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RESEARCH PAPER

Strategies to improve efficacy and safety of a novel class of antiviral hyper-activation-limiting therapeutic agents: the VS411 model HIV/AIDS

D De Forni[1], MR Stevens[2] and F Lori[1]

[1]*ViroStatics, Sassari, Italy, and* [2]*ViroStatics, Princeton, NJ, USA*

Correspondence
F Lori, ViroStatics srl, Viale Umberto I, 46, 07100 Sassari, Italy. E-mail: info@virostatics.com

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BACKGROUND AND PURPOSE

Antiviral hyper-activation-limiting therapeutic agents (AV-HALTs) are a novel experimental drug class designed to both decrease viral replication and down-regulate excessive immune system activation for the treatment of chronic infections, including human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome. VS411, a first-in-class AV-HALT, is a single-dosage form combining didanosine (ddI, 400 mg), an antiviral (AV), and hydroxyurea (HU, 600 mg), a cytostatic agent, designed to provide a slow release of ddI to reduce its maximal plasma concentration (C_{max}) to potentially reduce toxicity while maintaining total daily exposure (AUC) and the AV activity.

EXPERIMENTAL APPROACH

This was a pilot phase I, open-label, randomized, single-dose, four-way crossover trial to investigate the fasted and non-fasted residual variance of AUC, C_{max} and the oral bioavailability of ddI and HU, co-formulated as VS411, and administered as two different fixed-dose combination formulations compared to commercially available ddI (Videx EC) and HU (Hydrea) when given simultaneously.

KEY RESULTS

Formulation VS411-2 had a favourable safety profile, displayed a clear trend for lower ddI C_{max} ($P = 0.0603$) compared to Videx EC, and the 90% confidence intervals around the least square means ratio of C_{max} did not include 100%. ddI AUC_{∞} was not significantly decreased compared to Videx EC. HU pharmacokinetic parameters were essentially identical to Hydrea, although there was a decrease in HU exposure under fed versus fasted conditions.

CONCLUSIONS AND IMPLICATIONS

A phase IIa trial utilizing VS411-2 formulation has been fielded to identify the optimal doses of HU plus ddI as an AV-HALT for the treatment of HIV disease.

Abbreviations

3TC, lamivudine; ACTG, AIDS Clinical Trials Group; AIDS, acquired immunodeficiency syndrome; AUC, area under the plasma concentration–time curve; AUC_{∞}, area under the plasma concentration–time curve extrapolated to infinity; AUC_{last}, area under the plasma concentration–time curve from time of administration up to the last time point with a measurable concentration post-dosing; AV-HALT, antiviral hyper-activation-limiting therapeutic agent; CCR5, CC chemokine receptor 5; CI, confidence interval; C_{max}, maximal plasma concentration; CV, coefficient of variation; d4T, stavudine; DAIDS, Division of Acquired Immunodeficiency Syndrome; dATP, deoxyadenosine triphosphate; ddATP, dideoxyadenosine triphosphate; ddI, 2′, 3′-dideoxyinosine or didanosine; dNTP, deoxynucleotide triphosphate; EC, enteric coated; EFV, efavirenz; FDC, fixed-dose combination; GALT, gut-associated lymphoid tissue; HIV, human immunodeficiency virus; HU, hydroxyurea; IDV, indinavir; LC, liquid chromatography; LLOQ, lower limit of quantification; LS, least square; MS, mass spectrometry; NNRTI, non-nucleoside reverse transcriptase inhibitor;

NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; QD, 'quaque die', once daily; T_{lag}, time delay between dosing and time of appearance of measurable drug in the plasma; T_{max}, time to reach the maximal plasma concentration; ULN, upper limit of normal

Introduction

Although human immunodeficiency virus (HIV) mainly replicates in quiescent cells for the relatively short period of acute infection (Li *et al.*, 2005), the majority of viral production is sustained by actively proliferating cells during chronic infection. Chronic immune stimulation due to persistent HIV replication and microbial translocation across impaired gut-associated lymphoid tissues (GALTs) induces continuous T-cell activation and proliferation of both HIV-infected and bystander cells, ultimately resulting in the exhaustion of the immune system (Ameisen and Capron, 1991; Meyaard *et al.*, 1992; Finkel *et al.*, 1995; Grossman and Paul, 2000; McCune, 2001; Hellerstein *et al.*, 2003). This process is key to the pathogenesis of HIV infection and the progression to acquired immunodeficiency syndrome (AIDS). In turn, HIV-induced T lymphocyte cell activation and consequent cell proliferation provide new target cells for HIV replication (Zagury *et al.*, 1986; Stevenson *et al.*, 1990; Zack *et al.*, 1990; Lori *et al.*, 1992; Korin and Zack, 1998). There is a growing recognition that successful long-term therapy for the treatment of HIV infection should not only reduce viral replication, but also limit the excessive activation of the immune system now proposed as the cause of the eventual progression to AIDS (Douek, 2003; Hunt *et al.*, 2003; Kaufmann *et al.*, 2003; Brenchley *et al.*, 2006). Therefore, new antiviral hyper-activation-limiting therapeutic (AV-HALT) drugs and drug combinations are needed.

Here, we describe a pilot phase I, open-label, randomized, single daily dose of VS411, a single-capsule combination AV-HALT containing didanosine (ddI, 400 mg), an antiviral (AV) and hydroxyurea (HU, 600 mg), a cytostatic agent when given at this dosage. Several pilot studies (Biron *et al.*, 1996; Foli *et al.*, 1997; Montaner *et al.*, 1997; Vila *et al.*, 1997) and four randomized controlled studies (Lori *et al.*, 1997b; Rutschmann *et al.*, 1998; Frank *et al.*, 2004) enrolling more than 500 subjects have demonstrated that HU increased the efficacy of suboptimal backbone regimens (ddI or ddI plus stavudine, d4T).

HU is an antiproliferative agent currently used to treat many malignancies, as well as non-neoplastic diseases such as sickle cell anemia and psoriasis (Donehower, 1992; Charache *et al.*, 1995; Navarra and Preziosi, 1999; Smith, 1999; Davies and Gilmore, 2003). HU is not indicated for the treatment of HIV-infected individuals. However, there are indications that the action of certain nucleoside reverse transcriptase inhibitors (NRTIs) is enhanced by the administration of HU, particularly adenosine analogues such as ddI (2′, 3′-dideoxyinosine), a synthetic purine NRTI active against HIV (Gao *et al.*, 1993; Lori *et al.*, 1994; 1997a; Johns and Gao, 1998). Intracellularly, ddI is converted to the active metabolite dideoxyadenosine triphosphate (ddATP), which decreases viral replication through the inhibition of HIV reverse transcriptase. HU reduces the synthesis of cellular deoxynucleotide triphosphates (dNTPs), in particular deoxyadenosine triphosphate (dATP), by inhibiting ribonucleotide reductase (Yarbro, 1992). Therefore, by decreasing the availability of the natural substrates, HU produces a shift of the ddATP/dATP ratio, thereby favouring the incorporation of ddI over dATP into the nascent HIV DNA chain (Malley *et al.*, 1994; Gao *et al.*, 1995).

In addition, HU can limit virus replication by slowing down T-cell proliferation required for maximal HIV replication as HIV needs actively dividing cells to replicate (De Boer *et al.*, 1998; Ravot *et al.*, 1999; Lisziewicz *et al.*, 2003; Kelly *et al.*, 2004). Cell cycle arrest in G1 phase in response to HU also results in increased levels of CC (or β)-chemokines, the endogenous ligands of the HIV co-receptor CCR5, theoretically hampering HIV entry into the cell (Heredia *et al.*, 2003).

Historically, the combination of HU plus ddI has been used and studied most extensively with daily doses of between 1000 and 1500 mg of HU, and 400 mg of ddI per day. The clinical efficiency of this combination appears limited because of drug-induced toxicities. Moreover, HU is considered by many to increase ddI toxicity. This is in part true. In the Swiss HIV cohort study exploring the effects of ddI plus d4T, with or without HU, a trend in more frequent neuropathies was observed in the HU group (Rutschmann *et al.*, 1998). In the AIDS Clinical Trials Group (ACTG) 5025 study, subjects randomized to the HU arm progressed to a toxicity end point more quickly than those in the indinavir (IDV) + zidovudine + lamivudine (3TC) arm or in the IDV + d4T + ddI arm. The most frequently observed treatment-limiting toxicity was pancreatitis. Three subjects each in the IDV + ddI + d4T + HU and the IDV + ddI + d4T arms developed pancreatitis (Havlir *et al.*, 2001). In addition to pancreatitis,

neuropathy and elevated liver function tests were reasons for treatment discontinuation. Both ddI and d4T, alone, together or in combination with HU have been associated with increased risk of neuropathy. In part, the toxicity of HU and ddI combination has been over-interpreted because many additional contributing factors have been identified. Among these, the most relevant is the ddI dosage. Fatal and non-fatal pancreatitis has occurred during therapy with ddI used alone or in combination regimens in both treatment-naïve and treatment-experienced patients, regardless of degree of immunosuppression. Rates of pancreatitis in ddI arms seemed to be dose dependent (Jablonowski *et al.*, 1995; Alpha, 1996), as indicated in phase III trials using buffered formulations of ddI, with an incidence in adult patients of 1–10% in doses higher than currently recommended, and 1–7% with recommended doses (Electronic Source, Bristol-Myers-Squibb, 2006).

In a review of pancreatitis across 20 ACTG studies, the highest rates of pancreatitis were observed in studies using high daily doses of ddI of 500–750 mg (Reisler *et al.*, 2005). Another contributing factor to the observed toxicity was the lack of adherence to the weight-based ddI dose reduction. Moreover, HU itself did not increase toxicity because pancreatitis rates in ddI/HU arms were not significantly different from the rates for ddI alone. In fact, of the various combinations studied, the highest rates of pancreatitis were seen with the IDV/ddI/d4T were independent from the addition of HU. It is now well known that the d4T and ddI combination is toxic and it is no longer recommended.

For HU, the relationship between dosage and toxicity has already been established (Lori *et al.*, 2005b), although a clear relation between the maximal plasma concentration (C_{max}) and adverse events has not been proven. HU is generally well tolerated. In ACTG 307, the most common HU-associated adverse events were cytopenias which were observed in a dose-dependent manner with neutropenia and thrombocytopenia significantly more common and more severe in subjects who received high-dose HU (1500 mg) (Frank *et al.*, 2004). Recovery from bone marrow toxicity is usually rapid when HU is interrupted. Gastrointestinal (stomatitis, nausea, vomiting, anorexia, constipation, diarrhoea) and dermatological (maculopapular rash, facial erythema, pruritis, alopecia, hyperpigmentation of skin and nails, cutaneous leg ulcers) effects are all well-recognized side effects of HU-containing regimens. HU-induced toxicity is often successfully addressed by using lower HU doses.

In summary, many contributing factors have now been identified for the historical toxicity associated with the HU and ddI combination and, because each factor is avoidable, the use of these drugs can be made much safer.

The present study, VS411-C101, was a pilot phase I, open-label, randomized, single-dose, four-way crossover trial to investigate the fasted and non-fasted residual variance of the area under the plasma concentration–time curve (AUC) and C_{max}, and the oral bioavailability of ddI and HU co-formulated as VS411 and administered as two different fixed-dose combination (FDC) formulations compared to commercially available ddI (Videx EC) and HU (Hydrea) when given simultaneously. Through this formulation, designed to provide a slow release of ddI, we hoped to reduce ddI C_{max} (presumably reducing toxicity), while maintaining total daily exposure without significant changes in the overall AUC (thus maintaining the AV activity).

Methods

VS411 formulations

VS411 is designed as an FDC of 300 mg HU and 200 mg ddI in a single capsule. Each capsule contains two 150 mg HU tablets and two 100 mg enteric-coated ddI tablets. Two VS411 formulations were investigated in the present study, differing only in the content of one of the excipients (2 mg vs. 4 mg hypromellose) contained in the ddI tablets. Hypromellose (hydroxypropyl methylcellulose) is an inert semisynthetic polymer used as an excipient to control the rate of active drug delivery in pharmaceutical products. The first formulation was given the code VS411-2 (2 mg hypromellose) and the second one, VS411-4 (4 mg hypromellose).

Study design

VS411-C101 was a pilot phase I, open-label, randomized, single-dose, four-way crossover trial. All study participants gave written informed consent, and the local regulatory agency and ethical committee approved the study. The trial population consisted of 12 healthy non-HIV-infected adult subjects. Each subject received a single oral dose of VS411 containing 400 mg ddI and 600 mg HU in three sessions (as treatments A, B and D), and in one session a single oral dose of 400 mg ddI and 500 mg HU (as treatment C). Treatment A consisted of two capsules of formulation VS411-2, an FDC formulation of 200/300 mg ddI/HU, administered under fasted conditions. Treatment B consisted of two capsules of formulation VS411-4, an FDC formulation of 200/300 mg ddI/HU, administered under fasted conditions. Treatment C consisted of one capsule of 400 mg Videx EC, and one capsule of 500 mg

Hydrea administered simultaneously under fasted conditions. Randomization of treatments A, B and C in sessions I, II and III was done in such a way that in each session 4 out of 12 subjects received the same treatment, and each subject received a different treatment in each session.

An interim pharmacokinetic analysis was performed after session III to select formulation VS411-2 or VS411-4 for further investigation in session IV. In session IV, each subject received treatment D consisting of two capsules of VS411 as formulation VS411-2 administered following a high-fat breakfast. There was a washout period of at least 1 week between subsequent intakes of trial medication. In each session, full pharmacokinetic profiles of ddI and HU were obtained utilizing plasma samples obtained up to 24 h post-dose. Short-term safety and tolerability were assessed on an ongoing basis.

Study population

Eligible subjects were healthy males or females, aged between 18 and 55 years old, with normal weight (Quetelet index: 18.0–30.0 $kg \cdot m^{-2}$). Pregnant or breastfeeding women, HIV-positive subjects, subjects with current alcohol abuse, active substance abuse interfering with compliance were excluded, as were those subjects with hepatitis A, B or C infection at trial screening. Subjects with a history of pancreatitis, peripheral neuropathy, optic neuritis or renal insufficiency were also excluded. Subjects with laboratory abnormalities at screening such as serum creatinine $\geq$ 1.1 times the upper limit of normal (ULN), lipase or pancreatic amylase $\geq$ 1.1 times the ULN, haemoglobin $\leq$ 109 $g \cdot L^{-1}$, platelet count $\leq 124.999 \times 10^9$ L^{-1}, absolute neutrophil count $\leq 1.3 \times 10^9$ L^{-1}, aspartate aminotransferase or alanine aminotransferase $\geq$ 1.25 times the ULN, bilirubin $\geq$ 1.1 times the ULN were excluded, as were those with any other biochemical values of Division of Acquired Immunodeficiency Syndrome (DAIDS) grade 2 or above.

Study monitoring, data collection, analysis and statistical methods

Study monitoring, data collection and analysis were performed by an independent contract research organization, IATEC, Amsterdam, The Netherlands. Pharmacokinetic and statistical analyses were performed by Kinesis Pharma B.V., Breda, The Netherlands, using the validated computer program WinNonlin Professional (version 4.1; Pharsight Corporation, Mountain View, CA, USA). Non-compartmental analysis model 200 (extravascular input, plasma data) was applied for the pharmacokinetic analysis. Furthermore, Microsoft Excel (version 2007; Microsoft, Redmond, WA, USA) and/or SAS (version 9.1.3; SAS Institute Inc., Cary, NC, USA) were used.

Descriptive statistics were calculated for the plasma concentrations of ddI and HU at each time point and for the derived pharmacokinetic parameters. Statistics included sample size (n), mean, standard deviation, percentage of coefficient of variation (%CV), geometric mean, median, minimum and maximum.

Statistical analyses were performed for ddI and HU using treatment A as test and treatment C as reference, and using treatment B as test and treatment C as reference, respectively. The primary pharmacokinetic parameters were C_{max} (maximal plasma concentration), AUC_{last} (area under the plasma concentration–time curve from time of administration up to the last time point with a measurable concentration post-dosing), and AUC_∞ (area under the plasma concentration–time curve extrapolated to infinity) on the logarithmic scale. AUC_∞ was rejected as primary parameter for a treatment if more than half of the subjects did not have a reliable value for that treatment. Only paired observations for test and reference were included in the statistical analysis. The least square (LS) means of the primary parameters for each treatment group were estimated with a linear mixed effects model controlling for treatment, sequence and period as fixed effects and subject as a random effect. A 90% confidence interval (CI) was constructed about the difference between the LS means of test and reference. Both the difference between the LS means and the 90% confidence limits were retransformed to the original scale. Treatment and period effects were considered significant at the 5% level, and sequence effects were considered significant at the 10% level.

Statistical analyses were also performed for ddI and HU using treatment D as test and treatment A as reference. The primary pharmacokinetic parameters were C_{max}, AUC_{last} and AUC_∞ on the logarithmic scale. The same procedures were used as described above, except that the LS means of the primary parameters for each treatment group were estimated with a linear mixed effects model controlling for treatment as fixed effect and subject as a random effect. Treatment effects were considered significant at the 5% level.

The 90% CIs, the bioequivalence limits [80, 125%], were taken into account to evaluate the data; the P values of the treatment effects, and the sample size were also used.

T_{max} (time to reach the maximal plasma concentration) values of ddI and HU were subjected to the univariate procedure in SAS (two-sided Wilcoxon signed-rank test), comparing treatments A and C,

treatments B and C and treatments D and A as test and reference, respectively.

T_{lag} (the time delay between dosing and time of appearance of measurable drug in the plasma) was added as additional parameter to be calculated for ddI as the time point prior to the time point corresponding to the first measurable concentration.

As commercial Hydrea is supplied as 500 mg capsules and HU is believed to exhibit linear pharmacokinetics at the doses being investigated, the derived pharmacokinetic parameters of HU administered as VS411-2 and VS411-4 (treatment A and B, respectively) were dose-normalized to 500 mg for comparison with HU administered as Hydrea (treatment C) for statistical analysis and calculation of individual treatment ratios.

Measurement of ddI and HU concentrations

The bioanalysis of ddI and HU was performed by ABL B.V., Assen, The Netherlands. Plasma concentrations of ddI and HU were determined using a lower limit of quantification (LLOQ) of 10.0 ng·mL^{-1} for ddI and 20.0 ng·mL^{-1} for HU. Plasma concentrations were determined using a validated liquid chromatography–mass spectrometry/mass spectrometry (API4000 LC–MS/MS system, Applied Biosystems, Toronto, ON, Canada) method. The method consisted of a protein precipitation extraction with acetonitrile, followed by derivatization with dansyl chloride. After derivatization, the sample was diluted with water and injected in the LC–MS/MS system. HU and ddI were analysed in the same analytical batch, although ddI is not affected by the derivatization process. The validated analytical range of the assay in human heparin plasma was 20.0–10 000 ng·mL^{-1} for HU and 10.0–5000 ng·mL^{-1} for didanosine. Data acquisition was performed using Analyst (version 1.4) software of Applied Biosystems. A 9-point calibration curve was fitted to the model $Y = A + BX + CX^2$ using X^{-1} for HU and X^{-2} for ddI as weighting factor, where Y is the peak area ratio of the analyte and the internal standard, X is the nominal calibration level in ng·mL^{-1}, A is the intercept, B is the slope and C is a description of the curvature. Unknowns were calculated using the inverse of the response model. In total, three validation runs were performed for the determination of the validation parameters regarding calibration, recovery, accuracy, precision, specificity and stability. The quality control samples (QCs) used were at the LLOQ (LQC), low level (QC-L), medium level (QC-M), high level (QC-H) and 'over the curve' (OQC). Each validation run consisted of a set of nine calibrators. For each QC level, the overall accuracy, expressed as absolute bias, had to be ≤15%, except for the LQC where an absolute bias of ≤20% was allowed. Per QC level, the overall precision (total CV) and repeatability (intra-run CV) had to be ≤15%, except at the LQC, where a CV of ≤20% was allowed. The intra-batch CV for HU was 5.8, 4.9 and 3.2% at the low, medium and high levels, respectively. The inter-batch CV for HU was 9.3, 5.7 and 4.3% at the low, medium and high levels, respectively. The intra-batch CV for ddI was 5.7, 5.7 and 3.4% at the low, medium and high levels, respectively. The inter-batch CV for ddI was 7.1, 6.4 and 4.5% at the low, medium and high levels, respectively.

Results

Pharmacokinetics of ddI in VS411

Figure 1 shows the linear median plasma concentration–time curves of ddI after administration of a single dose of: 400/600 mg ddI/HU (two capsules of formulation VS411-2 under fasted conditions), 400/600 mg ddI/HU (two capsules of formulation VS411-4 under fasted conditions), 400 mg ddI + 500 mg HU (one capsule Videx EC and one capsule Hydrea under fasted conditions), 400/600 mg ddI/HU (two capsules of formulation VS411-2 after a high-fat breakfast).

A summary list of key pharmacokinetic parameters of ddI for all treatments is presented in Table 1.

Compared to Videx EC, both VS411-2 and VS411-4 formulations induced a delayed absorption, as well as a decrease in C_{max}. Median t_{max} observed was 3.0 (1.0–4.0) h with VS411-2, 4.0 (2.5–24.0) h with VS411-4, formulations of ddI and 2.0 (1.0–5.0) h for the commercially available formulation. T_{lag} was similar for all treatments administered under fasted conditions; however, there was an increase in median t_{lag} and t_{max} under fed compared to fasted conditions. Lower median values of C_{max} of ddI were observed for both the VS411-2 and VS411-4 formulations (890 and 832 ng·mL^{-1}, respectively) compared to the commercially available ddI formulation (1100 ng·mL^{-1}) when administered in the fasted state. Median AUC_{last} and AUC_{∞} values for Videx EC were 3518 and 3552 ng·h·mL^{-1}, respectively; the median values for the VS411-2 formulation were 3184 and 3245 ng·h·mL^{-1}, respectively; and for the VS411-4 formulation 3299 and 3338 ng·h·mL^{-1}, respectively. All the treatments showed comparable ddI half-life ($t_{1/2term}$) (approximately 2 h) in the fasted state. A higher inter-subject variability (based on the %CV of C_{max}, AUC_{last} and AUC_{∞}) for ddI was observed for the VS411-4 formulation compared to both the VS411-2 formulation and the commercially available formulation. When ddI was administered under fed

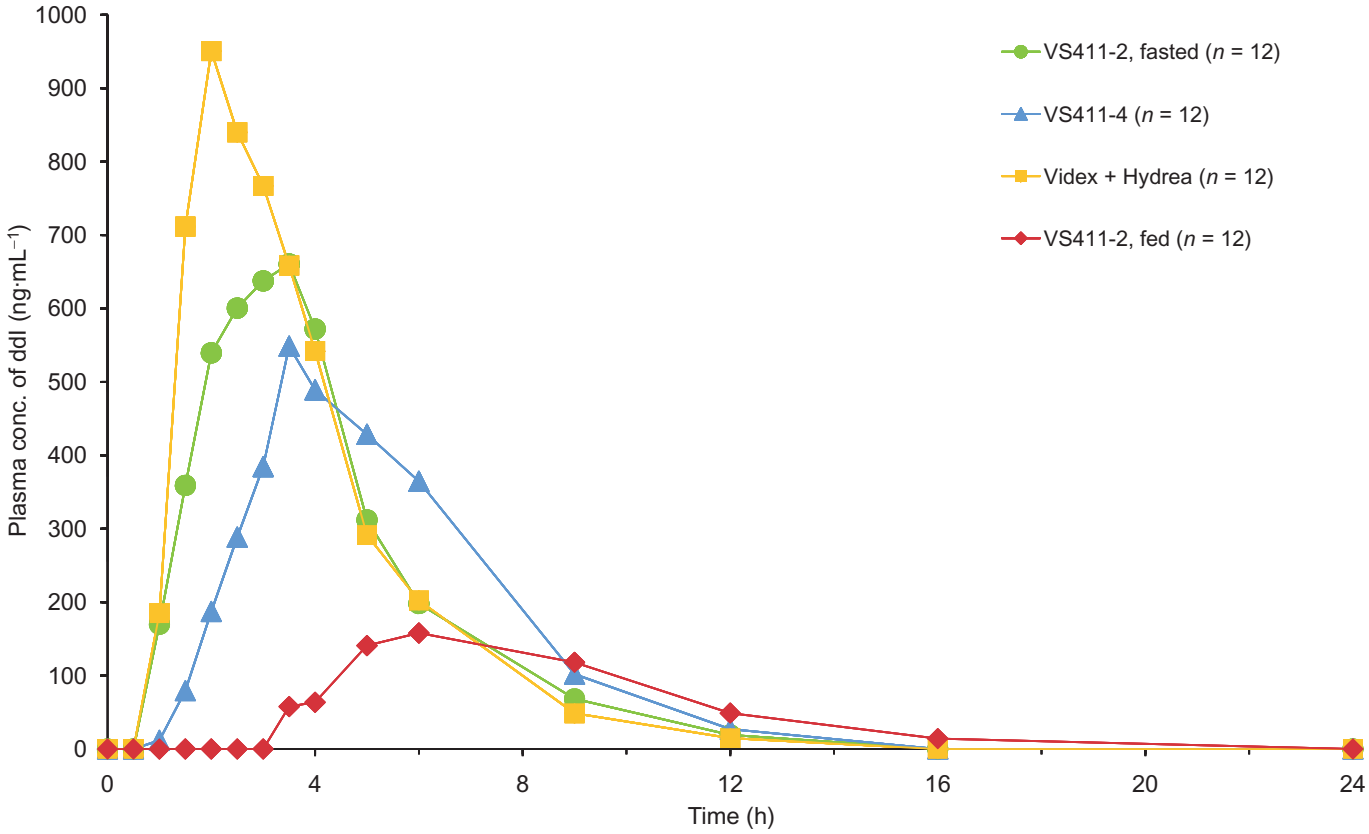

Figure 1

Median plasma concentration–time curves of ddI after administration of a single dose of 400/600 mg ddI/HU (two capsules of formulation VS411-2, fasted), 400/600 mg ddI/HU (two capsules of formulation VS411-4, fasted), 400 mg ddI plus 500 mg HU (one capsule Videx EC plus one capsule Hydrea, fasted), 400/600 mg ddI/HU (two capsules of formulation VS411-2, fed).

Table 1

Pharmacokinetic parameters of ddI

Pharmacokinetics of ddI median (range)	VS411-2	VS411-4	Videx EC + Hydrea	VS411-2, fed
C_{max} (ng·mL^{-1})	890 (325–2600)	832 (381–3150)	1100 (802–2240)	334 (114–1450)
t_{lag} (h)	0.50 (0.00–3.00)	0.75 (0.00–16.00)	0.50 (0.00–1.50)	3.00 (0.50–16.00)
t_{max} (h)	3.0 (1.0–4.0)	4.0 (2.5–24.0)	2.0 (1.0–5.0)	7.5 (2.5–24.0)
AUC_{last} (ng·h·mL^{-1})	3184 (968–6286)	3299 (1433–7588)[a]	3518 (2181–5872)	1591 (403–5383)[a]
AUC_{∞} (ng·h·mL^{-1})	3245 (1004–6334)	3338 (1493–7613)[a]	3552 (2208–5927)	2708 (1197–5489)[b]
$t_{1/2term}$ (h)	1.687 (1.431–2.360)	1.596 (1.390–4.730)[a]	1.599 (1.436–2.706)	1.494 (1.361–1.648)[b]

$n = 12$ unless otherwise specified.
[a]$n = 11$.
[b]$n = 7$.

conditions, inter-subject variability in t_{lag} and t_{max} further increased.

Table 2 provides a statistical comparison analysis of the pharmacokinetic parameters of ddI. A decrease in ddI C_{max}, AUC_{last} and AUC_{∞} was observed after treatment with VS411-2 under fasted conditions (test) compared to Videx EC plus Hydrea under fasted conditions (reference) (Table 2, upper panel). LS mean ratios (test/reference) for these parameters were 71.30, 84.28 and 84.75%, respectively, when ddI was administered as the VS411-2 formulation compared to the commercially available

Table 2

Summary of the statistical analysis of pharmacokinetic parameters for ddI

Parameter	LS means Videx EC + Hydrea (reference)	VS411-2 (test)	LS mean ratio (%)	90% CI (%)
C_{max} (ng·mL^{-1})	1209	862	71.30	53.41–95.17
AUC_{last} (ng·h·mL^{-1})	3547	2990	84.28	68.09–104.30
AUC_{∞} (ng·h·mL^{-1})	3593	3045	84.75	68.77–104.50
Parameter	**Median Videx EC + Hydrea (reference)**	**VS411-2 (test)**	**Treatment difference median**	**90% CI (%)**
t_{max} (h)	2.0	3.0	0.75	0.5–1.5
Parameter	**LS means Videx EC + Hydrea (reference)**	**VS411-4 (test)**	**LS mean ratio (%)**	**90% CI (%)**
C_{max} (ng·mL^{-1})	1209	885	73.24	49.37–108.70
AUC_{last} (ng·h·mL^{-1})	3498[a]	3247[a]	92.82	63.65–135.30
AUC_{∞} (ng·h·mL^{-1})	3545[a]	3322[a]	93.70	64.77–135.60
Parameter	**Median Videx EC + Hydrea (reference)**	**VS411-4 (test)**	**Treatment difference median**	**90% CI (%)**
t_{max} (h)	2.0	4.0	2.25[c]	1.5–3.0
Parameter	**LS means VS411-2, fasted (reference)**	**VS411-2, fed (test)**	**LS mean ratio (%)**	**90% CI (%)**
C_{max} (ng·mL^{-1})	862	351	40.78	23.84–69.75
AUC_{last} (ng·h·mL^{-1})	3110[a]	1663[a]	52.49	29.63–93.01
AUC_{∞} (ng·h·mL^{-1})	3137[b]	2644[b]	84.29	54.27–130.90
Parameter	**Median VS411-2, fasted (reference)**	**VS411-2, fed (test)**	**Treatment difference median**	**90% CI (%)**
t_{max} (h)	3.0	7.5	5.75[c]	2.0–9.5

n = 12 unless otherwise specified.
[a]n = 11.
[b]n = 7.
[c]Statistically significant difference.

formulation. For $C_{max,}$ the 90% CIs did not include 100% (the scaled LS means of the reference), while for AUC_{last} and AUC_{∞}, 100% was included within the 90% CIs. Although the observed differences were not statistically significant, the results show a clear trend for a lower C_{max} (P = 0.0603). Median t_{max} for ddI administered as VS411-2 under fasted conditions was 3 h, while median t_{max} for the reference ddI was 2 h, although this increase was not statistically significant.

Table 2, middle panel shows a summary of the statistical analysis of the pharmacokinetic parameters of ddI after treatment with VS411-4 (test) and Videx EC plus Hydrea (reference) formulations. LS mean ratios for C_{max}, AUC_{last} and AUC_{∞} were decreased by 27, 7 and 6% when ddI was administered as VS411-4 formulation compared to Videx EC plus Hydrea administration. The 90% CIs did include 100% (the scaled LS mean of the reference) for all the parameters. Also in this case, these

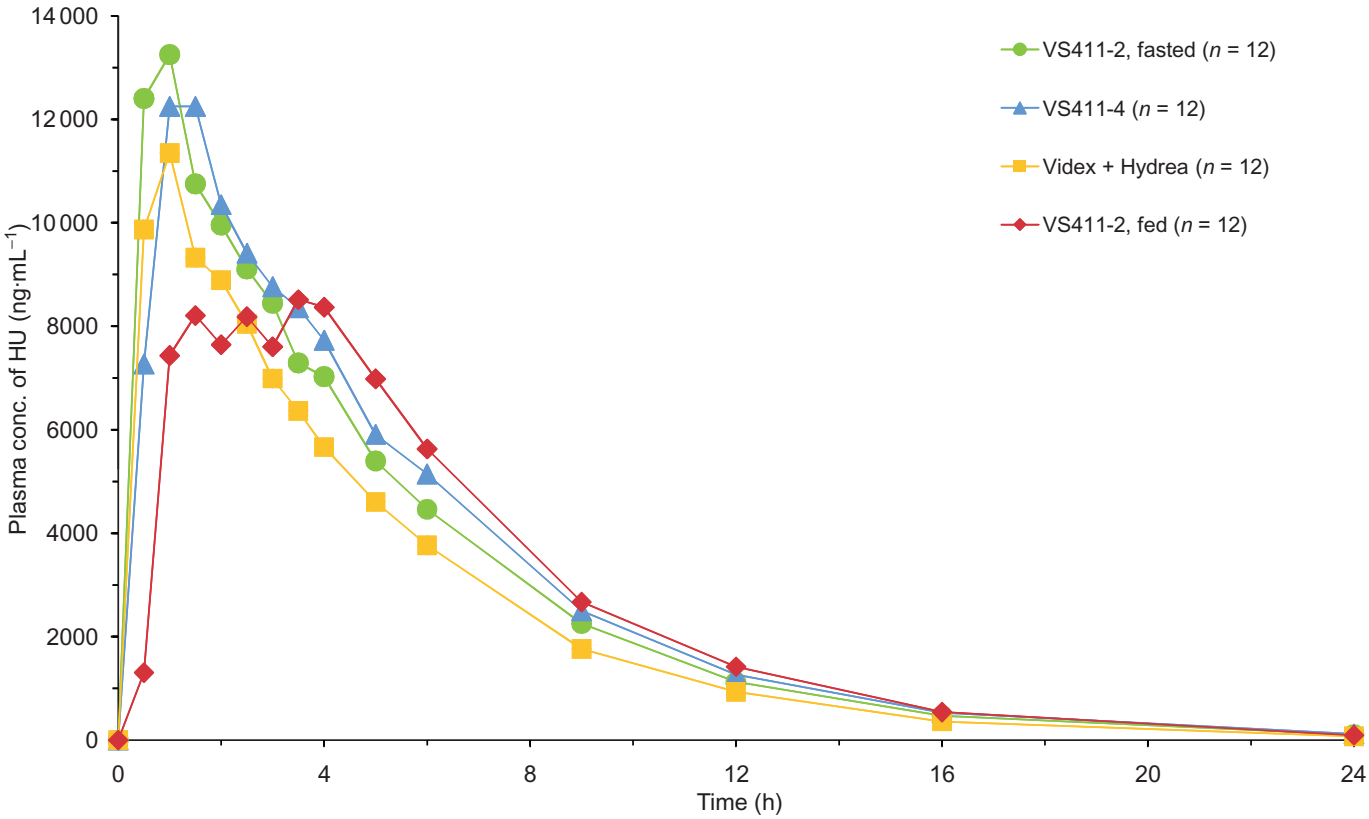

Figure 2

Median plasma concentration–time curves of HU after administration of a single dose of 400/600 mg ddI/HU (two capsules of formulation VS411-2, fasted), 400/600 mg ddI/HU (two capsules of formulation VS411-4, fasted), 400 mg ddI + 500 mg HU (one capsule Videx EC plus one capsule Hydrea, fasted), 400/600 mg ddI/HU (two capsules of formulation VS411-2, fed).

differences were not statistically significant. However, median t_{max} observed for ddI administered in the VS411-4 formulation (4 h) was significantly higher (P = 0.0020) when compared to Videx EC plus Hydrea administration (2 h).

The statistical analysis of the pharmacokinetic parameters of ddI after administration of VS411-2 under fasted (reference) or fed conditions (test) is shown in Table 2, lower panel. For C_{max} and AUC_{last}, the 90% CIs did not include 100% (the scaled LS mean of reference), while it was included for AUC_{∞}. Decreases in the pharmacokinetic parameters were observed, but only C_{max} was significantly lowered (LS mean ratio = 40.78%, P = 0.0120). When ddI was administered in the VS411-2 formulation under fed conditions, there was a significant increase in t_{max} compared to administration following a high-fat breakfast (t_{max} under fasted condition = 3.0 h, t_{max} under fed condition = 7.5 h, $P < 0.001$).

Pharmacokinetics of HU in VS411

Figure 2 shows the median plasma concentration–time curves of HU after administration of a single dose of: 400/600 mg ddI/HU (two capsules of formulation VS411-2 under fasted conditions), 400/600 mg ddI/HU (two capsules of formulation VS411-4 under fasted conditions), 400 mg ddI plus 500 mg HU (one capsule Videx EC plus one capsule Hydrea under fasted conditions), 400/600 mg ddI/HU (two capsules of formulation VS411-2 after a high-fat breakfast).

A summary list of key pharmacokinetic parameters of HU for all treatments is presented in Table 3.

As expected, due to difference in dose (600 vs. 500 mg), median C_{max}, AUC_{last} and AUC_{∞} of HU were higher after administration of the VS411-2 and VS411-4 formulations compared to the commercially available formulation. The median plasma concentration–time curves of all treatments under fasted conditions showed relatively fast absorption and elimination, while under fed conditions absorption was prolonged and C_{max} was lower. Variability was slightly increased when VS411-2 was administered under fed conditions. For all HU treatments administered under fasted conditions, t_{max} was comparable, while under fed conditions, median t_{max} was increased. There was a considerable decrease of C_{max} when HU was administered as VS411-2 under

Table 3

Pharmacokinetic parameters for HU

Pharmacokinetics of HU median (range)	VS411-2	VS411-4	Videx EC + Hydrea	VS411-2, fed
C_{max} (ng·mL^{-1})	16 200 (11 500–20 300)	14 900 (9740–21 900)	13 050 (10 600–18 800)	10 500 (7150–15 100)
t_{max} (h)	0.75 (0.50–2.00)	1.00 (0.50–3.00)	1.00 (0.50–2.00)	1.75 (0.50–5.00)
AUC_{last} (ng·h·mL^{-1})	69 110 (57 740–80 100)	69 870 (58 660–84 230)	55 480 (46 220–70 910)	64 300 (46 270–79 930)
AUC_{∞} (ng·h·mL^{-1})	69 770 (58 210–80 430)	70 370 (58 950–84 790)	55 910 (46 460–71 230)	65 020 (46 650–80 850)
$t_{1/2term}$ (h)	3.414 (2.879–3.733)	3.302 (2.703–3.904)	3.208 (2.781–3.794)	3.238 (2.761–3.747)

n = 12 for all tested parameters.

fed conditions. Also, AUC_{last} and AUC_{∞} were slightly lower under fed conditions compared to fasted. HU half-life was similar in all treatments (approximately 3 h).

In Table 4, a summary of the statistical analysis of the pharmacokinetic parameters of HU after test and reference treatments is shown. For statistical analysis and calculation of individual treatment ratios, C_{max}, AUC_{last} and AUC_{∞} of HU administered as VS411-2 and VS411-4 were dose-normalized to 500 mg for comparison with HU administered as Hydrea.

After administration of VS411-2 (test) and Videx EC plus Hydrea (reference) under fasted conditions, LS mean ratios for C_{max}, AUC_{last} and AUC_{∞}, as well as median t_{max}, of HU were similar for the two formulations (Table 4, upper panel), and the 90% CIs around LS mean ratios for these parameters did include 100% (the scaled LS mean of reference).

Table 4, middle panel shows a summary of the statistical analysis of the pharmacokinetic parameters of HU after administration of VS411-4 (test) and Videx EC plus Hydrea (reference) under fasted conditions. Also in this case, there was no statistically significant difference between the parameters between the two formulations; in fact, the 90% CIs around LS mean ratios for these parameters did include 100% (the scaled LS mean of reference).

A summary of the statistical analysis of the pharmacokinetic parameters of HU after administration of VS411-2 under fasted (reference) and fed (test) conditions is shown in the lower panel of Table 4. The differences between fasted and fed conditions for all parameters were statistically significant. Based on the LS mean ratios, C_{max}, AUC_{last} and AUC_{∞} of HU were decreased by 33% (P = 0.0004), 8% (P = 0.0093) and 8% (P = 0.0097), respectively, after administration of VS411-2 under fed conditions compared to administration under fasted conditions. The 90% CIs did not include 100% (the scaled LS mean of reference) for any of these parameters. T_{max} was significantly increased when VS411-2 was administered under fed conditions compared to fasted (treatment difference median was 1.0 h, P = 0.0244).

Adverse events

No pharmacokinetic–safety correlations were apparent as none of the subjects discontinued the trial prematurely nor experienced a severe adverse event (grade ≥3). Seven subjects had 24 non-serious adverse events. Only three adverse events (hypocholic stools, loose stools and diarrhoea) were found to be possibly related with the study treatment, and they were all mild. These adverse events occurred during treatments B, C and D, one in each of these groups. Among the adverse events not related to the treatment, only one was of a moderate severity and occurred during treatment with Videx EC plus Hydrea. The frequencies of subjects with adverse events and the frequencies of adverse events in all treatments were similar. Few subjects experienced changes in their blood chemistry tests. One of the more frequent changes was observed in the total cholesterol during treatment B, where three subjects out of 12 changed from a normal level to a grade 1 level. These three subjects also experienced grade 1 abnormalities during the other treatments. Another three subjects showed an increase in the pancreatic amylase levels during treatment C, one to grade 1 abnormal level and two to grade 2. For each subject, subsequent pancreatic amylase measurements were significantly lower; therefore, none of the elevated values were considered to be clinically significant or suspected of being a symptom of pancreatitis. Frequencies of subjects with abnormalities in blood chemistry tests were similar among the treatment groups. The group treated with Videx EC plus Hydrea had the highest number of subjects, 50%, experiencing an increase under DAIDS grading, while only 25% of the subjects treated with VS411-2 formulation under fed conditions had experienced increases in DAIDS grading.

Table 4

Summary of the statistical analysis of pharmacokinetic parameters for HU

Parameter[a]	LS means Videx EC + Hydrea (reference)	VS411-2 (test)	LS mean ratio (%)	90% CI (%)
C_{max} (ng·mL^{-1})	13 370	13 160	98.42	90.19–107.40
AUC_{last} (ng·h·mL^{-1})	56 370	56 810	100.80	97.22–104.50
AUC_{∞} (ng·h·mL^{-1})	56 760	57 290	100.90	97.48–104.50
Parameter	**Median Videx EC + Hydrea (reference)**	**VS411-2 (test)**	**Treatment difference median**	**90% CI (%)**
t_{max} (h)	1.0	0.75	0.0	0.0–0.0
Parameter[a]	**LS means Videx EC + Hydrea (reference)**	**VS411-4 (test)**	**LS mean ratio (%)**	**90% CI (%)**
C_{max} (ng·mL^{-1})	13 370	12 570	94.04	82.48–107.20
AUC_{last} (ng·h·mL^{-1})	56 370	58 990	104.60	99.97–109.50
AUC_{∞} (ng·h·mL^{-1})	56 760	59 500	104.80	100.20–109.70
Parameter	**Median Videx EC + Hydrea (reference)**	**VS411-4 (test)**	**Treatment difference median**	**90% CI (%)**
t_{max} (h)	1.0	1.0	0.25	0.0–1.5
Parameter	**LS means VS411-2, fasted (reference)**	**VS411-2, fed (test)**	**LS mean ratio (%)**	**90% CI (%)**
C_{max} (ng·mL^{-1})	15 790	10 570	66.97	57.91–77.44
AUC_{last} (ng·h·mL^{-1})	68 180	62 870	92.22	88.05–96.59
AUC_{∞} (ng·h·mL^{-1})	68 740	63 460	92.31	88.16–96.66
Parameter	**Median VS411-2, fasted (reference)**	**VS411-2, fed (test)**	**Treatment difference median**	**90% CI (%)**
t_{max} (h)	0.75	1.75	1.0[b]	0.0–3.0

n = 12 for all tested parameters.
[a]Parameter of test treatment dose normalized to 500 mg.
[b]Statistically significant difference.

Discussion

VS411 is the first-in-class of a new family of drugs known as AV-HALTs that have been designed to both suppress HIV replication and limit excessive immune activation. It combines in one capsule ddI, an AV, and HU, an immune system HALT agent. The results of this study are in agreement with the development plan for a formulation of ddI that results in a lower C_{max} (presumably reducing toxicity), but a similar total exposure compared to the reference Videx EC formulation. It has been shown for many drugs that the relationship between drug exposure and toxicity is extremely important. In the treatment of HIV disease, adverse events are a serious clinical problem and represent the predominant reason for treatment discontinuation in many clinical studies. Many studies have shown a relationship between various pharmacokinetic parameters and drug toxicity or efficacy of anti-HIV drugs such as protease inhibitors (PIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs). For example, the

renal toxicity induced by the PI IDV has been related to increased C_{max} (Gatti, 2001), and urological complications occurring during IDV treatment were found to be associated with elevated IDV plasma concentrations in 80% of patients in a clinical study (Dieleman *et al.*, 1999). In another PI study, it has been shown that high maximum saquinavir plasma concentration is correlated to side effects (Lotsch *et al.*, 2007). Moreover, individuals with higher ritonavir concentrations (both C_{max} and C_{min}, minimal plasma concentration) are at higher risk of experiencing neurological or gastrointestinal side effects (Gatti, 2001). In individuals treated with the NNRTI efavirenz (EFV) plus other antiretroviral agents, CNS toxicity has been reported to be three times more frequent in subjects with high EFV plasma levels (Marzolini *et al.*, 2001).

Among the two tested VS411 formulations, VS411-2 was selected for the following reasons: a clear trend for a lower C_{max} was observed for VS411-2 ($P = 0.0603$) with the lack of statistical significance most likely related to the sample size in relation to the observed variability; the 90% CIs around the LS mean ratio of C_{max} did not include 100%, another statistical indication that the C_{max} of VS411-2 was lower than for the Videx EC formulation; there was less variability among subjects seen with the VS411-2 compared to the VS411-4 formulation (based on the %CV of C_{max}, AUC_{last} and AUC_{∞}); the absorption of ddI from the VS411-4 formulation was less predictable (for instance, one subject had a lag time of 16 h), while the lag time of ddI for the VS411-2 formulation was similar to that of Videx EC; and the VS411-2 formulation had the most favourable safety profile.

The AUC_{last} and AUC_{∞} of ddI were also decreased when ddI was administered as the VS411-2 formulation compared to Videx EC, although the observed differences were not statistically significant. Additionally, the 90% CIs did include 100%, an observation in line with the intention to develop a formulation with a lower C_{max}, but with a similar exposure compared to Videx EC.

The LS mean ratios of C_{max}, AUC_{last} and AUC_{∞} of HU in the VS411-2 and VS411-4 formulation were almost identical to those of the commercially available Hydrea formulation when adjusted for differences in doses administered. In fact, 90% CIs around the LS mean ratios were within the commonly accepted 80–125% bioequivalence limits for both formulations. HU half-lives were comparable between all treatments (approximately 3 h).

This study also investigated the effect of food on ddI and HU absorption. Didanosine is degraded if it is not protected from stomach acid, resulting in low oral bioavailability. Commercial preparations of ddI have addressed this fact by either buffering the gastric pH to a higher value or protecting the drug using an enteric coating. Despite these approaches, it is known that food decreases ddI C_{max} and AUC by approximately 55% with the buffered formulation (Shyu *et al.*, 1991), and decreases the C_{max} and AUC of the enterically coated delayed-release capsules approximately by 46 and 19%, respectively (Damle *et al.*, 2002). All currently marketed ddI formulations require administration on an empty stomach. There is no contraindication to the administration of HU with food, although there are no data on the effect of food on the absorption of HU.

As expected, food decreased ddI exposures significantly and increased inter-subject variability in the test formulations. Based on the LS mean ratios, C_{max}, AUC_{last} and AUC_{∞} of ddI decreased by 59% ($P = 0.0120$), 48% ($P = 0.0684$) and 16% ($P = 0.4791$), respectively, under fed conditions compared to fasted conditions. For C_{max} and AUC_{last}, the 90% CIs did not include 100% (the scaled LS mean of reference), while it was included for AUC_{∞}. Moreover, food prolonged the median time to reach ddI maximum plasma concentration by 5.75 h ($P < 0.001$).

Unexpectedly, there was a decrease also in C_{max}, AUC_{last} and AUC_{∞} of HU, based on LS mean ratios, by 33% ($P = 0.0004$), 8% ($P = 0.0093$) and 8% ($P = 0.0097$), respectively, under fed conditions compared to fasted conditions. Although for AUC_{last} and AUC_{∞} the extent of decrease was relatively small, the decreases in C_{max}, AUC_{last} and AUC_{∞} were statistically significant, probably because the CIs around the LS mean ratios were narrow. Moreover, food prolonged the time to reach HU maximum plasma concentration by 1.0 h ($P = 0.0244$). To our knowledge, this is the first report showing a reduction in HU absorption when administered with food.

Through the flexibility of the VS411 formulation, it is possible to reduce the dose of HU in order to potentially limit toxicities. A reduction in HU dosage is supported by the results of the RIGHT 702 study which demonstrated that a lower HU dose of 600 mg daily achieved better antiretroviral activity than did higher doses of 800–1200 mg, together with a better CD4+ cell count increase and fewer adverse effects (Lori *et al.*, 2005b). This was consistent with the *in vitro* observation that decreasing HU concentrations are associated with the transition from cytotoxic (50–100 μM) to cytostatic (10 μM) effects (Lori *et al.*, 2005a), indicating that lowering HU doses may be an option in combination with ddI. Because combining ddI with HU reduces the 90% inhibitory concentration of ddI by approximately sixfold (Gao *et al.*, 1994), it is also reasonable to investigate dose reductions of ddI [e.g. from

400 mg once daily (QD) to 300 or possibly 200 mg QD)] to further improve the tolerability of HU plus ddI. In fact, a phase IIa trial utilizing the VS411-2 formulation has been fielded to identify the optimal doses of HU plus ddI for further evaluation in phase IIb trials in the treatment of HIV disease. In this study, subjects have been randomized to one of the following five study arms: ddI 400 mg QD plus HU 600 mg QD, ddI 400 mg QD plus HU 300 mg QD, ddI 200 mg QD plus HU 900 mg QD, ddI 200 mg QD plus HU 600 mg QD, ddI 200 mg QD plus HU 300 mg QD.

In conclusion, VS411 appears to be a suitable candidate to test the efficacy of AV-HALTs during chronic HIV-1 infection.

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Conflict of interest

The authors declare they are employees of, or consultants to, ViroStatics.

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