

Contract N01-AI-05415

**Specialized *In Vitro* Virological Evaluations
Of Strategies To Combat HIV/AIDS**

**Report for NIAID Compound:
11039**

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Results

Table 1. CD4-Dependent R5-Tropic Transmission Assay

Compound	Assay Type	IC ₅₀	IC ₉₀	TC ₅₀	TI ₅₀	Comments
TAK 779 (μM)	Standard	0.07	1.52	>10	>142.86	Active Control
	Standard	0.02	>10	>10	>500	
	pH	0.003	>10	>10	>3,333.33	
AMD 3100 (μM)	Standard	>10	>10	>10	NA	Inactive Control
	Standard	>10	>10	>10	NA	
	pH	>10	>10	>10	NA	
11039 (μM)	Standard	4.44	49.5	>100	>22.52	Active in standard and low pH assays
	Standard	0.58	60.4	>100	>172.41	
	pH	3.13	70.8	>100	>31.95	

Table 2. CD4-Dependent X4-Tropic Cell-Cell HIV Transmission Inhibition Assay

Compound	Assay Type	IC ₅₀	IC ₉₀	TC ₅₀	TI ₅₀	Comments
TAK 779 (μM)	Standard	>10	>10	>10	NA	Inactive Control
AMD 3100 (μM)	Standard	0.005	0.05	>10	>2,000	Active Control
NIAID 11039 (μM)	Standard	0.29	3.95	>100	>344.83	Active

Table 3. CCR5-Tropic Cell-Free (Attachment) HIV Transmission Inhibition Assay

Compound	IC ₅₀	IC ₉₀	TC ₅₀	TI ₅₀	Comments
TAK 779 (nM)	<0.002	34.2	>200	>100,000	Active Control Compound
AMD 3100 (μM)	>10	>10	>10	NA	Inactive Control
NIAID 11039 (μM)	0.01	0.34	>100	>10,000	Active

Table 4. *Lactobacillus sp.* Toxicity Testing.

Compound	Units	<i>L. crispatus</i> (ID ₅₀)	<i>L. jensenii</i> (ID ₅₀)	Comments
Penicillin/Streptomycin	Dilution 1	1:2,444,406	1:2,925,569	Control Compound
NIAID 11039	μM	>100	>100	Not Toxic

¹ The starting concentrations of Penicillin and Streptomycin are 1.25 U/ml and 1.25 μg/ml, respectively.

Table 5. X4-Tropic HIV-1 Attachment Inhibition Assay

Compound	IC₅₀	IC₉₀	TC₅₀	TI₅₀	Comments
Chicago Sky Blue (µg/mL)	0.24	2.28	>50	>208.33	Active Control
AMD 3100 (µM)	0.001	0.008	>10	>10,000	Active Control
NIAID 11039 (µM)	0.04	0.37	>100	>2,500	Active

Table 6. X4-Tropic HIV-1 Fusion Inhibition Assay

Compound	IC₅₀	IC₉₀	TC₅₀	TI₅₀	Comments
Chicago Sky Blue (µg/mL)	0.65	3.32	>50	>76.92	Active Control
AMD 3100 (µM)	0.002	0.02	>10	>5,000	Active Control
11039 (µM)	0.55	0.91	>100	>181.82	Active

Table 7. CD4-Independent Inhibition Assay

Compound	IC₅₀	IC₉₀	TC₅₀	TI₅₀	Comments
Dextran Sulfate (µg/mL)	0.004	91.8	>100	>25,000	Active Control
Dextran (µg/mL)	>100	>100	>100	NA	Inactive Control
11039 (µM)	0.35	80.1	>100	>285.71	Active

Summary

1. NIAID compound 11039 was received January 10, 2005 as a powder, solubilized in DMSO at 10mM, and stored at -80°C until tested in the standard panel of topical microbicide evaluation assays supplied by the NIAID under contract N01-A1-05415 (*Specialized In Vitro Virological Evaluations Of Strategies To Combat HIV/AIDS*). The compound is light brown and not very soluble. It was mixed well before each use.
2. NIAID 11039 was active in the CD4-dependent CCR5-Tropic HIV transmission inhibition assay. Activity was confirmed under standard conditions and not adversely affected by and low pH assay conditions (Table 1).
3. NIAID 11039 was active, in the CD4-dependent CXCR4-Tropic HIV transmission inhibition assay (Table 2)
4. NIAID 11039 was active in the cell-free CCR5-Tropic HIV transmission inhibition (R5-attachment) assay (Table 3).
5. NIAID 11039 did not demonstrate inhibition of growth of beneficial *Lactobacillus* species (Table 4).
6. NIAID 11039 was inhibitory to the processes of CXCR4-tropic HIV-1 attachment (Table 5)
7. NIAID 11039 was inhibitory to the processes of HIV-1 virus-cell fusion (Table 6).
8. NIAID 11039 was inhibitory in the CD4-independent HIV-1 cell-cell transmission assay.

Table 8. Overall Summary of Results for NIAID 11039

R5 cell-cell	R5 cell-cell pH	X4 cell-cell	R5 cell-free	X4 cell-free	X4 fusion	Lactobacillus
A	A	A	A	A	A	Not toxic

A=Active NA=Not active

NIAID compound 11039 appears to have potential as topical microbicide.

Introduction

Although great strides have been made in the development of post-infection treatment strategies for HIV-infected individuals, the development of effective topical microbicides to prevent sexual transmission of the virus has lagged behind. This deficiency has taken on a potentially catastrophic significance as the prevalence of AIDS-infected individuals in developing countries continues to rise in the absence of a socially acceptable and effective method to prevent sexual transmission of HIV. In addition, the increasing prevalence of viral resistance to first and second line therapeutic options increases the requirement for a topical microbicide capable of inhibiting a broad range of HIV-1 phenotypes and genotypes. Thus, there is an urgent need to supplement our current anti-AIDS strategies with topical microbicides capable of preventing virus transmission and the spread of AIDS through sexual contact. Therefore, under contract N01-AI-05415 (*Specialized In Vitro Virological Evaluations Of Strategies To Combat HIV/AIDS*), the NIAID has undertaken the development and application of a preclinical program to assess the applicability of previously characterized and newly identified compounds as topical microbicides.

Rationale

Sexual transmission of HIV-1 occurs via the transfer of bodily fluids containing virus from an infected donor to a recipient during sexual contact. Infectious HIV-1 has been found in the semen of infected individuals in both a cell-free and cell-associated state. Although the precise mode of initial virus infection *in vivo* (cell-free or cell-associated) is not known, in all cases infection and production of new virus proceeds through a series of defined steps. Virus must first attach to its new host cell and enter via interactions with host cell proteins (coreceptors and/or CD4) and virus proteins (gp120 and gp41). Once the virus has penetrated the cell reverse transcription proceeds in a stepwise manner. This occurs in concert with transport of the preintegration complex to the nucleus and integration of viral DNA into the host genome. Following integration, new transcription and translation of viral RNA and proteins initiates a complex series of interactions resulting in the formation and release of new virus by the cell. Given the possible modes of virus transmission, and the replication cycle of HIV-1, we hypothesize that optimal topical microbicides should block attachment or fusion of the virus to the cell membrane and/or interrupt virus replication prior to reverse transcription or

integration. Agents that alter virus infectivity, such as surfactants and p7 nucleocapsid protein inhibitors, are also considered to be potential topical microbicides.

Screening Methodologies

INITIAL CD4-DEPENDENT AND CD4-INDEPENDENT HIV TRANSMISSION INHIBITION SCREENS

Although there is currently some debate as to the mode of virus entry during sexual transmission, screening efforts are focused on the development of agents that prevent cell-to-cell transmission of HIV-1. Most HIV cell entry appears to proceed through a CD4-dependent pathway in the presence of coreceptors, and there is mounting evidence that primary infection occurs via CCR5 receptors in addition to CXCR4 receptor mediated pathways. These possibilities suggest that *in vivo* transmission of HIV could occur through a variety of routes, some requiring cell-to-cell contact and/or cell-free virus contact with cells in the presence or absence of CD4. Thus, in an attempt to model this complex process, an initial screen was devised which measures the inhibition of cell-to-cell virus transmission via CD4-dependent routes. The details of the individual assays are summarized and compared in **Table 9**. The CCR5- and CXCR4-tropic CD4-dependent assays use the human osteosarcoma derived cell line GHOST (3) R5 and GHOST (3) X4/R5 cells, respectively. These cell lines express CD4 and the CXCR4 (X4) and/or CCR5 (R5) coreceptors. (These assays were specifically developed for this screen to complement the CD4-independent X4-tropic cell-to-cell transmission assay previously developed by Phillips et al (*J Virological Meth*, 1995, **52**:1-13) that employs ME180 cells and is now used as an optional secondary screening assay in this program. ME180 cells are derived from cervical epithelia and express very low levels of X4 coreceptors in the absence of CD4.)

The final set of primary assays for active compounds includes assessment of the compound's ability to inhibit CCR5-tropic HIV-1 virus-cell attachment. The attachment assays are outlined and compared in **Table 10**. This attachment assay uses HeLa CD4 LTR β -gal cells, and measures the ability of compounds to inhibit the initial attachment of HIV-1 to the cell. These cells naturally express the CXCR4 coreceptor and are engineered to express cell surface CD4, CCR5 and the β -galactosidase enzyme under control of the HIV-1 LTR promoter. Infection of HeLa CD4 LTR β -gal cells with HIV-1 induces the expression of the β -galactosidase enzyme via *tat* transactivation of the HIV-1 LTR. Compounds that interact with any phase of

the virus cell attachment/entry process [binders to gp120, gp41 or new conformational targets, inhibitors of the gp120/CD4 interaction, or the interaction of the virus gp120/CD4 complex with the X4 or R5 coreceptor] score as positive in this assay. Specificity for these virus entry targets and not others in the replication cycle are maintained by washing out the compound before reverse transcription begins. Strongly virucidal compounds may also score positive in this assay through their ability to prevent a productive infection.

In the cell-cell transmission assays, compounds are initially assessed for their ability to prevent the transmission of virus from a chronically infected cell to a target cell under conditions which limit infection (4 h incubation) using a time interval compatible with antiviral targets associated with virus entry. In the cell-free transmission assays, compounds are assessed for their ability to inhibit attachment of virus to target cells during a 2 to 4 hour incubation. For each assay, defined quality control parameters involving total virus transmission, triplicate reproducibility and response to positive and negative controls are used to internally validate the results. Both antiviral efficacy (IC_{50} : concentration of compound that inhibits virus transmission by 50%) and compound cytotoxicity (TC_{50} : concentration of compound that results in 50% loss of cell viability) are determined by linear regression of the resulting data. From these parameters a therapeutic index (TI: TC_{50}/IC_{50}) is derived, and is used to identify active compounds (TI>10).

SECONDARY ANALYSIS OF ACTIVE COMPOUNDS

Active compounds are retested to verify and confirm initial activity in the appropriate CD4-dependent transmission assay with and without mucin, to assess the effects of a mucous-like environment on compound efficacy. Those that display consistent activity are advanced for further secondary analysis. Since the secretions of the vaginal vault are enriched in proteins and mucopolysaccharides, a successful topical microbicide must be able to mediate antiviral activity in a complex environment. The mucopolysaccharide-enriched environment of the vaginal vault is mimicked in our assay by the inclusion of 150 μ g/mL porcine mucin in the HIV transmission inhibition reactions. Failure of a compound to maintain blocking activity in the presence of mucin may be an indicator of potential problems with its future use as a topical microbicide.

Following verification of activity in the CD4-dependent HIV transmission inhibition assays, secondary analyses of the active compounds includes testing in *Lactobacillus spp.* to monitor the effect of the compound on beneficial vaginal bacteria. The rate of HIV-1 transmission has been correlated with the presence or

absence of beneficial H₂O₂-secreting *Lactobacillus* species. Thus, as part of the secondary tier of antiviral testing, we assess the effect of the compound on two common *Lactobacillus* species: *L. jensenii* and *L. crispatus*. Lack of toxicity to these beneficial bacteria of the vagina is expected of a topical microbicide candidate.

A final set of secondary assays for active compounds may include assessment of the compound's ability to inhibit CXCR4-tropic HIV-1 virus-cell attachment and HIV-1 Env/CD4 mediated syncytium formation. The attachment and fusion assays are outlined and compared in **Table 10**. The attachment assay uses HeLa CD4 LTR β -gal cells, and measures the ability of compounds to inhibit the initial attachment of HIV-1 to the cell. These cells naturally express the CXCR4 coreceptor and are engineered to express cell surface CD4 and the β -galactosidase enzyme under control of the HIV-1 LTR promoter. Infection of HeLa CD4 LTR β -gal cells with HIV-1 induces the expression of the β -galactosidase enzyme via *tat* transactivation of the HIV-1 LTR. Compounds that interact with any phase of the virus cell attachment/entry process [binders to gp120, gp41 or new conformational targets, inhibitors of the gp120/CD4 interaction, or the interaction of the virus gp120/CD4 complex with the X4 coreceptor] score as positive in this assay. Specificity for these virus entry targets and not others in the replication cycle are maintained by washing out the compound before reverse transcription begins. Strongly virucidal compounds may also score positive in this assay through their ability to prevent a productive infection.

The fusion assay uses HeLa CD4 LTR β -gal cells with HL2/3 cells as a fusion partner via the interaction of HIV gp120 with CD4. HL2/3 cells express HIV-1 Env on their cell surface and contain an HIV-1 Tat expression cassette. Upon syncytium formation and plasma membrane fusion, cytoplasmic mixing of the HL2/3 and HeLa CD4 LTR β -gal cell contents results in the transactivation of the LTR and β -Galactosidase expression is then directly related to inhibition of cell membrane fusion. Thus, these two mechanistic assays can be used to confirm and identify a virus entry-based target of the compound. Additionally, because the attachment assay uses cell-free virus this assay can be used to show applicability to cell-free virus transmission.

SUMMARY OF THE PROGRAM

The identification of potent topical microbicides for the prevention of HIV transmission is becoming a global necessity as the rates of transmission increase in developing countries and selected sectors of other

populations. Contractors to the NIAID have assembled a series of initial assays to test compounds for suitability as topical microbicides. This testing includes not only assessment of the ability of the compounds to prevent HIV transmission, but assessments of the compound's potential efficacy in the vaginal environment and its mechanism of action.

ALGORITHM FOR EVALUATING TEST AGENTS

As described above, the primary screening assays consist of two CD4-dependent cell-cell transmission inhibition assays (to assess activity of the compounds to inhibit cell to cell CCR5- and CXCR4-tropic infection) and a cell-free CCR5-tropic virus transmission assay (also known as the R5 attachment inhibition assay).

Confirmation of activity in the cell-cell transmission assays is performed in the presence and absence of mucin. This test is preferentially performed in the R5-tropic system, unless the compound showed activity only in the X4-tropic system.

Confirmed active compounds will be advanced for secondary testing in the Lactobacillus toxicity, the CD4-independent (ME180) X4-tropic HIV transmission inhibition assay (optional) and the CD4 dependent cell-associated HIV transmission assay with pH transition as appropriate.

For compounds that were only active in the X4-based cell-cell transmission assay, the X4 attachment, and X4 fusion assays will also be performed.

Compounds that only show activity in the cell-free R5 assay will be confirmed by repeat testing and advanced for secondary testing in the Lactobacillus toxicity and in the X4-based attachment and fusion assays.

Experimental Methods

Cells and viruses:

HIV-1_{IIIIB}, JR-CSF, BaL and the HeLa CD4 LTR β -gal, MAGI-R5, GHOST X4/R5, GHOST R5, MOLT4, and HL2/3 cell lines were obtained from the NIH AIDS Research and Reference Reagent Program (Bethesda, MD), and maintained as recommended. ME180 cells, *Lactobacillus jensenii* and *L. crispatus* were obtained from the American Type Culture Collection (Manassas, VA).

Test Material Handling and Storage:

Compounds are typically solubilized in 100% DMSO and stored at -80°C until tested, unless alternative solvents and storage conditions are specified by the sponsor. Frozen stocks are thawed at room temperature, pre-warmed for 15 min at 37°C and vortexed prior to preparation of working solutions in tissue culture medium. During all stages of compound dilution and handling, compounds are protected from incidental light by opaque coverings and by storage and dilution in opaque or amber-colored tissue culture plastics. Additionally, incidental room and laminar flow tissue culture hood light exposure is controlled by reducing total fluorescent lighting in the laboratory by 50%. The final DMSO concentration is 0.25% at the highest test concentration, which has no effect on antiviral activity (unpublished data).

CD4-Dependent HIV Transmission Inhibition Assays:

The CD4-dependent HIV transmission inhibition assays use the CD4 positive GHOST(3) X4/R5 or the CD4 positive GHOST(3) R5 cell line. These cell lines are derived from the HOS (human osteosarcoma) cell line that is negative for HIV coreceptor and CD4 expression. The cell line is engineered to express T4 (CD4), CCR5 and/or CXCR4 via non-selectable retroviral vectors and an HIV-2 LTR hGFP construct with a hygromycin selectable marker.

Twenty-four hours prior to the assay cells are trypsinized, washed and seeded in 96-well flat bottom microtiter plates. On the day of the assay, effector cells (H9 cells chronically infected with the SK1 clinical isolate of HIV-1 (H9-SK1) or MOLT4 cells chronically infected with the JR-CSF molecular clone) are treated with freshly made mitomycin C (200 $\mu\text{g}/\text{ml}$) for 60 minutes at 37°C . This concentration of mitomycin C is sufficient to result in cell death, but allows virus transmission to occur. After mitomycin C treatment the effector cells are washed repeatedly with tissue culture medium. Test compounds are added to the monolayer followed by effector cells. The cells are co-cultured with effector cells and test material for 4 hours, and the effector cells are removed by washing the monolayer repeatedly with RPMI. At 20 hours after assay initiation the wells are again washed to ensure removal of the effector cells, and virus replication is assessed via measurement of cell-associated HIV-1 gag p24 using an ELISA (Beckman-Coulter P24 ELISA). Compound toxicity and cell viability are assessed by MTS dye reduction. Compounds judged as active in the first test are retested with and without the addition of mucin. Testing in the presence of mucin is carried out by addition of 150 $\mu\text{g}/\text{ml}$ of porcine mucin (Sigma Chemical Co., St Louis, MO) in tissue culture medium to the transmission reactions. Transmission intervals and washing without replacement of mucin or compound are

carried out as described above. Compounds evaluated in the pH transition assay are set up essentially the same as described above with the exception that compounds are prepared in medium adjusted to a pH of 4-4.5 and added to target cells. Addition of effector cells prepared in a buffered medium results in a transition of the pH to neutrality. All determinations are performed in triplicate with serial Log₁₀ dilutions of the test materials.

CD4-Independent HIV Transmission Inhibition Assay (Optional):

The assay is performed similarly to the CD4-dependent assay described except that it uses ME180 cells and additional washing steps are included at 24 and 48 hour post infection and the culture is maintained for 6 days. ME180, a CD4 negative, X4 positive cervical epithelial cell line, is maintained in RPMI 1640 supplemented with 10% fetal bovine serum, glutamine and antibiotics (**Table 9**). At six days following co-cultivation, supernatants are collected and evaluated for HIV-1 gag p24 antigen expression by ELISA. Cell viability is assessed by XTT or MTS dye reduction. Compounds judged as active in the first test are retested with and without the addition of mucin. Testing in the presence of mucin is carried out by addition of 150 µg/ml of porcine mucin (Sigma Chemical Co., St Louis, MO) to the transmission reactions. Transmission intervals and washing without replacement of mucin or compound are carried out as described above. All determinations are performed in triplicate with serial ½ Log₁₀ or Log dilution of the test materials.

Lactobacillus Assay:

Lactobacillus crispatus and *Lactobacillus jensenii* were obtained from the American Type Culture Collection and grown in *Lactobacilli* MRS broth (Difco, Fisher Scientific, Pittsburgh, PA). This medium allows efficient growth of the *Lactobacilli* under facultative anaerobic conditions. Bacterial stocks are produced and frozen in 15% glycerol at -80°C for use in the sensitivity assay. To assess the effect of compounds on *L. crispatus* and *L. jensenii* growth, 10 ml of MRS media is inoculated with a stab from the glycerol bacterial stock and the culture is incubated for 24 h at 37°C. The next day the bacterial density is adjusted to an OD of 0.06 at a wavelength of 670 nm. Compounds are diluted and plated into a 96 well round bottomed plates and the diluted *Lactobacillus spp.* added. Commercially available Penicillin/Streptomycin solution at a high-test concentration of 1.25 U/ml and 1.25 µg/ml, respectively, is used as the positive control. The plates are incubated for 24 h at 37° C in a Gas Pak CO₂ bag and bacterial growth determined by measurement of optical density at 490 nm using a 96 well spectrophotometric plate reader. All determinations are performed with six ½ log dilutions from a high test concentration in triplicate.

Virus Attachment Assays (X4 and R5):

This assay is designed to detect compounds that block virus attachment using MAGI-R5 or HeLa CD4 LTR β -gal cells. HeLa CD4 LTR β -gal cells are routinely cultured with the required selection antibiotics (**Table 10**). Twenty-four h prior to initiation of the assay the cells are trypsinized, counted and plated in a 0.2 cm well in media without selection antibiotics. At 24 h media is removed and compound in media placed on the cells and incubated for 15 min at 37°C. A known titer of the IIB or BaL strain of HIV-1 is then added to the wells and the incubation continued for 2 to 4 h. At the end of the incubation the wells are washed 2 times with media and the culture continued for 40 to 48 h. At termination of the assay, media is removed and β -galactosidase enzyme expression determined by chemiluminescence per manufacturer's instructions (Tropix Gal-screen™, Bedford Mass.). Compound toxicity is monitored on a sister plate by XTT or MTS dye reduction. All determinations are performed in triplicate with serial $\frac{1}{2}$ Log₁₀ dilution of the test materials. The virus adsorption interval of 1 to 2 h is sufficiently short that AZT, which requires phosphorylation to achieve its active tri-phosphate form (AZT-TTP), is not active in this assay.

Fusion Assay:

The fusion assay assesses the ability of compounds to block cell-to-cell fusion mediated by HIV-1 Env and CD4 expressed on separate cells. This assay is sensitive to inhibitors of both the gp120/CD4 interaction and inhibitors of the X4 coreceptor. HeLa CD4 LTR β -gal cells are plated in microtiter wells and diluted compounds are added and allowed to incubate at 37°C for 1 hr prior to the addition of HL2/3 cells. The incubation is then continued for 40 to 48 h, after which fusion is monitored by measurement of β -galactosidase enzyme expression, detectable by chemiluminescence (Tropix Gal-screen™, Tropix, Bedford, MA). Compound toxicity is monitored on a sister plate using XTT or MTS dye reduction. All determinations are performed in triplicate with serial $\frac{1}{2}$ Log₁₀ dilution of the test materials.

P24 Antigen ELISA:

ELISA kits are purchased from Coulter Electronics, and detection of supernate or cell-associated p24 antigen is performed according to the manufacturer's instructions. For cell-associated p24, cell lysates are prepared by lysis of the well contents in 25 to 100 μ l of lysis buffer, and assayed following 1 round of freeze/thaw. All p24 determinations are performed following serial dilution of the samples to ensure absorbance values in the linear range of the standard p24 antigen curve. The standard curve is generated using manufacturer-supplied standards and instructions. Data are obtained by spectrophotometric analysis at 450 nm using a

Molecular Devices Vmax or SpectraMaxPlus plate reader. Final concentrations are calculated from linear regression analysis of the optical density values and expressed in pg/ml p24 antigen.

CellTiter96® Staining for Cell Viability and Compound Cytotoxicity:

In most assays cell viability and TC₅₀ values were derived using a commercially available soluble tetrazolium-based MTS reagent (CellTiter96® Reagent, Promega). MTS is metabolized like XTT by the mitochondrial enzymes of metabolically active cells to a soluble formazan product, allowing the rapid quantitative analysis of cell viability and compound cytotoxicity. In contrast to XTT, this reagent is a single stable solution that does not require preparation before use. At termination of the assay, MTS reagent is added to each well and incubated for 2 to 4 h at 37°C in 5% CO₂. Adhesive plate sealers are applied, the sealed plate is inverted several times to mix the soluble formazan product and the plate is read spectrophotometrically at 490/650 nm with a Molecular Devices Vmax or SpectraMaxPlus plate reader.

Data analysis

TAK 779 and AMD 3100 are used as positive and negative controls respectively for the cell to cell and cell-free R5 tropic HIV-1 transmission inhibition assays. Dextran sulfate (positive) and Dextran (negative) are used as controls for the CD4-independent HIV transmission inhibition assay. The sulfonic acid dye Chicago Sky Blue and the X4-specific AMD3100 are used as positive controls for the X4-tropic attachment and fusion assays (Clanton et al., J AIDS. 1992 5:771). Commercially available Penicillin (5,000U/ml) Streptomycin (5 mg/ml) solution is used as a positive control for the *Lactobacillus* sensitivity assay. For each assay, where appropriate, an IC_{50 or 90} (concentration inhibiting virus replication or transmission by 50 or 90%), ID₅₀ (concentration inhibiting 50% growth of *Lactobacilli*), TC_{50 or 90} (concentration resulting in a 50 or 90% reduction in cell viability) is calculated by linear regression. The therapeutic index (TI) is calculated by dividing the TC_{50 or 90} by the IC_{50 or 90}.

TABLE 9: Summary of CD4-Independent and CD4-dependent Transmission Assay Methodologies

Parameter	TRANSMISSION ASSAY	
	CD4-independent	CD4-dependent
Reference	Phillips et al. <i>J. Virological Meth.</i> (1995) 52:1.	Cecilia et al. <i>J. Virol.</i> (1998) 72:6988.
Target cell line	ME180	GHOST(3) X4and/or R5
Derived from	Human Cervical Epithelia	Human Osteosarcoma
Cell source	ATCC	NIAID AIDS Research and Reference Reagent Repository
Special characteristics of the cell lines	Adherent, HPV positive (Related to HPV-39)	Adherent, retroviral vector expressed: CD4, X4 and/or R5. GFP by LTR reporter construct
Cell phenotype	CD4-, R5-, minimal X4	CD4+, R5+ and/or X4+
Target cell pretreatment	Trypsinized and plated 18 to 48 hour prior to assay	Trypsinized and plated 30 to 48 hour prior to assay
Cells per well	2×10^3 in 200 μ L medium	1.6×10^4 in 200 μ L medium
Transmitting cell (effector)	H9 cells chronically infected with HIV-1 strain SK-1	H9 cells chronically infected with HIV-1 strain SK-1; or MOLT4/CCR5 cells chronically infected with JR-CSF
Virus source	SK-1, clinical isolate	SK-1, clinical isolate; or JR-CSF, R5-tropic molecular clone
Cell source	H9: clone of HUT 78, T cell lymphoma	H9: clone of HUT 78, T cell lymphoma; MOLT4/CCR5; T-cell engineered to express CCR5
H9/SK-1 & MOLT4/CCR5/JRCSF developed @:	Southern Research Institute	
Mitomycin C pretreatment	Freshly prepared 200 μ g/mL in culture medium for 1 hour	
H9/SK-1 culture medium	RPMI 1640, 10% FBS, L-glutamine, Pen/Strep, non-essential amino acids	
H9/SK-1 cells per well	2×10^4	5×10^2
Ratio target to effector cells	1 :10	50:1
Transmission assay media	RPMI, no phenol red, 10% FBS, Pen/Strep, L-glutamine, non-essential amino acids	DMEM, 10% FBS, Pen/Strep, L-glutamine
Transmission interval	4 hour co-culture	4 hour co-culture
Washes after transmission	3	3
Washes at 24 h	3	1
Washes at 48 h	3	Not applicable
Washing medium	PBS without Ca^{++} and Mg^{++}	DMEM without FBS or antibiotics
Assay terminated	6 days	20 hours
Readout	ELISA of supernatant p24	ELISA of cell-associated p24
Compounds assayed in:	triplicate	triplicate
Positive control	Dextran sulfate	Dextran sulfate for X4, TAK 779 and T20 for R5
Negative control	Dextran	Dextran (X4 and R5) and Dextran sulfate (R5)
Compound toxicity	MTS dye reduction	
Toxicity testing on	Efficacy plate	Replicate plate
Data analysis	Linear regression for IC_{50} , TC_{50} , IC_{90} , TC_{90} and calculated TI	

TABLE 10: Comparison of HIV Attachment and Fusion Assays

Parameter	Attachment Assay	Fusion Assay	
Assay Principal	HIV-1 virus infection results in production of Tat, which transactivates the LTR β -galactosidase reporter.	Co-incubation of HL2/3 with HeLa CD4 LTR β -gal cells results in gp120/CD4 interaction followed by gp41/X4 mediated cell-to-cell fusion. Cytoplasmic mixing of HL2/3 and HeLa CD4 LTR β -gal cells results in Tat transactivation of the β -galactosidase reporter.	
References	Buckheit et al. (1994) <i>AIDS Res. Hum. Retroviruses</i> 11:1497.	Buckheit et al. (1994) <i>AIDS Res. Hum. Retroviruses</i> 11:1497. Howard et al. (1998) <i>J. Med. Chem.</i> 41:2184.	
Cell Lines	HeLa CD4 LTR β -gal with or without CCR5 receptor	HeLa CD4 LTR β -gal	HL2/3
Cell Line Source	NIAID AIDS Research and Reference Reagent Repository		
Genetic Modifications	HeLa cells expressing cell surface CD4, CXCR4 and containing a Tat-responsive LTR β -galactosidase reporter construct. MAGI-R5: same cell line expressing CCR5	HeLa cells expressing cell surface CD4 and containing a Tat-responsive LTR β -galactosidase reporter construct.	HeLa cells expressing HIV-1 Env on their cell surface and HIV Tat in the cytoplasm.
Cell Maintenance Media	DMEM 10 % FBS, L-glutamine, Pen/Strep., G418 and hygromycin B (plus puromycin for MAGI-R5 cells).	DMEM 10 % FBS, L-glutamine, Pen/Strep., G418 and hygromycin B.	DMEM 10 % FBS, L-glutamine, Pen/Strep., G418
Assay Media	DMEM 10 % FBS, L-Glutamine, Pen/Strep.		
Cell Number	0.25 to 1 x 10 ⁴	5 x 10 ³	5 x 10 ³
Virus	HIV IIIB or HIV-1 BaL	None	
Assay preparation	Trypsinized and split 1:2 @ 24 hours before plating assay.	Trypsinized and split 1:2 @ 24 hours before assay.	Trypsinized and split 1:2 @ 24 hours before assay.
Assay	<ul style="list-style-type: none"> ❖ Pre-incubate test compounds for 15-30 min. ❖ Add pre-titered virus and incubate 37°C for 2 to 4 hour. ❖ Wash 2 times with plain media. ❖ Add 200 μL fresh culture media and incubate for 40 to 48 hour. 	<ul style="list-style-type: none"> ❖ Pre-incubate test compounds for 1 hour at 37°C (volume 100 μL). 	<ul style="list-style-type: none"> ❖ No pre-treatment.
	<ul style="list-style-type: none"> ❖ Add HL2/3 cells (100 μL) to HeLa CD4 LTR β-gal monolayer. Total Volume 200 μL. ❖ Incubate for 40 to 48 H. 		
Endpoint Detection	β -galactosidase production by Chemiluminescence		
Positive Control	Chicago Sky Blue, TAK 779	Chicago Sky Blue	
Compound Toxicity	MTS dye reduction	MTS dye reduction	
Data analysis	Linear regression for IC ₅₀ , TC ₅₀ IC ₉₀ , TC ₉₀ and calculated TI		

Results

The complete results for the evaluation of NIAID compound 11039 for activity in the topical microbicide screening assays are contained in the Appendices. Summary tables for all of the data appear at the beginning of this report. **Appendix I** contains graphical representations of the CCR5-Tropic Transmission Inhibition Assays, **Appendix II** contains the graphical representations of the CD4-dependent CXCR4-Tropic Transmission Inhibition Assays, **Appendix III** contains the graphs of the CCR5 Cell-Free (Attachment) Inhibition Assays, **Appendix IV** contains the graphs of the *Lactobacillus spp.* Testing, **Appendix V** contains the graphs of the CXCR4-Tropic Attachment Inhibition Assays, **Appendix VI** contains the graphs of the CXCR4-Tropic HIV-1 Fusion Inhibition Assays and **Appendix VII** contains the graphs of the CD4-independent HIV transmission inhibition assays. Each data representation uses the same general format of 2 tables representing the individual values for each well of the triplicate determinations and a graphical representation of the data. For all evaluations, except the *Lactobacillus* sensitivity testing, the upper table represents the antiviral efficacy of the compound in either p24 antigen (pg/ml) by ELISA for HIV transmission inhibition assays or relative light units for chemiluminescence detection of β -galactosidase expression for the attachment and fusion assays. The lower table represents the cell viability as determined by MTS dye reduction (OD 490/650) in replicate plates. For the *Lactobacillus* sensitivity testing the upper table represents the absorbance at 490nm for *L. crispatus* and the lower table *L. jensenii*, which is proportional to bacteria growth. The IC₅₀, ID₅₀ and TC₅₀ values are calculated by linear regression using a Microsoft Excel-based template developed specifically for these assays. The TI_{50 or 90} represents the ratio of the TC_{50 or 90} / IC_{50 or 90}, and is used to determine relative potency of the compounds. The graphical representation presents the relationship between antiviral efficacy (%VC) or cell survival (*Lactobacillus* testing) and compound toxicity (%CC) expressed as a percent of the control, virus no compound or cells no compound, respectively for each concentration tested.

The assays performed met internal validation and standardization criteria for performance of each of the assays. These criteria include triplicate variability, overall HIV virus transmission in the transmission inhibition assays, total relative light units produced in the attachment and fusion assays and the activity of the control compounds. Thus, the assays performed meet specific criteria set for these parameters and represent an experimentally valid result.

APPENDIX I

Results of the CD4-dependent R5-Tropic Transmission Assays

**CD4-R5 DEPENDENT HIV-1 TRANSMISSION ASSAY
COMPOUND: TAK 779**

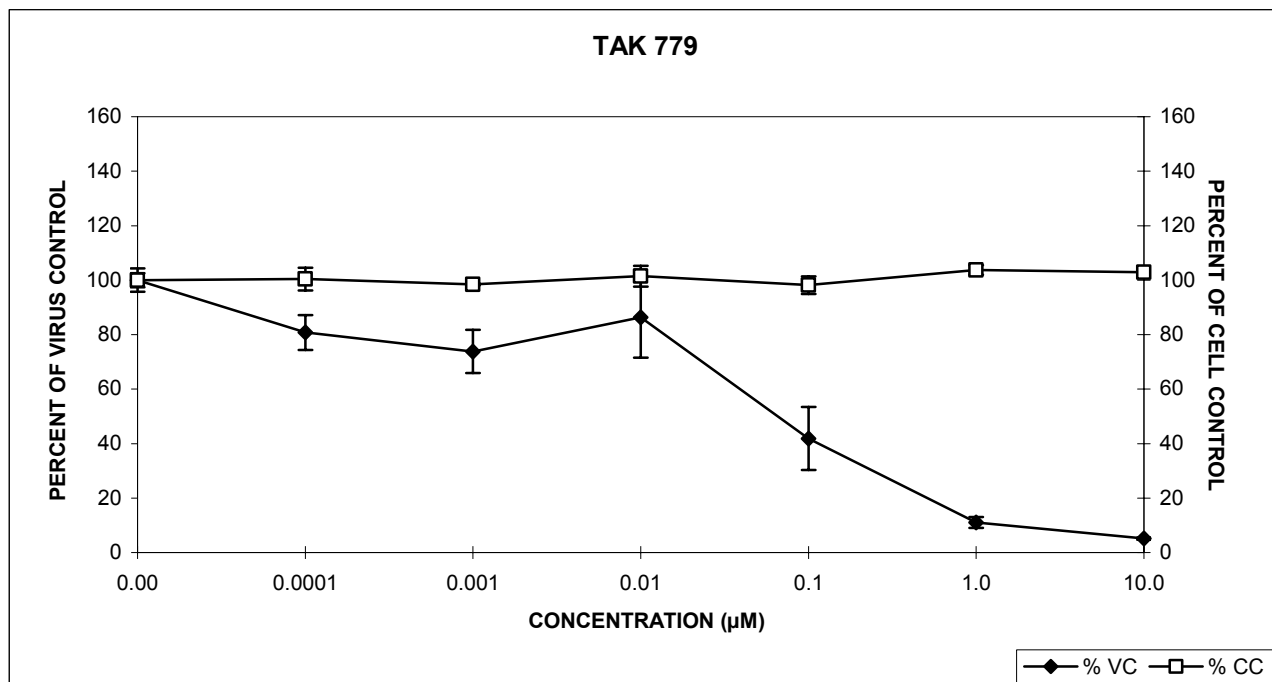
p24 VALUES (pg/ml)							
CONC (µM)	0.00	0.0001	0.001	0.01	0.1	1.0	10.0
SAMPLE 1	4368.52	3431.90	3518.66	4310.35	1797.25	567.82	233.59
SAMPLE 2	4180.70	3153.87	2936.97	3627.11	1273.73	416.97	196.13
SAMPLE 3	4178.73	3695.14	2937.96	3056.27	2253.73	426.83	220.77
MEAN	4242.65	3426.97	3131.20	3664.58	1774.91	470.54	216.83
% VC	100.0	80.8	73.8	86.4	41.8	11.1	5.1
STD DEV	2.6	6.4	7.9	14.8	11.6	2.0	0.4

TOXICITY VALUES (CellTiter96 - O. D. @ 490/650 nm)							
CONC (µM)	0.00	0.0001	0.001	0.01	0.1	1.0	10.0
SAMPLE 1	0.546	0.551	0.520	0.557	0.534	0.554	0.550
SAMPLE 2	0.502	0.531	0.517	0.518	0.519	0.549	0.535
SAMPLE 3	0.534	0.507	0.520	0.529	0.500	0.536	0.542
MEAN	0.527	0.529	0.519	0.535	0.518	0.547	0.542
% CC	100.0	100.4	98.5	101.4	98.2	103.7	102.9
STD DEV	4.3	4.2	0.3	3.8	3.3	1.7	1.4

IC50 (µM) = 0.07
IC90 (µM) = 1.52

TC50 (µM) = >10.0

TI = >142.86



**CD4-R5 DEPENDENT HIV-1 TRANSMISSION ASSAY
COMPOUND: TAK 779**

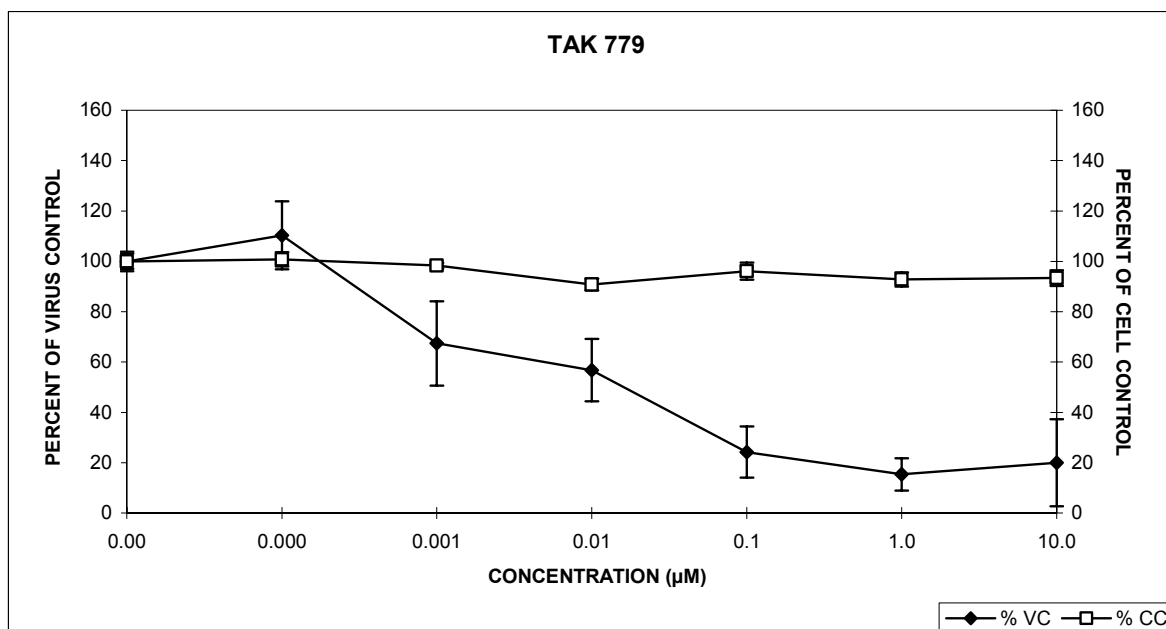
p24 VALUES (pg/ml)							
CONC (μM)	0.00	0.000	0.001	0.01	0.1	1.0	10.0
SAMPLE 1	2614.58	3080.92	2186.69	1072.61	901.06	205.00	967.11
SAMPLE 2	2419.37	2858.10	1478.80	1602.04	521.48	443.59	441.62
SAMPLE 3	2536.69	2411.48	1436.41	1623.73	413.03	513.59	103.45
MEAN	2523.54	2783.50	1700.63	1432.79	611.85	387.39	504.06
% VC	100.0	110.3	67.4	56.8	24.2	15.4	20.0
STD DEV	3.9	13.5	16.7	12.4	10.2	6.4	17.2

TOXICITY VALUES (CellTiter96 - O. D. @ 490/650 nm)							
CONC (μM)	0.00	0.000	0.001	0.01	0.1	1.0	10.0
SAMPLE 1	0.465	0.465	0.436	0.418	0.436	0.419	0.405
SAMPLE 2	0.442	0.454	0.446	0.397	0.444	0.429	0.432
SAMPLE 3	0.441	0.440	0.442	0.409	0.415	0.403	0.421
MEAN	0.449	0.453	0.441	0.408	0.431	0.417	0.419
% CC	100.0	100.8	98.3	90.8	96.0	92.8	93.3
STD DEV	3.0	2.8	1.1	2.4	3.4	2.8	3.1

IC50 (μM) = 0.02
IC90 (μM) = >10.0

TC50 (μM) = >10.0

TI = >500.00



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Tech: Osterling

CD4-R5 DEPENDENT HIV-1 TRANSMISSION ASSAY
GHOST X4R5

Southern Research Institute
Contract NO1-A1-05415

**CD4-R5 DEPENDENT HIV-1 TRANSMISSION ASSAY
COMPOUND: TAK 779 WITH PH TRANSITION**

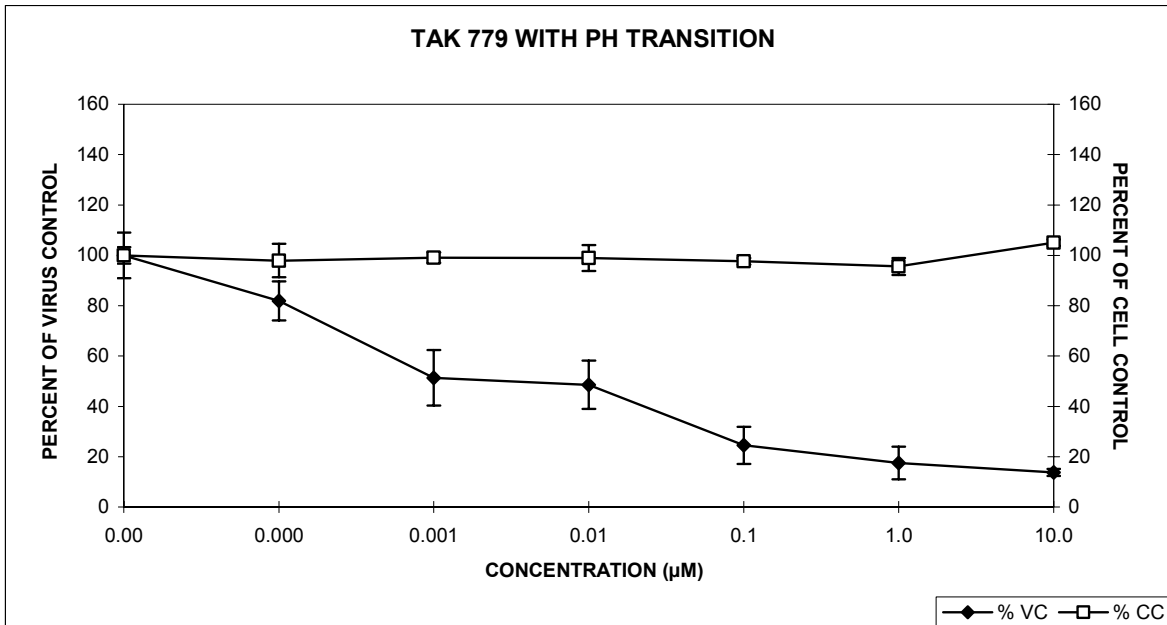
p24 VALUES (pg/ml)							
CONC (μM)	0.00	0.000	0.001	0.01	0.1	1.0	10.0
SAMPLE 1	3440.28	2450.92	1299.37	1285.56	552.04	479.08	409.08
SAMPLE 2	3003.03	2807.82	1839.65	1662.18	1036.13	834.01	465.28
SAMPLE 3	3585.21	2955.70	2006.66	1922.46	868.52	440.63	503.73
MEAN	3342.84	2738.15	1715.23	1623.40	818.90	584.58	459.37
% VC	100.0	81.9	51.3	48.6	24.5	17.5	13.7
STD DEV	9.1	7.8	11.1	9.6	7.4	6.5	1.4

TOXICITY VALUES (CellTiter96 - O. D. @ 490/650 nm)							
CONC (μM)	0.00	0.000	0.001	0.01	0.1	1.0	10.0
SAMPLE 1	0.440	0.416	0.425	0.437	0.429	0.411	0.455
SAMPLE 2	0.424	0.463	0.436	0.456	0.423	0.437	0.465
SAMPLE 3	0.453	0.411	0.443	0.411	0.434	0.412	0.464
MEAN	0.439	0.430	0.435	0.434	0.429	0.420	0.461
% CC	100.0	97.9	99.0	98.9	97.7	95.6	105.0
STD DEV	3.3	6.6	2.1	5.1	1.3	3.4	1.3

IC50 (μM) = 0.003
IC90 (μM) = >10.0

TC50 (μM) = >10.0

TI = >3333.33



**CD4-R5 DEPENDENT HIV-1 TRANSMISSION ASSAY
COMPOUND: AMD 3100**

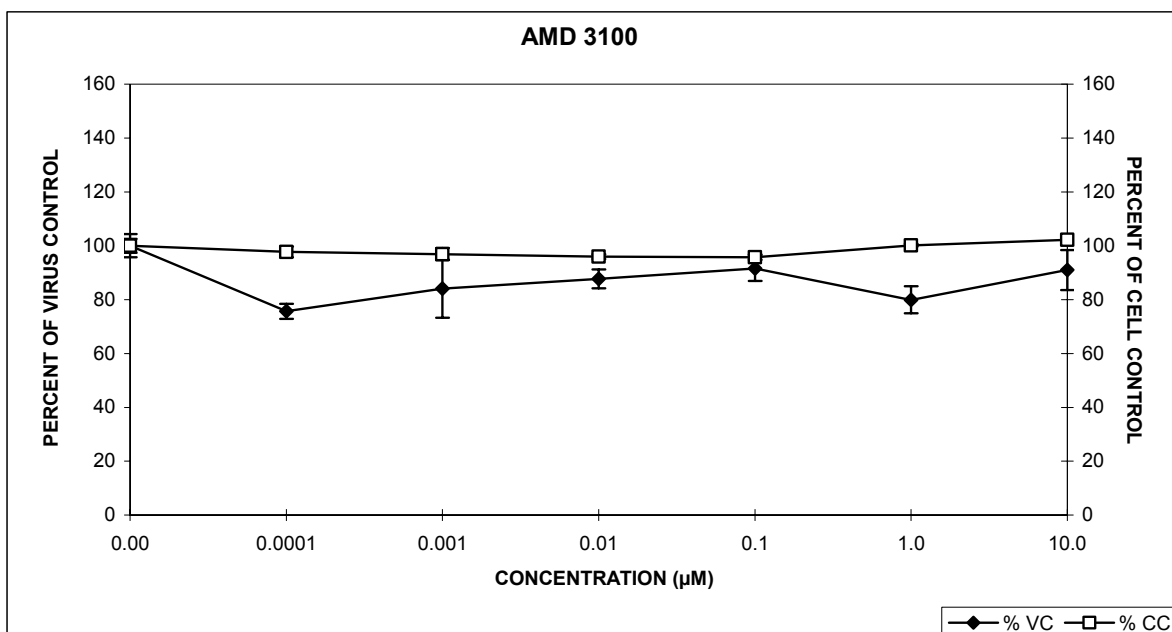
p24 VALUES (pg/ml)							
CONC (µM)	0.00	0.0001	0.001	0.01	0.1	1.0	10.0
SAMPLE 1	4368.52	3082.89	3987.96	3559.08	4067.82	3146.97	3596.55
SAMPLE 2	4180.70	3318.52	3078.94	3852.89	3681.34	3524.58	4208.80
SAMPLE 3	4178.73	3233.73	3633.03	3751.34	3899.23	3500.92	3773.03
MEAN	4242.65	3211.71	3566.64	3721.10	3882.79	3390.82	3859.46
% VC	100.0	75.7	84.1	87.7	91.5	79.9	91.0
STD DEV	2.6	2.8	10.8	3.5	4.6	5.0	7.4

TOXICITY VALUES (CellTiter96 - O. D. @ 490/650 nm)							
CONC (µM)	0.00	0.0001	0.001	0.01	0.1	1.0	10.0
SAMPLE 1	0.546	0.504	0.504	0.510	0.511	0.529	0.540
SAMPLE 2	0.502	0.525	0.524	0.508	0.501	0.530	0.531
SAMPLE 3	0.534	0.516	0.504	0.501	0.502	0.525	0.546
MEAN	0.527	0.515	0.511	0.506	0.505	0.528	0.539
% CC	100.0	97.7	96.9	96.0	95.7	100.2	102.3
STD DEV	4.3	2.0	2.2	0.9	1.0	0.5	1.4

IC50 (µM) = >10.0
IC90 (µM) = >10.0

TC50 (µM) = >10.0

TI = N/A



**CD4-R5 DEPENDENT HIV-1 TRANSMISSION ASSAY
COMPOUND: AMD 3100**

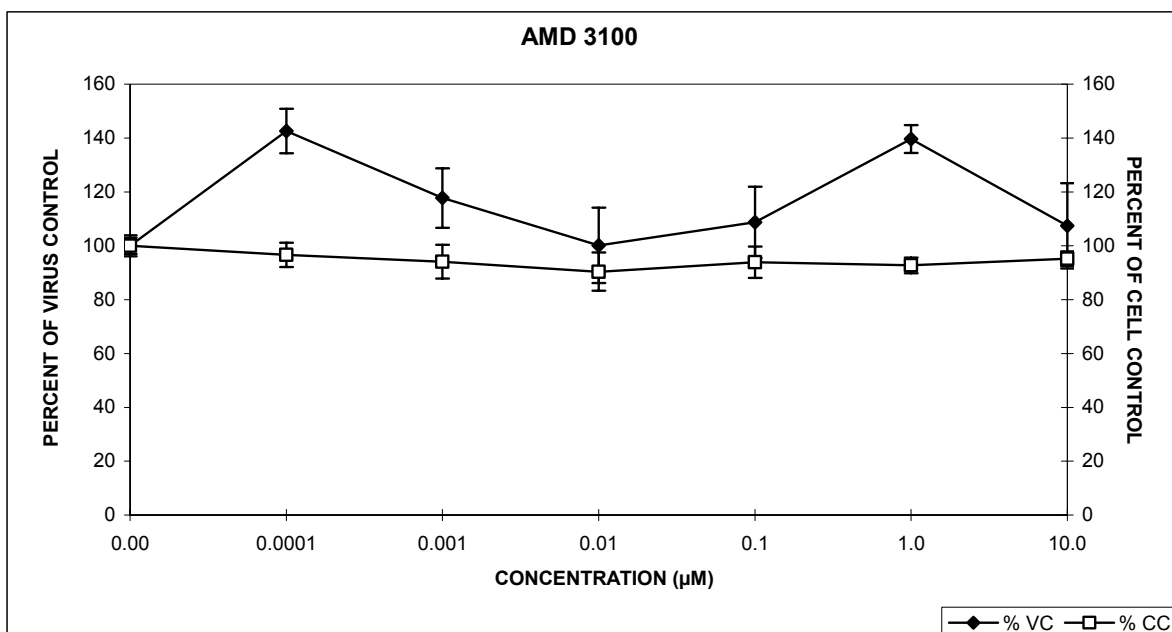
p24 VALUES (pg/ml)							
CONC (µM)	0.00	0.0001	0.001	0.01	0.1	1.0	10.0
SAMPLE 1	2614.58	3451.62	2648.10	2776.27	2952.75	3623.17	2494.30
SAMPLE 2	2419.37	3746.41	3129.23		2358.24	3375.70	3171.62
SAMPLE 3	2536.69		3133.17	2278.38	2916.27	3569.93	2464.72
MEAN	2523.54	3599.01	2970.16	2527.32	2742.42	3522.93	2710.21
% VC	100.0	142.6	117.7	100.1	108.7	139.6	107.4
STD DEV	3.9	8.3	11.1	14.0	13.2	5.2	15.8

TOXICITY VALUES (CellTiter96 - O. D. @ 490/650 nm)							
CONC (µM)	0.00	0.0001	0.001	0.01	0.1	1.0	10.0
SAMPLE 1	0.465	0.427	0.449	0.383	0.398	0.402	0.413
SAMPLE 2	0.442	0.457	0.426	0.443	0.450	0.428	0.434
SAMPLE 3	0.441	0.418	0.393	0.392	0.418	0.419	0.435
MEAN	0.449	0.434	0.423	0.406	0.422	0.417	0.428
% CC	100.0	96.6	94.1	90.4	93.9	92.7	95.2
STD DEV	3.0	4.5	6.3	7.1	5.9	2.9	2.8

IC50 (µM) = >10.0
IC90 (µM) = >10.0

TC50 (µM) = >10.0

TI = N/A



remediated
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CD4-R5 DEPENDENT HIV-1 TRANSMISSION ASSAY
GHOST X4R5

Southern Research Institute
Contract NO1-A1-05415

CD4-R5 DEPENDENT HIV-1 TRANSMISSION ASSAY COMPOUND: AMD 3100 WITH PH TRANSITION

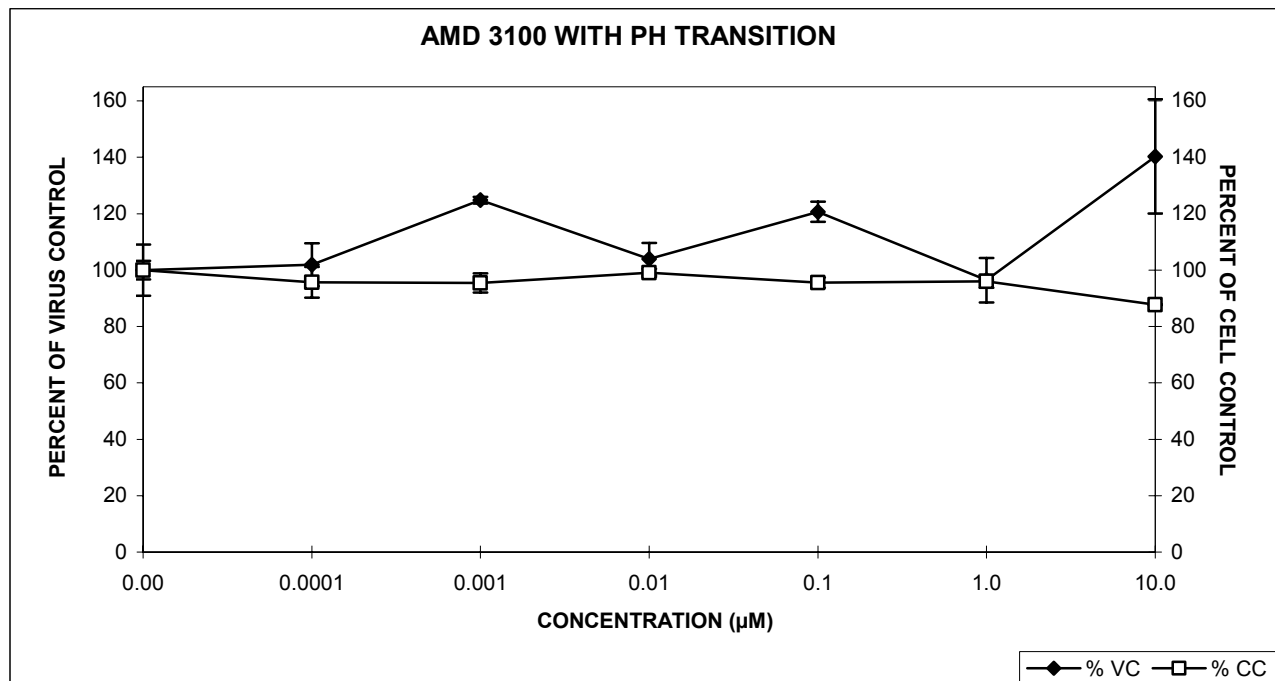
p24 VALUES (pg/ml)							
CONC (µM)	0.00	0.0001	0.001	0.01	0.1	1.0	10.0
SAMPLE 1	3440.28	3413.17	4210.77	3471.34	4001.76	3524.58	4211.76
SAMPLE 2	3003.03	3149.93	4173.31	3284.01	3933.73	3108.52	5169.08
SAMPLE 3	3585.21	3655.70	4128.94	3664.58	4167.39	3034.58	
MEAN	3342.84	3406.27	4171.01	3473.31	4034.30	3222.56	4690.42
% VC	100.0	101.9	124.8	103.9	120.7	96.4	140.3
STD DEV	9.1	7.6	1.2	5.7	3.6	7.9	20.3

TOXICITY VALUES (CellTiter96 - O. D. @ 490/650 nm)							
CONC (µM)	0.00	0.0001	0.001	0.01	0.1	1.0	10.0
SAMPLE 1	0.440	0.402	0.420	0.438	0.425	0.425	0.385
SAMPLE 2	0.424	0.411	0.434	0.438	0.423	0.410	0.385
SAMPLE 3	0.453	0.447	0.404	0.428	0.410	0.429	0.386
MEAN	0.439	0.420	0.419	0.435	0.420	0.422	0.385
% CC	100.0	95.7	95.5	99.0	95.6	96.0	87.7
STD DEV	3.3	5.5	3.4	1.4	1.9	2.3	0.1

IC50 (µM) = >10.0
IC90 (µM) = >10.0

TC50 (µM) = >10.0

TI = N/A



**CD4-R5 DEPENDENT HIV-1 TRANSMISSION ASSAY
COMPOUND: NIAID 11039**

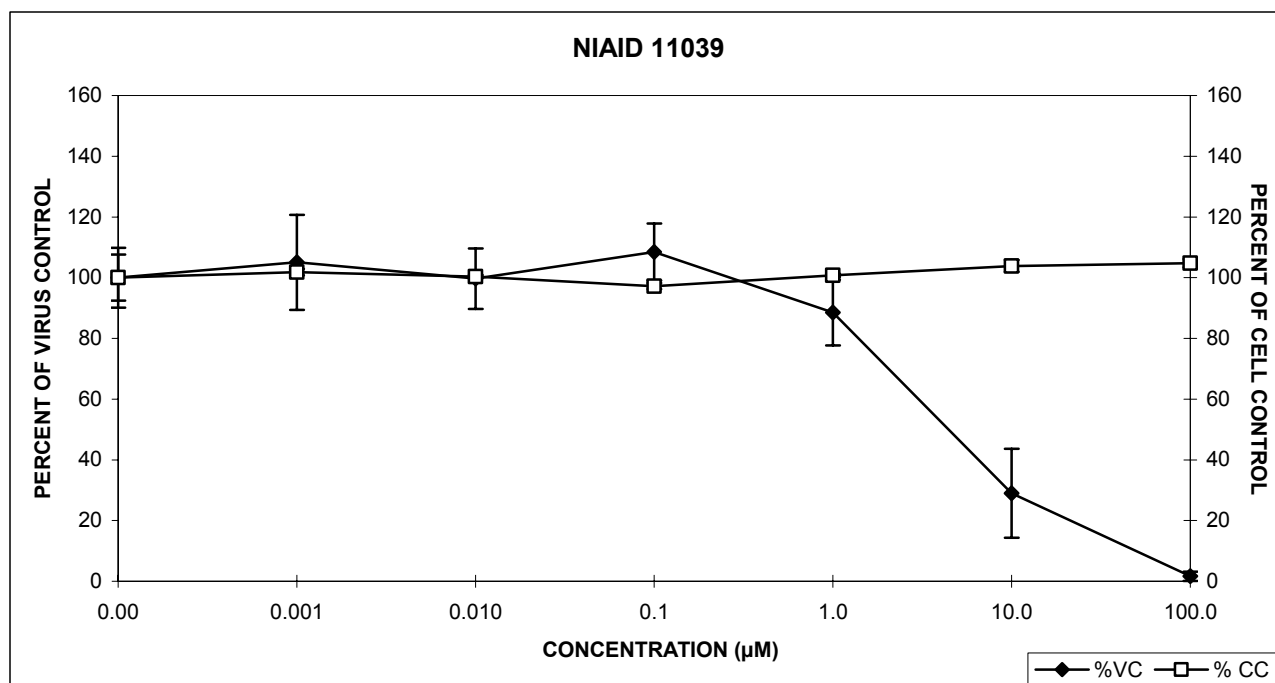
p24 VALUES (pg/ml)							
CONC (μM)	0.00	0.001	0.010	0.1	1.0	10.0	100.0
SAMPLE 1	4101.34	4532.18	4077.68	4072.75	3478.24	1690.77	110.35
SAMPLE 2	3488.59	3571.90	3366.83	4291.62	2797.96	744.30	0.00
SAMPLE 3	3456.55	3499.93	3567.96	3614.30	3499.93	768.94	72.89
MEAN	3682.16	3868.00	3670.82	3992.89	3258.71	1068.00	61.08
% VC	100.0	105.0	99.7	108.4	88.5	29.0	1.7
STD DEV	9.9	15.7	10.0	9.4	10.8	14.7	1.5

TOXICITY VALUES (CellTiter96 - O. D. @ 490/650 nm)							
CONC (μM)	0.00	0.001	0.010	0.1	1.0	10.0	100.0
SAMPLE 1	0.547	0.519	0.504	0.494	0.509	0.534	0.525
SAMPLE 2	0.491	0.513	0.509	0.484	0.505	0.522	0.524
SAMPLE 3	0.474	0.508	0.505	0.492	0.510	0.515	0.536
MEAN	0.504	0.513	0.506	0.490	0.508	0.524	0.528
% CC	100.0	101.8	100.4	97.2	100.8	103.9	104.8
STD DEV	7.6	1.1	0.5	1.0	0.5	1.9	1.4

IC50 (μM) = 4.44
IC90 (μM) = 49.5

TC50 (μM) = >100.0

TI = >22.52



**CD4-R5 DEPENDENT HIV-1 TRANSMISSION ASSAY
COMPOUND: NIAID 11039**

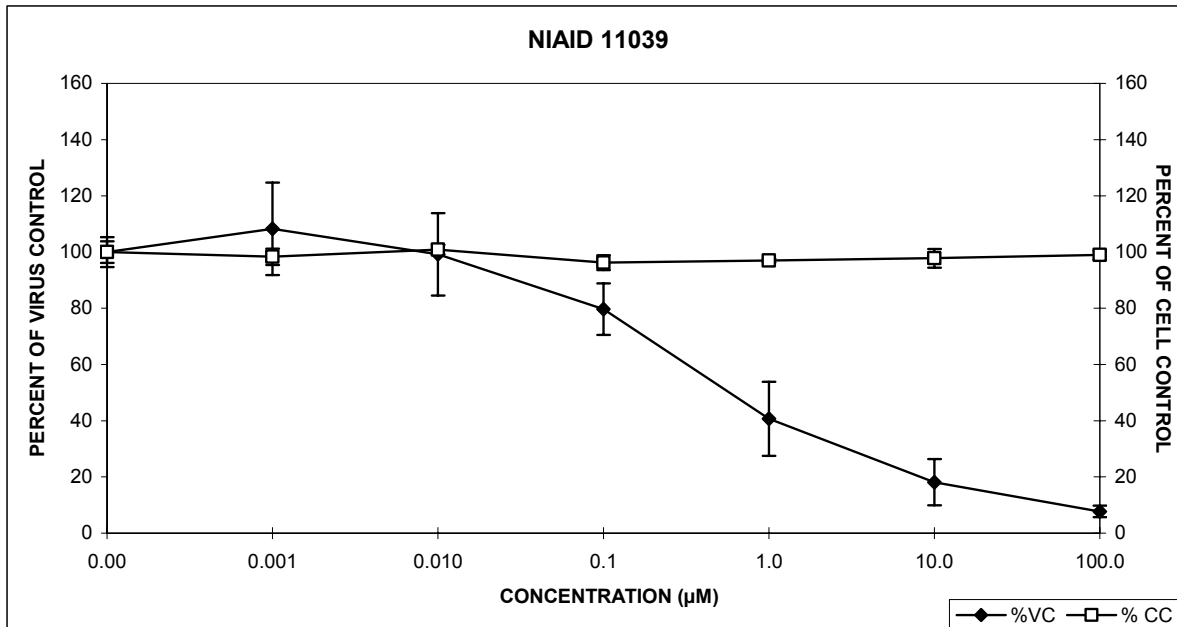
p24 VALUES (pg/ml)							
CONC (µM)	0.00	0.001	0.010	0.1	1.0	10.0	100.0
SAMPLE 1	3785.85	4600.21	3197.25	3442.75	1281.62	655.56	221.76
SAMPLE 2	4052.54	3465.42	4225.56	2729.93	2170.92	1045.00	379.51
SAMPLE 3	3804.58	4536.13	4125.99	3100.63	1286.55	410.07	297.68
MEAN	3880.99	4200.59	3849.60	3091.10	1579.69	703.54	299.65
% VC	100.0	108.2	99.2	79.6	40.7	18.1	7.7
STD DEV	3.8	16.4	14.6	9.2	13.2	8.2	2.0

TOXICITY VALUES (CellTiter96 - O. D. @ 490/650 nm)							
CONC (µM)	0.00	0.001	0.010	0.1	1.0	10.0	100.0
SAMPLE 1	0.469	0.453	0.464	0.461	0.447	0.440	0.452
SAMPLE 2	0.438	0.472	0.477	0.443	0.450	0.471	0.459
SAMPLE 3	0.487	0.445	0.464	0.437	0.454	0.452	0.467
MEAN	0.464	0.457	0.468	0.447	0.450	0.454	0.460
% CC	100.0	98.3	100.8	96.2	96.9	97.8	98.9
STD DEV	5.3	2.9	1.7	2.6	0.7	3.3	1.6

IC50 (µM) = 0.58
IC90 (µM) = 60.4

TC50 (µM) = >100.0

TI = >172.41



March 10, 2005
Tech: Osterling

CD4-R5 DEPENDENT HIV-1 TRANSMISSION ASSAY
GHOST X4R5

Southern Research Institute
Contract NO1-A1-05415

CD4-R5 DEPENDENT HIV-1 TRANSMISSION ASSAY COMPOUND: NIAID 11039 WITH PH TRANSITION

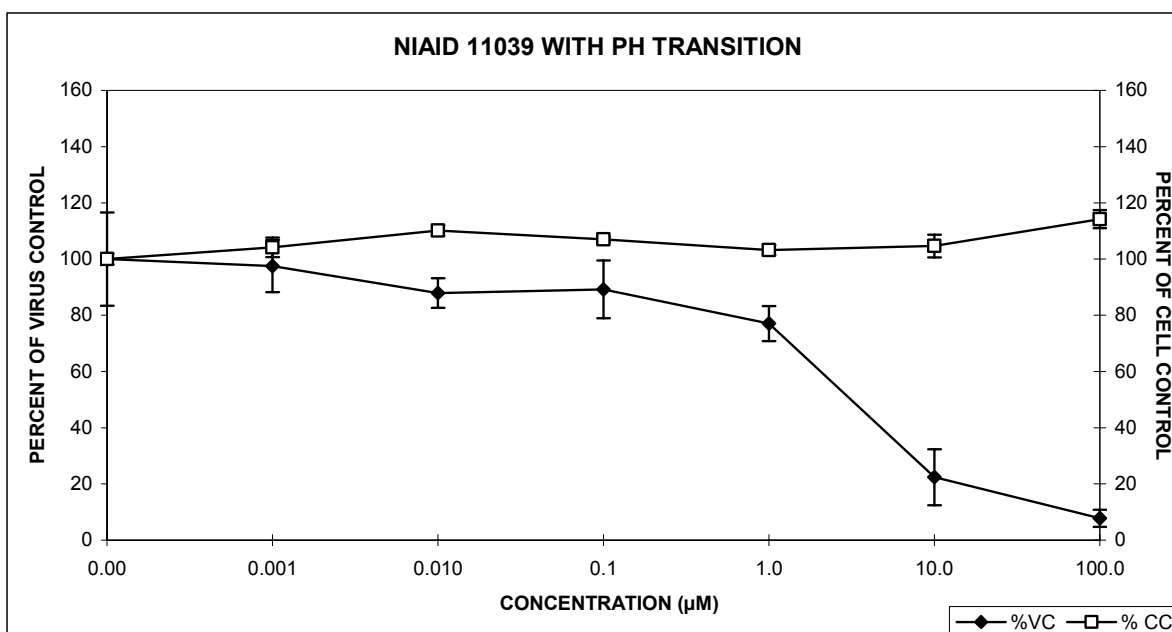
p24 VALUES (pg/ml)							
CONC (µM)	0.00	0.001	0.010	0.1	1.0	10.0	100.0
SAMPLE 1	4394.15	4206.83	3205.14	3371.76	3011.90	444.58	249.37
SAMPLE 2	3170.14	3721.76	3600.49	3931.76	3251.48	1197.82	440.63
SAMPLE 3	4159.01	3501.90	3503.87	3155.85	2766.41	987.82	225.70
MEAN	3907.77	3810.16	3436.50	3486.46	3009.93	876.74	305.23
% VC	100.0	97.5	87.9	89.2	77.0	22.4	7.8
STD DEV	16.6	9.2	5.3	10.2	6.2	9.9	3.0

TOXICITY VALUES (CellTiter96 - O. D. @ 490/650 nm)							
CONC (µM)	0.00	0.001	0.010	0.1	1.0	10.0	100.0
SAMPLE 1	0.444	0.464	0.478	0.468	0.457	0.470	0.512
SAMPLE 2	0.428	0.457	0.486	0.465	0.445	0.458	0.489
SAMPLE 3	0.431	0.435	0.470	0.460	0.443	0.435	0.486
MEAN	0.434	0.452	0.478	0.464	0.448	0.454	0.496
% CC	100.0	104.1	110.1	107.0	103.2	104.7	114.2
STD DEV	2.0	3.5	1.8	1.0	1.7	4.1	3.3

IC₅₀ (µM) = 3.13
IC₉₀ (µM) = 70.8

TC₅₀ (µM) = >100.0

TI = >31.95



March 10, 2005
Tech: Osterling

CD4-R5 DEPENDENT HIV-1 TRANSMISSION ASSAY
GHOST X4R5

Southern Research Institute
Contract NO1-A1-05415

APPENDIX II

Results of the CD4-dependent X4-Tropic Transmission Assays

**CD4-DEPENDENT HIV-1 TRANSMISSION ASSAY
COMPOUND: TAK 779**

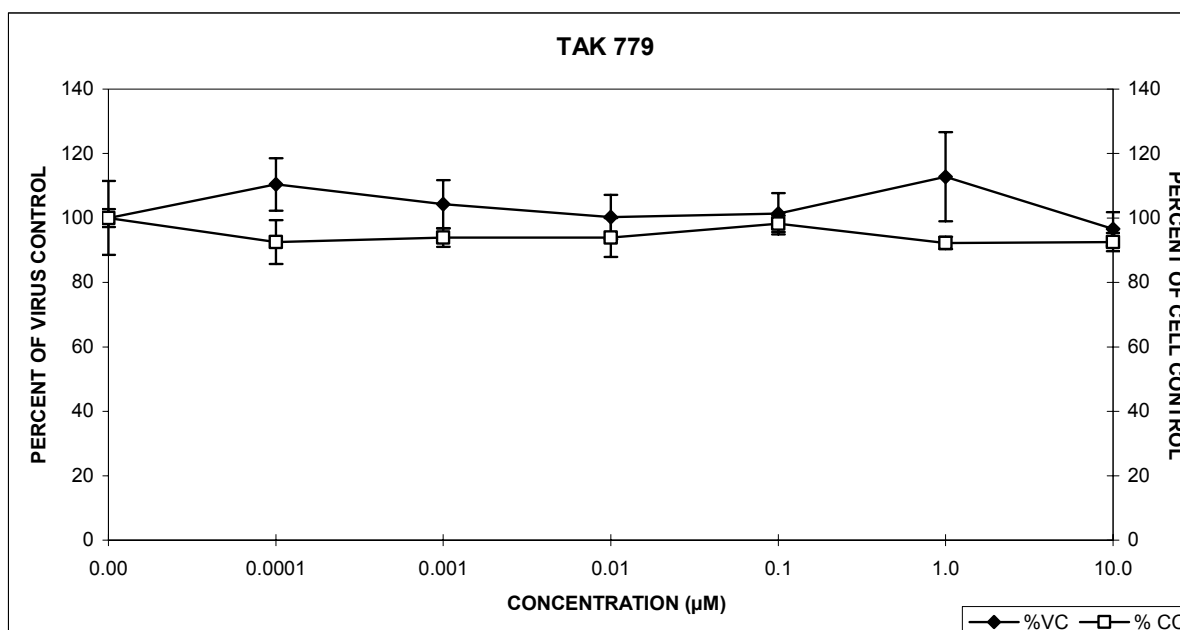
p24 VALUES (pg/ml)							
CONC (µM)	0.00	0.0001	0.001	0.01	0.1	1.0	10.0
SAMPLE 1	9252.91	10065.34	11153.83	9204.99	9369.48	9812.88	9187.11
SAMPLE 2	9257.20	11643.00	9714.19	10562.38	10129.70	11168.84	9371.62
SAMPLE 3	11221.05	11128.79	10133.99	10049.60	10628.89	12553.41	10161.17
MEAN	9910.38	10945.71	10334.00	9938.99	10042.69	11178.38	9573.30
% VC	100.0	110.4	104.3	100.3	101.3	112.8	96.6
STD DEV	11.5	8.1	7.5	6.9	6.4	13.8	5.2

TOXICITY VALUES (CellTiter96 - O. D. @ 490/650 nm)							
CONC (µM)	0.00	0.0001	0.001	0.01	0.1	1.0	10.0
SAMPLE 1	0.957	0.799	0.849	0.907	0.943	0.859	0.834
SAMPLE 2	0.940	0.867	0.881	0.813	0.897	0.845	0.876
SAMPLE 3	0.906	0.927	0.902	0.914	0.912	0.881	0.884
MEAN	0.934	0.864	0.877	0.878	0.917	0.862	0.864
% CC	100.0	92.5	93.9	94.0	98.2	92.2	92.5
STD DEV	2.8	6.8	2.9	6.0	2.5	1.9	2.9

IC50 (µM) = >10.0
IC90 (µM) = >10.0

TC50 (µM) = >10.0

TI = N/A



**CD4-DEPENDENT HIV-1 TRANSMISSION ASSAY
COMPOUND: AMD 3100**

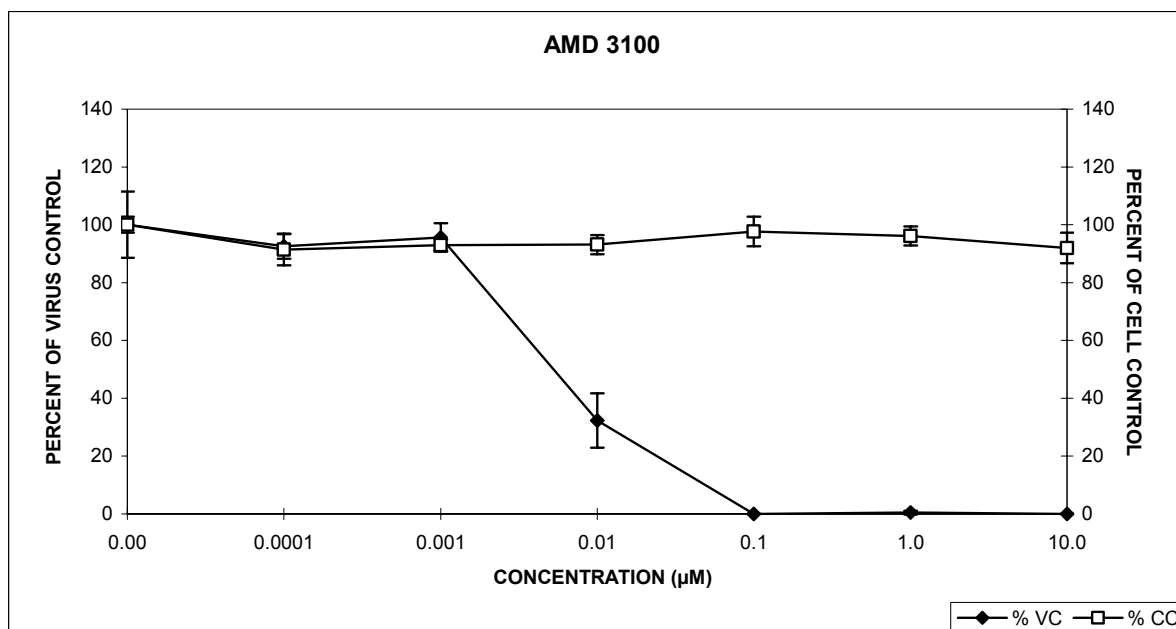
p24 VALUES (pg/ml)							
CONC (μM)	0.00	0.0001	0.001	0.01	0.1	1.0	10.0
SAMPLE 1	9252.91	8868.14	8916.78	3680.31	0.00	0.00	0.00
SAMPLE 2	9257.20	9479.61	9714.19	3799.74	0.00	123.77	0.00
SAMPLE 3	11221.05		9794.29	2126.96	0.00	18.64	0.00
MEAN	9910.38	9173.88	9475.08	3202.33	0.00	47.47	0.00
% VC	100.0	92.6	95.6	32.3	0.0	0.5	0.0
STD DEV	11.5	4.4	4.9	9.4	0.0	0.7	0.0

TOXICITY VALUES (CellTiter96 - O. D. @ 490/650 nm)							
CONC (μM)	0.00	0.0001	0.001	0.01	0.1	1.0	10.0
SAMPLE 1	0.957	0.910	0.862	0.905	0.958	0.922	0.915
SAMPLE 2	0.940	0.838	0.889	0.857	0.863	0.864	0.841
SAMPLE 3	0.906	0.813	0.854	0.848	0.917	0.908	0.822
MEAN	0.934	0.854	0.868	0.870	0.913	0.898	0.859
% CC	100.0	91.4	93.0	93.1	97.7	96.1	92.0
STD DEV	2.8	5.4	1.9	3.3	5.1	3.2	5.2

IC50 (μM) = 0.005
IC90 (μM) = 0.05

TC50 (μM) = >10.0

TI = >2000.00



Remediated
February 17, 2005
Mankowski

CD4-DEPENDENT HIV-1 TRANSMISSION ASSAY
GHOST(3)X4/R5

Southern Research Institute
Contract NO1-A1-05415

CD4-DEPENDENT HIV-1 TRANSMISSION ASSAY COMPOUND: NIAID 11039

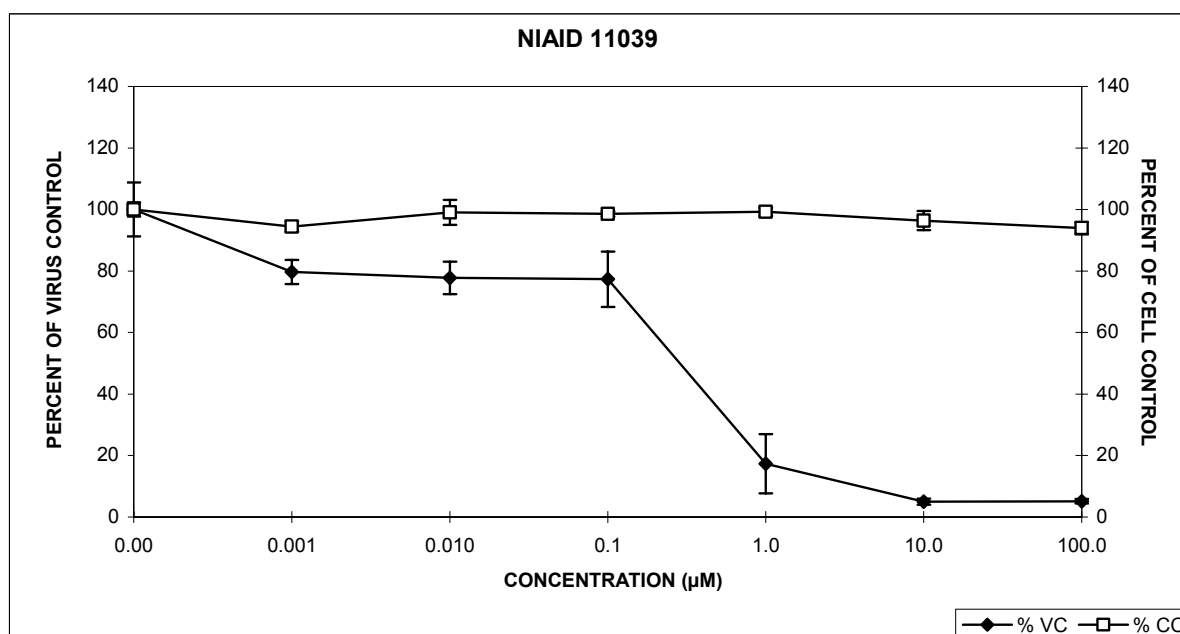
p24 VALUES (pg/ml)							
CONC (μM)	0.00	0.001	0.010	0.1	1.0	10.0	100.0
SAMPLE 1	7032.82	5415.33	5438.30	4823.70	761.01	423.26	310.68
SAMPLE 2	7904.18	5923.67	5332.04	5935.16	938.49	289.43	396.26
SAMPLE 3	6677.27	5882.31	6037.97	5958.71	2045.93	375.59	404.31
MEAN	7204.76	5740.44	5602.77	5572.52	1248.47	362.76	370.42
% VC	100.0	79.7	77.8	77.3	17.3	5.0	5.1
STD DEV	8.8	3.9	5.3	9.0	9.7	0.9	0.7

TOXICITY VALUES (CellTiter96 - O. D. @ 490/650 nm)							
CONC (μM)	0.00	0.001	0.010	0.1	1.0	10.0	100.0
SAMPLE 1	0.920	0.844	0.852	0.891	0.901	0.891	0.856
SAMPLE 2	0.884	0.862	0.884	0.876	0.886	0.863	0.822
SAMPLE 3	0.883	0.832	0.924	0.881	0.878	0.835	0.846
MEAN	0.895	0.846	0.887	0.883	0.888	0.863	0.841
% CC	100.0	94.5	99.0	98.6	99.2	96.4	93.9
STD DEV	2.3	1.7	4.0	0.9	1.3	3.1	2.0

IC₅₀ (μM) = 0.29
IC₉₀ (μM) = 3.95

TC₅₀ (μM) = >100.0

TI = >344.83



**APPENDIX III Results of the CCR5-Tropic Cell-Free (Attachment)
Assays**

INHIBITION OF R5-TROPIC HIV-1 ATTACHMENT BY TAK 779

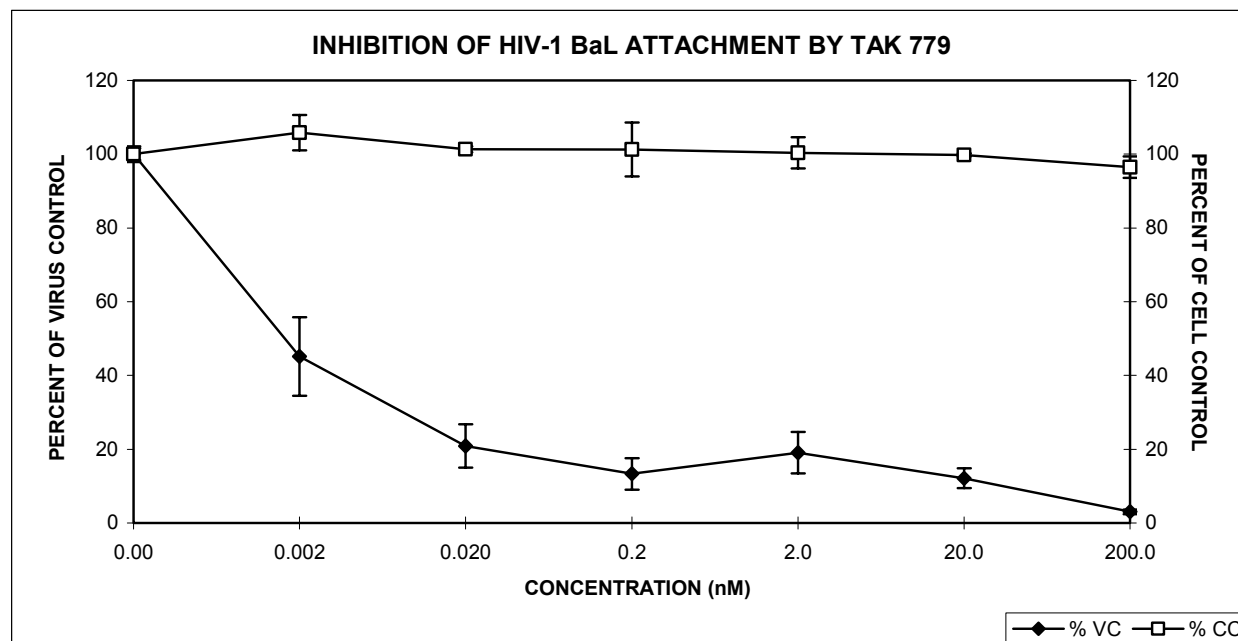
RLU (Relative Light Units)							
CONC (nM)	0.00	0.002	0.020	0.2	2.0	20.0	200.0
SAMPLE 1	64696.1	34415.6	17758.6	10922.6	14893.6	9772.6	1505.6
SAMPLE 2	63036.6	31246.6	11107.6	9205.6	13706.6	6680.6	2024.6
SAMPLE 3	64939.6	21302.6	11355.6	5538.6	8126.6	6881.6	2316.6
MEAN	64224.1	28988.3	13407.3	8555.6	12242.3	7778.3	1948.9
% VC	100.0	45.1	20.9	13.3	19.1	12.1	3.0
STD DEV	1.6	10.7	5.9	4.3	5.6	2.7	0.6

TOXICITY VALUES (CellTiter96 - O. D. @ 490/650 nm)							
CONC (nM)	0.00	0.002	0.020	0.2	2.0	20.0	200.0
SAMPLE 1	0.853	0.970	0.890	0.949	0.905	0.869	0.861
SAMPLE 2	0.881	0.915	0.889	0.884	0.890	0.876	0.814
SAMPLE 3	0.887	0.888	0.878	0.821	0.835	0.871	0.853
MEAN	0.874	0.925	0.885	0.885	0.877	0.872	0.843
% CC	100.0	105.8	101.3	101.3	100.4	99.8	96.5
STD DEV	2.1	4.8	0.8	7.3	4.2	0.4	2.9

IC50 (nM) = <0.002
IC90 (nM) = 34.2

TC50 (nM) = >200.0

TI = >100000.00



January 12, 2005
Tech: Snyder

Magi R5-Tropic Attachment Inhibition Assay
Virus: BaL

Southern Research Institute
Contract NO1-A1-05415

INHIBITION OF R5-TROPIC HIV-1 ATTACHMENT BY AMD 3100

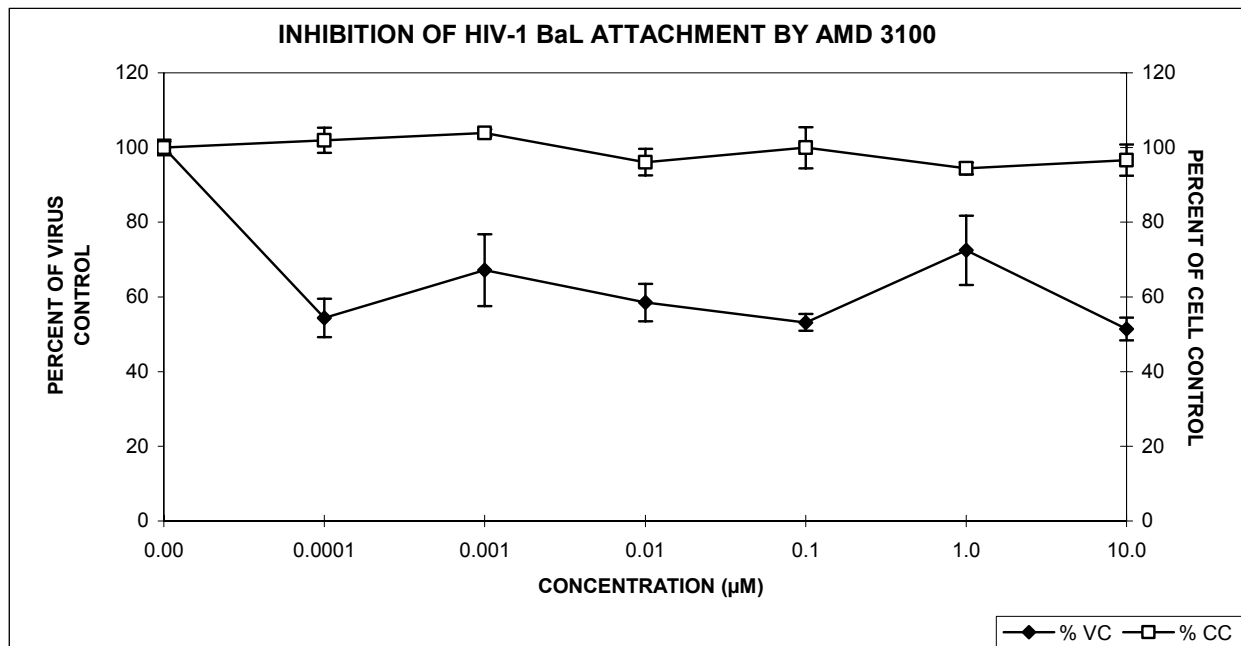
RLU (Relative Light Units)							
CONC (μM)	0.00	0.0001	0.001	0.01	0.1	1.0	10.0
SAMPLE 1	64696.1	31306.6	43439.6	37251.6	33285.6	49994.6	34684.6
SAMPLE 2	63036.6	35578.6	36807.6	34523.6	33322.6	39677.6	30910.6
SAMPLE 3	64939.6	37845.6	49145.6	40943.6	35825.6	50002.6	33506.6
MEAN	64224.1	34910.3	43130.9	37572.9	34144.6	46558.3	33033.9
% VC	100.0	54.4	67.2	58.5	53.2	72.5	51.4
STD DEV	1.6	5.2	9.6	5.0	2.3	9.3	3.0

TOXICITY VALUES (CellTiter96 - O. D. @ 490/650 nm)							
CONC (μM)	0.00	0.0001	0.001	0.01	0.1	1.0	10.0
SAMPLE 1	0.853	0.857	0.917	0.806	0.883	0.833	0.879
SAMPLE 2	0.881	0.903	0.901	0.867	0.916	0.832	0.847
SAMPLE 3	0.887	0.911	0.906	0.845	0.821	0.809	0.807
MEAN	0.874	0.891	0.908	0.839	0.873	0.825	0.844
% CC	100.0	101.9	103.9	96.1	99.9	94.4	96.6
STD DEV	2.1	3.3	0.9	3.5	5.5	1.6	4.1

IC50 (μM) = >10.0
IC90 (μM) = >10.0

TC50 (μM) = >10.0

TI = N/A



January 12, 2005
Tech: Snyder

Magi R5-Tropic Attachment Inhibition Assay
Virus: BaL

Southern Research Institute
Contract NO1-A1-05415

INHIBITION OF R5-TROPIC HIV-1 ATTACHMENT BY NIAID 11039

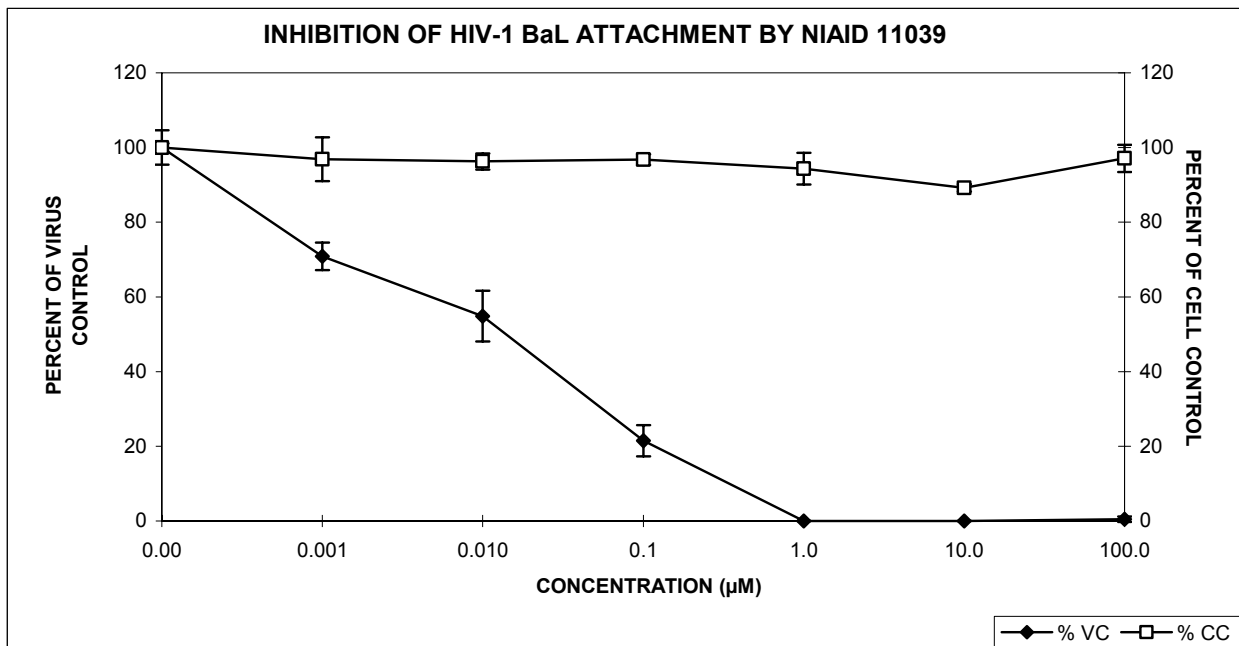
RLU (Relative Light Units)							
CONC (μM)	0.00	0.001	0.010	0.1	1.0	10.0	100.0
SAMPLE 1	65292.7	44136.9	29939.9	15449.9	0.0	0.0	813.9
SAMPLE 2	59897.2	41584.9	33796.9	10441.9	0.0	0.0	45.9
SAMPLE 3	60952.4	46143.9	38359.9	14085.9	0.0	0.0	0.0
MEAN	62047.4	43955.3	34032.3	13325.9	0.0	0.0	286.6
% VC	100.0	70.8	54.8	21.5	0.0	0.0	0.5
STD DEV	4.6	3.7	6.8	4.2	0.0	0.0	0.7

TOXICITY VALUES (CellTiter96 - O. D. @ 490/650 nm)							
CONC (μM)	0.00	0.001	0.010	0.1	1.0	10.0	100.0
SAMPLE 1	0.889	0.807	0.825	0.861	0.870	0.792	0.844
SAMPLE 2	0.873	0.835	0.863	0.851	0.815	0.783	0.825
SAMPLE 3	0.871	0.908	0.847	0.836	0.799	0.774	0.887
MEAN	0.878	0.850	0.845	0.849	0.828	0.783	0.852
% CC	100.0	96.9	96.3	96.8	94.3	89.2	97.1
STD DEV	1.1	5.9	2.2	1.4	4.2	1.0	3.6

IC50 (μM) = 0.01
IC90 (μM) = 0.34

TC50 (μM) = >100.0

TI = >10000.00



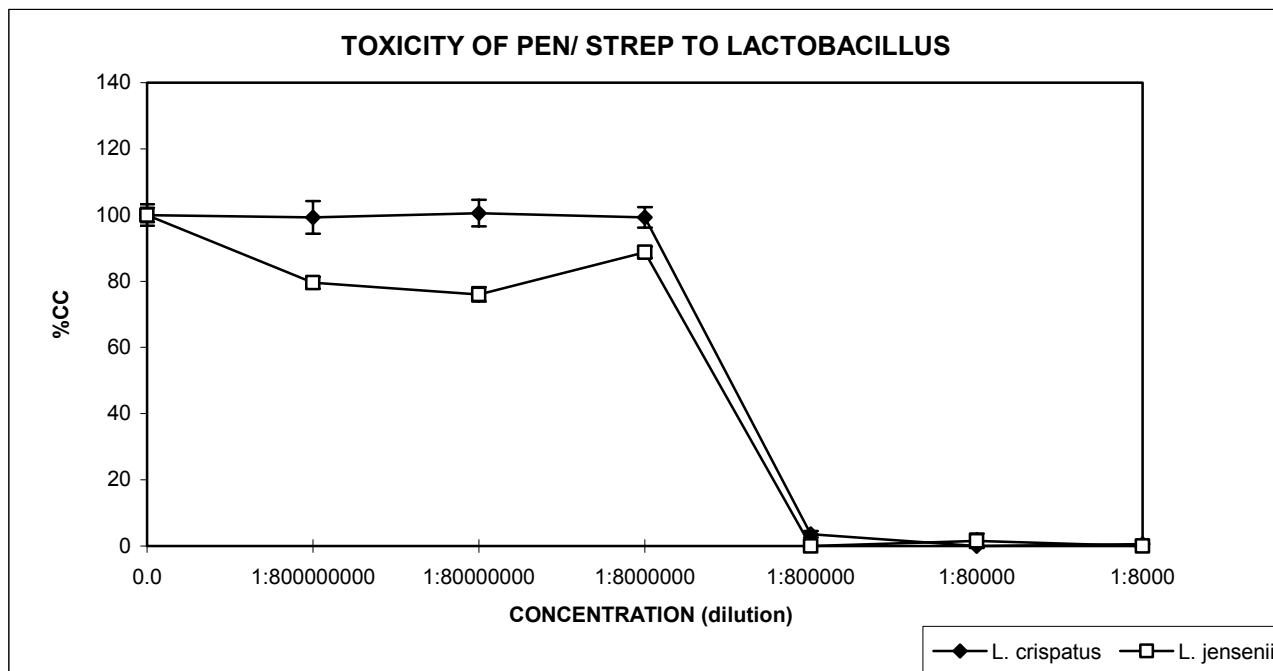
APPENDIX IV Results of the *Lactobacillus* Assay

TOXICITY OF PEN/ STREP TO LACTOBACILLUS

Toxicity to <i>L. crispatus</i> (O.D. @ 490 nm)							
CONC (dilution)	0.0	1:800000000	1:80000000	1:8000000	1:800000	1:80000	1:8000
SAMPLE 1	0.878	0.819	0.833	0.840	0.034	0.000	0.015
SAMPLE 2	0.826	0.837	0.856	0.833	0.036	0.000	0.000
SAMPLE 3	0.870	0.900	0.900	0.882	0.021	0.000	0.000
MEAN	0.858	0.852	0.863	0.852	0.031	0.000	0.005
% CC	100.0	99.3	100.6	99.3	3.6	0.0	0.6
STD DEV	3.2	4.9	4.0	3.1	0.9	0.0	1.0

Toxicity to <i>L. jensenii</i> (O.D. @ 490 nm)							
CONC (dilution)	0.00	1:800000000	1:80000000	1:8000000	1:800000	1:80000	1:8000
SAMPLE 1	1.065	0.840	0.803	0.935	0.000	0.043	0.000
SAMPLE 2	1.057	0.847	0.803	0.953	0.000	0.000	0.000
SAMPLE 3	1.100	0.876	0.844	0.973	0.000	0.008	0.000
MEAN	1.074	0.854	0.816	0.954	0.000	0.017	0.000
% CC	100.0	79.5	76.0	88.8	0.0	1.6	0.0
STD DEV	2.2	1.8	2.2	1.7	0.0	2.2	0.0

<i>L. crispatus</i> TC50 (dilution) = 1: 2444406
<i>L. jensenii</i> TC50 (dilution) = 1: 2925569

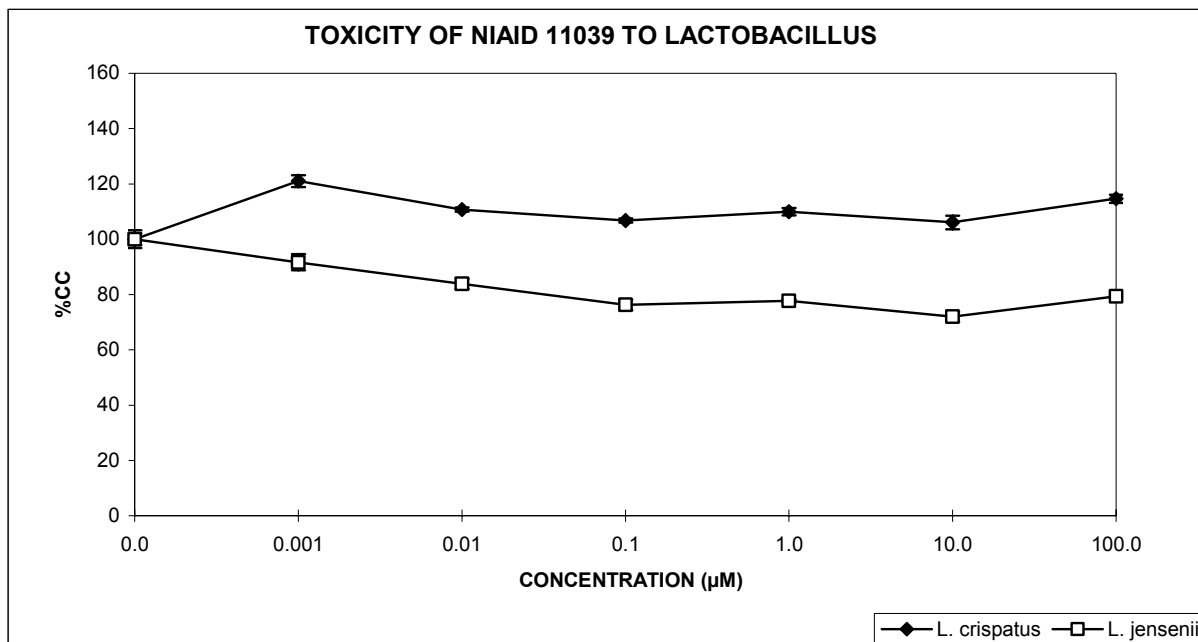


TOXICITY OF NIAID 11039 TO LACTOBACILLUS

Toxicity to <i>L. crispatus</i> (O.D. @ 490 nm)							
CONC (μM)	0.0	0.001	0.01	0.1	1.0	10.0	100.0
SAMPLE 1	0.878	1.058	0.952	0.922	0.951	0.931	0.995
SAMPLE 2	0.826	1.023	0.956	0.909	0.948	0.889	0.970
SAMPLE 3	0.870	1.034	0.942	0.917	0.930	0.912	0.986
MEAN	0.858	1.038	0.950	0.916	0.943	0.910	0.984
% CC	100.0	121.0	110.7	106.8	109.9	106.1	114.6
STD DEV	3.2	2.1	0.8	0.8	1.3	2.5	1.4

Toxicity to <i>L. jensenii</i> (O.D. @ 490 nm)							
CONC (μM)	0.00	0.001	0.01	0.1	1.0	10.0	100.0
SAMPLE 1	1.065	0.949	0.877	0.806	0.834	0.786	0.848
SAMPLE 2	1.057	0.998	0.911	0.843	0.841	0.783	0.857
SAMPLE 3	1.100	1.007	0.913	0.808	0.829	0.751	0.853
MEAN	1.074	0.985	0.901	0.819	0.835	0.773	0.853
% CC	100.0	91.7	83.8	76.2	77.7	72.0	79.4
STD DEV	2.2	2.9	1.9	1.9	0.6	1.8	0.5

<i>L. crispatus</i> TC50 (μM) = > 100
<i>L. jensenii</i> TC50 (μM) = > 100



APPENDIX V Results of the X4-Tropic Attachment Assay

INHIBITION OF X4-TROPIC HIV-1 ATTACHMENT BY Chicago Sky Blue

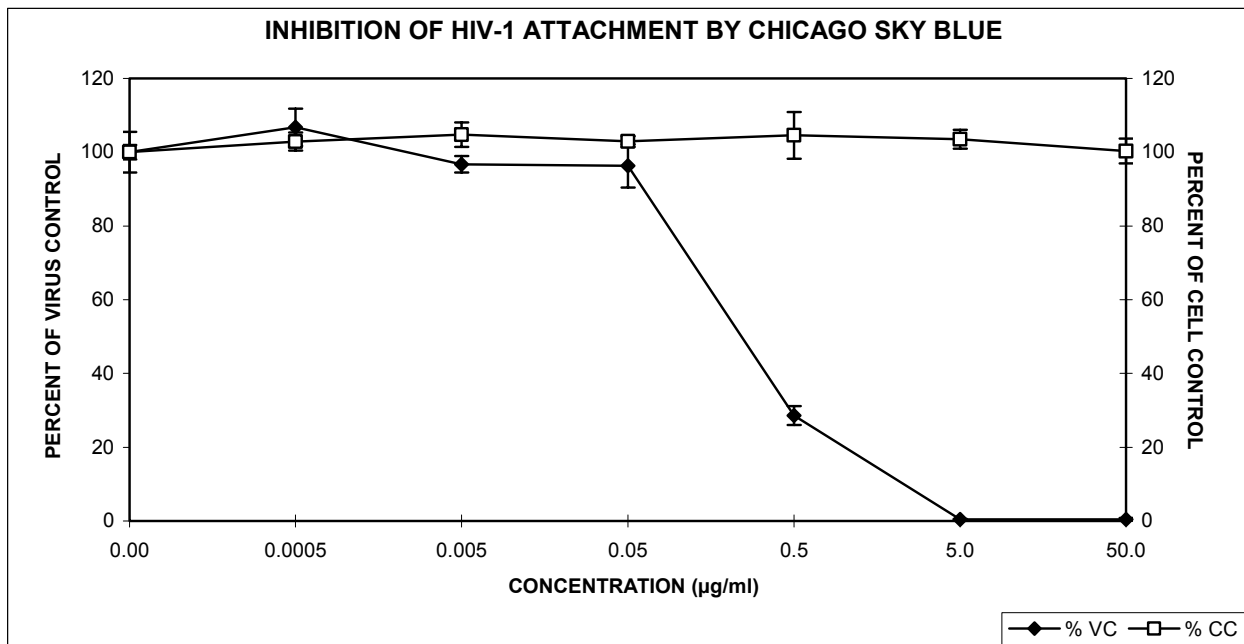
RLU (Relative Light Units)							
CONC (µg/ml)	0.00	0.0005	0.005	0.05	0.5	5.0	50.0
SAMPLE 1	125193.6	137593.1	127780.1	120769.1	34511.1	483.1	0.0
SAMPLE 2	137903.6	150350.1	132736.1	136344.1	41288.1	838.1	862.1
SAMPLE 3	137979.6	140165.1	127431.1	129062.1	38969.1	288.1	903.1
MEAN	133692.3	142702.8	129315.8	128725.1	38256.1	536.4	588.4
% VC	100.0	106.7	96.7	96.3	28.6	0.4	0.4
STD DEV	5.5	5.0	2.2	5.8	2.6	0.2	0.4

TOXICITY VALUES (CellTiter96 - O. D. @ 490/650 nm)							
CONC (µg/ml)	0.00	0.0005	0.005	0.05	0.5	5.0	50.0
SAMPLE 1	0.854	0.901	0.921	0.866	0.873	0.885	0.884
SAMPLE 2	0.839	0.877	0.900	0.890	0.955	0.907	0.860
SAMPLE 3	0.872	0.860	0.865	0.883	0.853	0.863	0.827
MEAN	0.855	0.879	0.895	0.879	0.894	0.885	0.857
% CC	100.0	102.9	104.8	102.9	104.5	103.5	100.3
STD DEV	1.9	2.4	3.3	1.4	6.3	2.6	3.4

IC50 (µg/ml) = 0.24
IC90 (µg/ml) = 2.28

TC50 (µg/ml) = >50.0

TI = >208.33



INHIBITION OF X4-TROPIC HIV-1 ATTACHMENT BY AMD 3100

RLU (Relative Light Units)							
CONC (μM)	0.00	0.0001	0.001	0.01	0.1	1.0	10.0
SAMPLE 1	125193.6	129947.1	68349.1	9768.1	2293.1	1779.1	679.1
SAMPLE 2	137903.6	124425.1	72891.1	6135.1	0.0	0.0	395.1
SAMPLE 3	137979.6	125767.1	53761.1	7705.1	1882.1	1441.1	18.1
MEAN	133692.3	126713.1	65000.4	7869.4	1391.7	1073.4	364.1
% VC	100.0	94.8	48.6	5.9	1.0	0.8	0.3
STD DEV	5.5	2.2	7.5	1.4	0.9	0.7	0.2

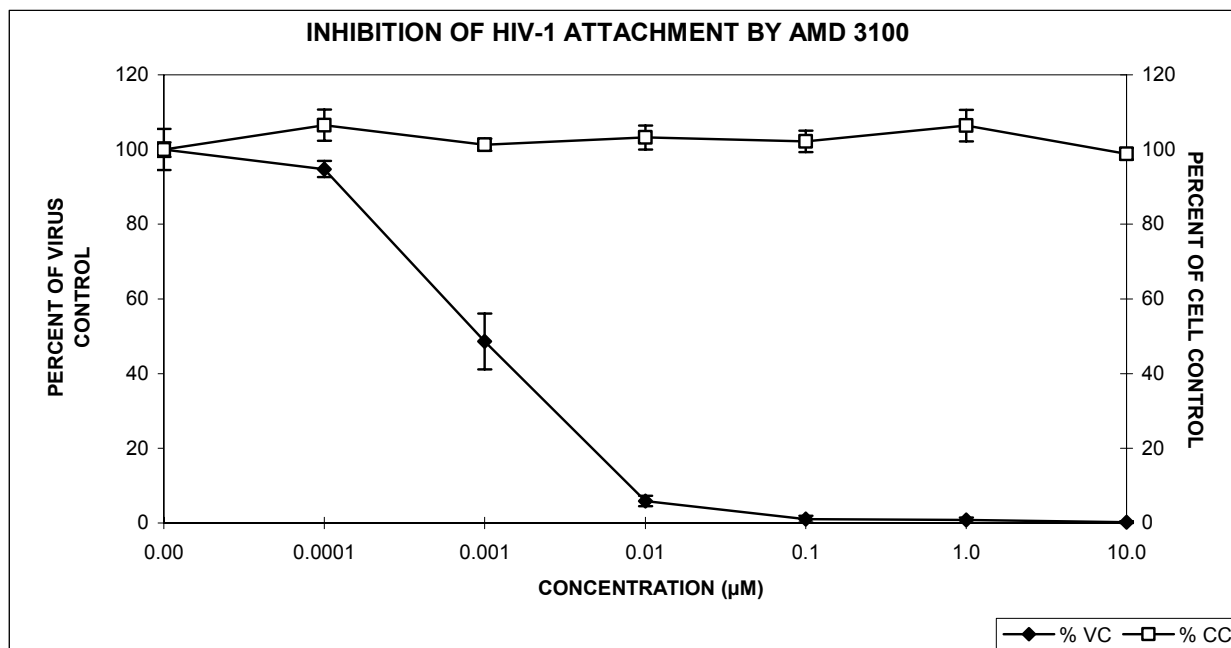
TOXICITY VALUES (CellTiter96 - O. D. @ 490/650 nm)							
CONC (μM)	0.00	0.0001	0.001	0.01	0.1	1.0	10.0
SAMPLE 1	0.854	0.894	0.875	0.884	0.888	0.869	0.850
SAMPLE 2	0.839	0.886	0.849	0.854	0.887	0.936	0.855
SAMPLE 3	0.872	0.951	0.872	0.909	0.845	0.924	0.830
MEAN	0.855	0.910	0.866	0.882	0.873	0.909	0.845
% CC	100.0	106.5	101.3	103.2	102.2	106.4	98.8
STD DEV	1.9	4.2	1.6	3.2	2.9	4.2	1.6

IC50 (μM) = 0.001

TC50 (μM) = >10.0

TI = >10000.00

IC90 (μM) = 0.008



March 16, 2005
Tech: Snyder

Beta-gal X4-Tropic Attachment Inhibition Assay
Virus: IIIB

Southern Research Institute
Contract NO1-A1-05415

INHIBITION OF X4-TROPIC HIV-1 ATTACHMENT BY NIAID 11039

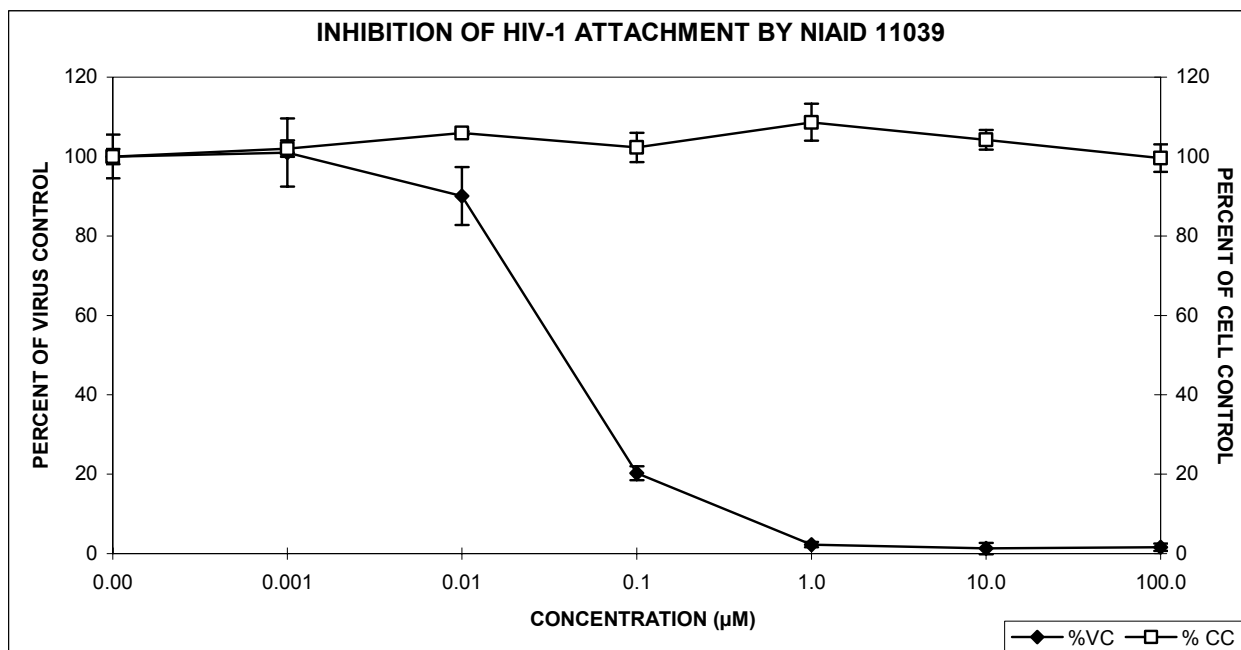
RLU (Relative Light Units)							
CONC (μM)	0.00	0.001	0.01	0.1	1.0	10.0	100.0
SAMPLE 1	125193.6	124709.1	111608.1	24304.1	2358.1	204.1	1281.1
SAMPLE 2	137903.6	133005.1	130947.1	28732.1	2668.1	1117.1	1540.1
SAMPLE 3	137979.6	147370.1	118622.1	28127.1	3986.1	3782.1	3555.1
MEAN	133692.3	135028.1	120392.4	27054.4	3004.1	1701.1	2125.4
% VC	100.0	101.0	90.1	20.2	2.2	1.3	1.6
STD DEV	5.5	8.6	7.3	1.8	0.6	1.4	0.9

TOXICITY VALUES (CellTiter96 - O. D. @ 490/650 nm)							
CONC (μM)	0.00	0.001	0.01	0.1	1.0	10.0	100.0
SAMPLE 1	0.854	0.852	0.901	0.850	0.937	0.876	0.842
SAMPLE 2	0.839	0.886	0.906	0.863	0.963	0.915	0.884
SAMPLE 3	0.872	0.878	0.909	0.910	0.885	0.881	0.828
MEAN	0.855	0.872	0.905	0.874	0.929	0.891	0.851
% CC	100.0	102.0	105.9	102.3	108.6	104.2	99.6
STD DEV	1.9	2.1	0.5	3.7	4.7	2.5	3.5

IC50 (μM) = 0.04
IC90 (μM) = 0.37

TC50 (μM) = >100.0

TI = >2500.00



APPENDIX VI Results of the X4-Tropic HIV-1 Fusion Assays

INHIBITION OF HIV-1 X4-TROPIC FUSION BY Chicago Sky Blue

RLU (Relative Light Units)							
CONC (µg/ml)	0.00	0.0005	0.0050	0.0500	0.5000	5.0	50.0
SAMPLE 1	45410.9	45261.4	42136.4	46475.4	29788.4	0.0	0.0
SAMPLE 2	42766.9	42548.4	42896.4	45503.4	24606.4	0.0	0.0
SAMPLE 3	47951.9	52317.4	44255.4	41503.4	22146.4	0.0	735.4
MEAN	45376.6	46709.1	43096.1	44494.1	25513.8	0.0	245.1
% VC	100.0	102.9	95.0	98.1	56.2	0.0	0.5
STD DEV	5.7	11.1	2.4	5.8	8.6	0.0	0.9

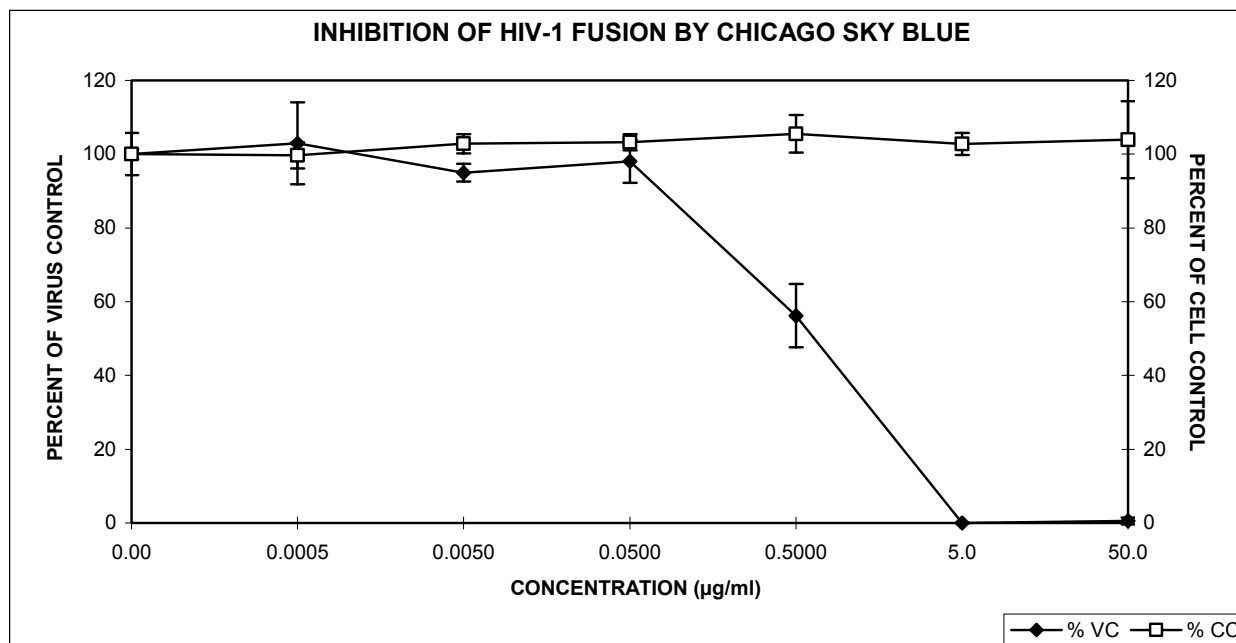
TOXICITY VALUES (CellTiter96 - O. D. @ 490/650 nm)							
CONC (µg/ml)	0.00	0.0005	0.0050	0.0500	0.5000	5.0	50.0
SAMPLE 1	0.957	0.988	0.986	1.001	0.987	0.988	0.953
SAMPLE 2	0.938	0.935	0.952	0.989	0.969	1.002	1.103
SAMPLE 3	0.962	0.925	1.000	0.960	1.060	0.947	0.914
MEAN	0.952	0.949	0.979	0.983	1.005	0.979	0.990
% CC	100.0	99.7	102.8	103.2	105.5	102.8	103.9
STD DEV	1.3	3.5	2.6	2.2	5.1	3.0	10.4

IC50 (µg/ml) = 0.65

TC50 (µg/ml) = >50.0

TI = >76.92

IC90 (µg/ml) = 3.32



March 15, 2005
Tech: Snyder

β-gal X4-Tropic Fusion Inhibition Assay

Southern Research Institute
Contract NO1-A1-05415

INHIBITION OF HIV-1 X4-TROPIC FUSION BY AMD 3100

RLU (Relative Light Units)							
CONC (μM)	0.00	0.0001	0.0010	0.0100	0.1000	1.0	10.0
SAMPLE 1	45410.9	45981.4	30474.4	5078.4	0.0	0.0	0.0
SAMPLE 2	42766.9	45086.4	35573.4	5106.4	0.0	0.0	0.0
SAMPLE 3	47951.9	34587.4	34734.4	7258.4	0.0	0.0	0.0
MEAN	45376.6	41885.1	33594.1	5814.4	0.0	0.0	0.0
% VC	100.0	92.3	74.0	12.8	0.0	0.0	0.0
STD DEV	5.7	14.0	6.0	2.8	0.0	0.0	0.0

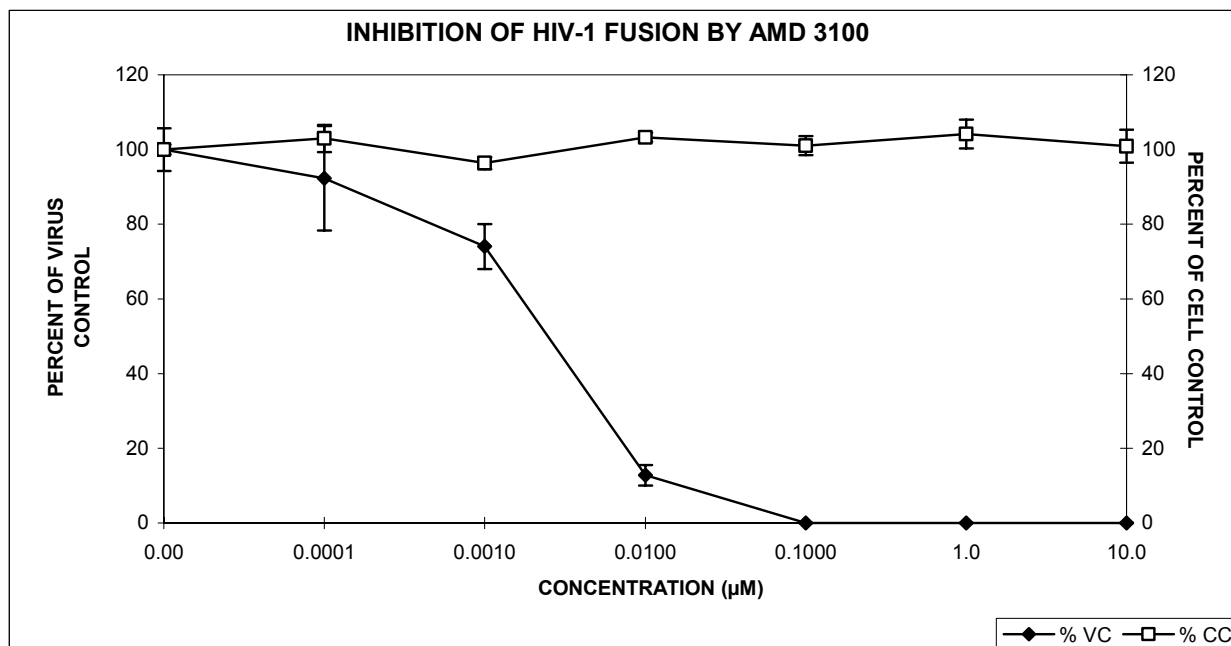
TOXICITY VALUES (CellTiter96 - O. D. @ 490/650 nm)							
CONC (μM)	0.00	0.0001	0.0010	0.0100	0.1000	1.0	10.0
SAMPLE 1	0.957	0.970	0.935	0.976	0.985	1.030	1.009
SAMPLE 2	0.938	1.020	0.908	0.979	0.965	0.988	0.934
SAMPLE 3	0.962	0.953	0.910	0.997	0.937	0.957	0.940
MEAN	0.952	0.981	0.918	0.984	0.963	0.992	0.961
% CC	100.0	103.0	96.3	103.3	101.1	104.1	100.9
STD DEV	1.3	3.6	1.6	1.2	2.5	3.9	4.4

IC50 (μM) = 0.002

TC50 (μM) = >10.0

TI = >5000.00

IC90 (μM) = 0.02



March 15, 2005
Tech: Snyder

β-gal X4-Tropic Fusion Inhibition Assay

Southern Research Institute
Contract NO1-A1-05415

INHIBITION OF HIV-1 X4-TROPIC FUSION BY NIAID 11039

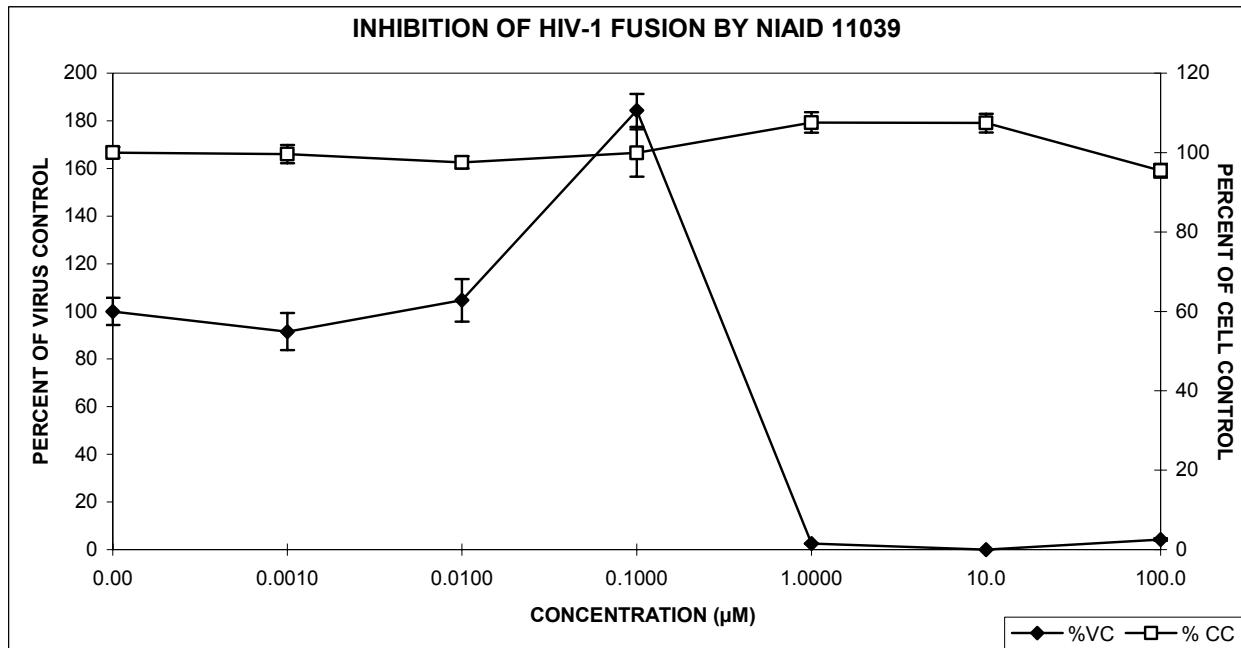
RLU (Relative Light Units)							
CONC (μM)	0.00	0.0010	0.0100	0.1000	1.0000	10.0	100.0
SAMPLE 1	45410.9	45106.4	46613.4	84009.4	1160.4	0.0	1743.4
SAMPLE 2	42766.9	38054.4	43947.4	80371.4	1116.4	0.0	1881.4
SAMPLE 3	47951.9	41432.4	51891.4	86615.4	1173.4	74.4	2203.4
MEAN	45376.6	41531.1	47484.1	83665.4	1150.1	24.8	1942.8
% VC	100.0	91.5	104.6	184.4	2.5	0.1	4.3
STD DEV	5.7	7.8	8.9	6.9	0.1	0.1	0.5

TOXICITY VALUES (CellTiter96 - O. D. @ 490/650 nm)							
CONC (μM)	0.00	0.0010	0.0100	0.1000	1.0000	10.0	100.0
SAMPLE 1	0.957	0.974	0.924	0.930	1.000	1.027	0.899
SAMPLE 2	0.938	0.941	0.945	1.016	1.024	0.999	0.900
SAMPLE 3	0.962	0.931	0.918	0.908	1.049	1.043	0.928
MEAN	0.952	0.949	0.929	0.951	1.025	1.023	0.909
% CC	100.0	99.6	97.5	99.9	107.6	107.4	95.4
STD DEV	1.3	2.3	1.5	6.0	2.6	2.3	1.7

IC50 (μM) = 0.55
IC90 (μM) = 0.91

TC50 (μM) = >100.0

TI = >181.82



APPENDIX VII

Results of the CD4-Independent (ME180) Assays

**CD4-INDEPENDENT X4-TROPIC HIV-1 CELL TO CELL TRANSMISSION INHIBITION ASSAY
COMPOUND: DEXTRAN SULFATE**

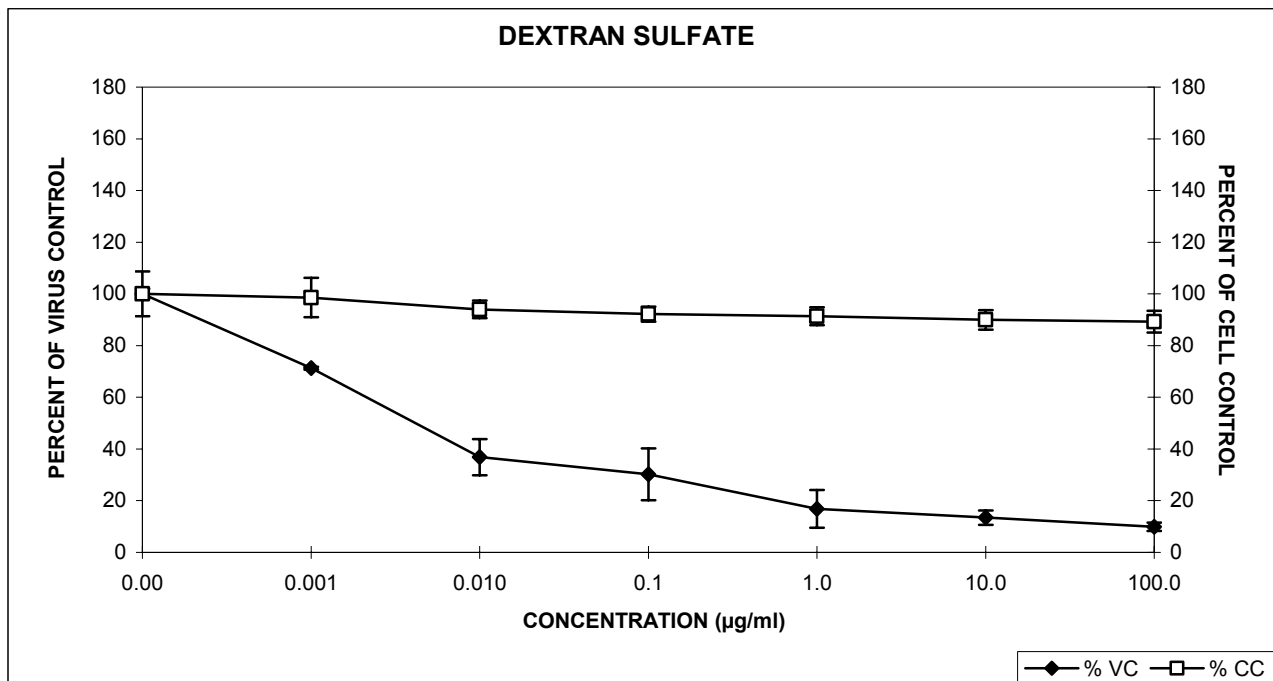
p24 VALUES (pg/ml)							
CONC (µg/ml)	0.00	0.001	0.010	0.1	1.0	10.0	100.0
SAMPLE 1	1229.48	863.92	475.00	263.21	177.36	126.89	113.68
SAMPLE 2	1305.78	869.34	513.21	499.29	131.13	167.92	103.30
SAMPLE 3	1098.00	855.90	351.42	333.49	303.07	193.63	141.51
MEAN	1211.08	863.05	446.54	365.33	203.85	162.81	119.50
% VC	100.0	71.3	36.9	30.2	16.8	13.4	9.9
STD DEV	8.7	0.6	7.0	10.0	7.3	2.8	1.6

TOXICITY VALUES (CellTiter96 - O. D. @ 450/650 nm)							
CONC (µg/ml)	0.00	0.001	0.010	0.1	1.0	10.0	100.0
SAMPLE 1	1.299	1.241	1.286	1.206	1.194	1.167	1.205
SAMPLE 2	1.346	1.265	1.199	1.201	1.183	1.167	1.125
SAMPLE 3	1.344	1.427	1.264	1.271	1.268	1.254	1.233
MEAN	1.330	1.311	1.250	1.226	1.215	1.196	1.187
% CC	100.0	98.6	94.0	92.2	91.4	90.0	89.3
STD DEV	2.0	7.6	3.4	2.9	3.5	3.8	4.2

IC50 (µg/ml) = 0.004
IC90 (µg/ml) = 91.8

TC50 (µg/ml) = >100.0

TI = >25000.00



**CD4-INDEPENDENT X4-TROPIC HIV-1 CELL TO CELL TRANSMISSION INHIBITION ASSAY
COMPOUND: DEXTRAN**

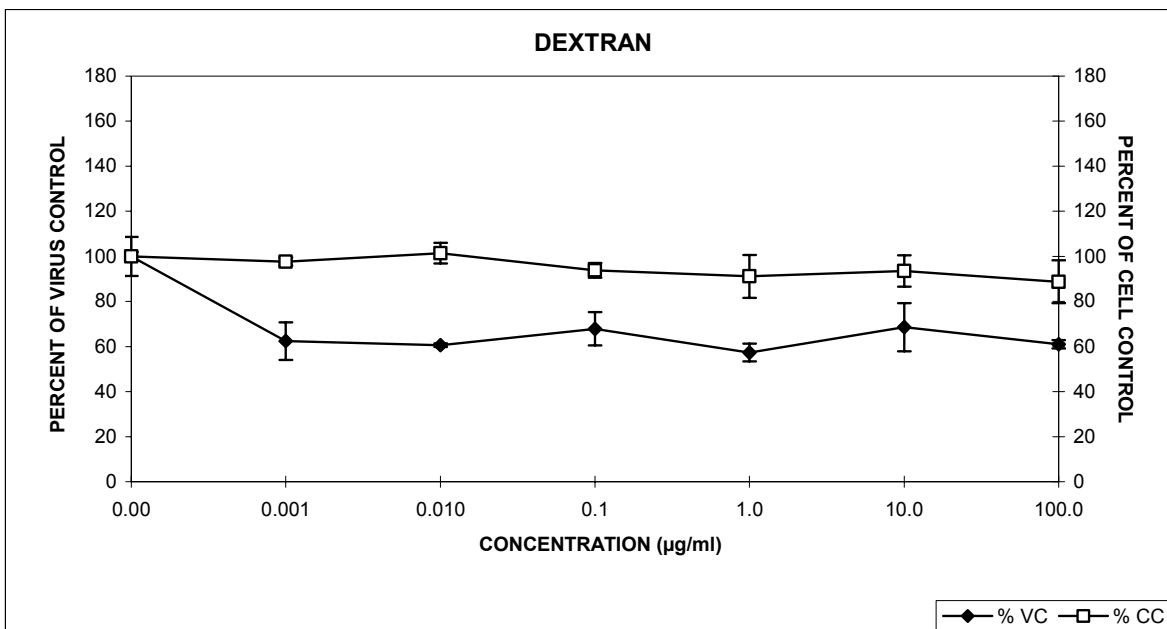
p24 VALUES (pg/ml)							
CONC (µg/ml)	0.00	0.001	0.010	0.1	1.0	10.0	100.0
SAMPLE 1	1229.48	654.01	740.80	754.95	706.37	900.47	738.21
SAMPLE 2	1305.78	758.02	726.89	786.32	641.51	680.42	717.45
SAMPLE 3	1098.00	854.72		923.58	733.96	909.91	761.79
MEAN	1211.08	755.58	733.84	821.62	693.95	830.27	739.15
% VC	100.0	62.4	60.6	67.8	57.3	68.6	61.0
STD DEV	8.7	8.3	0.8	7.4	3.9	10.7	1.8

TOXICITY VALUES (CellTiter96 - O. D. @ 450/650 nm)							
CONC (µg/ml)	0.00	0.001	0.010	0.1	1.0	10.0	100.0
SAMPLE 1	1.299	1.271	1.311	1.219	1.087	1.154	1.057
SAMPLE 2	1.346	1.308	1.314	1.225	1.209	1.239	1.175
SAMPLE 3	1.344	1.315	1.419	1.298	1.340	1.338	1.309
MEAN	1.330	1.298	1.348	1.247	1.212	1.244	1.180
% CC	100.0	97.6	101.4	93.8	91.2	93.5	88.8
STD DEV	2.0	1.8	4.6	3.3	9.5	6.9	9.5

IC50 (µg/ml) = >100.0
IC90 (µg/ml) = >100.0

TC50 (µg/ml) = >100.0

TI = N/A




SOUTHERN RESEARCH
FREDERICK
March 31, 2005
Tech: Osterling

Remediated
CD4-INDEPENDENT X4-TROPIC HIV-1 CELL TO CELL TRANSMISSION INHIBITION ASSAY
EFFECTOR CELLS: H-9/SK1
TARGET CELLS: ME-180

Southern Research Institute
Contract NO1-A1-05415

**CD4-INDEPENDENT X4-TROPIC HIV-1 CELL TO CELL TRANSMISSION INHIBITION ASSAY
COMPOUND: NIAID 11039**

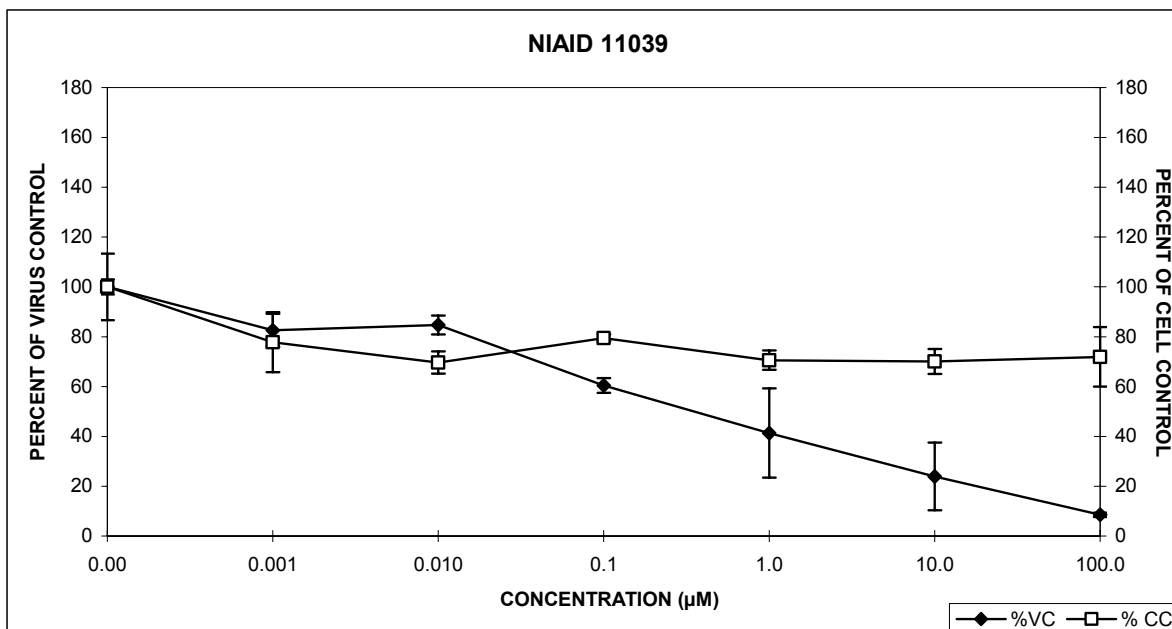
p24 VALUES (pg/ml)							
CONC (µM)	0.00	0.001	0.010	0.1	1.0	10.0	100.0
SAMPLE 1	1074.29	1102.59	1092.22	729.01	357.08	141.51	115.57
SAMPLE 2	1349.41	1092.69	1020.99	767.69	783.49	484.43	113.21
SAMPLE 3	1386.20	950.71	1113.21	804.48	434.43	284.20	95.75
MEAN	1269.97	1048.66	1075.47	767.06	525.00	303.38	108.18
% VC	100.0	82.6	84.7	60.4	41.3	23.9	8.5
STD DEV	13.4	6.7	3.8	3.0	17.9	13.6	0.9

TOXICITY VALUES (CellTiter96 - O. D. @ 450/650 nm)							
CONC (µM)	0.00	0.001	0.010	0.1	1.0	10.0	100.0
SAMPLE 1	1.712	1.539	1.097	1.376	1.124	1.250	1.451
SAMPLE 2	1.641	1.133	1.209	1.346	1.225	1.093	1.067
SAMPLE 3	1.742	1.293	1.242	1.330	1.249	1.229	1.146
MEAN	1.698	1.322	1.183	1.350	1.199	1.191	1.221
% CC	100.0	77.8	69.6	79.5	70.6	70.1	71.9
STD DEV	3.0	12.0	4.5	1.4	3.9	5.0	11.9

IC50 (µM) = 0.35
IC90 (µM) = 80.1

TC50 (µM) = >100.0

TI = >285.71




SOUTHERN RESEARCH
FREDERICK
March 31, 2005
Tech: Osterling

CD4-INDEPENDENT X4-TROPIC HIV-1 CELL TO CELL TRANSMISSION INHIBITION ASSAY
EFFECTOR CELLS: H-9/SK1
TARGET CELLS: ME-180

Southern Research Institute
Contract NO1-A1-05415