

Expert Opinion

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Antiretroviral drugs in development. A report from HIV DART 2008: frontiers in drug development for antiretroviral therapies

9 – 12 December 2008, Rio Grande, Puerto Rico

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HIV DART 2008, Frontiers in Drug Development for Antiretroviral Therapies, was held on 9 – 12 December 2008 in Puerto Rico. The stated purpose of this biannual international meeting is to assemble clinicians, researchers and basic scientists together to advance the knowledge of the ongoing drug development processes in antiretroviral research. However, there were also presentations of thematic reviews of clinical topics such as prevention and drug resistance; HIV therapy in developing countries; and HIV/hepatitis and other coinfections. This report focuses on the main theme of the conference, the antiretroviral drug development pipeline. The author is solely responsible for the selection of topics and presentations to be included in this report.

Expert Opin. Investig. Drugs (2009) 18(4):549-553

1. Introduction

Currently, there are 22 FDA-approved antiretroviral drugs, categorized into six classes according to their mechanism of action. Numerous additional agents are in various stages of preclinical and clinical development. Some of these new agents belong to existing drug classes but were developed with the intent of improving the pharmacokinetic and safety profile and providing a higher genetic barrier to the development of resistance without sacrificing antiviral potency. Others represent entirely new classes of agents directed against newer targets and with unique mechanisms of action.

2. Antiretroviral drugs in development

2.1 Amdoxovir

Amdoxovir (diaminopurine dioxolone, DAPD) is a selective guanosine analogue nucleoside reverse transcriptase inhibitor (NRTI) under clinical development. It is phosphorylated to its active moiety, DXG-TP, which has an intracellular half-life of 16 h. It has been shown to be synergistic in human lymphocytes with zidovudine (ZDV), and in combination prevents selection of thymidine analogue mutations (TAMs) and K65R mutations. Results of RFS-AMDX-203, a Phase I clinical trial that evaluated the pharmacokinetics and anti-HIV activity of DAPD, were presented [1]. Twenty-four HIV-infected participants with CD4⁺ cell counts > 200 cells/ml and plasma HIV-1 RNA \geq 5000 copies/ml but not receiving antiretroviral therapy in the preceding 90 days were randomized into six arms: DAPD 500 mg twice daily alone (n = 6); DAPD 500 mg twice daily plus ZDV 200 mg

b.i.d. ($n = 6$); DAPD 500 mg twice daily plus ZDV 300 mg ($n = 6$); placebo twice daily ($n = 2$); ZDV 200 twice daily ($n = 2$); ZDV 300 twice daily ($n = 2$). Treatment duration was 10 days with study participants followed up for an additional 10 days. Participants underwent daily HIV-1 RNA measurements as well as PK sampling on days 1 and 10. The overall mean baseline HIV-1 RNA was $4.5 \log_{10}$ copies/ml and the overall mean CD4⁺ cell count was 417 cells/mm^3 . At the end of 10 days, the combination of DAPD with ZDV reduced HIV-1 RNA by 2 logs and was significantly better than DAPD alone ($p \leq 0.0001$). Adverse events were reported as mild to moderate and transient. There were no serious adverse events or drug-related adverse events leading to study discontinuation and there was no significant difference in mean change of HIV-1 RNA at 10 days between DAPD 500 mg + ZDV 200 mg and DAPD 500 mg + ZDV 300 mg. The combination of DAPD and ZDV reduced the variability observed with DAPD monotherapy.

Results of the pharmacokinetic portion of the study were reported separately [2]. The coadministration of DAPD with ZDV did not affect the systemic or renal clearance of either drug.

2.2 GS-9148

GS-9148 is a new NRTI in early clinical development. Its prodrug, GS-9131, has been shown to have a favorable resistance profile against common NRTI mutations such as K65R, M184V, L74V, and TAMS. GS-9148 was evaluated for its potential to cause mitochondrial and renal toxicity [3]. Mitochondrial toxicity was assessed by measuring mitochondrial and chromosomal DNA levels and inhibition of mtDNA polymerase in the HepG2 cell line. Renal effect was assessed by determining the efficiency of GS-9148 transport in cell lines overexpressing human influx and efflux transporters. Uptake into the kidney was determined using human renal cortex tissue and accumulation in renal tissue was evaluated by measuring [^{14}C] GS-9131 in the kidney of dogs.

Treatment of HepG2 cells with GS-9148 did not result in a selective depletion of mitochondrial DNA. GS-9148 was a more efficient substrate for the renal efflux transporter MRP4 than for the influx (renal uptake) transporters hOAT1 and hOAT3. GS-9148 was inefficiently taken up by human renal cortex tissue *in vitro*. Low accumulation of [^{14}C] GS-9131 was also found in the kidneys of dogs.

2.3 TMC278

TMC278 is an investigational non-nucleoside reverse transcriptase inhibitor (NNRTI) with activity against HIV strains harboring key NNRTI resistance mutations. Results at 96 weeks of TMC278-C204, a 5-year Phase IIb dose-ranging study in treatment-naïve patients, were presented at HIV DART 2008 [4]. A total of 368 patients were randomized to receive three (25, 75 or 150 mg) blinded, once-daily doses of TMC278 or open-label efavirenz (EFV)

600 mg once daily, all in combination with coformulated zidovudine/lamivudine or tenofovir/emtricitabine. At 96 weeks, virological response (< 50 copies/ml; ITT-TLOVR) in the TMC arms (71 – 76%) was similar to that achieved with EFV (71%). Similar mean CD4⁺ cell count increases from baseline were also observed (from 146 to 172 vs 160 cells/mm³). Patients randomized to the TMC278 arms had significantly lower incidences of rash (9% vs EFV: 21%, $p < 0.01$) and nervous system adverse events (31 vs 48%, $p < 0.01$). The study duration has now been extended to 240 weeks.

2.4 VS411

Lori and colleagues [5] had previously proposed a new antiretroviral class, virostatics (antiviral + cytostatic), which contains both an antiviral (didanosine) and a cytostatic (hydroxyurea). VS411-C101, a pilot Phase I, open-label, randomized, single-dose, four-way crossover trial, compared the fasted and non-fasted oral bioavailability of two fixed-dose formulations of didanosine (ddI) and hydroxyurea (HU) (VS411 – 2 and VS411 – 4) to that of commercial ddI and HU (5). The results showed that the maximal concentration (C_{max}) of ddI was 30% lower for both the VS411 – 2 and VS411 – 4 formulations, compared with the commercial ddI formulation. The area under the curve (AUC) of ddI did not significantly differ between the virostatics and the commercial product. HU C_{max} and AUC in the virostatics products were identical to those of the commercial product. Unexpectedly, both the C_{max} and the AUC of HU were found to be statistically significantly decreased when administered with food. The VS411 – 2 formulation exhibited more predictable absorption of ddI and less intersubject variability than the VS411 – 4 formulation. Enrollment for a 4-week Phase IIa pharmacokinetic, pharmacodynamic, and preliminary safety and efficacy study using five dosage combinations of HU and ddI has been completed.

2.5 PRO 140

CCR5 chemokine receptors have become targets for antiretroviral drug development. One drug, maraviroc, has already received FDA approval; others are in various stages of development. Maraviroc represents what are known as small-molecule CCR5 antagonists that bind to a hydrophobic cavity and inhibit CCR5 through allosteric mechanisms. PRO 140 is a humanized CCR5 monoclonal antibody (mAb) in early clinical development as an antiretroviral drug. It is thought to exert its anti-HIV-1 effect through competitive mechanisms by binding to hydrophilic extracellular regions on CCR5.

Study 2301 enrolled and randomized 15 HIV-infected individuals with no antiretroviral therapy in the preceding 12 weeks and examined the tolerability, antiviral activity, and PK of two single i.v. doses (5 and 10 mg/kg) of PRO 140 to those of placebo. To be eligible, study participants were required to have R₅-only virus, HIV-1 RNA > 5000 copies/ml,

CD4⁺ cell count > 300 cells/mm³ (nadir > 250 cells/mm³) and no AIDS-defining illness. The study follow-up period was 58 days. An interim analysis on data from the first 15 subjects was presented Table 1 [6].

Both doses of PRO140 showed high-level antiviral activity compared with placebo. Additionally, the duration of antiviral effects was increased with PRO 140 10 mg/kg, showing a mean viral load reduction of > 1.5 log₁₀ through to day 22.

Study 2101 had the same design, objectives, and eligibility criteria as study 2301 but evaluated three different s.c. doses and dosing schedules of PRO 140 and compared them with placebo [6]. The antiviral effects of PRO 140 administered subcutaneously were dose-dependent and were significantly better than placebo for the PRO 140 324-mg dose arms (Table 2).

There were no serious adverse events or drug-related adverse events leading to study discontinuation. Injection-site reactions were similar among placebo and PRO 140 treatment groups, and were minimal to mild and self-limited.

2.6 Idx899

IDX899 is a once-a-day second-generation NNRTI in clinical development that has exhibited activity against HIV-1 isolates selected *in vitro* by efavirenz as well as etravirine.

NV-05A-002 is a Phase IIb/IIa study that assessed antiviral activity, safety and pharmacokinetics of once-daily IDX899 in treatment-naïve HIV-1-infected subjects with HIV-1 RNA ≥ 5000 copies/ml and CD4⁺ cell count ≥ 200 cells/mm³ [7]. Forty subjects were randomized to receive four different doses of IDX899 (800, 400, 200 or 100 mg) or placebo once daily for 7 days. The IDX899 arms showed mean log₁₀ decreases of 1.78 – 1.87 copies/ml in HIV-1 plasma RNA from baseline to day 8, compared with a mean log₁₀ decrease of 0.1 log₁₀ copies/ml (Table 3). There were no treatment discontinuations, treatment-emergent serious adverse events, or dose-limiting toxicities. No clear pharmacokinetic/pharmacodynamic relationship was observed.

2.7 BIT225

Vpu is an HIV-1 protein that is important in viral assembly and release. It is not present in HIV-2. Previous investigations have shown that Vpu forms ion channels in phospholipid membranes [8]. HMA [5-(N,N-hexamethylene)amiloride], an amiloride analogue later designated BIT009, blocks Vpu's ion channel activity and was also shown to inhibit the replication of HIV-1 in cultured human blood monocyte-derived macrophages [9]. A lead compound, BIT225, with significant improvements in anti-HIV-1 activity, was selected from a library of numerous small-molecule compounds that had subsequently been developed. Results from a series of experiments designed to further define BIT225's antiviral activity were presented [10]. BIT225 demonstrated a > 90% inhibition of HIV-1 release from human macrophages, with a 50% inhibitory concentration (IC₅₀) of 1.1 ± 0.4 μM and

a 50% toxic concentration (TC₅₀) of 212 μM. BIT225 exerted its effects post integration and had no effect on reverse transcriptase or protease enzymes. Morphological appearance of HIV-1 *de novo* synthesis viewed by electron microscopy suggested a defect in virion packaging and budding. The development of BIT225 is proceeding into its early clinical stage with a Phase I, standard single-dose, dose-escalation study in healthy volunteers.

2.8 Bryostatin-1

Isolated from the marine animal *Bugula neritina*, bryostatin-1 binds to and inhibits protein kinase C (PKC), a cell-signaling enzyme with a whole range of important functions including regulation of transcription, mediation of immune responses, and regulation of cell growth, learning and memory. These functions are achieved by PKC-mediated phosphorylation of other kinases and regulatory proteins. The effects of bryostatin-1 on HIV latency and on the biochemical targets IκBα, ERK, and JNK were investigated and reported [11]. IκBα is a cellular protein that inhibits the transcription factor NF-κB. ERK (extracellular signal-regulated kinase) and JNK (c-Jun NH2-terminal kinase) are proteins that belong to a family of ubiquitous kinases, which participate in signal transduction pathways that control intracellular events including cell differentiation, cell proliferation, and cell death. Bryostatin-1's effects were found to be concentration-dependent. At a concentration of 10 nM, bryostatin-1 fully reactivated HIV-1 latency but did not induce IκBα phosphorylation and degradation or JNK activation. This suggested a therapeutic role for bryostatin-1 for activating HIV latency without an accompanying activation of signal transduction pathways with possible negative consequences. The investigators also reported that the interaction between bryostatin-1 and histone deacetylases (HDAC) inhibitors such as valproic acid allows bryostatin-1 to reactivate HIV latency at a much reduced concentration of 1 nM, thus improving its therapeutic selectivity.

2.9 ATI-0917

Initially developed as an antifungal agent, ATI-0917 is now being evaluated as a candidate antiretroviral drug [12]. ATI-0917 inhibits the production of viral messenger RNA in infected cells, which prevents the infected cells from making new virions. It is thought that it exerts this effect through inhibition of Rev. Rev is an HIV-1 protein that is responsible for the export of unspliced and partially spliced viral RNA into the cytoplasm, thus shifting the HIV replication cycle from the early to the late phase. ATI-0917 causes an accumulation of multiply spliced HIV RNA in the cell with a corresponding decrease in singly spliced or unspliced viral RNAs, resulting in a reduction in the production of viral proteins and progeny RNA and causing a substantial decline in virus production from an infected cell. The toxicity profile of ATI-0917 is extrapolated from its previous evaluation as a systemic antifungal drug and is

Table 1. PRO 140 study 2301 interim analysis.

	Placebo (n = 5)	PRO 140 5 mg/kg (n = 5)	PRO 140 10 mg/kg (n = 5)
Baseline HIV-1 RNA (median) log ₁₀	4.52	4.62	5.00
Baseline CD4 count (median) mm ³	310	339	422
Mean maximum log ₁₀ reductions	0.48	1.90	2.17
mean log ₁₀ change from baseline in HIV RNA at day 12	+0.06	-1.88	-2.01

Table 2. PRO 140 study 2101 interim analysis.

	Placebo (n = 5)	PRO 140 162 mg s.c. once weekly (n = 4)	PRO 140 324 mg s.c. biweekly (n = 6)	PRO 140 324 mg s.c. once weekly (n = 5)
Baseline HIV-1 RNA (median) log ₁₀ copies/ml	4.03	4.47	4.89	4.27
Baseline CD4 count (median) mm ³	411	320	504	389
Mean maximum reductions log ₁₀ copies/ml	0.31	0.5	1.2	1.7

Table 3. NV-05A-002 results.

	IDX 100 (n = 8)	IDA 200 (n = 8)	IDX 400 (n = 8)	IDX 800 (n = 8)	Placebo (n = 8)
Baseline HIV-1 RNA (mean) log ₁₀ copies/ml	4.81	4.74	5.09	4.36	4.54
Baseline CD4 count (mean) mm ³	491	432	466	437	567
Mean change from baseline in HIV RNA at 7 days log ₁₀ copies/ml	-1.87	-1.84	-1.78	-1.78	+0.10

reported as favorable. Human clinical trials are anticipated in the near future.

2.10 IQP-0528

IQP-0528 is a novel anti HIV-1 candidate drug that belongs to a group of compounds known as pyrimidinediones. The pyrimidinediones inhibit HIV at two steps of replication, virus entry and reverse transcription, with activity at sub-nanomolar concentration [13]. This preintegration site of activity has led to the evaluation of several pyrimidinediones for potential microbicidal use to prevent the sexual transmission of HIV. IQP-0528 is the lead microbicide candidate among the pyrimidinediones [14]. IQP-0528 inhibited both cell-free and cell-associated virus transmission to CD4-expressing cells in virus transmission inhibition assays and was highly active in the microbicide transmission and sterilization assay (MTSA). However, it was not active against several viral, bacterial or fungal organisms known to cause sexually transmitted infections. *In vitro* tests did not demonstrate

toxicity to primary human cells, nor to the normal vaginal flora *Lactobacillus*. IQP-0528 is currently being formulated in an intravaginal ring for delivery of the compound.

3. Expert opinion

While the use of potent antiretroviral drugs in combination regimens has led to dramatic declines in HIV-related morbidity and mortality, treatment failures still occur and the emergence of resistance continues to present significant challenges to clinicians. Therefore, the ongoing search for new antiretroviral agents with unique and different mechanisms of HIV inhibition remains critical. Fortunately, the presentations at HIV DART 2008 indicate that the antiretroviral drug development pipeline is robust, with numerous candidate compounds in various stages of preclinical and clinical development. Importantly, various new components of the replication cycle of HIV have become targets for drug discovery, promising further expansion of the antiretroviral

drug armamentarium. Further elucidation of these new approaches and the progression of some of these candidate drugs into clinical development, and indeed clinical use, is eagerly anticipated.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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