COMMENTARY

Biowaiver Monographs for Immediate-Release Solid Oral Dosage Forms: Zidovudine (Azidothymidine)

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ABSTRACT: Literature data on the properties of zidovudine relevant to waiver of *in vivo* bioequivalence (BE) testing requirements for the approval of immediate-release (IR) solid oral dosage forms containing zidovudine alone or in combination with other active pharmaceutical ingredients (APIs) are reviewed. Solubility, dissolution, and permeability data for zidovudine, along with its dosing schedule, therapeutic index and pharmacokinetic properties, and reports related to BE/bioavailability were all taken into consideration. Data for solubility and permeability suggest that zidovudine belongs to Class I according to the Biopharmaceutics Classification System. Also, zidovudine is not a narrow therapeutic index drug. Although five out of 13 formulations tested *in vivo* (mostly of unreported composition) failed to show BE, it appears that *in vitro* studies performed according to biowaiver methods could predict *in vivo* behavior. Nevertheless, it is highly recommended that if a biowaiver is to be applied, excipient choices be limited to those found in IR drug products approved in International Conference on Harmonisation (ICH) or associated countries in the same dosage form (Table 2 of this monograph), in their usual amounts. These conclusions apply to products containing zidovudine as the only API and also to fixed combination products containing zidovudine with respect to the zidovudine component of the formulation. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:2409–2423, 2013

Keywords: zidovudine; biopharmaceutics classification system; absorption; solubility; permeability; bioavailability; bioequivalence; biowaiver; dissolution

INTRODUCTION

Zidovudine was the first drug with antiretroviral activity approved for marketing by the United States Food and Drug Administration (US FDA), having received approval in March of 1987. Zidovudine, also

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known as azidothymidine, is used for the treatment of human immunodeficiency virus (HIV).[1] It inhibits the HIV-1, HIV-2, and human T cells leukemia virus type 1, as well as other mammalian retroviruses.[2]

In this paper, a biowaiver monograph for zidovudine is presented. The risks of substituting *in vivo* with BCS-biowaiver-based bioequivalence (BE) testing for the approval of new multisource and/or reformulated immediate-release (IR) solid oral dosage forms containing zidovudine are evaluated under consideration of both the biopharmaceutical and clinical properties of zidovudine. Using the same approach as in previous biowaiver monographs (see www.fip.org/bcs[3] for examples), all relevant data available from the open literature for the given active pharmaceutical ingredient (API) are evaluated to assess the risk associated with biowaiving, with risk being defined as (a) the probability of arriving at an incorrect biowaiver decision, and (b) what impact an incorrect biowaiver decision would likely have on an individual patient and on public health. Bioequivalence Guidelines taken into consideration for the risk/benefit assessment include those from the World Health Organization (WHO),[4] US FDA,[5] and Europea Medicines Agency (EMA).[6] More than 25 biowaiver monographs have already been published and are also available online at http://www.fip.org/bcs_monographs.

METHODS

Information published in the open literature up to January 2013 was obtained from PubMed[7] and through the International Pharmaceutical Abstracts. Key words used were zidovudine, indication, solubility, polymorphism, partition coefficient, dose, permeability, bioavailability, BE, stereospecificity, absorption, distribution, metabolism, excretion, and dissolution. No other selection criteria were used.

GENERAL CHARACTERISTICS

Zidovudine is an inhibitor of the enzyme reverse transcriptase and therefore finds use as an antiretroviral agent. It is structurally similar to the endogenous compound thymidine, differing only by the presence of an azido group (N3) in the ribose ring of zidovudine instead of a hydroxyl group.[2,8] This drug is chemically defined as -[(2R,4S,5S)- 4-azido-5-(hydroxymethyl) oxolan-2-yl]-5-methyl-pyrimidine-2,4-dione, with the molecular formula $C_{10}H_{13}N_5O_4$ and a molecular weight of 267.25 g/mol. Chemically, the molecule is represented by an erythroisomeric moiety (3′-azido-3′-deoxythymidine) as shown in Figure 1.[9]

Zidovudine is a prodrug. To be converted into its active triphosphate form, zidovudine must be phosphorylated in three steps at the hydroxyl position (Fig. 2).

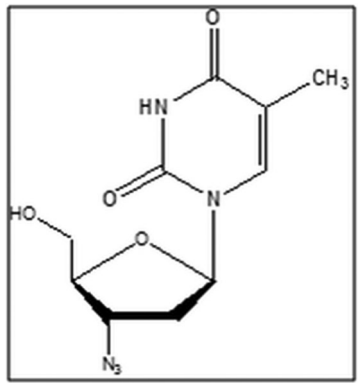

Figure 1. Structural formula of zidovudine.

The phosphorylation takes place in lymphocytes. The phosphorylated form, if administered, would have a lower bioavailability as this is a more polar molecule and would not cross membranes so easily.[10]

The active form has a high affinity for viral reverse transcriptase and competes with nucleotide triphosphates for incorporation into new viral DNA.[11] When zidovudine triphosphate is used as a substrate by retroviral DNA polymerase (reverse transcriptase), it is incorporated into the DNA chain. The substitution at position 3′ makes formation of the phosphodiester bond at the 5′ → 3′ position impossible. Zidovudine triphosphate thus acts as a chain terminator of DNA synthesis.[12]

Therapeutic Indications and Dose

Zidovudine inhibits the HIV-1, HIV-2, and human T cells leukemia virus type 1, as well as other mammalian retroviruses.[13] It is indicated for the initial treatment of HIV-infected adults with $CD4^+$ cell count less than 500 cells/mL. $CD4^+$ represents T lymphocytes that carry CD4 antigen (mostly helper cells) and is a crude indicator of immune status and susceptibility to certain AIDS-related conditions. For patients from 3 months to 12 years of age, it is currently recommended at a dosage of 180 mg/m^2 every 6 h, not exceeding 200 mg

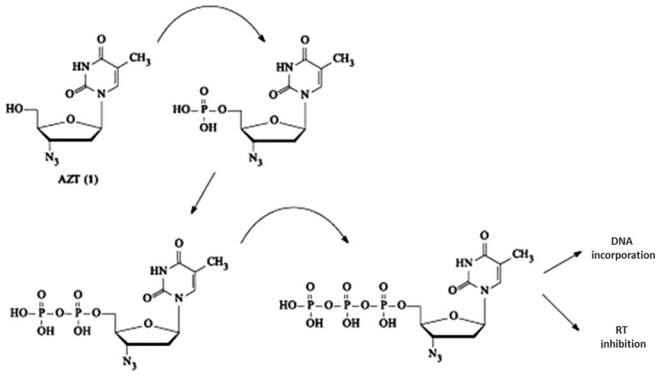

Figure 2. Intracellular phosphorylation of zidovudine.

every 6 h. It is also recommended for pregnant women infected with HIV and their newborns.[2] The neonatal dosage is 2 mg/kg orally every 6 h starting within 12 h after birth and continuing through 6 weeks of life.[14] Zidovudine is also used for opportunistic infections such as those caused by *Pneumocystis carinii* in preventive therapy for adult patients whose $CD4^+$ T cell counts fall below 200 cells/mL.

A number of studies have been performed to determine the optimal dosage regimen of zidovudine, and as a result, there has been a general trend to use lower doses for the treatment of HIV infection and the prophylaxis and treatment of AIDS. Doses studied in patients with AIDS have ranged from 400 to 1500 mg daily in a variety of divided-dose regimens.[15] These studies found that low-dose regimens were as effective as higher doses in terms of prolongation of survival and were better tolerated. Similar results were found in patients with AIDS-related complex (ARC)[16] and asymptomatic HIV infection,[17] and it was concluded that doses above 400–600 mg daily[18,19] offered no clinical advantage.

Antiretroviral therapy using zidovudine alone often leads to the selection of resistant strains. Dosing in combination with other antivirals, including fixed-dose combination (FDC) products, has proved to be more effective in suppressing viral replication. This is evidenced by studies comparing various treatments involving combinations or separate therapy with the following reverse transcriptase inhibitors: nevirapine, didanosine, zalcitabine, and zidovudine. These studies demonstrated that the use of triple therapy containing antiretroviral drugs resulted in a decrease in plasma levels of HIV RNA-1 to below the detection limit.[20–22] In combination with other antiretroviral agents, the daily oral dose of zidovudine is 600 mg divided in three doses of 200 mg or two doses of 300 mg in a day.[20]

Therapeutic Index and Toxicity

Zidovudine has demonstrated clinical efficacy, with reduced mortality, in patients with HIV. However, its clinical effectiveness is constrained because of the risk of adverse effects, especially during chronic therapy at high doses.

Included among the adverse effects commonly encountered with zidovudine therapy are hematological effects, such as anemia and neutropenia, hepatotoxicity, myopathy, and neurotoxicity.[23]

Haematotoxicity

In patients infected with HIV, zidovudine has been known to cause a severe hypoproliferative anemia that resolves promptly when the drug is stopped.[24] At the doses required for HIV treatment, zidovudine is toxic to myeloid and erythroid progenitor cells. The resulting suppression of red blood cells and circulating neutrophils can result in anemia and granulocytopenia. These side effects tend to appear at an advanced stage of HIV infection.[13] Anemia and granulocytopenia usually appear 4–6 weeks after beginning treatment in cases of advanced infection. The incidences of these effects are 2% and 37%, respectively, at a daily dose of 600 mg and 6% and 50%, respectively, at a daily dose of 1200 mg. Some of these effects are reversible after therapy interruption.[2] These are the most common serious toxicities associated with zidovudine therapy and may limit treatment in some patients with advanced disease.[25] As both are serious side effects, erythrocyte and neutrophil counts should be monitored.[26]

Myopathy and Hepatotoxicity

The exposure to zidovudine for a period of 2–12 months has been associated with skeletal muscle myopathy in up to 27% of patients and increased liver enzyme activity in about 7% of patients. Patients suffer, among other effects, from muscle weakness, decreased carnitine concentration, accumulation of lipid in muscle fibers, morphologically abnormal mitochondria, hepatomegaly with macrovesicular steatosis, and (a side effect that is frequently fatal) lactic acidosis.[27–29,30–34] A longer use of this drug (2 years or more) has been associated with cardiomyopathy[35]; 8% of patients had abnormal echocardiograms.[36] The myopathy and hepatotoxicity observed improved after the discontinuation of zidovudine.[27,31,36]

Adverse effects with zidovudine tend to be dose related and reversible. A study in patients with $CD4^+$ cell counts below 500 per milliliter was conducted to evaluate the safety and effectiveness of zidovudine. Patients who received the highest dose of the drug (1500 mg per day) had higher rates of anemia and neutropenia,[37] but severe hematologic toxic effects were not significantly different in patients receiving 500 mg per day than in those receiving a placebo.

A study with 365 patients that evaluated the activity and toxicity of oral zidovudine demonstrated that the benefits of zidovudine are limited to a few months for patients with ARC or AIDS. This study showed that after 6 months, the level of CD4 cell count, which increased in the first months, had returned to the pretreatment level. This was partly ascribed to the hematological toxicity of the drug, which led to treatment interruption in many patients.

Another study with 97 patients analyzed the long-term tolerance of zidovudine treatment. This study concluded that only a minority of patients with severe symptomatic HIV-1 related disease can tolerate full-dose zidovudine regimes for prolonged periods. Treatment with lower daily doses of zidovudine may lead to reduced toxicity, while maintaining efficacy.[38]

In more advanced disease, the risk of zidovudine toxicity is greater.[39] Although adverse effects are

uncommon in asymptomatic or nearly asymptomatic patients who have no immunological complication, especially those subjected to low daily doses, the incidence of adverse reactions appears to increase with disease progression, and patients should be monitored carefully.[40] For the most severely affected patients, reduced dosage of zidovudine may increase the therapeutic index.[41]

In addition to factors such as administered dose and stage of the disease, it is also apparent that individual patients exhibit substantial variability in their ability to tolerate chronic zidovudine treatment.[1] Because of the toxicities related to these and other antiretroviral therapy agents, patients in industrialized countries are routinely monitored for asymptomatic laboratory abnormalities.[42] Owing to its toxicity profile, zidovudine was listed in a 1992 draft report by Health Canada as a narrow therapeutic index (NTI) API.[43–45] However, that draft report has since been superseded by a 2006 Guidance from Health Canada,[46] in which zidovudine is no longer handled as a critical dose drug (a categorization that includes NTI drugs). This change may have been because of the further experience gained with zidovudine in the 14 years between the two reports, including the switch to lower doses and a combination strategy for dosing anti-HIV drugs. Indeed, the Prequalification Program of the WHO does not currently consider zidovudine to be a NTI drug.[47] Further, in the USA, zidovudine is not handled as an NTI compound and therapeutic drug monitoring is not routinely practiced.

CHEMICAL PROPERTIES

Salts, Esters, and Polymorphs

Zidovudine has a crystalline appearance, is colored white to yellow, and has a characteristic odor.[48] It may exhibit polymorphism,[49] but in the references available in the open literature, it is not clear which polymorphs are used therapeutically.

Solubility

Zidovudine has been reported to be sparingly soluble in water but readily soluble in ethanol.[49] However, it has also been reported that the solubility of zidovudine in the pH range from 1.2 to 6.8 at 37°C is about 20 mg/mL.[50] Further studies using zidovudine at the highest recommended single oral dose (300 mg) were performed using the shake flask method at 37°C. The volume needed to dissolve this amount of drug was 16.08, 12.9, 12.28, and 14.93 mL of buffer at pH 1.2, 4.5, 6.8, and 7.5, respectively (Table 1).[51]

Table 1. Solubility at 37°C of Zidovudine from Literature Data and the Corresponding Dose/Solubility (*D*/*S*) Ratio for *D* 300 mg*

pH	Solubility (mg/mL)	*D*/*S*[a] (mL)
1.2	18.65	16.1
4.5	23.25	12.9
6.8	24.43	12.3
7.5	20.10	14.9

*Highest tablet strength on the WHO List of Essential Medicines[54] (http://whqlibdoc.who.int/hq/2011/a95053_eng.pdf).

[a]Upper limit: 250 mL.

Partition Coefficient

The log *p* value for zidovudine has been reported as 0.05.[52] The clog *P* calculated by Kasim et al.[53] using clog *P* program (3.0 version) from BioByte Corporation (Claremont, California) is 0.04. No other data in the literature pertaining to zidovudine partition coefficient could be found.

p*K*a

As the conjugated base is a resonance hybrid, there is only one detectable p*K*a, at 9.8.[54]

Available Dosage Form Strengths

The oral product with the highest dosage strength according to the WHO Essential Medicines List is a 300 mg tablet.[4] Also listed on the 17th Essential Medicines List are capsules containing 100 or 250 mg and an oral liquid containing 50 mg/5 mL.[55] In the Brazilian market, the following zidovudine products are available: 100 mg hard gelatin capsules and 10 mg/mL oral solutions (syrup). In the USA, intravenous solutions containing 10 mg/mL are also approved for commercialization in addition to the tablets, capsules, and oral solutions.[4,56] In Europe, 100, 250, and 300 mg capsules and 300mg tablets have received marketing authorization.

PHARMACOKINETIC PROPERTIES

Absorption and Bioavailability

After oral administration, zidovudine is rapidly and almost completely absorbed from the gastrointestinal (GI) tract. The oral bioavailability of zidovudine is approximately 66%, although one study reported BA as high as 88%.[57] The incomplete bioavailability is attributable to first pass metabolism rather than incomplete absorption.[11,26,58–60]

Absorption is influenced by timing of the dose in relation to meal intake and composition of the meal, with an increase in time to maximum concentrarion (C_{max}) when administered postprandially.[51]

Permeability

Bidirectional transport studies of zidovudine using Madin Darby Canine Kidney Cells trasfected with

the human MDR1 gene (MDCK–MDR1) and Caco-2 cells were performed using radiolabeled digoxin as a reference compound.[61] The apparent permeability coefficient in apical (A) to basolateral (B) direction was 1.73×10^{-6} cm/s (MCDK) and 61.40×10^{-6} cm/s (Caco-2), which would indicate high permeability.[62] The B→A/A→B ratio was 5.79 in MDCK–MDR1 and 1.35 in Caco-2 cells, and slightly decreased to 4.25 and 1.09 when a P-glycoprotein (P-gp) inhibitor (GG918) was used. The authors concluded that zidovudine is a poor P-gp substrate because P-gp inhibitors do not significantly affect the permeability of the drug.[61]

Excised human jejunal mucosa, used to investigate the permeability of the small intestine, showed that steady-state fluxes across the intestinal mucosa for zidovudine were obtained after 3.1 h, a mean value that is higher than for the Class II drugs tested (didanosine—6.5 h and enalapril maleate—8.4 h), suggesting a high permeability across the small intestine. Although zidovudine has a slightly higher molecular weight (267.24 g/mol) than didanosine (236.23 g/mol), it showed a higher mean flux over the entire experiment. In these experiments, transport of zidovudine by the Na^+/nucleoside carrier was reported in addition to passive diffusion, and the high permeability of this drug through the intestinal membrane was attributed to a combination of uptake mechanisms.[63]

Studies investigating the site of absorption of zidovudine using four segments of the rat intestine and the *in situ* recirculating perfusion technique concluded that absorption was greater in the upper GI tract than in the lower portions.[64] It was hypothesized that this observation was because of the gradient of surface area for absorption, commensurate with a passive transport mechanism. The process followed first-order kinetics with peak plasma concentrations achieved within 0.5–1.5 h after oral administration.[64]

Distribution, Metabolism, and Elimination

The administration of a single oral dose of zidovudine results in a wide range of plasma concentrations in adults. There is variability in absorption, distribution, metabolism, and clearance of the drug among patients.[11] The percentage of HIV patients receiving daily doses of zidovudine 600, 1000, and 1500 mg that reach a steady state with plasma concentrations of more than 0.8 mg/L is 6%, 15%, and 32%, respectively. For individuals who receive daily doses of 600 and 1000 mg, about 38% and 18%, respectively, do not reach the effective plasma concentration, which is 0.2 mg/L. These studies demonstrate the high variability of zidovudine and the need to individually tailor anti-HIV therapy with this API.[65]

Zidovudine plasma levels decay in a biexponential manner, indicating two-compartment pharmacokinetics.[58]

Only about 25% of circulating zidovudine is bound to plasma proteins, primarily albumin. Tissue uptake is assumed to occur by passive diffusion. Pharmacokinetic studies using noncompartmental model analysis determined that the apparent volume of distribution is 121.2 L, and the mean volume of distribution is 1.3 L/kg.[66]

The elimination of zidovudine is dose dependent. At doses between 2 and 15 mg/kg, zidovudine exhibits linear pharmacokinetics. However, at high doses, saturation of the main route of elimination can occur and there is a subsequent decrease in the ratio of clearance to fraction absorbed (CL/F).[58,67,68] The plasma elimination of zidovudine is rapid, with elimination half-life ranging from 1 to 1.5 h. However, the depletion of intracellular zidovudine nucleotides is slower, resulting in the recommended dosage interval of every 8 h.[13]

Zidovudine undergoes presystemic elimination in the liver. The main metabolic pathway is glucuronidation by UGT2B7,[69,70] with formation of the metabolite 5′-O-glucuronide (GAZT), which is then excreted through the kidneys. This inactive metabolite (GAZT) is rapidly cleared from plasma with a half-life of 1 h. Some researchers have suggested that the large interindividual variability in the kinetics of zidovudine, as well as variability in the ability to convert zidovudine to its glucuronide, indicates that there are functional polymorphisms in UGT2B7 affecting zidovudine disposition.[69,71,72] Some drugs such as probenecid, naproxen, and fluconazole, which are occasionally coadministered to HIV patients, can interfere with glucuronidation and lead to increased activity and toxicity of zidovudine.[8]

On the basis of data from urinary recovery, the renal clearance of zidovudine is estimated to be approximately 350 mL/min/70 kg, indicating that both glomerular filtration and active secretion are involved in the renal elimination.[58] About 60%–70% of the drug is excreted in the urine as the inactive metabolite and about 1% is eliminated in the active form.[58] A third clearance mechanism is via a reduction reaction with formation of the 3′-amino-3′-deoxythymidine metabolite, which has potential cytotoxicity.[8] Other sources report that about 90% of the drug is recovered in the urine as zidovudine or GAZT.[58,73]

The antiviral effect of zidovudine is dependent not only on dose and rate of elimination but also on the rate and extent of intracellular phosphorylation. Zidovudine phosphorylation is a saturable process, and therefore, increases in plasma concentration do not result in parallel increases in the concentration of phosphorylated zidovudine.[74]

The pharmacokinetics of nucleoside reverse transcriptase inhibitors differ significantly among preterm infants, full-term infants, and older children.

The lower hepatic glucuronide formation capacity in neonates is responsible for the pharmacokinetic differences in zidovudine disposition when compared with older children. To avoid treatment failure, the pediatric dosage is based on age, weight, and/or body surface area. It is recognized that the diversity of pharmacokinetic and pharmacodynamic properties of antiretrovirals could influence choices of pharmacotherapy in children.

DOSAGE FORM PERFORMANCE

Excipients

Excipients present in the Brazilian reference product of IR dosage form of Zidovudine are: corn (maize) starch, magnesium stearate, microcrystalline cellulose, and sodium starch glycolate. Apart from the reference product, four other IR zidovudine products are registered in Brazil. Two of them have the same excipients as those of the reference product and the other two use talc instead of corn starch. The approval of these medicines was based on *in vitro* testing only, as it occurred at a time before BE studies became mandatory.

For the products listed in Table 2, it is reasonable to assume that the Marketing Authorization indicates that these formulations successfully passed an *in vivo* BE study and thus that the excipients do not have any substantial influence on the *in vivo* performance in the quantities used. Indeed, most of these excipients have no known interactions with permeability or motility. Although PEG 400, a macrogol, may influence the absorption of Class III drugs, as shown by Basit and coworkers[75,76] for ranitidine, the amounts used in those studies (0.5–10 g) were much higher than would commonly be the case in solid oral dosage forms, and it is also not yet clear whether Class I drugs are also susceptible to such effects.

Excipients and Manufacturing Effects on BA

Several reports in the literature have investigated BE among products containing zidovudine.

A BE and pharmacokinetic study was performed using the comparator Retrovir® (GlaxoSmithKline, Research Triangle Park, North Carolina) and the test formulation (FURP, São Paulo, Brazil).[77] Two 100 mg zidovudine capsules were compared using 24 healthy volunteers. The 90% of confidence intervals (CI) of the treatment ratios for the logarithmic transformed values of C_{max} and Area Under the Curve (AUC_{0-t})were 80%–113.6% [with a coefficient of variability (CV) of 44,43% for test drug and 40.64% for reference drug] and 93.9%–109.7% (with a CV of 27.53% for test drug and 23.93% for reference drug), respectively. The values for the test and reference formulations were within the BE definition intervals of 80%–125%.

Another comparative bioavailability study was performed between a generic product from Ranbaxy Laboratories Limited and Retrovir® from GlaxoSmithKline, with a total of 68 healthy adult volunteers receiving a 300 mg oral dose of zidovudine.[78] The 90% CIs obtained from 65 volunteers were 100.4%–107.3% for AUC_{0-t} and 91.6%–113.7% for C_{max}. Overall, with a sample size of 65 subjects, the study was powered to detect a 20% difference between test and reference formulations, considering an intraindividual CV% of approximately 50% for the C_{max} parameter of zidovudine, a power of 80%, and an α-level of 0.05.

In a further study, the BE of a test formulation, Antivir (Government Pharmaceutical Organization, Bangkok, Thailand) to the reference formulation, Retrovir® (GlaxoSmithKline), was evaluated.[79] Both formulations contained 100 mg zidovudine. The products were orally administered as a single dose of three capsules according to a randomized two-way crossover design to 28 healthy fasted Thai male volunteers. The comparative bioavailability was determined by the analysis of variance using logarithmic transformed data. The 90% CI for the difference of mean C_{max} was 90.76%–120.81%. The 90% CI for the difference of mean AUC_{0-t} was 91.83%–103.99%. Thus, BE was demonstrated between test and reference products.

By contrast, a study with Aspen zidovudine 300 mg versus Retrovir®, as the reference formulation, failed to demonstrate BE between the products. The excipients in Aspen formulation (colloidal silicon dioxide, magnesium stearate, microcrystalline cellulose, polyethylene glycol, polyvinyl alcohol, sodium starch glycollate, talc, and titanium dioxide) are well known and were used in their usual functions and at usual amounts. The study was performed in 26 volunteers. The 90% CI for the difference of mean AUC_{0-t} was 93.6%–108%, whereas for C_{max}, the 90% CI was 76.3%–121%. The results of the study show that present acceptance limits of 80%–125% were met only for AUC.[80]

In addition to studies reported for single-API products containing zidovudine, several studies to evaluate BE of generic and brand name FDCs of anti-HIV drugs containing zidovudine have also been performed and are summarized below. Of nine studies, four failed to meet the C_{max} criterion for BE, which in some cases could have been attributable to the high individual pharmacokinetic variability and lack of an adequate number of subjects to compensate for this variability.

A comparative study of products containing lamivudine and zidovudine in 24 healthy volunteers was performed.[81] The relative bioavailability of two Brazilian test formulations of lamivudine/zidovudine

Table 2. Excipients Used in Zidovudine Products with Marketing Authorizations (MA) in the USA, Canada, and European Countries

Excipient	Drug Products Containing that Excipient with a MA Granted by the Named Country	Range Present in Solid Oral Dosage Forms with a MA in the USA (mg)
Cellulose	BE[1,2] CA[3–5] CZ[6] DE[7,8] DK[9,10] ES[11–17] FI[18,19] FR[20] GR[21,22] HU[23] IE[24,25] NL[26,27] NO[28] PT[29,30] RO[31] SE[32,33] SK[34] UK[35,36] USA[37–53]	4.6–1385[a]
Hypromellose	ES[17] USA[38,40,45–53]	0.8–537
lactose	USA[45]	23–1020[a]
Macrogols (Polyethylenglycols)	ES[17] USA[38,40,45–48,50–53]	0.12–961[a]
Magnesium stearate	BE[1,2] CA[4,5] CZ[6] DE[7,8] DK[9,10] ES[11–17] FI[18,19] FR[20] GR[21,22] HU[23] IE[24,25] NL[26,27] NO[28] PT[29,30] RO[31] SE[32,33] SK[34] UK[35,36] USA[37–53]	0.15–401[a]
Polydextrose	USA[45]	3.8–8.1
Povidone	BE[2] DE[8] DK[10] ES[12,15] FI[19] FR[20] GR[22] IE[25] NL[27] NO[28] PT[29] SE[33]	0.17–80
Silica	CA[3] USA[43,45,49]	0.5–100
Sodium starch glycolate	BE[1,2] CA[4,5] CZ[6] DE[7,8] DK[9,10] ES[11-17] FI[18,19] FR[20] GR[21,22] HU[23] IE[24,25] NL[26,27] NO[28] PT[29,30] RO[31] SE[32,33] SK[34] UK[35,36] USA[37,53]	2–876[a]
Starch	BE[1] CA[3-5] CZ[6] DE[7] DK[9] ES[11,14] FI[18] GR[21] HU[23] IE[24] NL[26] PT[30] RO[31] SE[32] SK[34] UK[35] USA[37,39,43,49]	0.44–1135[a]
Starch, pregelatinised	ES[13,16] UK[36] USA[41,42]	5.0–600
Stearic acid	CA[3]	0.9–72[a]
Talc	USA[43,49]	0.1–220[a]
Triacetin	USA[45]	0.72–15

Table 2 shows the excipients[b] used in IR zidovudine solid oral drug products, with a Marketing Authorization (MA) in Belgium (BE), Canada (CA), Czech Republic (CZ), Germany (DE), Denmark (DK), Spain (ES), Finland (FI), France (FR), Greece (GR), Hungary (HU), Ireland (IE), The Netherlands (NL), Norway (NO), Portugal (PT), Romania (RO), Sweden (SE), Slovakia (SK), United Kingdom (UK), and the United States (USA)[c], and the minimal and maximal amount of that excipient present pro dosage unit in solid oral drug products with a MA in the USA[d].

[a] The upper range value reported seems unusually high for solid oral dosage forms and the authors question its validity.

[b] Colorants, water, and ingredients present in the coating and/or the printing ink are not included.

[c] Sources of data: BE, www.bcfi.be/ (accessed January 31, 2012); CA, www.hc-sc.gc.ca (accessed January 31, 2012); CZ, www.sukl.cz/ (accessed January 31, 2012); DE, www.rote-liste.de; (accessed January 31, 2012); DK, www.dkma.dk (accessed January 31, 2012); ES, www.aemps.es (accessed January 31, 2012); EU, www.ema.europa.eu (accessed January 30, 2012); FI, www.fimea.fi (accessed January 31, 2012); FR, www.vidal.fr/; (accessed January 31, 2012); GR, www.eof.gr (accessed January 31, 2012); HU, www.ogyi.hu (accessed January 31, 2012); IE, www.imb.ie/ (accessed February 6, 2012); NL, www.cbg-meb.nl/cbg/nl (accessed January 31, 2012); NO, www.legemiddelverket.no/ (accessed February 01, 2012); PT, http://www.infarmed.pt/ (accessed January 31, 2012); RO, www.anm.ro/ (accessed January 31, 2012); SE, www.lakemedelsverket.se (accessed February 01, 2012); SK, www.sukl.sk (accessed February 01, 2012); UK, www.medicines.org.uk/emc/ (accessed February 01, 2012); US, http://dailymed.nlm.nih.gov/ (accessed February 01, 2012).

[d] US: FDA's Inactive Ingredient Database, http://www.fda.gov/Drugs/InformationOnDrugs/ucm113978.htm (version date Dec. 31, 2011)

1. RETROVIR 100 mg capsules.
2. RETROVIR 300 mg filmomhulde tabletten.
3. APO-ZIDOVUDINE Zidovudine Capsules 100 mg.
4. NOVO-AZT (Zidovudine) 100 mg Capsules.
5. PrRETROVIR® (AZT™) Zidovudine Capsules USP, 100 mg.
6. Retrovir 250.
7. Retrovir® 100 mg/250 mg Hartkapseln.
8. Retrovir® 300 mg Filmtabletten.
9. Retrovir, hårde kapsler.
10. Retrovir, filmovertrukne tabletter.
11. Retrovir 250 mg cápsulas duras.
12. Retrovir 300 mg comprimidos recubiertos con película.
13. ZIDOVUDINA AUROBINDO 100/250 mg cápsulas duras EFG.
14. ZIDOVUDINA COMBINO PHARM 100/250/300 mg cápsulas EFG.
15. ZIDOVUDINA COMBINO PHARM 300 mg Comprimidos EFG.
16. ZIDOVUDINA G.E.S. 100/250/300 mg Cápsulas duras EFG.
17. ZIDOVUDINA IDIFARMA 300 mg Comprimidos recubiertos con película EFG.
18. RETROVIR 100/ 250 mg kapseli, kova.
19. RETROVIR 300 mg kalvopäällysteinen tabletti.
20. RETROVIR 100/-250 mg gél.
21. RETROVIR 300 mg cp pellic.
22. Retrovir καψάκιασκληρά 100/250 mg (Retrovir 100/250 mg hard capsules).
23. Retrovir επικαλυμμένα με λεπτό υμένιο δισκία 300 mg (Retrovir film-coated tablets 300 mg).
24. Retrovir 250 mg kemény kapszula.
25. Retrovir 100 mg capsules, hard.
26. Retrovir 300 mg film-coated tablets.
27. Retrovir 100 mg capsules, hard.
28. Retrovir 300 mg filmomhulde tabletten.
29. Retrovir 300 mg tablett, filmdrasjert.
30. Retrovir 300 mg comprimidos revestidos por película.
31. Retrovir 100/250 mg cápsulas.
32. Retrovir 100 mg, capsule.
33. Retrovir 100/250 mg, kapslar, hårda.
34. Retrovir 300 mg, filmdragerade tabletter.
35. Retrovir 100/250 mg.
36. Retrovir 100/250 mg capsules, hard.

Continued

Table 2. Footnote Continued.

37. Zidovudine 100/250 mg capsules, hard.
38. RETROVIR (zidovudine) capsule [GlaxoSmithKline LLC].
39. RETROVIR (zidovudine) tablet, film coated [GlaxoSmithKline LLC].
40. RETROVIR (zidovudine) capsule [ViiV Healthcare Company].
41. RETROVIR (zidovudine) tablet, film coated [ViiV Healthcare Company].
42. ZIDOVUDINE capsule [American Health Packaging].
43. ZIDOVUDINE capsule [Aurobindo Pharma Limited].
44. ZIDOVUDINE capsule [Cipla Limited].
45. ZIDOVUDINE tablet [Camber Pharmaceuticals].
46. ZIDOVUDINE tablet [Roxane Laboratories, Inc].
47. ZIDOVUDINE tablet, film coated [American Health Packaging].
48. ZIDOVUDINE tablet, film coated [Aurobindo Pharma Limited].
49. ZIDOVUDINE tablet, film coated [Bryant Ranch Prepack].
50. ZIDOVUDINE tablet, film coated [Cipla Limited].
51. ZIDOVUDINE tablet, film coated [Greenstone LLC].
52. ZIDOVUDINE tablet, film coated [Mylan Pharmaceuticals Inc.].
53. ZIDOVUDINE tablet, film coated [Rebel Distributors Corp].
54. ZIDOVUDINE tablet, film coated [State of Florida DOH Central Pharmacy].

tablets in comparison to the reference product Biovir® (Glaxo-Wellcome, Rio de Janeiro, Brazil) was calculated. On the basis of statistical results, the authors concluded that test and reference products met requirements for BE with respect to AUC_{0-t} and C_{max} for lamivudine and with respect to AUC_{0-t} for zidovudine.[82,83] The 90% CI of C_{max} calculated for zidovudine for one test formulation was 84%–116% and for the other was 76%–124%. In all cases, products were bioequivalent in rate and extent of absorption for lamivudine and in extent of absorption for zidovudine.

An open, randomized, two-period crossover study used 18 Rwandan healthy volunteers to evaluate BE between tablets of a novel FDC (300 mg zidovudine/160 mg lamivudine) and Duovir® (300 mg zidovudine/150 mg lamivudine; Cipla, Mumbai, India).[84] The test formulation composition per tablet was 300 mg zidovudine, 160 mg lamivudine, 470 mg Avicel® PH 102 g, 40 mg Explotab®, 5 mg Aerosil®, and 5 mg magnesium stearate. Statistical analysis of C_{max} and AUC parameters [parametric t-test ($p < 0.05$)] showed that they were within the 95% CI, complying with the limits 80%–125%.

In a further study, the BE of a combination product containing abacavir 300 mg, lamuvidine 150 mg, and zidovudine 300 mg (A/L/Z) with the reference products of the single-API components administered together was determined. This was a single-center, open-label, three-way crossover study in 24 healthy subjects. In the course of the study, the effect of food on the bioavailability of the drugs from the combination tablet was also determined.[85] The combined A/L/Z tablet was found to be bioequivalent with respect to both the extent (AUC) and rate of absorption (C_{max} and T_{max}) to the individual reference (brand name) drug components. The geometric least squares (LS) ratios and 90% CI for log-transformed $AUC_{0-\infty}$ and C_{max} under fasted conditions were 0.99 (0.96, 1.03) and 1.00 (0.90, 1.11), respectively, for abacavir; 0.95 (0.91, 0.99) and 0.90 (0.84, 0.99), respectively, for lamivudine; and 0.95 (0.89, 1.02) and 0.96 (0.80, 1.15), respectively, for zidovudine. When the combination product was administered with a meal, there was no change in the extent of absorption of abacavir, lamivudine, or zidovudine. However, food slowed the rate of absorption, delayed the T_{max}, and reduced the C_{max} of all three APIs.

Similarly, a crossover study in 24 healthy subjects was conducted to assess the BE of a tablet combining lamivudine 150 mg with zidovudine 300 mg with the separate reference (brand name product) components administered concurrently. Also, in this study, the effect of food on the bioavailability of the drugs from the combination tablet was assessed.[86] The 90% CI for the ratio of LS means for the lamivudine and zidovudine $AUC_{0-\infty}$ and C_{max} fell entirely within the range 0.80–1.25 for log-transformed parameters under fasted conditions.

The pharmacokinetics of 150 mg lamivudine, 300 mg zidovudine, and 200 mg nevirapine were assessed following single oral administration of a FDC tablet versus simultaneous administration of the separate innovator products in healthy male subjects ($n = 64$) under fasting conditions in an open-label, randomized, two-way crossover study.[87] The ratio of the LS means (FDC to individual products) and 90% CI of AUC_{0-t} and C_{max} for lamivudine, zidovudine, and nevirapine were all within 80.0%–125.0%.

Another open-label, two-way crossover study was conducted in 24 healthy subjects to assess the BE between a combined lamivudine/zidovudine test tablet (FURP) and the reference product Biovir® (GlaxoSmithKline, Rio de Janeiro, Brazil).[88] After overnight fasting, the subjects were given one single dose of each formulation in a randomized design. For zidovudine, the geometric mean ratio and 90% CI for AUC_{0-t} was 109% and 97%–120%, respectively. For C_{max}, the values were 116% and 90%–141%, exceeding the 90% CI limit of 80–125. The standard deviation from

zidovudine was higher than 30%, suggesting a high variability of absorption.

A further randomized, two-way crossover study enrolled 12 male subjects who received 150/300 mg lamivudine/zidovudine innovator or test tablets.[89] Geometric LS mean ratios and 90% CI of test with respect to the reference product for C_{max} and AUC_{0-t} for zidovudine were 78.0% (63.4–95.8) and 88.3% (68.0–114.8), respectively. Hence, the results did not fall within the specified 80%–125% interval needed to exhibit average BE between the products. The authors suggested that the result could be attributed to the limited individual sample size used in the BE study.

A randomized, crossover BE study of generic and brand name FDC tablets of nevirapine, zidovudine, and lamivudine was conducted in 15 HIV-negative Indian women.[90] The women were administered single doses of all formulations, with each phase separated by a 14-day washout period. Average BE was determined using natural log-transformed C_{max} and AUC mean ratio data. The 90% CI for nevirapine (14 subjects) and lamivudine (15 subjects) for both C_{max} and AUC_{0-t} mean ratios and the 90% CI for zidovudine (15 subjects) AUC_{0-t} mean ratio were all within the range of 0.80–1.25. However, the 90% CI for the zidovudine C_{max} mean ratio was 0.70–1.46.

A comparative study of oral solutions of zidovudine, lamivudine, and abacavir versus tablets of Combivir (zidovudine and lamivudine) and abacavir was conducted in 19 HIV-1-infected Ugandan children. Dose-normalized AUC and C_{max} of the tablet formulation were bioequivalent to those of the oral solution with respect to zidovudine and abacavir. All the formulations tested were made by the pharmaceutical company holding marketing authorization (GlaxoSmithKline).[91]

A randomized, single-dose, open-label, two-way crossover design under fasting conditions used 24 healthy adults to compare the bioavailability of FDC granules for reconstitution comprising lamivudine/zidovudine/nevirapine 30/60/50 mg per 5 mL to the bioavailabilities of the single entities of the reference products when administered simultaneously. The clinical batch of the test product was manufactured by Emerson Resources Inc. (Norristown, Pennsylvania) Xylitol, sucralose, microcrystalline cellulose, carboxymethylcellulose sodium, colloidal silicon dioxide, methylparaben, and propylparaben were used as excipients. The 90% CI for the geometric mean ratio of test/reference for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ for lamivudine, zidovudine, and nevirapine were within the 80%–125% BE limits.[92]

Dissolution

The United States Pharmacopeia advocates the use of the apparatus II (paddle), with 900 mL degassed water as the dissolution medium, at a speed of 50 rpm, for 30 min in the case of zidovudine tablets and 45 min for zidovudine capsules. The percentage of drug dissolution specified within this time frame is 80% and 75%, respectively.

The dissolution properties of novel FDC tablets and commercially available zidovudine/lamivudine tablets (Duovir®, Cipla) ($n = 6$) were determined according to USP 27 using a Vankel dissolution tester, type VK7000 (Edison City, New Jersey) at a paddle speed of 50 rpm and distilled water at 37°C as dissolution medium. The composition of the test tablets was 300 mg zidovudine, 160 mg lamivudine, 470 mg Avicel® PH 102, 40 mg Explotab®, 5 mg Aerosil®, and 5 mg magnesium stearate. Both products dissolved greater than 85% in 15 min. *In vivo* studies demonstrated BE of the products.[84]

Dissolution profiles of test and innovator lamivudine/zidovudine tablets were compared using the dissolution equipment (Vankel Industries, Inc., Cary, North Carolina) and the USP method described above at 37°C. No information regarding the formulation composition was provided by the authors. The percentage of zidovudine dissolved in 30 min was greater than 80% for generic and innovator and the f2 similarity factor of the profiles was 53.21, even though the products were not found to be bioequivalent, with a point estimate for C_{max} of 91.00% (90% CI 73.5–112.6) and for AUC_{0-t} of 85% (90% CI 68.5–105.6).[89] Note that the quality control test of the USP does not correspond to the dissolution test conditions required for a biowaiver assessment. The test conditions for a biowaiver require dissolution in at least three different media, including 0.1 N HCl and phosphate buffers at pH 4.5 and 6.8.

A comparative study of dissolution profiles of three brands of lamivudine and zidovudine combinations was performed.[93] Samples from one batch of each of the following products were tested: Virex-LZ® (a registered trade name of Fidson Healthcare, a Nigerian pharmaceutical company), Combivir® (manufactured by GlaxoSmithKline of India and marketed by GlaxoSmithKline, Nigeria), and Lazid® (a product name registered by Emcure of India and marketed by FIL Pharmaceuticals, Lagos, Nigeria). No information regarding the composition was provided by the authors. The three different dissolution media employed were 0.1 N HCl and phosphate buffers at pH 4.5 and 6.8. Dissolution of tablets was carried out in six vessels, each containing 900 mL of dissolution media, at 50 ± 1 rpm. The similarity of dissolution profiles was determined using the f2 factor. The values of f2 calculated between Combivir® and Virex-LZ® or between Combivir® and Lazid® in pH 4.5 phosphate buffer were 47.9 and 48, respectively, indicating a lack of similarity of these products. Although the three products have the same composition and

the same label strength, Virex-LZ® and Lazid® differ from Combivir® in their dissolution characteristics. Virex-LZ® and Lazid® reached more than 85% dissolved in 15 min in both pH 4.5 and 6.8 phosphate buffers, and can be classified as very rapidly dissolving products. Therefore, these two products can be considered to have similar dissolution characteristics. The Combivir® did not meet all criteria for a rapidly dissolving product. No PK data were generated for these products.

Results from other dissolution studies performed using Biovir® (coated tablet containing 150 mg of lamivudine and 300 mg of zidovudine) and a Brazilian generic candidate showed that the test and reference products released the drug similarly in pH 6.8 phosphate buffer (more than 85% of the drug was dissolved in 15 min for both products), but not in water or hydrochloric acid, wherein more than 85% of the drug was dissolved in 15 min for the test product and in 30 min for the reference.[94] The *in vitro* test conditions were 50 rpm per paddle per 900 mL. No information regarding the formulation composition was provided by the authors. In this study, an *in vivo* comparative study was conducted in 24 healthy volunteers. Pharmacokinetic parameters estimates were analyzed following log transformation. Geometric LS mean ratios and 90% CI for AUC_{0-t} and C_{max} for zidovudine were 109% (97%–120%) and 116% (90%–141%), respectively. In this case, *in vitro* tests corresponding to the biowaiver dissolution tests were able to predict the bioinequivalence result.[88]

Tablets of 300 mg zidovudine purchased from Indian market were submitted to dissolution testing.[50] No information regarding the composition was provided by the authors. The drug release rate was characterized using USP apparatus II at 50 rpm, 900 mL of dissolution medium (water, 0.01 N HCl, USP acetate buffer pH 4.5 and USP phosphate buffer pH 6.8) at 37°C. All tablets dissolved more than 85% of the drug in 15 min. No *in vivo* studies were performed.

A dissolution test was carried out with Zidovir (marketed tablet from Cipla) and two other zidovudine formulations, F1 [with 8.5% Hydroxypropylmethylcellulose (HPMC)] and F2 (with 8.5% acacia).[95] Other ingredients in F1 and F2 were gelatin, magnesium stearate, and talc. Six tablets from each formulation were tested in 900 mL 0.1 N HCl using USP II dissolution test apparatus at 100 rpm. Zidovir showed a higher rate of drug release, 97.8% in 30 min. Tablets containing HPMC and acacia showed 26.1% and 76.5% release in 30 min, respectively. The authors concluded that the slow *in vitro* dissolution of formulation F1 might be because of the strong, rigid complex formed by HPMC with drug. No *in vivo* studies were performed.

Another study compared *in vitro* dissolution characteristics (in simulated gastric fluid, acetate buffer pH 4.5 USP, and simulated intestinal fluid prepared according to the USP) and other quality measures of different zidovudine products purchased in the United States with a comparator pharmaceutical product (CPP). All investigated zidovudine products were found to be equivalent to the CPP *in vitro*, as all investigated products showed greater than 85% dissolution within 15 min.[96]

DISCUSSION

Solubility

Solubility data show that the volume of aqueous media necessary to dissolve 300 mg of zidovudine over the pH range of 1.2–6.8 at 37°C is less than the limit of 250 mL. Thus, zidovudine can be classified as a highly soluble drug.

Permeability

Although most studies reported BA of zidovudine as less than 85%,[11,26,58–60] urine recovery of 90% indicates that the low BA is a consequence of first-pass metabolism rather than incomplete absorption.[58,73]

Experiments in Caco-2 cells, rat perfusions, and excised human jejunal mucosa provide additional supporting evidence for a high permeability.[61–64]

BCS Classification

Zidovudine has been classified by Kasim et al. as BCS Class III.[53] However, the definition of permeability was based on clog *P* data, which does not take active transport into consideration. A more recent review assigns zidovudine as BCS Class I, based on absolute BA studies.[97] The balance of evidence in the literature, as presented in this monograph, indicates that zidovudine exhibits high solubility and high permeability and, thus, can be classified as BCS Class I.

Risk of Bioinequivalence Caused by Excipient and/or Manufacturing

Among the 13 BE studies found in the literature, eight formulations were shown to be bioequivalent to the comparator, but five did not meet the regulatory requirements for C_{max} and/or AUC. The formulation composition was revealed in only two studies, in which the products tested were bioequivalent. Some authors blame the high within-individual variability of the drug for the lack of BE with respect to C_{max}.[81,88] Indeed, some references point to zidovudine as a highly variable (interindividual) drug.[86–88] Furthermore, in the studies that failed to show BE, numbers of subjects were typically lower than in studies that were able to demonstrate BE.

Nevertheless, in should be pointed out that to conclude that a drug is highly variable (within-individual

variability of 30% or more), it is not sufficient to merely compare different formulations.[98] More convincing evidence would be provided if the same formulation was to be given to the same subjects in different periods: this would enable calculation of the intraindividual variability. Indeed, it has been suggested by Davit et al.[99] that for 50% of inconsistently highly variable drugs (drugs that were highly variable in some drug product BE studies but not in others), formulation effects could contribute to the high variability.

Taking into account that all products listed in Table 2 have complied with the BE criteria, one can conclude that these excipients in usual amounts do not affect BA of zidovudine. In view of the fact that other formulations (mostly of unreported composition) have failed to show BE, it is highly recommended that excipient choices be limited to those found in Table 2, and that the excipients chosen be used in their usual amounts, if a biowaiver is to be applied.

Risk of Not Detecting Bioinequivalence by *In Vitro* Testing

Several studies were found in the literature addressing dissolution testing of oral IR zidovudine solid dosage forms. Of these, only three of the studies compared test and comparator formulations *in vivo* as well as *in vitro*.[84,88,89,94] In one study, the dissolution rates of both test and reference products in water were very rapid (more than 85% of the drug dissolved in 15 min), indicating pharmaceutical equivalence (in terms of the release characteristics) between them.[84] In another study, even though the products were not bioequivalent, the f2 similarity factor of the dissolution profiles in water was higher than 50.[89] In this case, the result of the compendial *in vitro* test would lead to a wrong decision. However, the compendial test does not comply with the tests required in the biowaiver procedure, so the reliability of the biowaiver methods for predicting lack of equivalence *in vivo* cannot be assessed from this study. In the third study, the *in vivo* tests confirmed the bioinequivalence result predicted from *in vitro* tests in water and 0.01 N HCl.[88,94]

In summary, data are too scanty to verify that the BCS biowaiver dissolution tests can accurately forecast the result of a pharmacokinetic proof of BE. However, very rapid dissolution or rapid dissolution in an acidic medium (i.e., tests more aligned with the biowaiver methods) appears to be more reliable than the compendial method for this purpose.

Patient Risks Associated with Bioinequivalence

The evaluation of $CD4^+$ cell count and viral load should occur before the start and after the first and third months of treatment. Monitoring should continue every 4 months as long as treatment success. The pharmacological ramifications of incorrect dosing or lack of BE are (a) unexpected toxicity, if the test formulation is more bioavailable than the reference product, which could lead to effects such as anemia and neutropenia, hepatotoxicity, myopathy, and neurotoxicity; or (b) subtherapeutic concentrations, if the bioavailability of the test product is lower than its comparator, which would be associated with an increase in viral load and more rapid disease progression.[100–102]

CONCLUSIONS

According to the current BCS criteria, zidovudine is a Class I drug. Although this is one of the requirements to waive an *in vivo* BE study, other aspects must also be considered, such as therapeutic index, clinical relevance, and therapeutic use. Although at one time zidovudine was considered to be an NTI drug in certain jurisdictions, this is no longer the case. Taking into account the clinical relevance and severe consequences of a bioinequivalent product containing this API, it is highly recommended, for a biowaiver to be applied, that excipient choices be limited to those found in Table 2, and that the excipients chosen be used in their usual amounts. Careful selection of excipients is consistent with current WHO recommendations and is crucial, as several formulations (mostly of unspecified composition) have failed to show BE. With respect to the *in vitro* dissolution studies, it should be demonstrated that the test product and its comparator (a) are both "rapidly dissolving" and meet similarity of the dissolution profiles at pH 1.2, 4.5, and 6.8 or (b) are both "very rapidly dissolving" at the same pH values.[4,6]

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