

# Biologic Activity of an Orally Bioavailable CXCR4 Antagonist in Human Subjects

N Stone<sup>1\*</sup>, S Dunaway<sup>2</sup>, C Flexner<sup>1</sup>, G Calandra<sup>3</sup>, I Wiggins<sup>1</sup>, J Conley<sup>2</sup>, S Synder<sup>4</sup>, C Tierney<sup>5</sup>, C Hendrix<sup>1</sup>, and the Adult ACTG A5191 Study Group

<sup>1</sup>Johns Hopkins University, Baltimore, MD, USA; <sup>2</sup>University of Washington, Seattle, WA, USA; <sup>3</sup>AnorMED, Inc., Langley, BC, Canada; <sup>4</sup>Social and Scientific Systems, Inc., Silver Spring, MD, USA; <sup>5</sup>Harvard University, Boston, MA, USA

## Introduction

- New classes of antiretroviral drugs are needed to exploit previously unchallenged viral targets and keep ahead of the emergence of viral resistance to existing drugs.
- AMD3100, an inhibitor of the CXCR4 chemokine receptor, acts as a co-receptor for mediating HIV entry into the lymphocyte. AMD3100 had anti-HIV activity *in vitro* in the SCID-Hu Thy/Liv animal model and in clinical studies in HIV-infected persons, thus establishing the legitimacy of CXCR4 inhibition as an antiretroviral strategy. In clinical studies, AMD3100 also demonstrated dose dependent leukocytosis attributed to CXCR4 antagonism. Absence of oral absorption and side effects halted its anti-HIV development.
- AMD070, a new chemical entity, also inhibits CXCR4, with an IC<sub>50</sub> similar to AMD3100 (6.6 nM and 4.3 nM, respectively) against HIV-1 NL4.3 in MT-4 cells. The protein binding adjusted EC<sub>90</sub> for AMD070 is 125nM against HIV-1 in MT-4 cells. AMD070 also demonstrated good bioavailability orally in animals (5).
- Given the need for ongoing development of new anti-HIV drugs, the establishment of CXCR4 inhibition as a viable antiretroviral strategy, and the need for orally bioavailable drugs, we studied the safety, tolerability and pharmacokinetics of AMD070.

## Methods

### Study Design

- Phase 1, single dose arms followed by multiple dose arms, open label, inpatient dose escalation

### Study Population

- Healthy HIV seronegative men, 18 years of age or older
- No active medical illness by history, physical, or laboratory evaluation

### Study Procedures

- Dosing regimen and dose escalation as below
- Blood collected for AMD070 analysis: pre-dose, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 20, and 24 hours post dose (coincident with 7th dose for multiple dose cohorts)
- Safety assessed via telemetry, lab tests, history and physical while inpatient and at 2 week follow-up visit.

Cohort	Subjects	Dose	Frequency	# of Doses
A	3	50 mg	Once	1
B	3	100 mg	Once	1
C	3	200 mg	Once	1
D	3	400 mg	Once	1
E	6	100 mg	Q12 hours	7
F	6	200 mg	Q12 hours	7
G	6	400 mg	Once (with food)	1

## Abstract

**Background:** AMD070 is an oral CXCR4 antagonist with *in vitro* inhibitory activity against X4 virus. Its predecessor, AMD3100, also a CXCR4 antagonist, reduced X4 viral load in a phase II study and produced dose-dependent leukocytosis, but its further development for HIV infection was halted due to apparently limited antiretroviral success and the desire to use orally bioavailable compounds.

**Objective:** Evaluate AMD070 safety, pharmacokinetics (PK) and pharmacodynamics.

**Methods:** Four cohorts of 3 healthy volunteers received escalating single oral doses of AMD070 ranging from 50 mg to 400 mg. We monitored subjects for adverse effects and collected blood for AMD070 PK sampling and white blood cell (WBC) counts to monitor for CXCR4 antagonism.

**Results:** AMD070 was well-tolerated; 3 of 12 subjects reported a transient mild/moderate headache. The drug appears well absorbed, reaching peak plasma concentrations between 0.5 and 4 hours post-dose. Changes in peak plasma concentration (C<sub>max</sub>) and area under the curve (AUC<sub>0-24</sub>) were dose-proportional. C<sub>max</sub> ranged from 169 to 530 nM for the 50 mg cohort and from 3,849 to 9,561 nM for the 400 mg cohort. The concentration 12 hours following a single oral dose for the 400 mg cohort ranged from 79 to 155 nM. (The protein-binding adjusted *in vitro* EC<sub>90</sub> in MT-4 cells is 125 nM.) The degree of change from baseline of white blood cell counts ranged from 1.3 to 1.6-fold with the 50 mg dose and from 1.5 to 2.9-fold with the 400 mg dose. The WBC increase over baseline, best fit to C<sub>max</sub>, was 2.4-fold (95% CI 1.9 - 3.0).

**Conclusions:** In healthy volunteers, one oral dose of AMD070 was well tolerated, well absorbed, and demonstrated dose-proportional PK. AMD070 also had a dose-proportional effect on WBC count attributed to CXCR4 blockade, consistent with a previous CXCR4 antagonist used in human subjects. If leukocytosis can serve as a surrogate marker for inhibition of CXCR4, these findings suggest that the doses used in this study are active *in vivo* and demonstrate a dose-response. In most of the subjects, the 12-hour concentrations following the 400 mg dose were in excess of the antiviral EC<sub>90</sub> of AMD070 *in vitro*.

## Summary

- AMD070 was well tolerated by the healthy volunteers in this study; all adverse events recorded (several headaches) were mild and reversible.
- Pharmacokinetic data are consistent with dose proportionality, adequate absorption, and significant variability in pharmacokinetic parameter estimates.
- Multiple dosing (BID) results in ~2 fold increase in the 12 & 24 hour concentration, though no statistically significant difference in C<sub>max</sub> or AUC.
- Fed cohort data suggests no adverse impact of food on bioavailability.
- The AMD070 concentrations at 12 hours following one 400 mg oral dose stayed above the *in vitro* antiviral EC<sub>90</sub> (protein-binding adjusted) for nearly all subjects.
- Based on leukocytosis as a surrogate for CXCR4 inhibition, the doses used in this study are active *in vivo* and demonstrate a dose-response.

## Results

Figure 1. Single dose pharmacokinetics of AMD070 by dose cohort (median and range). Terminal half-life was estimated at 6-10 hours.

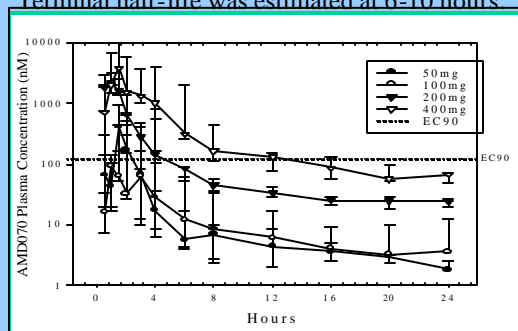


Figure 2. C<sub>max</sub> (left) and AUC (center) are dose proportional across escalating single doses of AMD070, though all pharmacokinetic parameter estimates, including terminal half-life (right), are quite varied.

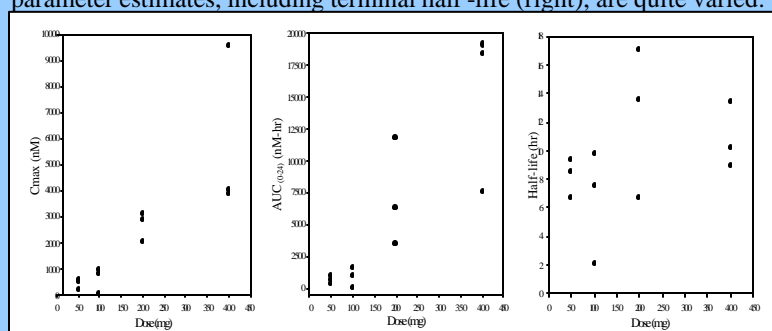


Figure 3. Multiple dose cohort (100 mg) when compared to 100 mg single dose cohort shows ~2-fold increase in terminal concentrations.

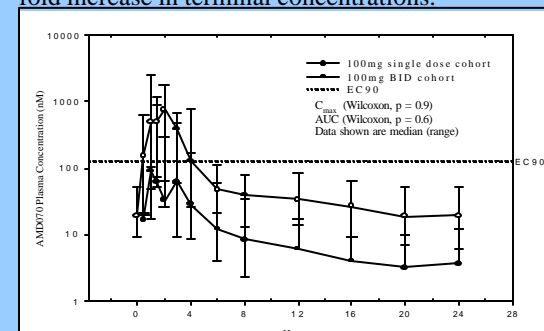


Figure 4. Six subjects received 2 doses, one with food and one fasting. Terminal concentrations were greater with food, but there was no difference in C<sub>max</sub> or AUC.

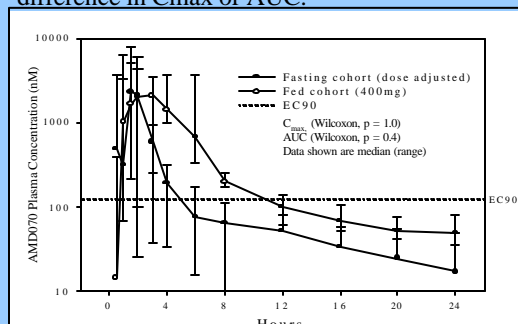


Figure 5. Pharmacodynamics. Dose-dependent leukocytosis followed AMD070 dosing (left). Counterclockwise hysteresis was seen, consistent with delayed equilibrium between plasma and the "effect site" (center). The maximum WBC-to-baseline ratio well with dose, C<sub>max</sub>, and AUC in an E<sub>max</sub> model; E<sub>max</sub> estimates ranged from a 2.4 – 3.2 fold WBC increase (right).

