

"New Drugs, New Targets"
AMD070 - A new class of HIV
Entry Inhibitors that target
CXCR4, a chemokine receptor

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Clinical Trial of AMD3100 in HIV+ Patients

Objectives: Safety, pharmacokinetics, antiviral activity.

Design

Open label, dose-escalation, 10-day IV infusion

HIV-infected subjects with vRNA >5,000 copies/ml

assayed twice at least 2 weeks apart, within 0.5 log₁₀

Doses: 2.5, 5, 10, 20, 40, 80, 160 µg/kg/hr

Stable ART/no ART

Two arms - 40 Patients enrolled

Strata 1 - NSI (R5) or SI (X4)

Strata 2 - SI⁺ (by MT-2 assay)

Status

One patient receiving the highest dose of AMD3100 administered (160 $\mu\text{g}/\text{Kg}/\text{hr}$), responded with a 0.89 \log_{10} reduction in viral load by day 11 (1.34 \log_{10} by day 18).

Steady-state plasma concentration of AMD3100 at a dose of 160 $\mu\text{g}/\text{Kg}/\text{hr}$ was $\sim 6.5 \mu\text{M}$.

Trial discontinued in May 2001.

Retrospective Analysis of HIV Samples

A retrospective analysis of blood samples from screen or baseline (day 1) and day 11 samples were performed using the following HIV assays:

- PBMC co-culture phenotyping assays.
- PhenoSense Assay performed at ViroLogic Inc.

Screen or baseline and day 11 lymphocyte samples from 30 patients were available for testing.

Screen or baseline and day 11 plasma samples were available from 39 patients.

Assay Methods

- PBMC Co-Culture Assay

Co-culture of HIV generated by mixing patient lymphocytes with PHA-stimulated lymphocytes from HIV negative donors.

Virus stock was tested for replication in U87.CD4.CXCR4 or U87.CD4.CCR5 cells using p-24 Ag ELISA.

Ability of AMD3100 to inhibit viral replication was measured in PBMC's.

Assay Methods

- PhenoSense Assay (ViroLogic Inc.)

HIV envelope sequences are amplified from patient plasma samples and inserted into an expression vector.

Virus particles expressing patient virus envelope proteins are produced by transfecting HEK293 cells with the envelope expression vector plus an HIV genomic vector containing a firefly luciferase indicator gene.

The derived “pseudotyped” viruses are used to infect U87 cells expressing CD4 and CCR5 or CXCR4.

Infection is monitored by measuring luciferase activity.

Sensitivity of Assay Methods to Detect X4 HIV

At entry into the study:

12 of 30 patients tested SI-positive in the MT-2 assay.
Retrospectively, 19 of 39 patients were X4 or dual-tropic by the PhenoSense assay.

17 Patient samples from screen or baseline were tested in all three assays (PhenoSense, PBMC and MT-2):

Of the 10 patient samples that were designated X4 or dual-tropic by the PhenoSense Assay and confirmed by PBMC co-culture assays, only 6 were SI positive by the MT-2 assay at the time of inclusion into the study.

Conclusion: The PhenoSense and PBMC co-culture assays are more sensitive for detection of X4 virus.

Quantitative PhenoSense Assay to Measure Antiviral Effects

Samples from 19 of 39 patients at entry into the study contained CXCR4-using variants of HIV, but with varying composition in the total virus population.

9 of 19 patients were dual-tropic at entry (exhibited virus that uses CXCR4 and CCR5) but were pure CCR5-using at day 11.

3/9 were confirmed by the MT-2 assay: SI positive at entry but NSI at day 11 after treatment with AMD3100.

The ability of AMD3100 to completely eliminate CXCR4-using variants of HIV was dose-dependent.

Patients Whose CXCR4-Using HIV Variants were Less Than 10% at Entry

	Dose ($\mu\text{g}/\text{Kg}/\text{hr}$)	Dual to R5 switch Day 1 to Day 11	# ARV Conmeds	% X4 Day 1	% X4 Day 11
6-73	80	YES	2	0.2	0
2-28	20	YES	NONE	0.3	0
1-31	20	YES	NONE	0.5	0
3-32	40	YES	NONE	0.8	0
2-9	5	YES	NONE	1.0	0
2-10	10	YES	NONE	1.0	0
3-11	5	YES	NONE	1.5	0
3-26	20	NO	NONE	2.0	0.05
1-4*	2.5	NO	2	2.4	1.2*
6-30**	20	NA	3	4.4	----
1-35	40	YES	NONE	8.6	0
1-33	40	YES	4	9.8	0

* Treated for 9 days, day 9 sample tested.

** Treated for 8 days, sample at conclusion of treatment or day 11 not available.

Patients Whose CXCR4-Using HIV Variants were 25-100% at Entry

	Dose (µg/Kg/hr)	Dual to R5 switch Day 1 to Day 11	# ARV Conmeds	% X4 Day 1	% X4 Day 11
6-70	40	NO	3	25.0	22.8
6-71	40	NO	NONE	31.7	18.4
1-2	2.5	NO	NONE	41.0	32.0
4-72*	40	NO	NONE	47.3	43.8*
2-3**	2.5	NA	6	65.0	-----
1-21	10	NO	NONE	94.0	26.0
1-40	160	vRNA reduction 0.89 log ₁₀	NONE	100	100

* Treated for 3 days, sample from day 4 of study.

** Day 11 sample not available.

The Ability of AMD3100 to Eliminate CXCR4-Using Variants was Dose-Dependent

Cohort Receiving AMD3100 at a Dose of 40 $\mu\text{g}/\text{Kg}/\text{hr}$

Patient	Dual to R5 switch (Day 1 to Day 11)	# ARV Conmeds	% X4 Day 1	% X4 Day 11
3-32	YES	NONE	0.8	0
1-35	YES	NONE	8.6	0
1-33	YES	4	9.8	0
6-70	NO	3	25	22.8
6-71	NO	NONE	31.7	18.4
4-72*	NO	NONE	47.3	43.8*

* Treated for 3 days, sample from day 4 of study.

When the CXCR4-using component of the total HIV population exceeded ~ 10 - 20%, the 40 $\mu\text{g}/\text{Kg}/\text{hr}$ dose of AMD3100 was insufficient to completely eliminate X4 virus.

Patient 1-40

Patient #: 1-40

Sex: M

Age: 40

Yrs with dx of HIV Infection: 6 MT-2 Status at entry: SI

Current HIV Meds: None

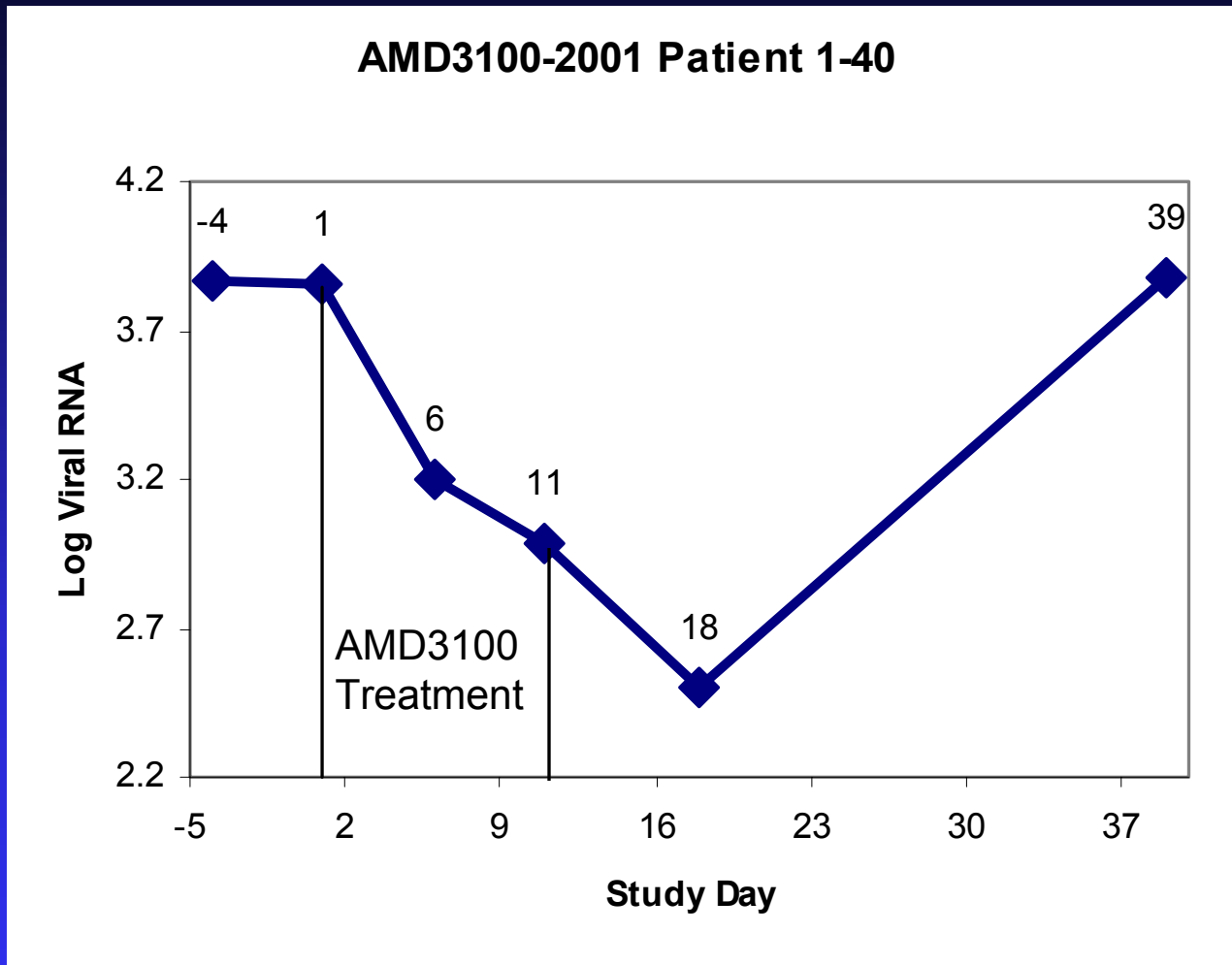
CD4 Count at Day 1: 520 Day 11: 1,425

WBC at Day 1: 6,200 Day 11: 29,700

Viral Load:

Day 1	Day 6	Day 11	Day 18
7,089	1,584	965	<322

Patient 1-40



Patient 1-40

ViroLogic PhenoSense Assay

Visit	Growth in cells*		Co-receptor Designation	AMD3100 IC ₅₀ (μM)
	Expressing CD4+ CCR5	CXCR4		
Screen	270	119990	X4	0.0097
Day 6	104	149436	X4	0.0140
Day 11	103	292528	X4	0.0095
Day 18	180	17996	X4	0.0333

* Luciferase counts (Relative Light Units) for a single round of replication. Counts in U87.CD4.CCR5 cells are less than the control infection with NL4.3 or HXB2 (X4 strains).

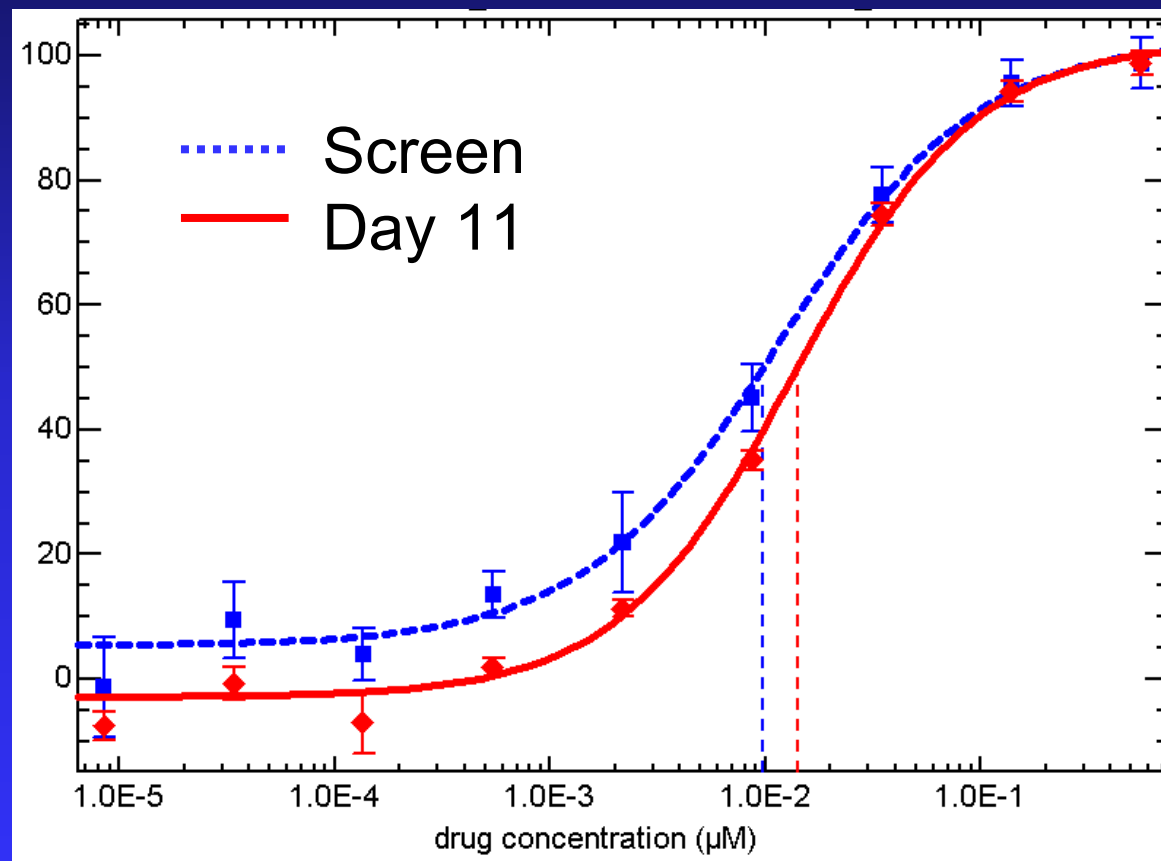
PBMC Co-Culture Assay

Visit	Growth in cells		AMD3100 IC ₅₀ (μM) in PBMC	MT-2 Assay
	Expressing CD4+ CCR5	CXCR4		
Screen	-	++	0.019	SI
Day 6				
Day 11	-	++	0.022	SI
Day 18				

Patient 1-40

PhenoSense Assay

AMD3100 Inhibits replication of a pseudo-virus expressing patient envelope proteins at Screen and Day 11.



Conclusions

One patient, whose virus was pure CXCR4-using at entry into the study, responded to AMD3100 treatment at a dose of 160 $\mu\text{g}/\text{Kg}/\text{hr}$ with a 0.89 \log_{10} reduction in viral load by day 11 (1.34 \log_{10} by day 18).

9 of 19 Patients that exhibited dual-tropic HIV at entry into the study, were pure CCR5-using by day 11, suggesting that AMD3100 treatment eliminated X4 HIV variants.

The ability of AMD3100 to eliminate X4 variants was dose-dependent.

The PhenoSense Assay detected the presence and composition of CXCR4-using HIV in the total virus population.

CXCR4 is a validated target for HIV therapy

Oral CXCR4 Antagonist: **AMD070**

Physical Properties

- Molecular weight less than 400
- 100% Protonated at physiological pH
- Solubility at pH 7.0 of > 100 mg/mL

Oral CXCR4 Antagonist: **AMD070**

Virology

- Activity of AMD070 against HIV-1 NL4.3:

	EC ₅₀ (nM)		EC ₉₀ (nM)	
	MT-4	PBMC	MT-4	PBMC
AMD070	6.6	7.9	12.5	31.4
AMD3100	4.3	12.3	8.2	36.6

- Inhibits X4 and dual-tropic clinical isolates in PBMC with comparable EC₅₀'s to AMD3100.
- Not active against M-tropic strains in PBMC.

In vitro Profile of AMD070

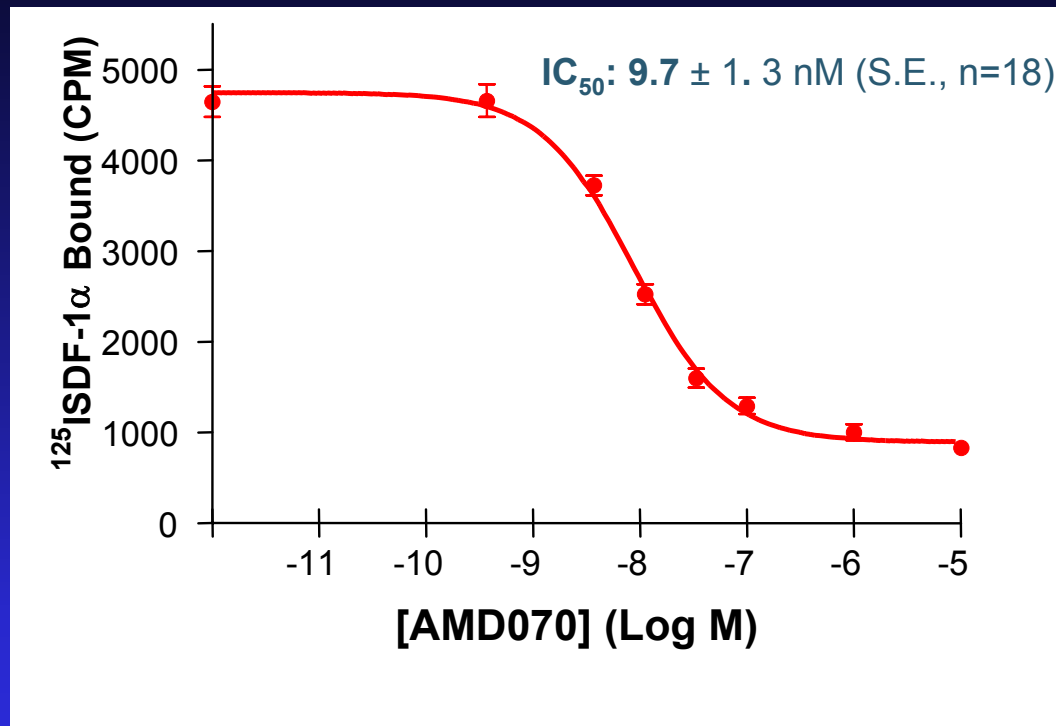
- Effect of increasing virus inoculum on the activity of AMD070.

Inhibition of HIV-1 NL4.3 in MT-4 cells:

TCID ₅₀	EC ₅₀ (nM)
100	2.5
500	5.0
2500	6.9

- Activity of AMD070 unaffected by the presence of human serum.
- Specific antagonist of the chemokine receptor CXCR4 IC₅₀'s (Inhibition of Ca flux, ligand binding) > 10,000 nM for CCR1, CCR2b, CCR4, CCR5, CCR6, CCR9, CXCR1, CXCR2, CXCR3.

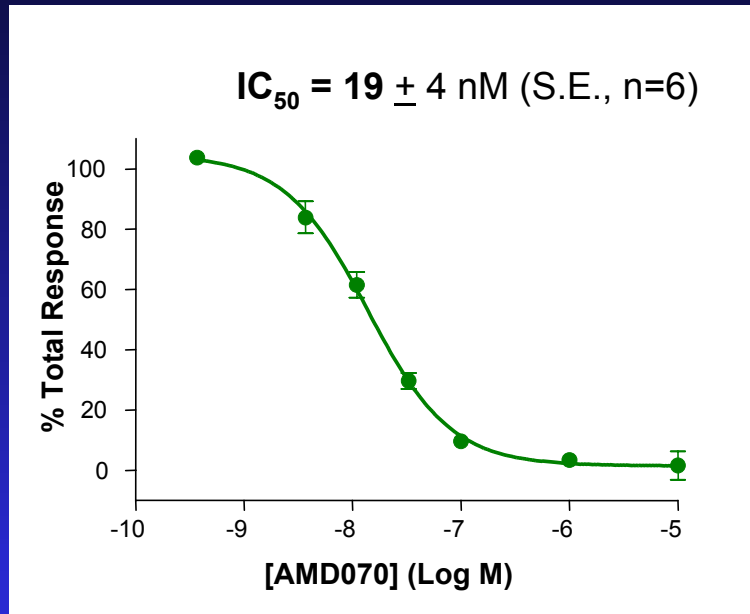
Inhibition of SDF-1 α Binding by AMD070



- CCRF-CEM Cells were incubated on filter plates (3 hours at 4 °C) with 100 pM of ^{125}I -SDF-1 α and varying concentrations of AMD070.
- Bound ^{125}I -SDF-1 α was separated from free by washing, and was measured by scintillation counting.

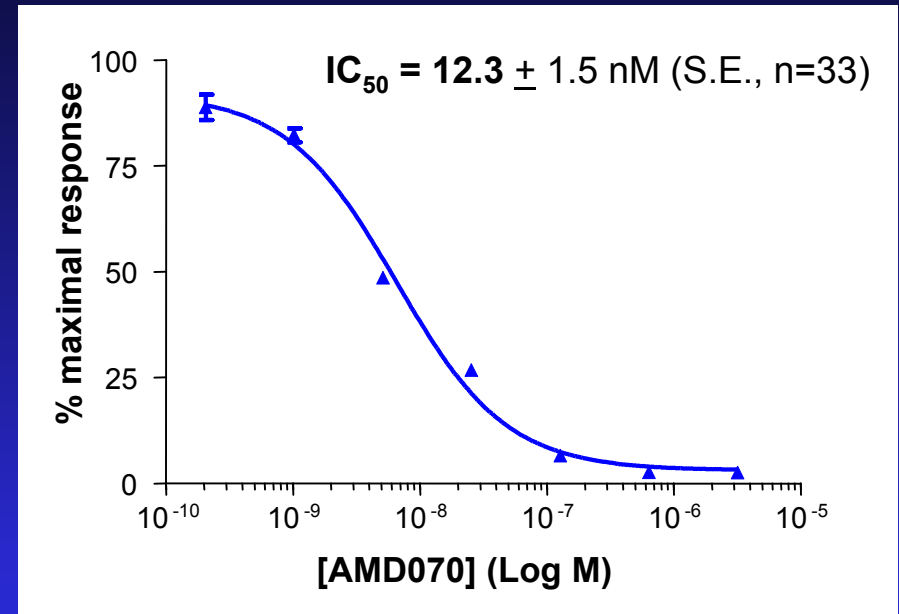
Antagonist Activity of AMD070

Inhibition of SDF-1 α induced ^{35}S -GTP γS binding



- CCRF-CEM membranes were incubated on filter plates with 10 nM SDF-1 α , 1nM ^{35}S -GTP γS and varying concentrations of AMD070 for 1 hour at room temperature.
- Bound ^{35}S -GTP γS was separated from free by washings, and was measured by scintillation counting.

Inhibition of SDF-1 α induced Ca flux



- Cells loaded with Fluo-4/AM were preincubated with a concentration range of AMD070 for 15 min.
- Changes in intracellular calcium concentration upon SDF-1 α addition (25 nM) were monitored using a FLEXstation™.

Pharmacokinetic Profile of AMD070

Oral bioavailability: Rat (26%), Dog (79%).

Half-life: Rat (3.4 hr), Dog (10 hr).

Dose-proportional increase in AUC up to 500 mg/Kg, single-dose in rats.

14-day repeat dose toxicity testing in rats: Tolerated up to 200 mg/Kg/day.

AMD070 penetrates the blood-brain barrier.

Binding Site of AMD070 in the CXCR4 Receptor

AMD3100 binds to Asp171 and Asp262 in the CXCR4 receptor.

Gerlach et al. J. Biol. Chem. 2001, 276, 14153-14160.

The ability of AMD3100 to inhibit HIV-1 NL4.3 or III_B replication in U87. CD4 cells expressing CXCR4[D171N] or CXCR4[D262N] was reduced by 4- to 20-fold compared to CXCR4[WT].

U87.CD4.CXCR4[D171N,D262N] failed as a co-receptor for infection.

Hatse et. al. Mol. Pharmacol. 2001, 60, 164-173.

Activity of AMD070 against X4 HIV-1 strains was reduced 10-20 fold on cells expressing CXCR4[D171N] but was unaffected by D262N.

HIV-1 NL4.3 AMD3100-resistant virus is cross-resistant to AMD070.

AMD070 and AMD3100 bind to a similar site in CXCR4

Conclusions-2

- CXCR4 is a validated target for HIV therapy
- Oral CXCR4 antagonist in advanced pre-clinical testing

Specific antagonist of the CXCR4 receptor

Potent anti-HIV activity against X4 laboratory strains and clinical isolates

Suitable PK / toxicity profile

- Multiple back-up candidates in testing

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