corp, Indianapolis, IN, USA).
and 1–5 µg/ml of phytohaemagglutinin from Phaseolus vulgaris (PHA-P; Sigma-Aldrich, Inc) for 48–72 h at 37°C and subsequently cultured in the same medium without PHA-P.

Intracellular metabolism
TFV or ABC either alone or in combination at concentrations of 10–100 µM were added to CEM cells seeded at 0.2 million/ml or PBMCs seeded at 0.5 million/ml. At 2 and 24 h, aliquots of cells taken for analyses were separated from the media and extracellular drug by spinning through oil [7]. Isolated cells were resuspended in 70% methanol in order to extract intracellular metabolites. After drying by vacuum, samples were resuspended in an injection buffer containing 10 mM tetrabutyl ammonium hydroxide (TBAH) and 10 mM ammonium phosphate pH 7.0 at concentrations of 1–5 million cells per 20 µl, and used for analysis of intracellular anabolites.

Transient ion-pairing high performance liquid chromatography (HPLC) coupled to positive ion electrospray tandem mass spectrometry (LC/MS/MS) was used to analyse TFV and ABC in their phosphorylated forms for uptake and egress studies. Earlier methods for nucleoside triphosphate analysis using TBAH as an ion pair [8] were modified to include an acetonitrile step gradient in order to attain sufficient retention of TFV and TFV-MP on an Xterra MS, C18, 3.5 µm, 1.0x100 mm reverse phase column (Waters Corp, Milford, MA, USA). Mobile phases included 0.25 mM TBAH, 4 mM ammonium phosphate pH 6.5 and acetonitrile concentrations of 6 and 20%. A flow rate of 50 µl/min was maintained using HPLC with an Agilent 1100 chromatographic system (Agilent Technologies, Palo Alto, CA, USA). Parent/daughter mass transitions of 274.2/162.4, 354.0/162.4, 448.3/162.4, 488.0/135.3 and 434.0/162.4 m/z for TFV, TFV-MP, TFV-DP, CBV-TP and adeovir-DP (internal standard), respectively, were monitored on an API-4000 triple quadrupole mass spectrometer purchased from Applied Biosystems/MDS Sciex (Foster City, CA, USA). Typical lower limits of quantitation of 20–200 fmols on column (0.01–0.04 pmols/million cells in a 20 µl injection of lysate from 1–5 million cells) were achieved. Stable isotope methods for the measurement of intracellular dATP and dGTP will be described in detail elsewhere (manuscript in preparation). Briefly, standard curves in cellular matrix were performed utilizing stable isotope-labelled dATP and dGTP (mass transition of 506.7/145.6 and 522.6/161.8 m/z) while, concurrently, the amount of dATP and dGTP (mass transition of 492.1/136.2 and 507.7/151.6 m/z) in the cellular sample were monitored. The LC/MS/MS methods described above for TFV-DP and CBV-TP detection were used for these analyses.

Antiviral synergy
TFV and ABC were tested in combination for antiviral activity against the HXB2 laboratory strain of HIV-1 in PBMCs. Stimulated PBMCs were infected with virus at a multiplicity of infection of 0.001 for 4 h, washed once with complete media and plated in 96-well plates containing various concentrations of test compound. The final concentration of cells was 1 million PBMC per well. Compounds were plated in a checkerboard fashion with the starting concentration for each compound fixed at three- to fourfold above the EC₅₀ value for the specific compound. Subsequent twofold serial dilutions were then performed across the plate. Five plates for each drug combination were tested. The plates were incubated for 4 days at 37°C in 5% CO₂ and the level of virus replication monitored by evaluation of HIV-1 p24 antigen in the cell culture supernatant of each well (Coulter HIV-1 p24 antigen assay). Combinations of 3TC and 3TC were included as controls for additivity. Combinations of TFV with 3TC and ABC with 3TC were also evaluated as positive controls as these compounds have demonstrated good clinical activity when used in combination.

Results from the antiviral assays were analysed using the isobologram method [9] and the MacSynergy program [10] to determine potential synergistic and/or antagonistic activities. The isobologram evaluation is based on the Lowe additivity model. Dose response curves were generated for each drug alone and in combination and used to determine EC₅₀ values for each drug alone or in the presence of the fixed concentration of the second drug. Fractional inhibitory concentrations (FICs) were calculated by dividing the EC₅₀ of drug 1 with a fixed overlay of drug 2 by the EC₅₀ of drug 1 alone (the x coordinate). The y coordinate is the fixed concentration of drug 2 divided by the EC₅₀ of drug 2 alone. These points are plotted on a graph to generate the isobologram. On this same graph, a line representing additivity is included [coordinates (1,0) to (0,1)]. Data points that are above the additivity line represent antagonism between the compounds whereas data points below the additivity line represent synergy between the compounds. The intensity of the interaction can be quantitated and the statistical difference from dose-wise additivity tested by a 1-tailed t-test [11].

The MacSynergy program calculates a theoretical additive value for each drug combination based on the values generated by the drugs alone using an independent effects model (Bliss independence). The theoretical additive values are subtracted from the experimental values generated by each drug combination to give a value of synergy (positive value) or antagonism (negative value). These synergy and/or antagonism values are plotted on a three-dimensional graph with their
corresponding drug combinations. Areas of the graph below zero indicate antagonism whereas areas above zero indicate synergy. A synergy volume is calculated by adding all of the synergy values (positive values) for each drug combination. Likewise, all of the antagonistic values (negative values) are added to give an antagonistic volume. These synergy and antagonism volumes are then statistically evaluated using the 95% confidence level and are expressed in µM²%. Values between −25 µM²% and +25 µM²% are considered additive, values below −25 µM²% represent antagonism and values above 25 µM²% represent synergy.

**Results**

**Metabolism of TFV and ABC in CEM cells**

To address whether ABC and TFV interacted with one another’s phosphorylation, initial experiments were done examining the phosphorylation of TFV and ABC in the T-cell line CEM-CCRF. The cells were incubated with TFV (10 µM) or ABC (10 µM) and the level of phosphorylated anabolites formed were determined using LC/MS/MS analysis after 24 h of incubation. CEM cells accumulated levels of TFV-MP and TFV-DP of 0.53 and 1.49 pmol/million cells, respectively, after 24 h (Table 1). The addition of 10 µM ABC did not markedly affect the formation of TFV-DP (1.44 pmol/million cells). The conversion of ABC to CBV-TP was found to be less efficient than TFV, reaching levels of 0.12 pmols/million after 24 h. The anabolism of ABC at 24 h was unaffected by the presence of TFV (Table 1). To test if supra-pharmacological concentrations of the drugs could affect their metabolism, experiments were repeated in CEM cells incubated with 100 µM TFV and ABC. While levels of intracellular metabolites for both drugs were increased by approximately 10-fold, no difference in the accumulation of TFV or ABC anabolites were noted upon co-administration (data not shown). To verify that it is possible to elicit a nucleoside drug interaction at the level of phosphorylation in CEM-CCRF cells, studies were done with 3TC and zalcitabine (ddC) incubated at 10 µM. Consistent with previous reports [12], levels of ddC-TP were reduced fivefold (from 102 to 22.6 pmol/million cells) upon the addition of 10 µM 3TC to the incubation media (data not shown).

**Effect of TFV and ABC on 2′ deoxynucleotide pool sizes**

After phosphorylation to their respective triphosphate analogues, TFV and ABC compete with the dATP or dGTP deoxynucleotide pools for incorporation by HIV-1 reverse transcriptase. Alterations in the competing pool sizes could negatively affect the antiviral activity of TFV and ABC. The observation of the inhibition of an enzyme involved in the regulation of nucleotide pools (purine nucleoside phosphorylase) by phosphorylated metabolites of TFV [7] has led Kakuda and colleagues to hypothesis that TFV may alter nucleotide pools [13]. To test if TFV or ABC can alter dATP or dGTP pools, 10 µM incubations were carried out either alone or in combination with CEM-CCRF cells and levels of dATP and dGTP were determined after 24 h by LC/MS/MS. CEM cells were chosen for these studies because it has been shown that they are highly sensitive to pool size alterations by inhibitors of purine nucleoside phosphorylase [14]. Results in Table 1 show that neither ABC nor TFV, either alone or in combination, significantly reduced the competing deoxynucleotide pool sizes from the 42.7 ±10.7 and 23.7 ±10.9 pmols/million cell levels measured in untreated cells for dATP and dGTP, respectively.

**Metabolism of TFV and ABC in stimulated PBMCs**

We extended these combination studies of TFV and ABC in the cell type most physiologically relevant for HIV therapy, human PBMCs. PBMCs from two separate donors were stimulated with IL-2 and PHA. Twenty-four h incubations with 10 µM TFV and 30 µM ABC resulted in the intracellular activation of both analogue. The results summarized in Table 1 demonstrate that there was no difference in the levels of TFV-DP or CBV-TP formed after 24 h whether the drugs were incubated alone or together. Similar to

### Table 1. In vitro metabolism of 10 µM TFV and 30 µM ABC either alone or in combination and their effects on dATP and dGTP pool sizes

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Nucleotide</th>
<th>TFV alone</th>
<th>ABC alone</th>
<th>TFV + ABC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEM</td>
<td>TFV</td>
<td>1.42 ±0.41</td>
<td>1.59 ±0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TFV-MP</td>
<td>0.53 ±0.12</td>
<td>0.47 ±0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TFV-DP</td>
<td>1.49 ±0.07</td>
<td>1.44 ±0.20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CBV-Tp</td>
<td>0.12 ±0.07</td>
<td>0.18 ±0.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>dATP</td>
<td>40.3 ±7.6</td>
<td>40.3 ±4.8</td>
<td>41.7 ±12.2</td>
</tr>
<tr>
<td></td>
<td>dGTP</td>
<td>20.5 ±7.3</td>
<td>22.8 ±2.6</td>
<td>23.8 ±14.6</td>
</tr>
<tr>
<td>PBMC</td>
<td>TFV</td>
<td>1.11 ±0.47</td>
<td>0.81 ±0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TFV-MP</td>
<td>0.15 ±0.06</td>
<td>0.16 ±0.10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TFV-DP</td>
<td>0.21 ±0.06</td>
<td>0.22 ±0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CBV-Tp</td>
<td>0.11 ±0.03</td>
<td>0.11 ±0.01</td>
<td></td>
</tr>
</tbody>
</table>

*Concentrations expressed in pmol/10⁶ cells for all measurements after 24 h of drug incubation. Values for CEM cells are the mean ± SD of n=4 experiments. Values for stimulated human PBMCs are the mean ± SD of n=4 experiments done in cells from two separate donors. Intracellular concentrations of dATP and dGTP in untreated CEM cells were 42.7 ±10.7 and 23.7 ±10.9 pmols/million cells, respectively. dATP, 2′-deoxyadenosine triphosphate; dGTP, 2′-deoxycytidine triphosphate; PBMC, peripheral blood mononuclear cell; ABC, abacavir; CBV, carbovir; TFV, tenofovir; MP, monophosphate; DP, diphosphate; TP, triphosphate.
results obtained in CEM cells, quantitation of data for TFV and TFV-MP also showed that there were no significant differences beyond standard experimental error (Table 1).

Antiviral interaction between TFV and ABC in the presence or absence of 3TC
To expand upon these intracellular metabolism studies, TFV and ABC were analysed for synergistic or antagonistic anti-HIV activity in PBMCs infected with the HXB2 strain of HIV-1. Using MacSynergy analysis, the combination of TFV and ABC was found to be additive with regards to inhibition of HIV-1 replication (Table 2). The combination of TFV and 3TC and the combination of ABC and 3TC showed minor synergy with net synergy volumes >25 µM^2%. Triple combinations were performed by addition of 3TC at concentrations of 25 nM, 50 nM and 150 nM to graded concentrations of TFV and ABC. The addition of 3TC at any of these concentrations had no effect on the observed additive anti-HIV activity of TFV and ABC. Higher concentrations of 3TC in combination with TFV and ABC resulted in complete suppression of HIV growth and thus synergy calculations were not attainable (data not shown). As expected, the combination of 3TC and 3TC showed additive anti-HIV activity.

The combination anti-HIV assays were also analysed for synergy or antagonism by the isobologram method. A representative isobologram for the combination of ABC and TFV is shown in Figure 2. At each drug concentration, the fractional inhibitory concentrations were below the additivity line indicating a lack of antagonism and possible synergy. The other two-drug combinations and the three-drug combination showed similar results with negative mean deviations from additivity (Table 3). Statistical analysis indicated that deviation from additivity was significant only for the two-drug combination of TFV and 3TC. No combinations showed antagonistic anti-HIV activity.

Table 2. Summary of MacSynergy analysis

<table>
<thead>
<tr>
<th>Drug 1</th>
<th>Drug 2</th>
<th>3TC overlay</th>
<th>Synergy volume, µM^2%</th>
<th>Antagonism volume, µM^2%</th>
<th>Net effect*, µM^2%</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABC</td>
<td>3TC</td>
<td>None</td>
<td>45.1</td>
<td>-14.3</td>
<td>30.8 (minor synergy)</td>
</tr>
<tr>
<td>TFV</td>
<td>3TC</td>
<td>None</td>
<td>33.8</td>
<td>0</td>
<td>33.8 (minor synergy)</td>
</tr>
<tr>
<td>ABC</td>
<td>TFV</td>
<td>None</td>
<td>6.1</td>
<td>-20.6</td>
<td>-14.5 (additive)</td>
</tr>
<tr>
<td>ABC</td>
<td>TFV</td>
<td>25 nM</td>
<td>1.7</td>
<td>-0.5</td>
<td>1.2 (additive)</td>
</tr>
<tr>
<td>ABC</td>
<td>TFV</td>
<td>50 nM</td>
<td>2.8</td>
<td>-9.8</td>
<td>-7 (additive)</td>
</tr>
<tr>
<td>ABC</td>
<td>TFV</td>
<td>150 nM</td>
<td>10.3</td>
<td>0</td>
<td>10.3 (additive)</td>
</tr>
<tr>
<td>3TC</td>
<td>3TC</td>
<td>None</td>
<td>19.1</td>
<td>-1.8</td>
<td>17.3 (additive)</td>
</tr>
</tbody>
</table>

*Net effect calculated as the sum of synergy and antagonism volumes. Values below -25 µM^2% are antagonistic and values greater than 25 µM^2% are synergistic; values between -25 µM^2% and +25 µM^2% are additive. 3TC, lamivudine; ABC, abacavir; TFV, tenofovir.

Figure 2. Isobologram for the inhibition of HIV replication by the combination of ABC and TFV

Theoretical additivity is represented by the straight line which intercepts both FIC at axes at a value of 1. Error bars show the standard error of the two FICs plotted. FIC, fractional inhibitory concentrations; ABC, abacavir; TFV, tenofovir.

Table 3. Summary of isobologram synergy analysis

<table>
<thead>
<tr>
<th>Drug combination</th>
<th>Average deviation from additivity</th>
<th>P value</th>
<th>Net effect*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABC + TFV</td>
<td>-0.2137</td>
<td>0.057</td>
<td>Additive</td>
</tr>
<tr>
<td>TFV + 3TC</td>
<td>-0.2206</td>
<td>0.027</td>
<td>Synergistic</td>
</tr>
<tr>
<td>ABC + 3TC</td>
<td>-0.0417</td>
<td>0.323</td>
<td>Additive</td>
</tr>
<tr>
<td>ABC + TFV + 3TC</td>
<td>-0.0531</td>
<td>0.410</td>
<td>Additive</td>
</tr>
</tbody>
</table>

*Mean deviation from theoretical additivity line for all drug concentrations.
†Negative mean deviation indicates additivity. For P values <0.05, synergy is indicated. SEM, standard error of the mean; 3TC, lamivudine; ABC, abacavir; TFV, tenofovir.
Conclusions

Triple NRTI therapies have been clinically explored because they could theoretically have several advantages over non-NRTI (NNRTI)- and protease inhibitor (PI)-containing regimens. Triple NRTI treatments could allow for infrequent dosing with a small number of pills, avoidance of the unwanted side effects and drug interactions associated with the other classes of HIV therapy, and, in first-line treatment, leave options open for later administration of NNRTI- or PI-containing regimens. Unfortunately, the promising potential for triple NRTI therapies has not been realized and poor clinical outcomes have been observed in several studies [15].

The goal of the experiments described in this article was to determine if there is an intracellular drug interaction between ABC and TFV that could potentially explain the poor performance of triple NRTI therapies containing these two agents. In order to accomplish this, studies were conducted in cell types relevant for HIV infection. The results described in this article found no evidence for an intracellular drug interaction between TFV and ABC using both a direct intracellular drug metabolism analysis and antiviral drug combination analysis in PBMCs. The recently reported measurements of intracellular CBV-TP and TFV-DP in PBMCs from HIV-infected patients undergoing therapy with these two drugs in combination have led to similar conclusions [16]. These results are consistent with the distinct anabolic pathways presented in Figure 1 for these two NRTIs.

The result of these in vitro studies, as well as data reported elsewhere showing a lack of detectable intracellular interaction in vivo [16] and no systemic plasma drug interaction in vivo [4], suggest that the poor efficacy of triple NRTI combinations including TDF and ABC cannot be attributed to a drug–drug interaction between TDF and ABC. Furthermore, the results presented here show that neither ABC, TFV or their combination altered the intracellular pool sizes of dATP or dGTP, illustrating that perturbations in the natural dNTP pools is an unlikely explanation for the poor efficacy observed.

The poor performance of other triple NRTI regimens not including TDF and ABC together argues against a specific mechanism for suboptimal response. Combinations of didanosine/stavudine/ABC and TDF/3TC/didanosine have also performed poorly [17,18]. The early termination of the zidovudine/ABC/3TC arm in the ACTG 5095 study also raises concerns about this triple NRTI combination [19]. These results lend indirect evidence for the hypothesis that there is weaker potency in triple NRTI regimens, which can lead to the development of resistance and treatment failure in some patients. Further support for this hypothesis comes from the clinical observations that patients with high viral loads are more likely to fail on these regimens with a step-wise development of resistance [20]. Better understanding of the underlying mechanism responsible for the poor efficacy of these regimens may help guide the development of more effective combination regimens.

Acknowledgements

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Conflicts of interest

All authors are employees of Gilead Sciences, Inc, the marketer of tenofovir disoproxil fumarate (Viread).

References

9. Elion GB, Singer S & Hetchings GH. Antagonists of nucleic acid derivatives. VIII. Synergism in combinations of...


