Development of an Optimized Dose for Co-formulation of Zidovudine with Drugs that Select for the K65R Mutation Using a Population Pharmacokinetic and Enzyme Kinetic Simulation Model

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\textbf{Running title:} Optimum ZDV dose for co-formulation with K65R selecting NRTI

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Abstract.

*In vitro* selection studies and data from large genotype databases from clinical studies have demonstrated that tenofovir disoproxil fumarate and abacavir sulfate select for the K65R mutation in the HIV-1 polymerase region. Furthermore, other novel non-thymine nucleoside reverse transcriptase (RT) inhibitors also select for this mutation *in vitro*. Studies performed *in vitro* and in humans suggest that viruses containing the K65R mutation remained susceptible to zidovudine (ZDV) and other thymine nucleoside antiretroviral agents. Therefore, ZDV could be co-formulated with these agents as a “resistance repellent” agent for the K65R mutation. The approved ZDV oral dose is 300 mg bid and is commonly associated with bone marrow toxicity thought to be secondary to zidovudine-5’-monophosphate (ZDV-MP) accumulation. A simulation study was performed *in silico* to optimize the ZDV dose for bid administration with K65R selecting antiretroviral agents in virtual subjects using population pharmacokinetic and cellular enzyme kinetic parameters of ZDV. These simulations predicted that a reduction in ZDV dose from 300 to 200 mg bid should produce similar amounts of zidovudine-5’-triphosphate (ZDV-TP) associated with antiviral efficacy (> 97% overlap), and reduced plasma ZDV and cellular amounts of ZDV-MP associated with toxicity. The simulations also predicted reduced peak and trough amounts of cellular ZDV-TP after treatment with ZDV 600 mg qd than 300 or 200 mg ZDV bid, indicating that once a day dosing with ZDV should be avoided. These *in silico* predictions suggest that 200 mg bid ZDV is an efficacious and safe dose that could delay the emergence of the K65R mutation.
Introduction.

Current first line highly active antiretroviral therapy (HAART) for the treatment of human immunodeficiency virus (HIV-1) infections combines two nucleoside RT inhibitors (NRTI) together with either a protease inhibitor (PI) or non-nucleoside RT inhibitor (NNRTI) (18, 19, 54). These drug combinations have markedly decreased mortality and morbidity from HIV-1 infections in the developed world (11). Existing therapeutic modalities cannot eradicate HIV-1 infection because of the compartmentalization of the virus and its latent properties (80, 81). Therefore, chronic therapy remains the standard of care for the foreseeable future. HAART regimens are selected in part to minimize cross resistance, and thereby delay the emergence of resistant viruses. However, all regimens eventually fail, due primarily to lack of adherence to strict regimens, delayed toxicities and/or the emergence of drug-resistant HIV-1 strains (68). Thus, it is a major imperative to develop regimens that delay, prevent or attenuate the onset of resistance for second line treatments for infected individuals who have already demonstrated mutations in the systemic circulation. The occurrence of common resistance mutations, including thymine analog mutations (TAM, specifically, D67N, K70R, T215Y, T219Q), K65R and M184V, need to be the continued focus in the rationale design of HIV-1 NRTI drug development (79).

Data from large genotype databases demonstrated an increased incidence of the K65R mutation from 0.8% in 1998 to 3.8% in 2003, presumably as a result of increased use of K65R selecting drugs (76, 88). This mutation produces a single amino acid change from lysine to arginine in the HIV-1 RT gene. *In vitro* selection of K65R, accompanied with moderate resistance, has been demonstrated for non-thymine NRTI including
abacavir sulfate (ABC), tenofovir disoproxil fumarate (TDF), zalcitabine, didanosine, adefovir dipivoxil and lamivudine (3TC), β-D-2',3'-didehydro-2',3'-dideoxy-5-fluorocytidine (D-d4FC, dexelvucitabine, Reverset), and β-D-(2R,4R)-1,3-dioxolane guanosine (DXG) (39, 76, 88, 89). The guanosine nucleoside prodrug of DXG, Amdoxovir [(-)-β-D-2,6-diaminopurine dioxolane; AMDX; DAPD] (12, 25), is being developed by RFS Pharma, LLC, primarily for the second line treatment of HIV-1 infections (35, 52). Advantages of DXG include an increased sensitivity to M184V/I strains in vitro and activity against TAM that may have been selected during previous antiretroviral therapy and 69SS double insert (36, 37, 55). DXG is synergistic with several NRTI including ZDV, 3TC, and nevirapine (36). In vitro studies with HIV-1 in culture with MT-2 cells demonstrated a slow onset of resistance to DXG that was associated with the K65R mutation (31, 67, 89). An in vitro study demonstrated that ZDV alone selected for a mixture of K70K/R mutations at week 25, and DAPD alone selected for a mixture of K65R and L74V mutations at week 20. However, when DAPD and ZDV were used in combination in HIV-infected primary human lymphocytes, no drug resistant mutations were detected through week 28 (71.). Furthermore, mechanistic studies demonstrated that K65R mutants remained susceptible to thymine NRTI, including ZDV and stavudine (d4T) (8, 29, 38, 39, 66). Regimens containing TDF in combination with lamivudine and abacavir have demonstrated high failure rates due to the emergence of drug resistance mutations including K65R. A composite analysis of data from those regimens revealed a success rate of 86% when the regimen contained ZDV compared to 62% when it did not. Furthermore, no K65R mutations were observed in subjects on regimens containing ZDV, suggesting that ZDV has value in preventing selection for this
mutation (33). A previous study has also demonstrated a reduced selection of the K65R mutation when ZDV was added to a regimen containing ABC (50). Therefore, co-formulation of ZDV with K65R mutation selecting drugs is warranted (33, 53, 69), since ZDV has the potential to serve as a “resistance repellent” agent for the K65R mutation. The addition of ZDV may not be appropriate if it competes for rate limiting enzyme phosphorylation with other NRTI contained in the HAART regimen. However, the enzyme used for the rate limiting phosphorylation step of ZDV differs from those of TDF, ABC and DXG (3, 4, 20, 22-25, 30, 41, 55, 59, 85).

ZDV was the first antiretroviral drug tested in the clinic, initially, as a monotherapy drug and later as a component of HAART regimens (11, 17, 26) and was approved as a generic formulation in September 2005 by the United States Food and Drug Administration (FDA). Like other NRTI, ZDV undergoes three intracellular phosphorylation steps to form the active ZDV-5’-triphosphate (ZDV-TP) which inhibits wild-type HIV-1 RT with a median inhibitory concentration (IC₅₀) value of about 0.035 µM (72). The single dose plasma pharmacokinetics of ZDV have been well described in HIV-1 infected individuals following intravenous and oral administration (1, 10, 20, 34, 78, 90).

ZDV treatment is limited by toxic side effects, including nausea and malaise, as well as serious bone marrow cytotoxicities including anemia and neutropenia (15, 73, 82). The bone marrow cytotoxicities of ZDV are believed to be associated with mitochondrial damage, and correlate with the ZDV-MP content (87). The current approved oral dose of ZDV in most of the world is 300 mg bid. A double-blind, parallel group multicenter study involving 474 HIV infected patients, comparing ZDV monotherapy daily doses of
400, 800 and 1,600 mg was published in 1992 (62). A dose dependent statistically significant increase in the incidence of anemia and leucopenia (p = 0.008) and neutrophilemia (p = 0.0005), neuropathy (p = 0.03) was observed, and the percentage of subjects who failed to complete the study due to side effects was dose related (21%, 31%, 32%, for the 400, 800 and 1600 mg daily doses of ZDV, respectively). Although, there was a trend towards fewer cases AIDS demential complex, it was not statistically significant. It was concluded that lower ZDV doses reduced toxicity, and doses > 400 mg to 600 mg a day doses offered no clinical advantage (62). Furthermore, study by Barry, et. al., (7) reported that a reduced dose with ZDV 100 mg tid produced similar amounts of cellular ZDV-TP, which mediates its antiviral effect, with significantly decreased ZDV plasma concentrations and intracellular amounts of ZDV-MP, lending support to enzymatic studies that suggest thymidylate kinase (TMPK) may be over-saturated at clinical doses (30). There are conflicting reports regarding the clinical relevance of saturation of TMPK at clinical ZDV doses. Fletcher, et al., reported higher average amounts of cellular ZDV-TP and decreased variance (0.62 nM, 32% CV versus 0.76 nM, 16% CV, respectively), when ZDV doses were adjusted to maintain a target plasma concentration (27), suggesting that between subject variability in pharmacokinetics is important. However, the accumulation of ZDV-TP is further complicated, since phosphorylation is cell cycle dependant and the fraction of dividing cells may vary between HIV infected individuals (49, 58, 86); and once activated, cells from different hosts may have varying TMPK activities (59). Given the large variation in cellular ZDV-TP concentrations measured in peripheral blood mononuclear (PBM) cells (CV approximately 100%) (2, 27), large clinical trials would be needed to statistically
demonstrate whether ZDV phosphorylation is saturable. A potentially useful approach could be to merge the enzyme kinetic data, which is best characterized in vitro, with the in vivo derived population pharmacokinetic parameters of ZDV in HIV infected individuals, in an in silico simulation study. This approach would allow large populations of virtual subjects to be simulated and compared with actual clinical data.

The results of Barry, et al. (7), suggest the potential for dose saturation of ZDV phosphorylation, and in vitro data indicate that virus containing the K65R mutation is more sensitive to ZDV (36, 37, 55). Furthermore, ZDV 600 mg qd regimen was recently shown to produce a less pronounced and slower onset of viral depletion was observed than at the standard 300 mg bid regimen (77). Therefore, the objectives of this study were: to develop a population pharmacokinetic and pharmacodynamic (PK/PD) model that combines population PK parameters and population statistics of cellular enzyme levels in HIV-1 infected individuals to determine whether dose saturation is supported mechanistically; to develop an optimal dosage regimen of ZDV for co-formulation as a K65R resistant repellent with DAPD and other NRTI when the initial load of K65R is low; and to assess whether the outcomes of the ZDV 600 mg qd trial were predictable based on plasma pharmacokinetics and enzyme dynamic data derived in vitro. Since ZDV reduces overall viral load by < 1 log, it was considered prudent to target cellular contents of ZDV-TP similar to those observed presently in the clinic.

Materials and Methods.

The structure of the population pharmacokinetic and the cellular pharmacology models used in this study are summarized in Figure 1.
Population pharmacokinetics of ZDV:

Population characteristics and pharmacokinetic parameters are summarized in Table 1. The 2-compartment model of Zhou et al. was fitted using data from 175 individuals who received 200 mg ZDV tid. However, only 93 subjects were used to model ZDV in the absence of nevirapine, which produced a pharmacokinetic interaction with ZDV in that study. Drug absorption was approximated as zero-order, since insufficient data were available to characterize the absorption phase, and subjects were partitioned into fast or slow absorbers. Fast absorbers (41.7% of individuals) absorbed ZDV over 0.25 hr, while slow absorbers (the remainder) absorbed ZDV over 1.57 hr. The systemic clearance (CL/F, L/hr) was related to the covariates age (years) and body weight (kg) using the following equations:

For individuals < 30 years old, Eq. 1a:

\[
CL/F = 127 + 0.93 \times \text{body weight -70}
\]

Otherwise Eq. 1b:

\[
CL/F = 127 + 0.93 \times \text{body weight -70} + 6.52 \times \text{age-25}
\]

The steady-state volume of distribution \((V_{ss}/F, \text{L})\) was related body weight using Eq. 2:

\[
V_{ss}/F = 464 + 9.83 \times \text{body weight - 70}
\]

Similarly, the volume of the central compartment \((V_{1}/F, \text{L})\) is calculated using a constant fitted ratio of \(V_{1}/V_{ss} (R_{V1/Vss})\) using Equation 3:

\[
V_{1}/F = R_{V1/Vss} \times V_{ss}/F
\]

The volume of the peripheral tissue compartment \((V_{2}/F)\) was calculated by difference.
Both CL/F and Vss/F were assumed to follow log-normal distributions, with variance equal to $\omega^2_{CL/F}$ and $\phi \times \omega^2_{CL/F}$, respectively, where $\phi$ was the fixed ratio between variances. The residual/inter-individual variance was modeled as $\varepsilon_{ij} \times b \times C_p$, where $\varepsilon_{ij}$ is normally distributed with mean 0 and variance $\sigma^2$. The factor $b$ was a constant parameter that is assumed equal to 1 after the absorption phase (> 2 hr) and allowed for a greater variability during the drug absorption phase. Given the limited number of subjects, large number of covariates, and lack of a reported variance-covariance matrix, the available data might not have supported successful estimation of all potentially important covariate effects and co-variances. It is also possible that linking inter-subject variances in CL/F and V/F could result in a reduced overall variability in the simulation. The covariate distributions from Zhou et al (90) were used unchanged in the simulation.

The plasma pharmacokinetics of ZDV are characterized by a bi-exponential decay after intravenous injection (9). However, some pharmacokinetic studies have utilized a 1-compartment model to describe ZDV disposition (65), in part due to some obscuring of the distribution phase of by the absorption phase. Panhard, et al., fitted a 1-compartment model in 10 infected individuals receiving ZDV alone (65). The residual/inter-individual variance was described using a combined proportional and additive error model ($\sigma^2_{total} = \sigma^2 \times (a + C_{ij})$, where variance $\sigma^2$ is normally distributed, “a” is a fitted constant and $C_{ij}$, is the predicted plasma concentration. Since much of the variance occurs during drug absorption, this approach may tend to overestimate variance in the post-absorption phase, resulting in an over-predicted variability of ZDV-MP and -TP. The parameters for both pharmacokinetic models are presented in Table 1.
Accumulation of ZDV and its nucleotides in human PBM cells:

Plasma and cytosolic concentrations of ZDV were assumed to equilibrate instantaneously, since ZDV is not appreciably protein bound and equilibration between plasma and cytoplasm is achieved rapidly due to the action of equilibrative nucleoside transporters present on the cell membranes of lymphocytes (5, 70). The initial intracellular phosphorylation step of ZDV is catalyzed by cellular thymidine kinase (TK). The primary enzyme for 5’-monophosphorylation to ZDV-MP is TK\textsubscript{1}, which is located primarily in the cytosol of cells in S-phase. However, mitochondrial TK (TK\textsubscript{2}) has also been shown to phosphorylate ZDV in cultured monocytes/macrophages that do not express TK\textsubscript{1}, but to a much lesser degree (3, 4, 22, 23, 30, 59). The K\textsubscript{m} (concentration at 50% of maximal metabolism rate) of ZDV versus TK\textsubscript{1} is 3 µM (23, 30), and the activity of TK\textsubscript{1} versus ZDV is 0.6 of the activity versus thymidine. Thymidylate kinase (TMPK) catalyzes the subsequent phosphorylation to ZDV-5’-diphosphate (ZDV-DP) and is rate limiting with a K\textsubscript{m} of 7.6 to 8 µM versus ZDV-MP (30, 45). Distributions describing maximal rates of conversion of phosphorylation of ZDV (V\textsubscript{max,TK1}) and ZDV-MP (V\textsubscript{max,ZDV-MP}), respectively, were estimated using data from previous enzymatic studies conducted on PBM cells isolated from cohorts of infected individuals (48, 49). These and other studies have reported a decreased average level of TK\textsubscript{1} and TMPK in cells activated in vitro, of individuals treated with ZDV for more than 6 month. Therefore, distribution of TK\textsubscript{1} and TMPK activities were calculated using the TK\textsubscript{1} activities of 24 HIV positive individuals not previously exposed to AZT (48), and 27 HIV positive individuals with varying exposures to ZDV (49), respectively. Since TK\textsubscript{1} and TMPK are cell cycle dependent, PBM cells of infected individuals were stimulated ex vivo using
phytohemagglutinin (PHA) (32, 48, 49), followed by lysing and measurement of enzyme activities. Activities of TK\textsubscript{1} were measured using ZDV (20 μM), which is higher than concentrations used in vivo, but sufficient to saturate the enzyme, allowing the capacity of the enzyme (V\textsubscript{max,TK1}) to be measured. TMPK activities were measured using thymidine-MP (T-MP) as a substrate (50 μM). V\textsubscript{max,ZDV-MP} to be calculated by assuming a TMPK efficiency of 1% that of ZDV-MP relative to T-MP (45). The relatively slow V\textsubscript{max} of ZDV-MP is believed to be related to steric hindrance in the binding of ZDV-MP to TMPK (51). V\textsubscript{max,ZDV-MP} and V\textsubscript{max,TK1} were assumed to be distributed in a log-normal manner. To ensure that V\textsubscript{max} values remained in the physiological range, maximal values were constrained to be less than the largest values reported in non-infected individuals in the study, noting that values were higher in non-infected than in HIV infected individuals (48, 49). Units of the apparent V\textsubscript{max} were converted to μmol of ZDV-DP/hr/L, using an average of two reported measurements of protein content of PHA stimulated PBM cells (0.053 mg/10\textsuperscript{6}) (4, 43). The aqueous volume of activated lymphocytes was calculated using a mean projected cell surface area of (80 μm\textsuperscript{2}) (84), assuming a specific gravity of 1.06, 70% water, and spherical geometry. This volume was then used to convert pmol/10\textsuperscript{6} cells to μM. Parameters used for modeling the cellular phosphorylation and dephosphorylation of ZDV are presented in Table 2. It was assumed that PHA stimulates about 40% of the lymphocytes population in vivo (86).

The cytoplasmic accumulation of ZDV-MP is described by Eq. 4:

\[
dZDV-MP/dt = C_1 \times V_{max,TK1}/(C_1 + K_{m,TK1}) + ZDV-DP \times k_{DP,MP} - ZDV-MP \times k_{MP} - V_{max,TMP} \times ZDV-MP/(ZDV-MP + K_{m,TMP})
\]
The final phosphorylation step to active ZDV-TP is catalyzed by nucleoside diphosphate kinase and is not rate limiting under physiological conditions, and takes place too rapidly to be easily characterized in vitro (86). Considering the AUC ratio of ZDV-TP to ZDV-MP in vivo, \((0.42 \pm 0.42 : 0.52 \pm 0.32\) at 100 mg dose and \(0.61 \pm 0.81 : 0.56 \pm 0.57,\) pmol/10^6 x hr ± SE), for a 300 mg dose, respectively (7), a constant 1:1 ratio of ZDV-TP to -DP was assumed. The rates of dephosphorylation of ZDV-DP to ZDV-MP \((k_{DP,MP})\), and ZDV-MP to ZDV \((k_{MP})\) were calculated using steady-state data from an in vitro study of ZDV nucleotides measured in PHA stimulated PBM cells following a 4 hr incubation with various concentrations of ZDV (86). Thus, at steady-state the rate of accumulation of ZDV-MP \(\left[\frac{C_1 \times V_{\text{max},\text{TK1}}}{C_1 + K_{m,\text{TK1}}}\right]\) equals the rate of decomposition of ZDV-MP to ZDV \((ZDV-MP \times k_{MP})\), allowing \(k_{MP}\) to be calculated as the regression slope of \(C_1 \times V_{\text{max},\text{TK1}}/(C_1+K_{m,\text{TK1}})\) versus ZDV. Likewise, the first-order decomposition rate of ZDV-DP to ZDV-MP was calculated as the regression slope of \(V_{\text{max},\text{TMP}} \times ZDV-MP/(ZDV-MP + K_{m,TMP})\) versus ZDV-DP (86).

The accumulation of ZDV-DP + ZDV-TP \((ZDV-DP,TP)\) was described by Eq. 5:

\[
\frac{d(ZDV-DP,TP)}{dt} = V_{\text{max},\text{TMP}} \times ZDV-MP/(ZDV-MP + K_{m,TMP}) - ZDV-DP \times k_{DP,MP}
\]

Where \(\frac{d(ZDV-MP)}{dt}\) and \(\frac{d(ZDV-DP)}{dt}\) are the rates of change in cellular ZDV-MP and -DP, respectively.

Cellular contents of the various nucleotides of ZDV in activated cells were used to estimate the ZDV nucleotide contents of cells in vivo, assuming an in vivo activated cell fraction of 8 percent (40), and phosphorylation in active cells is minimal in non-activated cells (86). It was previously shown that the ratio between ZDV-MP, -DP and -TP were similar in resting and PHA-stimulated PBM cells, while the absolute
concentrations were 60 to 150 times higher in stimulated cells (86). Thus, assuming an 8% stimulation of PBM cells \textit{in vitro}, activated PBM cells would contribute between 84 to 92 percent of the total ZDV nucleotides in humans. The average percentage (88 %) was used to calculate the total amounts of ZDV-MP, -DP and -TP using the simulated amounts in activated cells. Plasma concentrations of ZDV and cellular amounts of ZDV-MP were compared with data from existing clinical studies (7, 28, 65, 90).

Computer Simulations.

Monte Carlo population pharmacokinetic and enzyme dynamic simulations were conducted for 5,000 individuals per simulated dose regimen, using Trial Simulator™ version 2.2.1, 2006 (Pharsight Corp., Mountain View, CA), which utilizes a 5\textsuperscript{th} order Runge-Cutta algorithm for numerical integration. This program allows customized differential equations, together with probability distributions of each parameter in the equations to be entered. The simulated results were then analyzed using routines in the S-Plus computer program (\textit{version} 6.0 Professional, Insightful Corp., Seattle, WA, 1988) embedded in Trial Simulator™ software. Medians, 25\textsuperscript{th} and 75\textsuperscript{th} percentiles \textit{versus} time of ZDV concentrations in plasma, and cytoplasmic content of ZDV-MP and ZDV-TP were calculated. Three simulations of 5,000 individuals each were performed to ensure reproducibility of the output (< 5% differences in medium and inter-quartile ranges).

Results.

The outline of the model is shown in graphic form in Fig. 1. Simulations were performed using both pharmacokinetic models at ZDV 50, 100, 200 and 300 mg bid and
600 mg qd doses. Median and interquartile ranges of plasma ZDV and cellular ZDV-MP and -TP were plotted for the bid doses (Figs. 2-4). Box plots of between dose peak and trough amounts of ZDV-TP (pmol/10^6 cells) (median and inter-quartile ranges) for all doses using the one- and two-compartment population pharmacokinetic models, (Fig. 5A and B, respectively). Levels of ZDV, and ZDV-MP, -DP and -TP essentially achieved steady-state within 48 hr. Plasma concentrations were assumed to increase linearly with dose in both models. The two-compartment model (90), produce higher C_{max} values, a more gradual terminal slope, and a smaller variance after the absorption phase (t > 2 hr) than the one compartment model (Fig. 2). Likewise, peak amounts of cellular ZDV-MP were higher and terminal decline slopes were slower for the two-compartment than the one-compartment model. Both models predicted a noticeable increase in predicted ZDV plasma concentrations and amounts of ZDV-MP when doses were increased from 200 to 300 mg bid (Fig. 3).

A high degree of overlap of peak amounts of ZDV-TP was predicted using the two-compartment model of Zhou, et al., as well as the one-compartment model of Panhard, et al., for ZDV-TP at the 200 and 300 mg bid doses (98% and 97%, respectively, Fig. 4 and Fig. 5A and B). The respective peak amounts of ZDV-TP at 300 mg bid were 0.061 pmol/10^6 cells (inter-quartile range 0.031 – 0.12), and 0.062 pmol/10^6 cells (0.032 – 0.12) However, the one-compartment model predicted lower trough amounts of ZDV-TP between doses (Fig. 4). Comparison of median peak amounts of ZDV-TP at steady-state, in the dose range 50 to 300 mg bid, with linear predictions using median peak values of the 50 and 100 mg bid doses, suggests some saturation of phosphorylation to ZDV-TP, demonstrated by the lower than expected median peak
ZDV-TP amount at 300 mg bid. Furthermore, the simulation based on the two-compartment model suggested higher amounts of cellular ZDV-TP (Fig. 5A), than the one-compartment model (Fig. 5B). A less than linear increase in between dose minimal (trough) amounts of ZDV-TP was suggested by the simulation using the two-compartment model (Fig. 5A) that was not evident for the one-compartment simulation (Fig. 5B). Both simulations predicted reduced peak and trough amounts of cellular ZDV-TP after treatment with ZDV 600 mg qd than 300 or 200 mg ZDV bid.

Discussion.

Pharmacokinetic and pharmacodynamic simulations are useful tools for consolidating all available drug information in a usable form, and are gaining favor in the pharmaceutical industry to design clinical trials, since they allow detailed analyses of dosage regimens in silico before the actual studies are conducted (13, 21, 56, 57, 63, 64). Rosario, et al., recently utilized clinical trial simulations to streamline the phase 2a development of the CCR5 receptor blocking agent maraviroc (75). Furthermore, Hurwitz, et al., recently developed a pharmacodynamic model of viral depletion for use with lamivudine (46).

The objective of this study was to superimpose previously reported population pharmacokinetic parameters together with distribution summaries of the cellular enzyme kinetics of ZDV metabolism derived from HIV-1 infected individuals, who were not previously treated with ZDV, using a simulation model. Activated CD4+ lymphocytes are the dominant substrates for HIV-1 infection and could be a significant site for the selection of the K65R mutant virus. The model was then used to predict the accumulation
of ZDV nucleotides in activated CD4\(^+\) lymphocytes versus dose regimen. Predicted concentrations of ZDV-TP in activated CD4\(^+\) PBM cells may be useful for possible incorporation into virus PK-PD model that relates virus depletion profiles versus time and dose of administration (46, 47).

Simulations were repeated using a one and a two-compartment population pharmacokinetic models, with different error structures (65, 90). The higher C\(_{\text{max}}\) values and a smaller variance after the absorption phase predicted by the two-compartment model of Zhou et al. (90), compared to the one compartment model, may result in part from the different variance structures used, which allowed for a boosted variance during the absorption phase in the two-compartment model, whereas the variance structure was not boosted for any particular portion of the concentration versus time curve. Furthermore, the more gradual terminal slope was expected from a two-compartment model, which predicts a more gradual elimination rate in the latter part of the dosing interval.

The peak cellular levels of ZDV-MP (associated with bone marrow toxicity), predicted by the two-compartment model were higher and the terminal elimination slightly slower than those predicted by the one compartment model in accordance with the plasma concentration versus time profiles of ZDV (Fig. 2). Peak amounts of ZDV-MP were higher in the simulation compared to those reported by Barry, et al., in 10 HIV infected subjects given 300 mg ZDV bid (2.25 ± 1.5, pmol/10\(^6\) cells, mean ± SD, n = 10). Calculation of ZDV-MP levels assumed a stimulated fraction of PBM cells in humans of 8%. Therefore, ZDV-MP levels in activated cells are expected to be much larger, and
would exceed the $K_m$ value versus thymidylate kinase (7.6 µM) for much of the dose interval.

Simulations using the two- and one-compartment pharmacokinetic models, predicted a high degree of overlap in cellular ZDV-TP between the 200 and 300 mg bid doses (98% and 97%, respectively) (Fig. 4). Peak amounts of predicted ZDV-TP at the 300 mg bid dose were 0.061 pmol/10^6 cells (inter-quartile range 0.031 – 0.12), and 0.062 pmol/10^6 cells (0.032 – 0.12), respectively, and were comparable to clinical observations from the study by Barry et al., in 10 HIV infected individuals given 300 mg ZDV bid (0.07 ± 0.09 pmol/10^6 cells, mean ± SD, n = 10) (7). The corresponding terminal-half-life for ZDV-TP, estimated using the median simulated curves were 9.8 and 5.4 hr, respectively, compared to 6.5 hr half-life estimated using the mean curve for the same dose regimen (7). Interestingly, similar peak amounts of ZDV-TP were reported for 100 and 300 mg bid doses in the same study. The more rapid terminal decline in cellular ZDV-TP observed for the lower dose could result from a declining replacement of decaying cellular ZDV-TP from phosphorylation of ZDV entering cells when concentrations of ZDV are low. Similarly, the more rapid decline in ZDV-TP is expected for the one-compartment model, since it predicts a more rapid terminal elimination of ZDV from the plasma. A decreased replacement in ZDV-TP could also explain the more rapid cellular half-life for ZDV-TP reported in vitro (< 2.5 hr) (86), than in vivo observations (7 to 10 hr) (2, 28, 74, 83), since cellular half-lives are typically measured in vitro after re-suspended cells in media in the absence of extra-cellular ZDV.

The higher median ZDV-TP levels predicted by the two compartment model (Fig. 5A) compared to the one-compartment model (Fig. 5B), could be related to the higher
predicted plasma $C_{\text{max}}$ values of ZDV. Comparisons of median peak amounts of ZDV-TP at steady-state, in the dose range 200 to 300 mg bid, compared to predictions using a linear regression of median peak values of the 50 and 100 mg bid doses, suggested at least partial saturation of phosphorylation to ZDV-TP. The less than linear increase in between dose trough ZDV-TP predicted using the two compartment model (Fig. 5A), but not the by the one compartment simulation (Fig. 5B), may be explained in part by the more rapid decline in ZDV-TP during the terminal phase of the dosing interval. ZDV-TP acts as a competitive inhibitor and chain terminator during reverse transcription. Therefore, the trough concentrations of ZDV-TP are of critical importance, since CD4$^+$ cells are maximally sensitive to HIV infection when ZDV-TP concentrations are lowest. Both models suggested that similar peak amounts of ZDV-TP following 600 mg bid and 300 mg bid, which could result from enzyme saturation or a lack of accumulation of ZDV-TP resulting from the prolonged dose interval. The median peak amount of ZDV-TP predicted for the 600 mg qd dose, was 0.061 and 0.065 pmol/10$^6$ cells, using the two- and one-compartment pharmacokinetic models, respectively and the corresponding median trough amounts of ZDV-TP amounts were 0.003 and 0.0033 pmol/10$^6$ cells, respectively. The trough amounts of ZDV-TP were comparable with amounts reported by Flyn et al., at the same dose when co-administered with 3TC in 27 HIV infected adolescents: 0.035 pmol/10$^6$ cells (95 % CI: 0.03 to 0.065, n = 27), and 0.01 pmol/10$^6$ cells (95% CI: 0.01 to 0.02), respectively (28).

Both pharmacokinetic models produced similar overall conclusions for ZDV phosphorylation. However, the population enzymology component of the model was limited by the availability of only one data set each, describing the phosphorylation
potential ($V_{\text{max}}$) for TK$_1$ and TMPK, respectively, in the PBM cells of infected subjects following \textit{ex vivo} stimulation (48, 49). These studies indicated substantial variances in the respective $V_{\text{max}}$ values between HIV infected individuals. A decrease in TK$_1$ and TMPK activities in PBM cells of infected individuals was reported in individuals treated with ZDV for more than 6 months. However, the significance of this finding is not clear, since statistically different cellular amounts of ZDV-TP were not demonstrated in a study of subjects exposed to ZDV over a similar time intervals (6, 44). Amounts of ZDV-MP levels tended to be larger while amounts of ZDV-TP agreed closely with in vivo amounts. However, \textit{in silico} predictions depend on the model structure together with parameter estimates and their associated variance structures. An average estimate for the aqueous cell volume in CD4$^+$ cells was used in these simulations. In reality cell volumes in an activated lymphocyte population vary according to stage in the cell cycle. The primary substrates for HIV infection are activated T-CD4$^+$ lymphocytes. In most studies the ZDV-TP content of is measured in PBM cells obtained by centrifugation using a Ficoll gradient, without further enriching for activated CD4$^+$ cells (7, 28, 83). Since TK$_1$ and TMPK expressions are cell cycle dependent, consideration of data from these measurements underestimates the ZDV-TP content in activated lymphocytes. In this study the ratio of activated PBM cells was assumed to be constant. However, the activated lymphocyte fraction tends to be elevated in untreated HIV infected individuals and may decrease towards more normal levels once the infection is stabilized using HAART (58). Therefore, it may be useful for future studies to measure amounts in activated cells if feasible, or to provide a cell cycle distribution together with the ZDV-TP content. The parameter distributions were from different studies, so that statistical
correlations between parameter covariates could not be assessed. The large variances in enzyme levels between infected individuals makes it difficult to estimate ratios of activities between TK₁ (producing ZDV-MP), and TMPK (producing ZDV-DP) (48, 49).

This simulation relied on the interplay between the various parameters, and it is possible that there could be a compounding or compensation in the overall error associated with the variance parameters.

The Thai national guidelines for the management of HIV recommended ZDV doses be reduced from 300 to 200 mg bid, in patients weighing less than 60 kg, which has resulted in fewer side effects and improved long term tolerability, without evidence of reduced efficacy (61). A pharmacokinetic study conducted in Thailand demonstrated similar plasma concentrations in subjects weighing less than 60 kg treated at the 200 mg bid dose, compared with a historical data set from heavier subjects administered 300 mg bid (16). Although the oral clearance of ZDV may be reduced in individuals with body weights < 60 kg (10, 90), other studies have failed to demonstrate a linear relationship between body weight and ZDV clearance (78). Furthermore, the wide interindividual variance in cellular TK₁ and TMPK (45, 48), suggests that plasma concentrations of ZDV alone, would be insufficient to predict levels of the active ZDV-TP. The in silico findings of this study suggest that the current ZDV dose could also be lowered from 300 to 200 mg bid in subjects with body weights more typical of Western populations, to reduce toxicities and still maintain adequate ZDV-TP concentrations. However, it would not be prudent to decrease the dose to levels much lower than 200 mg bid, as demonstrated by Fig. 5A and B, since a more linear decrease was predicted for median peak and trough amounts of ZDV-TP between 50 to 200 mg bid, but not for 300 mg bid.
However, the simulations assumed ZDV was not co-administered with agents that alter its metabolism. Therefore, ZDV doses may need modification when co-administered with protease inhibitors, including nevirapine which may induce glucuronidation and/or reduce absorption (Zhou, et al., 1999, Panhard, et al., 2007), resulting in lower plasma concentrations. The nucleoside reverse transcription inhibitors TDF, 3TC and DAPD are not known to affect ZDV metabolism (42, 60).

TDF is administered once a day in accordance with the long cellular half-life of tenofovir-DP (> 60 hr). Although the addition of ZDV to once a day TDF should be beneficial, a co-formulation with ZDV would result in a less effective ZDV response. The half-life of DXG-TP and carbovir-TP (the active metabolite of ABC) are ~16 hr and 12–24 hr, respectively (42, 79). DAPD is administered twice a day, while ABC demonstrated similar efficacy when administered once or twice a day (14). Therefore, ZDV may be a candidate for co-formulation at an optimal bid dose regimen with NRTI such as DAPD, the prodrug of DXG or ABC.

Based on these *in silico* results, an intensive pharmacokinetic Phase 2 clinical study in 24 HIV-1 infected subjects receiving ZDV 200 or 300 mg bid in combination with DAPD 500 mg bid for 10 days has recently been completed and support our *in silico* findings that ZDV 200 mg bid produces the same antiviral response as the approved ZDV dose of 300 mg bid without any untoward effects (60). The data generated from this clinical study will be important for positioning DAPD co-formulated with ZDV 200 mg bid in advanced Phase 2/3 studies. Furthermore, the utility of a lower effective dose of ZDV without its associated toxicity, particularly bone marrow, would be beneficial in future HAART combinations, when used with drugs that select for the K65R mutation.
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References:


Table 1. Population demographics and pharmacokinetic parameters of oral ZDV used for the simulation study.

<table>
<thead>
<tr>
<th>Population parameter</th>
<th>Estimate (%CV)</th>
<th>95% CI and/or ranges</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Zhou parameters (90)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>39.2 (20)</td>
<td>Range 20.5-60.8</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>73.9 (22)</td>
<td>Range 41-109</td>
</tr>
<tr>
<td>Cl/F (L/hr)</td>
<td>127.2 (4.9)</td>
<td>114.9-139.1</td>
</tr>
<tr>
<td>$^{1}\text{wgt-CL/F}$ (L/hr/kg)</td>
<td>0.93 (35.7)</td>
<td>0.28-1.58, Range 0-4</td>
</tr>
<tr>
<td>$^{2}\text{age-CL/F}$ (L/hr/yr)</td>
<td>6.52 (19.9)</td>
<td>3.97-9.07</td>
</tr>
<tr>
<td>$V_{SS}$ (L)</td>
<td>463.8 (17.4)</td>
<td>306.2-625.0</td>
</tr>
<tr>
<td>$^{3}\text{wgt-V}_{SS}$ (L/kg)</td>
<td>9.83 (28.9)</td>
<td>4.26-15.4</td>
</tr>
<tr>
<td>$\phi$</td>
<td>0.610 (46.4)</td>
<td>0.0553-1.165</td>
</tr>
<tr>
<td>$^{4}\text{CL}_{d}$ (L/hr)</td>
<td>27.0 (15.2)</td>
<td>19.0-35.0</td>
</tr>
<tr>
<td>$\omega_{\text{CL/F}}^2$</td>
<td>0.0703 (28.2)</td>
<td>0.0315-0.109</td>
</tr>
<tr>
<td>$^{5}\text{R}_{V1/V2}$</td>
<td>0.374 (15.1)</td>
<td>0.263-0.485</td>
</tr>
<tr>
<td>$\sigma^2$</td>
<td>0.261 (9.2)</td>
<td>0.216-0.308</td>
</tr>
<tr>
<td>$b$</td>
<td>1.56 (8.3)</td>
<td>1.311-1.809</td>
</tr>
<tr>
<td><strong>Panhard parameters (65)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{6}k_a$ (h$^{-1}$)</td>
<td>2.66</td>
<td>1.38-5.12</td>
</tr>
<tr>
<td>Cl/F (L/h)</td>
<td>195</td>
<td>148-256</td>
</tr>
<tr>
<td>V/F (L)</td>
<td>344</td>
<td>220-537</td>
</tr>
<tr>
<td>$\omega_{\text{CL/F}}^2$</td>
<td>0.446</td>
<td></td>
</tr>
<tr>
<td>$\sigma^2$</td>
<td>0.1102</td>
<td>0.0801-0.151</td>
</tr>
<tr>
<td>a (ng/ml)</td>
<td>120</td>
<td></td>
</tr>
</tbody>
</table>

$^{1}\text{wgt-CL/F}$, $^{2}\text{age-CL/F}$ and $^{3}\text{wgt-V}_{SS}$ were used to calculate Cl/F and $V_{SS}$/F using body weight in equations 1 and 2. $^{4}\text{CL}_{d}$ is the inter-compartment clearance between compartments 1 and 2. $^{5}\text{R}_{V1/V2}$ is the ratio $V_1$: $V_2$. $^{6}b$ is the factor by which residual variance is boosted during the absorption phase (< 2 hr). $^{7}k_a$ is a first-order absorption rate constant.
Table 2. Population enzyme kinetic parameters for cellular metabolism of ZDV used for the simulation study.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median</th>
<th>Interquartile range (ref)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{\text{max,TK1}}$ vs ZDV ($\mu$mol.mg$^{-1}$.min$^{-1}$)</td>
<td>35</td>
<td>(IQ range: 13-65) Range 0 - 300) (48)</td>
</tr>
<tr>
<td>$K_{M,\text{TK1}}$ vs ZDV (uM)</td>
<td>3</td>
<td>(30)</td>
</tr>
<tr>
<td>$V_{\text{max,PK}}$ vs T ($\mu$mol.mg$^{-1}$.min$^{-1}$)</td>
<td>53</td>
<td>(IQ range: 22-116) (Range 0 &lt; 723) (49)</td>
</tr>
<tr>
<td>$K_{M,\text{PK}}$ vs ZDV-MP (uM)</td>
<td>7.6</td>
<td>(45)</td>
</tr>
<tr>
<td>Protein per $10^6$ cells (mg)</td>
<td>0.053</td>
<td>mean of 0.040 (43) and 0.066 (4)</td>
</tr>
<tr>
<td>Relative PK efficiency: ZDV-MP/dT-MP</td>
<td>1%</td>
<td>1% (45)</td>
</tr>
<tr>
<td>Activated lymphocytes (in vivo)</td>
<td>5%</td>
<td>(40)</td>
</tr>
<tr>
<td>Projected cell area (µm$^2$)</td>
<td>80</td>
<td>(84)</td>
</tr>
<tr>
<td>$K_{MP} = K_{DP,MP}$ (h$^{-1}$)</td>
<td>0.28</td>
<td>constant (86)</td>
</tr>
<tr>
<td>$\omega^2 V_{\text{max,TK1}}$</td>
<td>1.42</td>
<td>(48)</td>
</tr>
<tr>
<td>$\omega^2 V_{\text{max,TMPK}}$</td>
<td>0.995</td>
<td>(49)</td>
</tr>
</tbody>
</table>

$V_{\text{max}}$ values were constrained to be less than the maximal value observed in non-infected individuals, which tend to have higher activities than infected individuals. $\omega^2$ refers to the variance of a log-normal distribution.
Figure legends:

FIG. 1. Overall schematic representation of the pharmacokinetic and viral dynamic model of ZDV phosphorylation.

FIG. 2. Simulated plasma concentrations of ZDV (n = 5,000), at the 200 and 300 mg doses (grey and black, respectively, according to the models of Zhou et al., (median solid line, p25 and p75, dashed lines), and Panhard et al, (median dot and dash line, p25 and p75 dotted lines) over 60 hr.

FIG. 3. Simulated cellular levels of ZDV-MP per $10^6$ cells (n = 5,000), at the 200 and 300 mg doses (grey and black, respectively, according to the models of Zhou et al., (median solid line, p25 and p75, dashed lines), and Panhard et al, (median dot and dash line, p25 and p75 dotted lines) over 60 hr.

FIG. 4. Predicted levels of ZDV-TP per $10^6$ cells (n = 5,000), at the 200 and 300 mg doses (grey and black, respectively, according to the models of Zhou et al., (median solid line, p25 and p75, dashed lines), and Panhard et al, (median dot and dash line, p25 and p75 dotted lines) over 60 hr.

FIG. 5. Box plots (medians and inter-quartile ranges, whisker plots are p10 - p90 ranges), of peak (checkered) and trough (shaded) levels of ZDV-TP in lymphocytes in vivo following ZDV doses of 50, 100, 150, 200 and 300 mg bid and 600 mg qd, using the pharmacokinetic model of Zhou, et al. and Panhard et al., (B), respectively. Prediction lines were derived by a regression of median values for the 50 and 100 mg bid doses.
**ZDV population PK:**

**Zhou, et al., 1999 vs Panhard et al., 2007**

**Zhou, et al., 1999**
- Fitted using n = 175.
- Zero-order absorption.
- 2-compartment distribution.
- First order elimination.

**Inter-individual variance:**
- CL/F related to body weight and age, log-normally distributed (variance = $\omega^2_{\text{CL/F}}$).
- $V_{\text{ss/F}}$ related to body weight log-normal distribution. $\omega^2_{V_{\text{ss/F}}}$ linked to $\omega^2_{\text{CL/F}}$ using variance ratio ($\phi$).

**Residual/intra-individual variance**
- Absorption-boosted proportional error model for plasma concentration ($C_p$).

**Panhard et al., 2007**
- Fitted using n = 12.
- First-order absorption.
- 1-compartment distribution.
- First order elimination.

**Inter-individual variance:**
- CL/F log-normally distributed with variance ($\omega^2_{\text{CL/F}}$).
- $V/F$ log-normally distributed with $\omega^2_{V/F}$

**Residual/intra-individual variance**
- Combined proportional & additive error model.

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**Cellular phosphorylation of ZDV:**

- $V_{\text{max,TK}}$

  - Kinetics in cells stimulated *ex vivo* (n = 49 HIV-positive).
  - (Jacobsson, 1998).

  - Assuming $V_{\text{max,TK}}$ values log-normally distributed.

  - Assumed 1:1 DP:TP ratio (see method for details).

- $K_{M,TMP}$

  - Kinetics measurements in S-phase cells stimulated *ex vivo* (n = 27 HIV-positive).
  - (Jacobsson, 1998).

  - Assuming $V_{\text{max}}$, population distribution was log-normal.

  - $K_m = 1\%$ of TMP (Hsu, et al., 2007)

---

**ZDV phosphorylation occurs primarily in stimulated Lymphocytes. ZDV –MP, -DP and –TP levels in lymphocytes in vivo = stimulated conc. X activated cell fraction**
Figure 2

Hours

ZDV, ng/ml, p25, median, p75

p75
p50
p25

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Figure 3

ZDV-MP, pmol/10^6 cells, p25, median, p75

Hours

0 10 20 30 40 50 60

p_{75}
p_{50}
p_{25}