

A Double-Blind Placebo-Controlled Study in HIV-1-infected Subjects on the Safety, Pharmacokinetics and Antiviral Effect of Cyclophilin A Targeting DEBIO-025

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Introduction

The host cell protein cyclophilin A (CypA) interacts with HIV-1 Gag protein, resulting in the packaging of CypA into HIV-1 virions. It is thought that CypA binding confers a replication advantage to HIV-1 by enabling the virus to escape post-entry host cell restrictions. DEBIO-025 is a synthetic non-immunosuppressive cyclophilin currently tested in humans as a potential anti-HIV drug. It binds strongly to CypA. DEBIO-025 is devoid of immunosuppressive activity and demonstrates a potent inhibitory activity on HIV replication^{1,2}. These properties were obtained through structural modifications of the molecule in positions 3 and 4, resulting in increased CypA and decreased calcineurin binding affinity (Fig 1)³. In contrast to the calcineurin binding domain that mediates immunosuppression, the cyclophilin binding domain is the determinant site for antiviral activity. The anti-HIV-1 effect of DEBIO-025 has been documented *in vitro*, in particular against multidrug resistant HIV-1 isolates⁴ and *in vivo* in the XCD18a mouse model⁵. A first clinical evaluation of DEBIO-025 in healthy adult male and female volunteers revealed a good tolerance of the drug. DEBIO-025 is therefore a first-in-class candidate for development as a new anti-HIV CypA inhibitor drug.

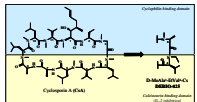


Figure 1. Structure of DEBIO-025

The aims of the study were:

- To assess the safety of repeated oral doses at three dose levels of DEBIO-025 in asymptomatic HIV-1 infected volunteers.
- To determine the pharmacokinetic (PK) profile of DEBIO-025 in whole blood and plasma.
- To determine the anti-HIV-1 activity of DEBIO-025.

Methods

A double blinded 10 day course of once daily oral DEBIO-025 or placebo was administered to HIV-1-positive antiretroviral naive subjects (n=8, 24 males/24 females) randomized to receive placebo or DEBIO-025 50mg (cohort 1), 400mg (cohort 2) or 1200mg (cohort 3). Inclusion criteria included CD4 count ≥ 250 cells/mm³ and plasma viral load ≥ 2000 copies/mL. Safety was assessed on incidence of adverse events, changes in vital signs, ECG and laboratory parameters. DEBIO-025 in whole blood, plasma and urine was quantified using validated HPLC/MS methods. Pharmacokinetics was investigated over 30 days and PK metrics were computed for DEBIO-025 levels according to non-compartmental analysis. HIV plasma RNA levels were assessed with a signal amplification nucleic acid probe assay using the Bayer System 340 MNA Analyser. HIV-1 load changes between Day -1, Day 10 and recovery period were compared by ANOVA using SAS PROC MIXED. HIV gag coding region for CypA binding was sequenced using PCR.

Results

Safety

DEBIO-025 was well tolerated at all doses levels. Adverse events included headache, abdominal distension and stomach pain, all mild to moderate in severity and of short duration. Neither deaths nor serious adverse events occurred during the study, other than an episode of epileptic seizure in a female failing to report her condition at screening. This event was considered unlikely to be study drug related.

Table 2. Mean liver transaminases, creatinine and leucocyte count values (SD) on Day -1 and Day 10

COFAVAT (U/L)	DAY	PLACEBO (n=12)	DEBIO-025 50MG (n=12)	DEBIO-025 400MG (n=12)	DEBIO-025 1200MG (n=12)
Day -1	31.1 ± 5.5	27.3 ± 5.6	28.1 ± 3.3	27.6 ± 1.2	28.3 ± 6.3
Day 10	29.6 ± 6.7	29.3 ± 5.3	28.3 ± 1.1	28.3 ± 6.3	28.3 ± 6.3
CPALAT (U/L)	Day -1	26.5 ± 14	19.7 ± 4.8	23.8 ± 4.2	24.3 ± 11.8
Day 10	23.6 ± 6.8	20.7 ± 6.6	23.3 ± 6.4	18.9 ± 6.6	
Leucocytes (10 ⁹ /L)	Day -1	694 ± 113	749 ± 113	713 ± 117	714 ± 119
Day 10	678 ± 109	681 ± 115	628 ± 83	668 ± 121	
Day -1	4.9 ± 1.1	5.1 ± 1.0	4.9 ± 1.7	5.1 ± 1.1	
Day 10	4.5 ± 0.9	5.7 ± 1.7	4.6 ± 1.1	5.1 ± 1.1	

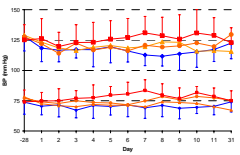


Figure 2. Mean blood pressure values (SD) over time (SBP, systolic blood pressure; DBP, diastolic blood pressure)

Pharmacokinetics

DEBIO-025 was rapidly absorbed after oral administration, with peak concentrations (C_{max}) on days 1 and 10 generally reached at 1-2 h (T_{max}) post-dosing. Most of the drug was immediately trapped into blood cells, as indicated by significantly higher blood than plasma levels (Figure 3). Table 3 summarises mean PK parameters.

In whole blood, drug exposure (C_{max} and AUC) was not proportional to dose with a less than dose-proportional increase in the systemic DEBIO-025 availability (Figure 3), which was consistent with a saturation of blood cell uptake occurring over this concentration range. This non-linearity in DEBIO-025 whole blood exposure was not apparent in plasma, which was not submitted to saturable binding.

DEBIO-025 concentrations declined with time in a multi-exponential way, with a mean apparent terminal half-life (T_{1/2}) estimated at about 120 h in plasma, which was roughly consistent among the different doses (Table 3), confirming linear elimination. Therefore, steady-state was not completely reached after 10-day repeated dosing, when accumulation was between 1.1 and 2.5-fold.

Only less than 0.5% of the dose was excreted as unchanged DEBIO-025 in urine within 24 h after dosing, suggesting that DEBIO-025 undergoes mainly hepatic elimination. DEBIO-025 PK was similar for both genders.

Table 3. DEBIO-025 mean pharmacokinetic parameters after single and repeated doses

WHOLE BLOOD	C _{max} (ng/ml)	T _{max} (h)	AUC ₀₋₂₄ (ng·h/ml)	Terminal T _{1/2} (h)	V _d (L)	Cl _{CR} (L/h)
50 mg	665	1.0	4420	116	65.1	11.6
400 mg	1306	1.0	12360	116	65.1	11.6
1200 mg	1610	1.0	23860	116	65.1	11.6
50 mg	668	1.5	8390	121	1139	6.6
400 mg	1396	1.0	18900	116	3610	21.4
1200 mg	2210	2.0	32400	116	7290	38.8
PLASMA	C _{max} (ng/ml)	T _{max} (h)	AUC ₀₋₂₄ (ng·h/ml)	Terminal T _{1/2} (h)	V _d (L)	Cl _{CR} (L/h)
50 mg	42	1.0	219	116	65.1	11.6
400 mg	112	1.0	1410	116	65.1	11.6
1200 mg	160	2.0	3270	116	65.1	11.6
50 mg	77	1.5	521	69.9	1050	127
400 mg	125	1.0	3270	90.8	1026	147
1200 mg	190	2.0	12360	63.5	1026	117

Table 1. Treatment emergent adverse events

ADVERSE EVENT (SUSPECTED CAUSE)	ALL TREATMENT GROUPS (n=36)	PLACEBO (n=12)	DEBIO-025 50MG (n=12)	DEBIO-025 400MG (n=12)	DEBIO-025 1200MG (n=12)
Total number of subjects (incidence %)	33 (91.4%)	4 (33.3%)	17 (141.7%)	10 (83.3%)	10 (83.3%)
Headache	1 (2.8%)	1 (8.3%)	2 (16.7%)	1 (8.3%)	1 (8.3%)
Stomach pain	1 (2.8%)	1 (8.3%)	1 (8.3%)	2 (16.7%)	1 (8.3%)
Abdominal distension	1 (2.8%)	1 (8.3%)	1 (8.3%)	1 (8.3%)	1 (8.3%)
Stomach pain	1 (2.8%)	1 (8.3%)	1 (8.3%)	1 (8.3%)	1 (8.3%)
Stomach pain	1 (2.8%)	1 (8.3%)	1 (8.3%)	1 (8.3%)	1 (8.3%)
Stomach pain	1 (2.8%)	1 (8.3%)	1 (8.3%)	1 (8.3%)	1 (8.3%)
Stomach pain	1 (2.8%)	1 (8.3%)	1 (8.3%)	1 (8.3%)	1 (8.3%)
Stomach pain	1 (2.8%)	1 (8.3%)	1 (8.3%)	1 (8.3%)	1 (8.3%)
Stomach pain	1 (2.8%)	1 (8.3%)	1 (8.3%)	1 (8.3%)	1 (8.3%)

Vital signs did not show any clinically significant changes from pre-dosing, nor were there any discernible differences between treatment groups. In general, the mean blood pressure values were all within their respective normal ranges, and there were no clinically significant changes from pre-dosing or any discernible differences between treatment groups. However, there seemed to be a higher blood pressure profile in the 1200mg group (although not significant) (Figure 2). Similarly, mean values for liver transaminases, creatinine and leucocyte counts were all within the respective normal reference ranges for all dose groups with no significant changes from pre-dosing (Table 2).

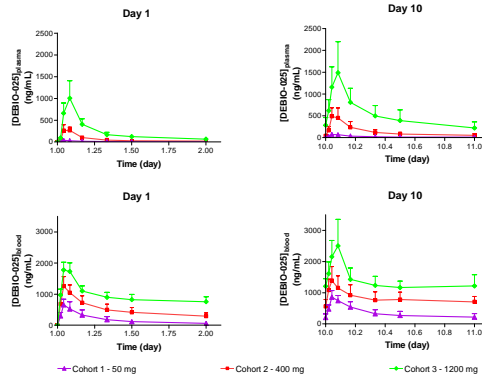


Figure 3. DEBIO-025 mean values (ng/ml) whole blood and plasma levels

Pharmacodynamics

Response had been defined as a HIV-1 plasma RNA viral load decrease of at least 0.5 log₁₀ units for values per- or post-treatment. Nine subjects showed a ≥ 0.5 log₁₀ viral load reduction, 2 of which ≥ 1.0 log₁₀ (Figures 4, F013 and F018). Twenty seven subjects had no relevant changes. Mean reduction was not significantly different between groups (p=0.05): -0.24 (placebo), -0.41 (50mg), -0.55 (400mg) and -0.44 (1200mg) (see Table 4).

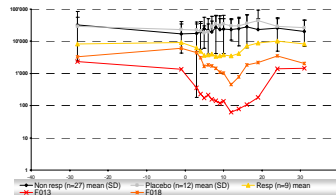


Figure 4. HIV-1 RNA copy/mL mean values (± SD) study day 1. For clarity SDs were not shown for comparison.

Table 4. HIV-1 RNA copy/mL mean values (± SD) study day 1. For clarity SDs were not shown for comparison.

Largest decrease between Day -1 and lowest pre- and post-treatment values	DURATION	MEAN ± SD	MIN	MAX
Largest decrease between Day -1 and lowest pre- and post-treatment values	Placebo	4.2 ± 0.4	3.4	4.8
	DEBIO-025 50 mg	3.9 ± 0.4	2.9	4.5
	DEBIO-025 400 mg	4.0 ± 0.5	3.1	5.0
	DEBIO-025 1200 mg	4.1 ± 0.4	3.2	4.7
Largest increase between Day -1 and highest pre- and post-treatment values	Placebo	-0.2 ± 0.1	0.0	-0.5
	DEBIO-025 50 mg	-0.4 ± 0.6	0.1	-2.2
	DEBIO-025 400 mg	-0.6 ± 0.6	0.1	-2.2
	DEBIO-025 1200 mg	-0.4 ± 0.3	-0.1	-1.1

Mean CD4 counts in all treatment groups were not different before and after treatment, and between groups. Interestingly, the mean CD4 count in the DEBIO-025 1200mg group was significantly higher at end-of-treatment with an increase of 118 cells per μL (p=0.03).

Table 5. CD4 values on Day -1 and lowest increase pre- and post-treatment

CD4 (cells/mm ³)	DURATION	MEAN ± SD	MIN	MAX
Largest increase between Day -1 and highest pre- and post-treatment values	Placebo	430 ± 149	230	731
	DEBIO-025 50 mg	445 ± 92	261	599
	DEBIO-025 400 mg	395 ± 173	234	711
	DEBIO-025 1200 mg	340 ± 94	202	509
Largest increase between Day -1 and highest pre- and post-treatment values	Placebo	94 ± 127	-128	296
	DEBIO-025 50 mg	63 ± 64	-62	129
	DEBIO-025 400 mg	107 ± 99	12	362
	DEBIO-025 1200 mg	115 ± 111	-111	290

Table 6. HIV-1 CypA polymorphisms in the CypA Caprip1 binding region observed in study subjects

Substitution	Amino Acid 86				Amino Acid 87		Amino Acid 91		Amino Acid 96	
	POLYMERASE	%	POLYMERASE	%	POLYMERASE	%	POLYMERASE	%	POLYMERASE	%
Y	14	0	0	11	7	44	8	79		
K	6	0	0	11	7	26	7	8		
F	0	0	0	0	0	0	0	0	1	11
Other	80	100	100	78	76	87	83	113	100	100

Discussion

It was recently demonstrated that HIV-1 isolates from the Main group naturally develop CypA-independent strategies to replicate in human cells⁶. Saturation experiments suggested that capsid substitutions (Y86R/H87Q/R191M/R96I) within the CypA-binding site renders the capsid core "invisible" to the potential host restriction factor. An analysis of 2599 HIV-1 capsid sequences available from the Los Alamos Database indicated that ~46% had no change from the consensus, whereas ~4% of the capsid sequences had a change in all four locations. The H87Q substitution, which was found in ~20% of the capsid sequences, rendered HIV-1 totally DEBIO-025-resistant and CypA-independent in human cells, but only in the context of the triple (H87Q/R191M/R96I) or the quadruple (Y86R/H87Q/R191M/R96I) substitutions. This sequence may be the determinant for sensitivity or natural resistance to DEBIO-025.

The distribution of the HIV-1 CypA-binding site polymorphism observed in the study population was similar (Table 6) to the frequencies previously reported⁶. The H87Q polymorphism was found in six subjects; however, no quadruple substitution was found in any subject. As recently suggested other polymorphisms⁷ may be important and reflect a bimodal effect of CypA on HIV-1 replicative ability.

Conclusions

Repeated oral doses of 50, 400 or 1200 mg of the cyclophilin inhibitor DEBIO-025 were safe and well tolerated in asymptomatic chronically HIV-1 infected subjects. No evidence of renal, cardiovascular or other toxicity was found. A limited anti-HIV-1 activity was observed in some subjects, however without a clear dose-response relationship. Exposure at a longer duration and/or at a higher dose level might be required to obtain a stronger viral response. A clinical study is presently underway to assess the potential of DEBIO-025 as an anti-HIV-1 compound. Unknown host or viral factors may explain differences in response, and these need to be identified to allow the development of DEBIO-025 as a potential treatment of HIV-1 infection.

1. Richman A, Coffin B, et al. (1997) Evidence of changes in HIV-1 RNA levels and symptoms of an oral nucleoside (DDI) used against HIV-1 and HIV-2. *JAMA*, 277(13):1603-1609.
 2. Steyn D, Richman A, et al. (2007) Safety and efficacy of the immunosuppressive cyclophilin A inhibitor DEBIO-025 in HIV-1-infected subjects. *Antiviral Res*, 72(1):1-10.
 3. Richman A, Coffin B, et al. (1997) Evidence of changes in HIV-1 RNA levels and symptoms of an oral nucleoside (DDI) used against HIV-1 and HIV-2. *JAMA*, 277(13):1603-1609.
 4. Richman A, Coffin B, et al. (1997) Evidence of changes in HIV-1 RNA levels and symptoms of an oral nucleoside (DDI) used against HIV-1 and HIV-2. *JAMA*, 277(13):1603-1609.
 5. Steyn D, Richman A, et al. (2007) Safety and efficacy of the immunosuppressive cyclophilin A inhibitor DEBIO-025 in HIV-1-infected subjects. *Antiviral Res*, 72(1):1-10.
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