CONTRACT NUMBER HHSN272201400002C

HUMANIZED MOUSE MODELS FOR HIV THERAPEUTICS DEVELOPMENT

Report of Completed Study

AV-235

Evaluation of the antiviral activity of fullerene polyaminocaproic acid (FPACA) against HIV-1 NL4-3 in PHA-stimulated human PBMC

Experimental period: 12/2/18 – 12/9/18

Drug sponsor: Lev Rasnetsov (ZAO Intelpharm, Nizhny Novgorod, Russia)

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December 11, 2018
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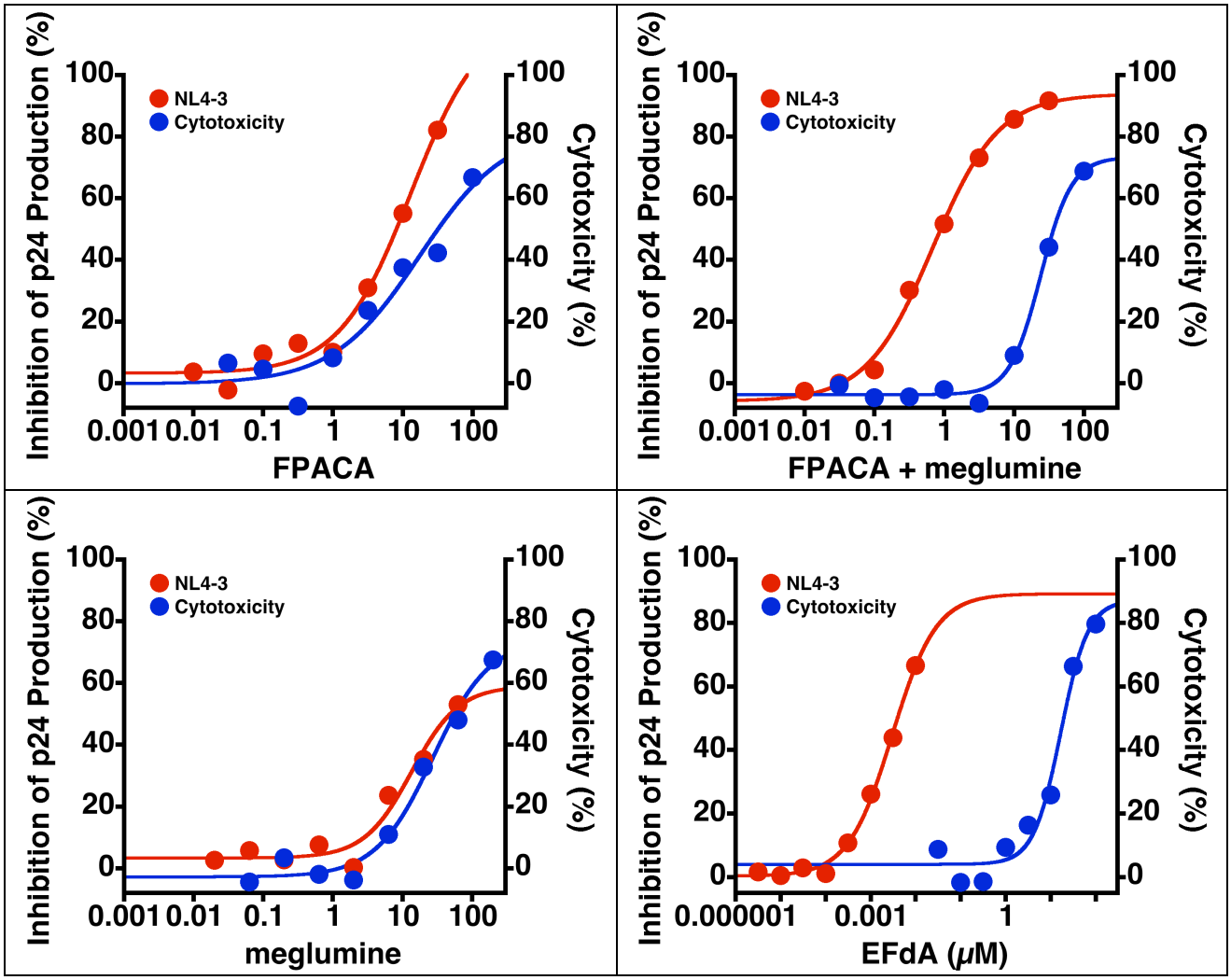
Drug	NL4-3 Antiviral (PBMC) (µg/ml)		PBMC Toxicity (µg/ml)		SI
	IC_{50}	IC_{90}	CC_{50}	CC_{90}	CC_{50}/IC_{50}
FPACA	8.2	47	33	>100	4.0
meglumine	50	>32	58	>200	1.2
FPACA + meglumine (1:2)	0.91	24	38	>100	41
EFdA (µM)	0.0041	0.061	20	>100	4,800


Inhibition of p24 Production (%)
Cytotoxicity (%)
NL4-3
Cytotoxicity
FPACA
FPACA + meglumine
meglumine
EFdA (μM)
0.001
0.01
0.1
1
10
100
0.000001
0.001
0
20
40
60
80

MATERIALS AND METHODS

Drug preparation. Fullerene polyaminocaproic acid (FPACA) and the meglumine excipient were received on 11/27/18 from Lev Rasnetsov (ZAO Intelpharm, Nizhny Novgorod, Russia) and were stored at room temperature in the dark. 4'-ethynyl-2-fluoro-2'-deoxyadenosine (EFdA, lot #F9995-1751) was received on 10/20/15 from Michael Parniak (University of Pittsburgh) and stored at room temperature in the dark. FPACA was dissolved in water at 10 mg/ml, meglumine was dissolved in water at 20 mg/ml, and EFdA was dissolved in methanol at 10 mg/ml. Dissolved compounds were diluted in complete cell culture medium with serial half-log dilutions made by adding 0.5 ml to 1.08 ml medium. A 2-fold dilution occurred when solutions were added to assay wells, resulting in a final concentration range of 0.010–100 µg/ml for FPACA, 0.020–200 µg/ml for meglumine, and 0.0000032–100 µM for EFdA.

Antiviral determination. The HIV-1 molecular clone NL4-3 was prepared by lipofectamine transfection of 293T/17 cells.

Phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMC) (batch Oct. 2018; pooled from the buffy coats of 6 donors) were thawed from liquid nitrogen, placed in a 75-cm^2 flask at a density of 2 x 10^6 cells per ml, and cultured for 48 h in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum and 5 U recombinant human IL-2 (Roche) per ml (complete cell culture medium).

On the day of the assay, PBMC were inoculated with HIV-1 NL4-3 at an MOI of 0.001 in bulk in a 15-ml conical tube for 2 h at 37°C. (Specifically, 10 x 10^6 cells were mixed with 10,000 $TCID_{50}$ HIV-1 in 2 ml total volume, which corresponds to 100 $TCID_{50}$ per well). Cells were pooled and the unabsorbed virus was removed by centrifugation at 400 x g for 5 min, cells were resuspended in medium at 1 x 10^6 cells per ml in complete cell culture medium, and 100 µl (100,000 cells) were added to triplicate wells of a round-

bottom 96-well plate that contained 100 µl of serially diluted test agent or medium alone (200 µl total), and the plates were incubated at 37°C in a humidified 5% CO_2 atmosphere for 7 days. Plates were then centrifuged at 400 x *g* for 5 min, and supernatants were collected and assayed for p24 antigen at a 1:800 dilution in HIV-1 p24 antibody-coated microplates (PerkinElmer) by quantitative ELISA using the p24 standard supplied by the manufacturer.

Data are expressed as % of inhibition of p24 production relative to untreated infected wells [(treated p24/untreated p24) x 100]. The 50% and 90% inhibitory concentrations (IC_{50}) were calculated by use of a 4-parameter fit (SOFTmax PRO 3.0, Molecular Devices) and are the concentrations of drug that reduced p24 production by 50% or 90%. At day 7, untreated virus control wells had mean supernatant p24 concentrations of 360 and 390 ng/ml.

Cytotoxicity determination. On the day of assay, 10^5 PBMC in 100 µl per well were seeded into round-bottom-96-well plates into triplicate wells containing 100 µl of serially diluted test agent or medium alone, and the plates were incubated at 37°C in a humidified 5% CO_2 atmosphere. After 7 days, 25 µl of 5 mg 3-(4,5,-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) per ml in phosphate-buffered saline was added to each well, and plates were incubated at 37°C for 4 h. Plates were then centrifuged at 400 x *g* for 5 min and supernatants were aspirated. Formazan-containing cell pellets were solubilized in 100 µl acid alcohol (0.04 N HCl in isopropanol), and absorbance was measured in a microplate reader at 570 nm (recording wavelength) minus 650 nm (reference wavelength). Data are expressed as % inhibition of formazan OD relative to untreated uninfected wells as described above. The 50% cytotoxic concentration (CC_{50}) is the concentration of test agent that reduced formazan OD values by 50%.

RESULTS

The IC_{50}, IC_{90}, CC_{50}, and CC_{90} values for FPACA, meglumine, FPACA + meglumine, and EFdA are shown in the table on p. 2 and the graphs on p. 3. Antiviral and cytotoxicity dose-response curves and detailed spreadsheets containing p24 values, optical densities, and percentage inhibition of p24 production and cytotoxicity for the assays are on pp. 8–31.

EFdA was highly active against HIV-1 NL4-3 (IC_{50}: 0.0041 µM), as we have reported previously. FPACA was 9 times more potent and had a higher selectivity index (IC_{50}: 0.91 µg/ml and SI: 41) when mixed 1:2 with meglumine than FPACA alone (IC_{50}: 8.2 µg/ml and SI: 4.0).

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In Vitro AV Assay

AV-235

Set up date: December 2, 2018

PBMC batch: PBMC Oct. 2018

Virus: HIV-1 NL4-3 (6/10/11) $10^{4.11}$ $TCID_{50}$/25 µl 20 µl virus + 2,980 µl + 10 x 10^6 PBMCs

Test agent: FPACA (fullerene polyaminocaproic acid)
Meglumine low in endotoxins EMPROVE
FPACA + Meglumine (1:2)
EFdA batch #2 (recd. 10-20-2015)

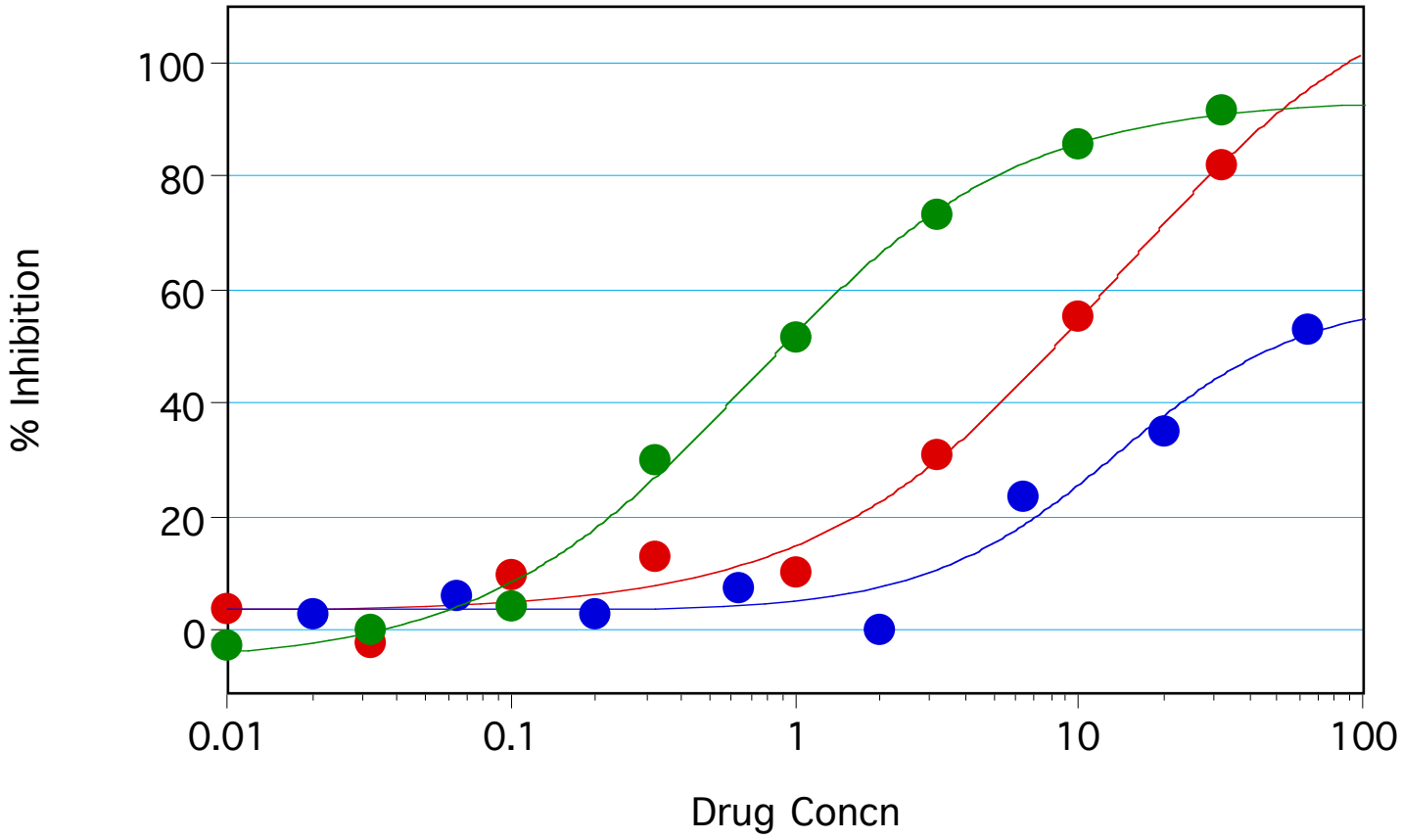
Procedure:
1. Infect PBMC blasts in bulk (100 $TCID_{50}$/10^5 cells; MOI = 0.001) for 2 hours with the specified virus.
2. Wash out unabsorbed virus and resuspend cells at 1 x 10^6/ml.
3. Seed 10^5 cells per well into plate containing drug dilutions (100 µl + 100 µl).
4. 1:2 dilution occurs when 100 µl infected cells mixed with 100 µl drug diluted in complete media (CM).
5. Assay supernatant p24 7 days after inoculation.

FPACA (µg/ml)		Meglumine (µg/ml)		FPACA + Meglumine 1:2 (µg/ml)		EFdA (µM)	
NL4-3	MTT	NL4-3	MTT	NL4-3	MTT	NL4-3	MTT
100	100	200	200	100 + 200	100 + 200	100	100
32	32	64	64	32 + 64	32 + 64	32	32
10	10	20	20	10 + 20	10 + 20	10	10
3.2	3.2	6.4	6.4	3.2 + 6.4	3.2 + 6.4	3.2	3.2
1.0	1.0	2.0	2.0	1.0 + 2.0	1.0 + 2.0	1.0	1.0
0.32	0.32	0.64	0.64	0.32 + 064	0.32 + 064	0.32	0.32
0.10	0.10	0.20	0.20	0.10 + 0.20	0.10 + 0.20	0.10	0.10
0.032	0.032	0.064	0.064	0.032 + 0.064	0.032 + 0.064	0.032	0.032
0.010	0.010	0.020	0.020	0.010 + 0.020	0.010 + 0.020	0.010	0.010
						0.0032	0.0032
						0.0010	0.0010
						0.00032	0.00032
						0.00010	0.00010
						0.000032	0.000032
						0.000010	0.000010
						0.0000032	0.0000032
supplied 1000 mg		supplied 1000 mg				MW: 293.26 solubility: 10 mg/ml in MeOH	
10 mg/ml solution in water		20 mg/ml solution in water		10 mg FPACA + 20 mg Meglumine in 1 ml water		(mM x MW = µg/ml) 34.1 x 293.26 = 10,000 µg/ml	
20 µl + 980 µl CM = 200 µg/ml		20 µl + 980 µl CM = 400 µg/ml		20 µl + 980 µl CM = 200 µg/ml of FPACA and 400 µg/ml Meglumine		3.41 mM 10,000 µg in 10,000 µl MeOH 30 µl + 770 µl CM = 0.1 mM 10 µl + 9,990 µl CM = 0.1 µM 200 µl + 800 µl CM = 20 nM For MTT: 586 µl + 9,414 µl CM = 200 µM	
half-log dilutions in CM 500 µl + 1,080 µl		half-log dilutions in CM 500 µl + 1,080 µl		half-log dilutions in CM 500 µl + 1,080 µl		half-log dilutions in CM 500 µl + 1,080 µl	

Assay date: 12/10/2018
FPACA, Meglumine & FPACA + Meglumine (1:2) against NL4-3 (6/10/2011) in PBMC for 7 days

FPACA IC50 (ug/ml) = 8.233
FPACA IC90 (ug/ml) = 47.110
Meglumine IC50 (ug/ml) = 49.700
Meglumine IC90 (ug/ml) = Range?
FPACA + Meglumine IC50 (ug/ml) = 0.908
FPACA + Meglumine IC90 (ug/ml) = 23.768

ANTIVIRAL ACTIVITY



4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
1 (FPACA: Drug Concn vs % Inhibition)	3.2	0.857	12.8	118	0.982
2 (Meglumine: Drug Concn vs % Inhibition)	3.28	1.29	13.5	58.7	0.959
3 (FPACA + Meglumine (1;2): Drug Conc...	-5.92	0.92	0.695	93.7	0.997

Weighting: Fixed

FPACA (ug/ml)

Sample	Wells	OD	•	p24 (pg/ml	Mean p24	SD	CV	Drug Concn	% Inhibition
A	A1	0.134		36.8	43.0	5.5	12.9	32.0000	82.1
	A2	0.167		44.7					
	A3	0.179		47.4					
A01	B1	0.422		105.4	108.1	7.2	6.6	10.0000	55.1
	B2	0.411		102.7					
	B3	0.468		116.3					
A02	C1	0.758		185.1	166.3	17.5	10.5	3.2000	30.9
	C2	0.666		163.2					
	C3	0.612		150.5					
A03	D1	0.865		210.6	216.5	6.5	3.0	1.0000	10.0
	D2	0.885		215.4					
	D3	0.919		223.6					
A04	E1	0.857		208.8	209.8	6.7	3.2	0.3200	12.9
	E2	0.835		203.6					
	E3	0.891		216.9					
A05	F1	0.841		205.0	217.8	20.0	9.2	0.1000	9.5
	F2	0.992		240.8					
	F3	0.853		207.7					
A06	G1	1.018		246.9	246.0	3.8	1.5	0.0320	-2.2
	G2	0.996		241.8					
	G3	1.027		249.2					
A07	H1	0.924		224.6	231.7	9.8	4.2	0.0100	3.7
	H2	0.937		227.7					
	H3	1.001		242.9					

Meglumine (ug/ml)

Sample	Wells	OD	•	p24 (pg/ml	Mean p24	SD	CV	Drug Concn	% Inhibition
B	A4	0.472		117.1	113.1	3.7	3.2	64.0000	53.0
	A5	0.451		112.1					
	A6	0.442		110.0					
B01	B4	0.668		163.7	155.9	8.4	5.4	20.0000	35.2
	B5	0.639		156.9					
	B6	0.598		147.1					
B02	C4	0.751		183.6	183.9	9.3	5.0	6.4000	23.6
	C5	0.714		174.8					
	C6	0.792		193.3					
B03	D4	1.040		252.2	240.3	10.4	4.3	2.0000	0.2
	D5	0.971		235.9					
	D6	0.958		232.7					
B04	E4	0.948		230.4	222.7	7.3	3.3	0.6400	7.5
	E5	0.887		215.9					
	E6	0.913		222.0					
B05	F4	0.850		207.1	234.1	24.8	10.6	0.2000	2.7
	F5	1.055		255.9					
	F6	0.985		239.3					
B06	G4	0.924		224.7	226.6	13.7	6.1	0.0640	5.8
	G5	0.994		241.3					
	G6	0.879		214.0					
B07	H4	0.965		234.3	234.5	3.0	1.3	0.0200	2.6
	H5	0.953		231.5					
	H6	0.978		237.5					

FPACA + Meglumine (1;2) (ug/ml)

Sample	Wells	OD	•	p24 (pg/	Mean p24	SD	CV	Drug Concn	% Inhibition
C	A7	0.073		22.4	20.1	2.1	10.6	32.0000	91.6
	A8	0.063		19.8					
	A9	0.055		18.1					
C01	B7	0.125		34.7	34.7	0.6	1.7	10.0000	85.6
	B8	0.128		35.3					
	B9	0.123		34.1					
C02	C7	0.248		63.9	64.8	0.8	1.3	3.2000	73.1
	C8	0.254		65.3					
	C9	0.254		65.3					
C03	D7	0.479		118.8	116.2	6.4	5.5	1.0000	51.7
	D8	0.487		120.8					
	D9	0.437		108.9					
C04	E7	0.673		165.0	168.3	5.4	3.2	0.3200	30.1
	E8	0.713		174.5					
	E9	0.675		165.4					
C05	F7	0.912		221.9	230.3	7.3	3.2	0.1000	4.3
	F8	0.968		235.0					
	F9	0.963		234.0					
C06	G7	1.011		245.3	240.8	4.6	1.9	0.0320	-0.0
	G8	0.992		240.8					
	G9	0.972		236.2					
C07	H7	0.994		241.4	246.9	6.2	2.5	0.0100	-2.6
	H8	1.046		253.6					
	H9	1.013		245.8					

VC mean p24 (pg/ml): 140.3

Comp X plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.134	0.167	0.179	0.472	0.451	0.442	0.073	0.063	0.055	0.878	1.664	1.691
B	0.422	0.411	0.468	0.668	0.639	0.598	0.125	0.128	0.123	0.951	0.814	0.798
C	0.758	0.666	0.612	0.751	0.714	0.792	0.248	0.254	0.254	0.958	0.361	0.367
D	0.865	0.885	0.919	1.040	0.971	0.958	0.479	0.487	0.437	0.934	0.175	0.185
E	0.857	0.835	0.891	0.948	0.887	0.913	0.673	0.713	0.675	1.247	0.081	0.095
F	0.841	0.992	0.853	0.850	1.055	0.985	0.912	0.968	0.963	0.966	0.039	0.042
G	1.018	0.996	1.027	0.924	0.994	0.879	1.011	0.992	0.972	1.094	0.019	0.020
H	0.924	0.937	1.001	0.965	0.953	0.978	0.994	1.046	1.013	0.903	0.010	0.010

Endpoint
Absorbance
Lm1 490
Lm2 650
Automix: Off
Calibrate: On
Column Priority
C. Speed: Normal

Start Read:
5:50 PM 12/10/18

Wavelength Combination: !Lm1-!Lm2
Mean Temperature: 23.8
Data Type: Absorbance
Reader: SPECTRAmax M3 ROM v3.0.22 16Feb11

Virus Control (pg/ml)

Sample	Wells	OD	•	p24 (pg/ml)	Mean p24	SD	CV	Virus p24
vir01	A10	0.878		213.8	240.7	28.8	12.0	385.131
	B10	0.951		231.2				
	C10	0.958		232.8				
	D10	0.934		227.0				
	E10	1.247		301.4				
	F10	0.966		234.7				
	G10	1.094		265.1				
	H10	0.903		219.8				

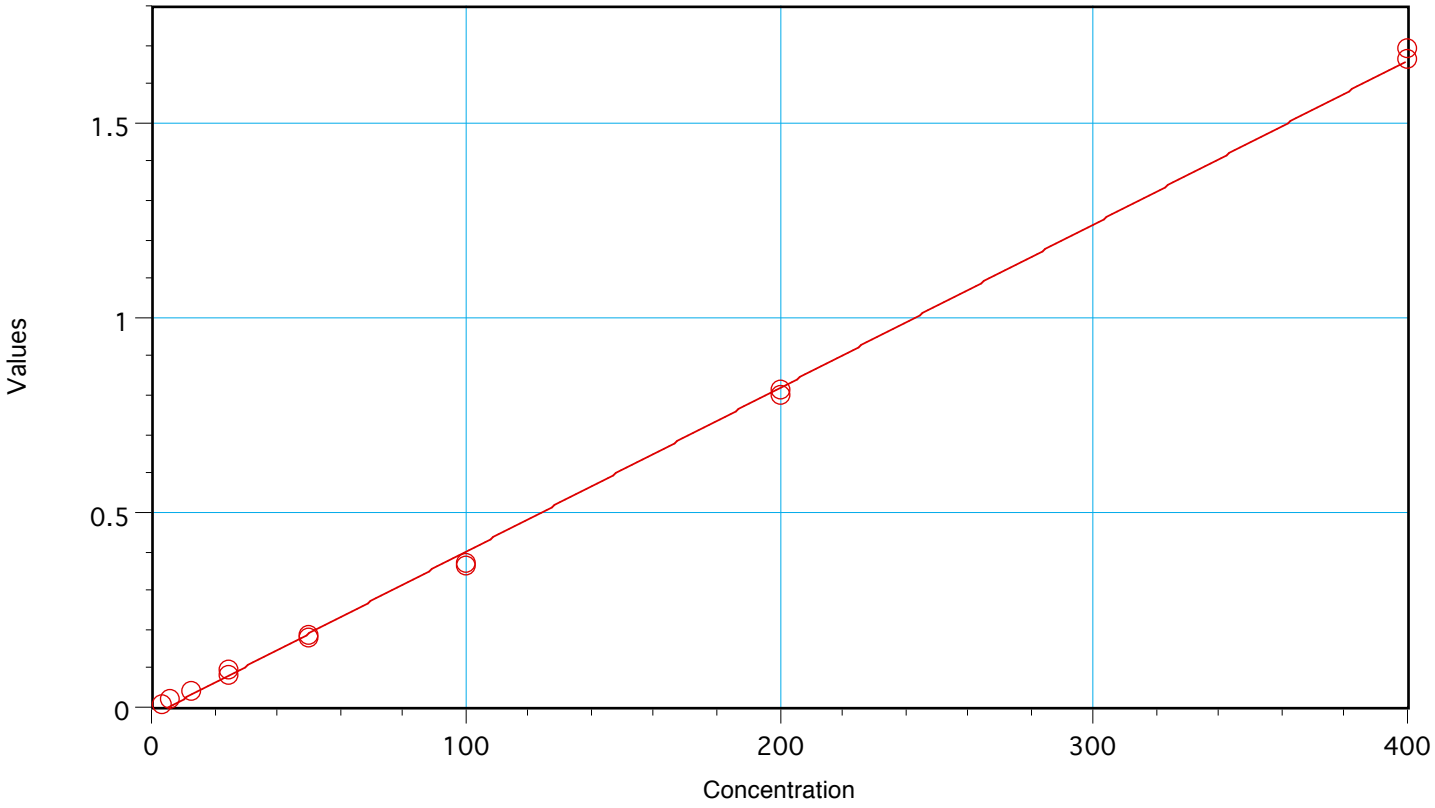
VC mean p24 (pg/ml): 240.7

Standards (pg/ml)

Standard	Concentration	Wells	Values	MeanValue	Std.Dev	CV%
Sta01	400.000	A11	1.664	1.678	0.019	1.1
		A12	1.691			
Sta02	200.000	B11	0.814	0.806	0.012	1.4
		B12	0.798			
Sta03	100.000	C11	0.361	0.364	0.004	1.1
		C12	0.367			
Sta04	50.000	D11	0.175	0.180	0.007	4.0
		D12	0.185			
Sta05	25.000	E11	0.081	0.088	0.010	11.1
		E12	0.095			
Sta06	12.500	F11	0.039	0.040	0.002	6.0
		F12	0.042			
Sta07	6.250	G11	0.019	0.019	0.001	3.3
		G12	0.020			
Sta08	3.125	H11	0.010	0.010	0.000	3.6
		H12	0.010			

Smallest standard value: 0.010
Largest standard value: 1.678

Graph#1



	Linear Fit: y = A + Bx:	A	B	R^2
O	STD (Standards: Concentration vs Values)	-0.0207	0.0042	0.999

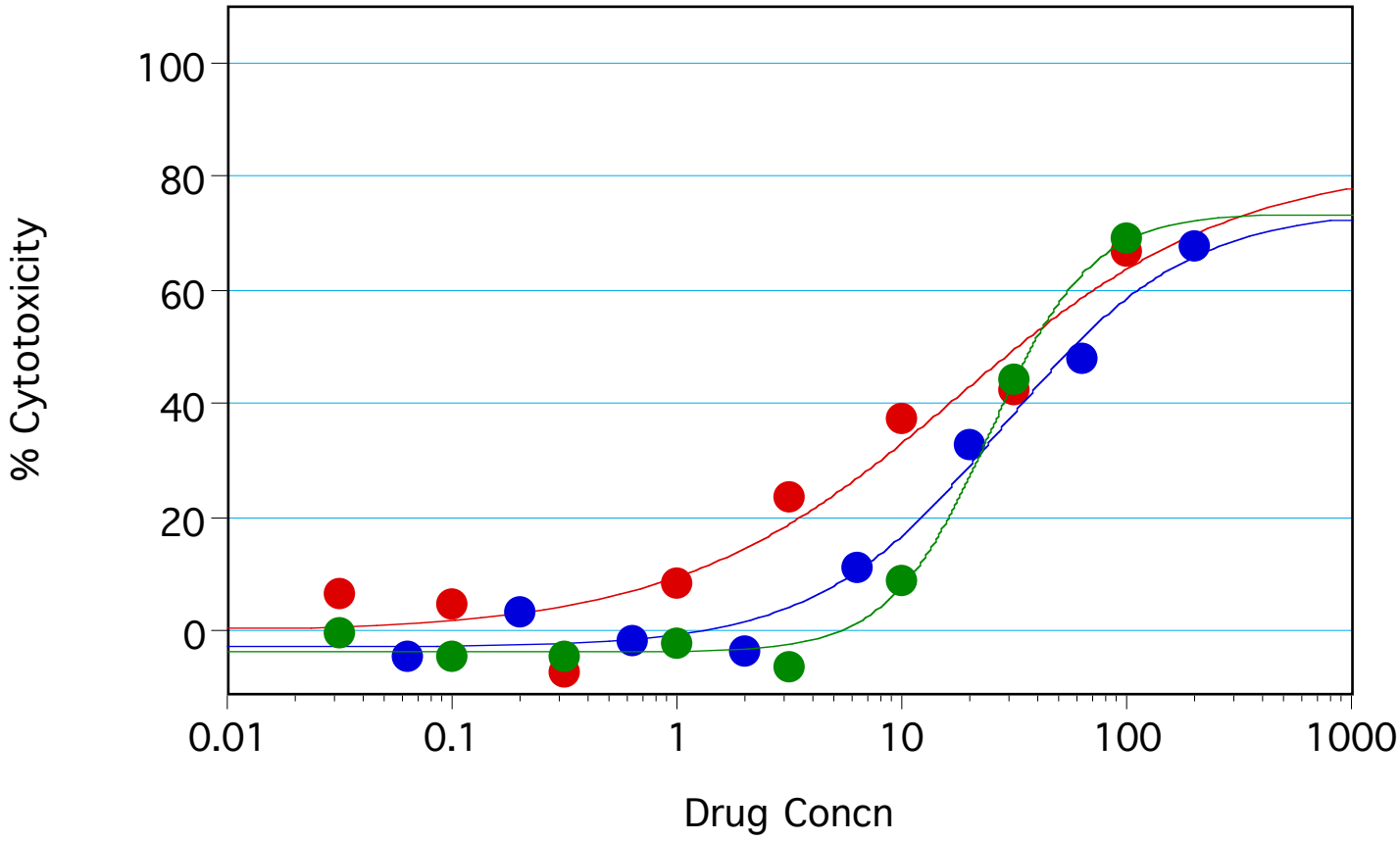
MTT Assay

Assay date: 12/9/2018

FPACA, Meglumine & FPACA + Meglumine (1:2) in PBMC for 7 days

FPACA CC50 (ug/ml) = 32.594
FPACA CC90 (ug/ml) = Range?
Meglumine CC50 (ug/ml) = 57.771
Meglumine CC90 (ug/ml) = Range?
FPACA + Meglumine CC50 (ug/ml) = 37.739
FPACA + Meglumine CC90 (ug/ml) = Range?

CYTOTOXICITY



4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
1 (FPACA: Drug Concn vs % Cytotoxicity)	-0.287	0.702	17.5	82.5	0.933
2 (Meglumine: Drug Concn vs % Cytotoxi...	-2.89	1.05	27.8	74.4	0.981
3 (FPACA + Meglumine: Drug Concn vs ...	-3.81	1.93	24.6	73.6	0.994

Weighting: Fixed

FPACA (ug/ml)

Sample	Wells	OD	Mean OD	SD	Drug Concn	% Cytotoxicity
A	A1	0.123	0.150	0.028	100.000	66.7
	A2	0.148				
	A3	0.180				
A01	B1	0.225	0.260	0.030	32.000	42.3
	B2	0.277				
	B3	0.279				
A02	C1	0.249	0.282	0.032	10.000	37.4
	C2	0.283				
	C3	0.314				
A03	D1	0.345	0.345	0.004	3.200	23.6
	D2	0.340				
	D3	0.349				
A04	E1	0.347	0.414	0.083	1.000	8.2
	E2	0.506				
	E3	0.389				
A05	F1	0.546	0.484	0.056	0.320	-7.4
	F2	0.437				
	F3	0.470				
A06	G1	0.436	0.431	0.038	0.100	4.5
	G2	0.390				
	G3	0.466				
A07	H1	0.448	0.422	0.034	0.032	6.5
	H2	0.434				
	H3	0.384				

Meglumine (ug/ml)

Sample	Wells	OD	Mean OD	SD	Drug Concn	% Cytotoxicity
B	A4	0.170	0.147	0.028	200.000	67.5
	A5	0.155				
	A6	0.115				
B01	B4	0.239	0.235	0.008	64.000	48.0
	B5	0.239				
	B6	0.226				
B02	C4	0.299	0.304	0.014	20.000	32.7
	C5	0.293				
	C6	0.319				
B03	D4	0.393	0.402	0.008	6.400	10.9
	D5	0.407				
	D6	0.405				
B04	E4	0.505	0.468	0.032	2.000	-3.8
	E5	0.457				
	E6	0.443				
B05	F4	0.465	0.460	0.026	0.640	-1.9
	F5	0.482				
	F6	0.432				
B06	G4	0.435	0.436	0.017	0.200	3.4
	G5	0.453				
	G6	0.419				
B07	H4	0.513	0.471	0.045	0.064	-4.4
	H5	0.477				
	H6	0.424				

FPACA + Meglumine (ug/ml)

Sample	Wells	OD	Mean OD	SD	Drug Concn	% Cytotoxicity
C	A7	0.136	0.141	0.020	100.000	68.8
	A8	0.163				
	A9	0.123				
C01	B7	0.247	0.252	0.013	32.000	44.1
	B8	0.243				
	B9	0.267				
C02	C7	0.409	0.411	0.018	10.000	9.0
	C8	0.393				
	C9	0.429				
C03	D7	0.439	0.481	0.036	3.200	-6.5
	D8	0.503				
	D9	0.500				
C04	E7	0.376	0.461	0.074	1.000	-2.1
	E8	0.503				
	E9	0.504				
C05	F7	0.500	0.471	0.051	0.320	-4.4
	F8	0.501				
	F9	0.412				
C06	G7	0.515	0.473	0.043	0.100	-4.7
	G8	0.429				
	G9	0.473				
C07	H7	0.441	0.454	0.012	0.032	-0.6
	H8	0.464				
	H9	0.457				

CC plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.123	0.148	0.180	0.170	0.155	0.115	0.136	0.163	0.123	0.423	0.435	0.348
B	0.225	0.277	0.279	0.239	0.239	0.226	0.247	0.243	0.267	0.484	0.484	0.456
C	0.249	0.283	0.314	0.299	0.293	0.319	0.409	0.393	0.429	0.520	0.457	0.402
D	0.345	0.340	0.349	0.393	0.407	0.405	0.439	0.503	0.500	0.452	0.507	0.536
E	0.347	0.506	0.389	0.505	0.457	0.443	0.376	0.503	0.504	0.552	0.486	0.389
F	0.546	0.437	0.470	0.465	0.482	0.432	0.500	0.501	0.412	0.497	0.407	0.502
G	0.436	0.390	0.466	0.435	0.453	0.419	0.515	0.429	0.473	0.413	0.445	0.430
H	0.448	0.434	0.384	0.513	0.477	0.424	0.441	0.464	0.457	0.484	0.421	0.299

Endpoint
Absorbance
Lm1 570
Automix: Off
Calibrate: On
Column Priority
C. Speed: Normal

Start Read:
2:56 PM 12/9/18

Wavelength Combination: !Lm1
Mean Temperature: 23.2
Data Type: Absorbance
Reader: SPECTRAmax M3 ROM v3.0.22 16Feb11

Cell Control

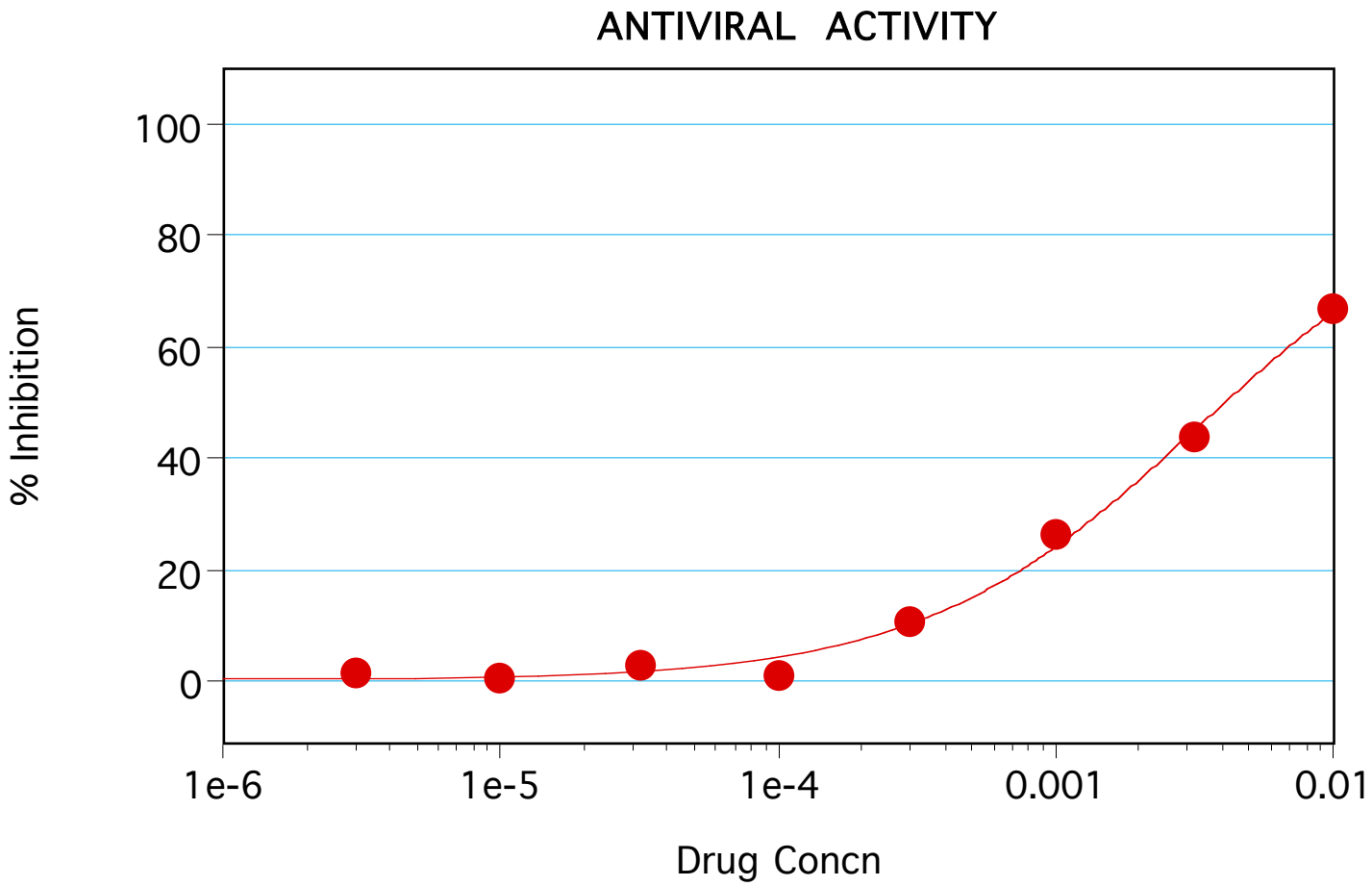
Sample	Wells	OD	Mean OD	SD
Cell Cont	A10	0.423	0.451	0.059
	A11	0.435		
	A12	0.348		
	B10	0.484		
	B11	0.484		
	B12	0.456		
	C10	0.520		
	C11	0.457		
	C12	0.402		
	D10	0.452		
	D11	0.507		
	D12	0.536		
	E10	0.552		
	E11	0.486		
	E12	0.389		
	F10	0.497		
	F11	0.407		
	F12	0.502		
	G10	0.413		
	G11	0.445		
	G12	0.430		
	H10	0.484		
	H11	0.421		
	H12	0.299		

CC mean : 0.451

AV-235 EFdA

Assay date: 12/10/2018
EFdA against NL4-3 (6/10/2011) in PBMC for 7 days

EFdA IC50 (uM) = 0.00406
EFdA IC90 (uM) = 0.611



4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
1 (EFdA (batch #2): Drug Concn vs % In...	0.183	0.87	0.00324	90.9	0.995

Weighting: Fixed

EFdA (batch #2) (uM)

Sample	Wells	OD	•	p24 (pg/ml	Mean p24	SD	CV	Drug Concn	% Inhibition
A	A1	0.257		65.6	74.3	10.1	13.5	0.0100000	66.6
	A2	0.283		71.9					
	A3	0.340		85.3					
A01	B1	0.510		125.5	124.8	11.4	9.1	0.0032000	43.9
	B2	0.457		113.1					
	B3	0.553		135.8					
A02	C1	0.681		166.2	164.5	5.7	3.4	0.0010000	26.1
	C2	0.694		169.2					
	C3	0.648		158.2					
A03	D1	0.868		210.5	198.8	10.2	5.1	0.0003000	10.7
	D2	0.798		194.0					
	D3	0.790		191.9					
A04	E1	0.900		218.1	220.1	1.9	0.9	0.0001000	1.1
	E2	0.910		220.4					
	E3	0.916		221.9					
A05	F1	0.909		220.1	216.0	4.0	1.8	0.0000320	2.9
	F2	0.890		215.7					
	F3	0.875		212.2					
A06	G1	0.970		234.7	221.3	12.5	5.7	0.0000100	0.5
	G2	0.866		210.0					
	G3	0.905		219.2					
A07	H1	0.991		239.5	218.9	18.1	8.3	0.0000030	1.6
	H2	0.875		212.1					
	H3	0.846		205.2					

Comp X plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.257	0.283	0.340							0.774	1.674	1.697
B	0.510	0.457	0.553							0.823	0.816	0.799
C	0.681	0.694	0.648							0.912	0.363	0.368
D	0.868	0.798	0.790							0.752	0.177	0.184
E	0.900	0.910	0.916							0.853	0.087	0.090
F	0.909	0.890	0.875							1.166	0.042	0.044
G	0.970	0.866	0.905							0.959	0.021	0.021
H	0.991	0.875	0.846							1.113	0.011	0.011

Endpoint
Absorbance
Lm1 490
Lm2 650
Automix: Off
Calibrate: On
Column Priority
C. Speed: Normal

Start Read:
5:49 PM 12/10/18

Wavelength Combination: !Lm1-!Lm2
Mean Temperature: 23.8
Data Type: Absorbance
Reader: SPECTRAmax M3 ROM v3.0.22 16Feb11

Virus Control (pg/ml)

Sample	Wells	OD	•	p24 (pg/ml)	Mean p24	SD	CV	Virus p24
vir01	A10	0.774		188.2	222.5	36.2	16.3	356.013
	B10	0.823		199.7				
	C10	0.912		220.9				
	D10	0.752		182.9				
	E10	0.853		206.8				
	F10	1.166		281.1				
	G10	0.959		232.1				
	H10	1.113		268.6				

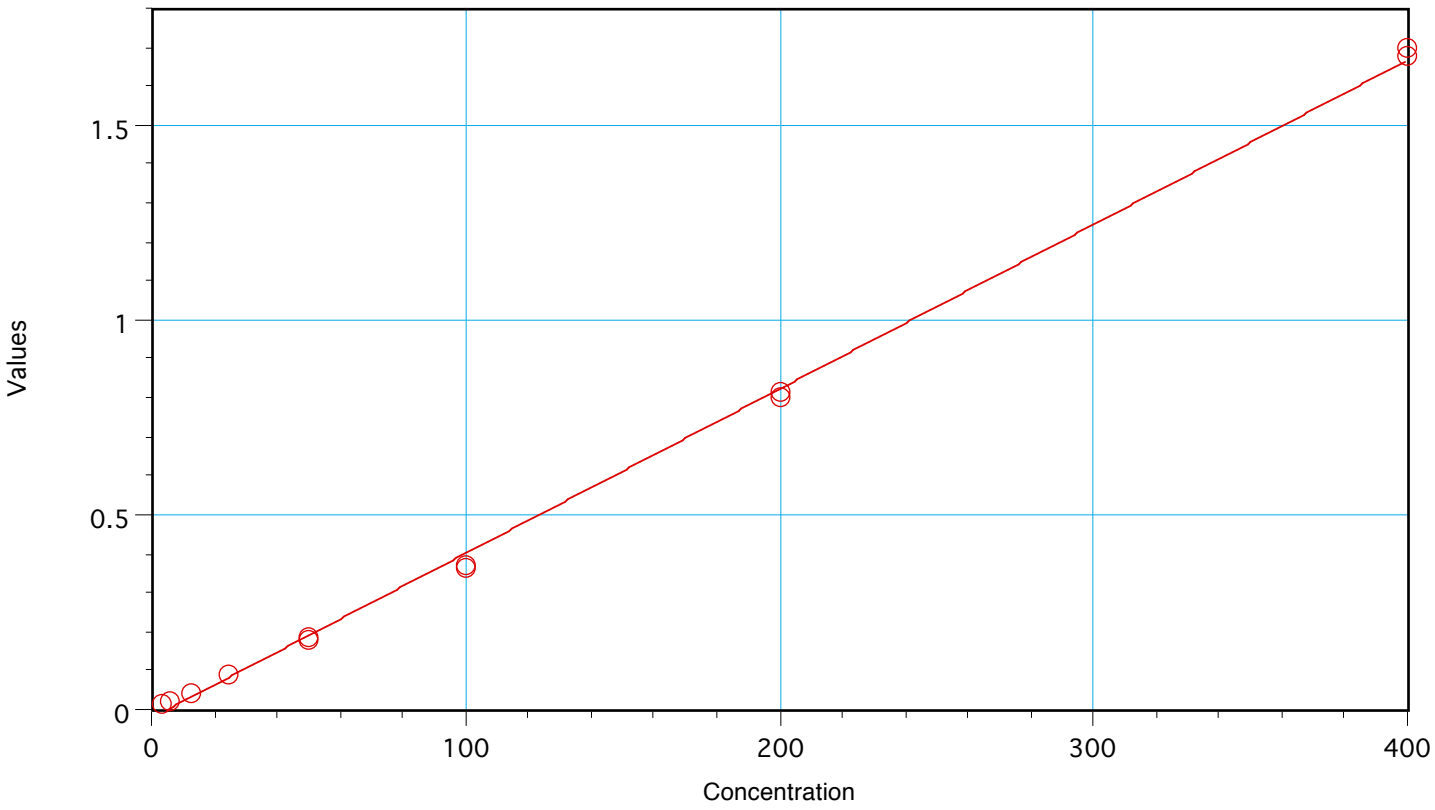
VC mean p24 (pg/ml): 222.5

Standards (pg/ml)

Standard	Concentration	Wells	Values	MeanValue	Std.Dev	CV%
Sta01	400.000	A11	1.674	1.686	0.016	1.0
		A12	1.697			
Sta02	200.000	B11	0.816	0.807	0.012	1.5
		B12	0.799			
Sta03	100.000	C11	0.363	0.365	0.004	1.0
		C12	0.368			
Sta04	50.000	D11	0.177	0.180	0.005	2.8
		D12	0.184			
Sta05	25.000	E11	0.087	0.089	0.003	2.9
		E12	0.090			
Sta06	12.500	F11	0.042	0.043	0.002	4.3
		F12	0.044			
Sta07	6.250	G11	0.021	0.021	0.001	2.7
		G12	0.021			
Sta08	3.125	H11	0.011	0.011	0.001	5.8
		H12	0.011			

Smallest standard value: 0.011
Largest standard value: 1.686

Graph#1



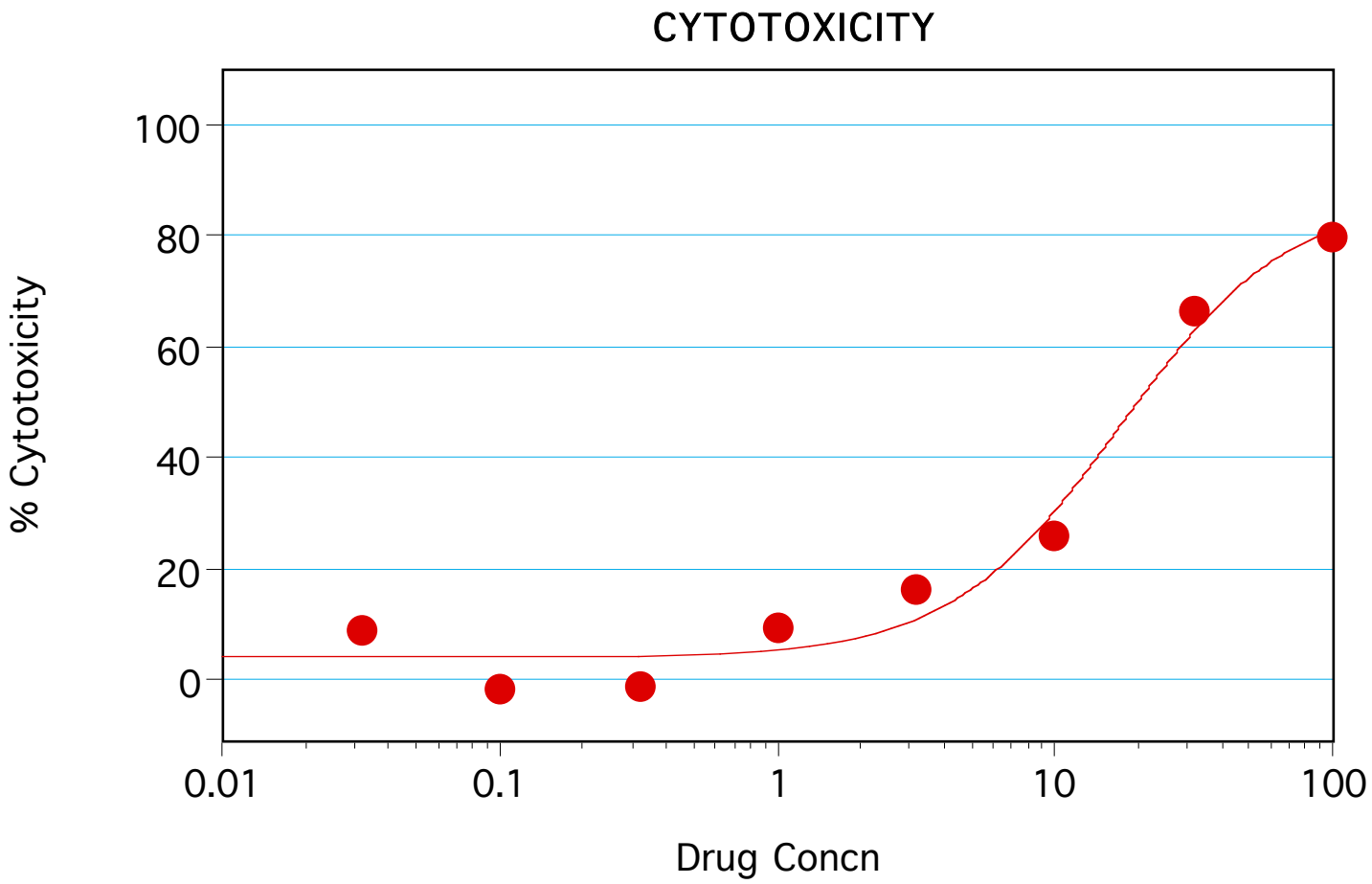
	Linear Fit: y = A + Bx:	A	B	R^2
○	STD (Standards: Concentration vs Values)	-0.0201	0.00422	0.999

AV-235 EFdA MTT Assay

MTT Assay

Assay date: 12/9/2018
EFdA in PBMC for 7 days

EFdA CC50 (uM) = 19.806
EFdA CC90 (uM) = Range?



4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
1 (EFdA (batch #2): Drug Concn vs % C...	3.9	1.42	17.1	87.3	0.975

Weighting: Fixed

EFdA (batch #2) (uM)

Sample	Wells	OD	Mean OD	SD	Drug Concn	% Cytotoxicity
A	A1	0.095	0.105	0.008	100.000	79.7
	A2	0.109				
	A3	0.110				
A01	B1	0.165	0.174	0.008	32.000	66.3
	B2	0.180				
	B3	0.176				
A02	C1	0.354	0.382	0.030	10.000	25.8
	C2	0.414				
	C3	0.378				
A03	D1	0.416	0.431	0.013	3.200	16.3
	D2	0.436				
	D3	0.441				
A04	E1	0.477	0.466	0.030	1.000	9.4
	E2	0.489				
	E3	0.432				
A05	F1	0.454	0.522	0.075	0.320	-1.4
	F2	0.510				
	F3	0.602				
A06	G1	0.476	0.523	0.096	0.100	-1.7
	G2	0.460				
	G3	0.634				
A07	H1	0.467	0.470	0.008	0.032	8.7
	H2	0.464				
	H3	0.479				

CC plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.095	0.109	0.110							0.405	0.431	0.303
B	0.165	0.180	0.176							0.605	0.447	0.482
C	0.354	0.414	0.378							0.570	0.416	0.498
D	0.416	0.436	0.441							0.448	0.530	0.498
E	0.477	0.489	0.432							0.533	0.673	0.589
F	0.454	0.510	0.602							0.552	0.639	0.470
G	0.476	0.460	0.634							0.600	0.659	0.464
H	0.467	0.464	0.479							0.584	0.587	0.370

Endpoint

Absorbance

Lm1 570

Automix: Off
Calibrate: On
Column Priority
C. Speed: Normal

Start Read:
2:59 PM 12/9/18

Wavelength Combination: !Lm1
Mean Temperature: 23.3
Data Type: Absorbance
Reader: SPECTRAmax M3 ROM v3.0.22 16Feb11

Cell Control

Sample	Wells	OD	Mean OD	SD
Cell Cont	A10	0.405	0.515	0.095
	A11	0.431		
	A12	0.303		
	B10	0.605		
	B11	0.447		
	B12	0.482		
	C10	0.570		
	C11	0.416		
	C12	0.498		
	D10	0.448		
	D11	0.530		
	D12	0.498		
	E10	0.533		
	E11	0.673		
	E12	0.589		
	F10	0.552		
	F11	0.639		
	F12	0.470		
	G10	0.600		
	G11	0.659		
	G12	0.464		
	H10	0.584		
	H11	0.587		
	H12	0.370		

CC mean : 0.515