



**Oncolytic Viruses as a
Potential Approach to
Eliminate the HIV Reservoir**

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HIV Reservoirs



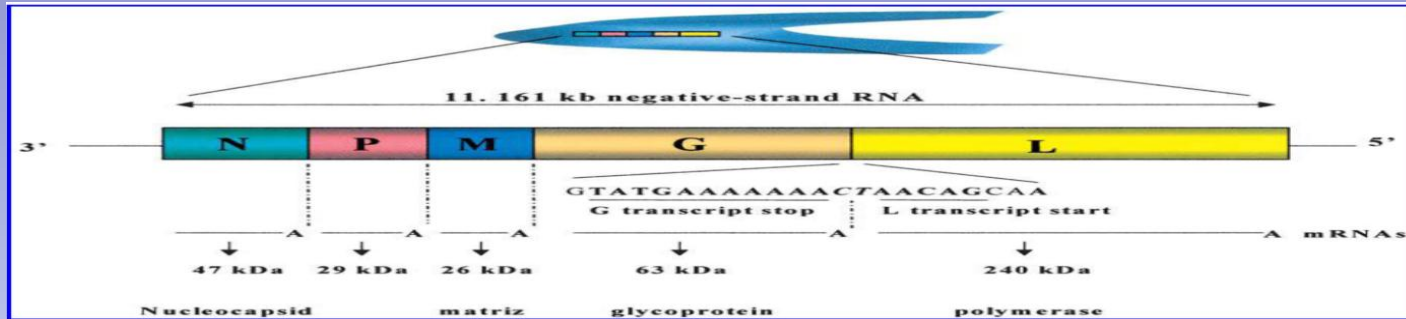
- **Viral Reservoirs**

- Cell type or anatomical site
- Replication-competent form of virus accumulates and persists

- **Implications**

- *A major factor contributing to our inability to cure HIV*









Recombinant Maraba virus (MG1)*



- Oncolytic virus (OV) which selectively targets cancer cells
- Double mutant
 - G protein (Q242R) and M protein (L123W)
- Undergoes complete lifecycle in cytoplasm → No genotoxicity

**Kindly provided by Dr D. Stojdl and Dr J Bell*

IFN-related Abnormalities Shared by Cancer Cells and HIV-infected Cells

Abnormality	Cancer Cells	HIV-infected Cells
• IFN- α / β receptor (IFNAR) expression)		
• IFN-mediated signaling (STAT 1 & STAT2)		
• IFN-inducible genes (protein kinase RNA (PKR))		
• IFN regulatory factor (IRF3)		

and others...

Rationale: HIV & Oncolytic Virotherapy

- Both cancer cells and HIV-infected cells have abnormal interferon signaling pathways, and thus differ from normal, healthy cells
- Similar to OV_s as a cancer therapeutic, OV_s can be designed to selectively target HIV-infected cells with these abnormalities, while sparing healthy cells

Hypothesis

Oncolytic viruses will have a greater propensity to target and kill HIV-infected cells compared to HIV-uninfected cells

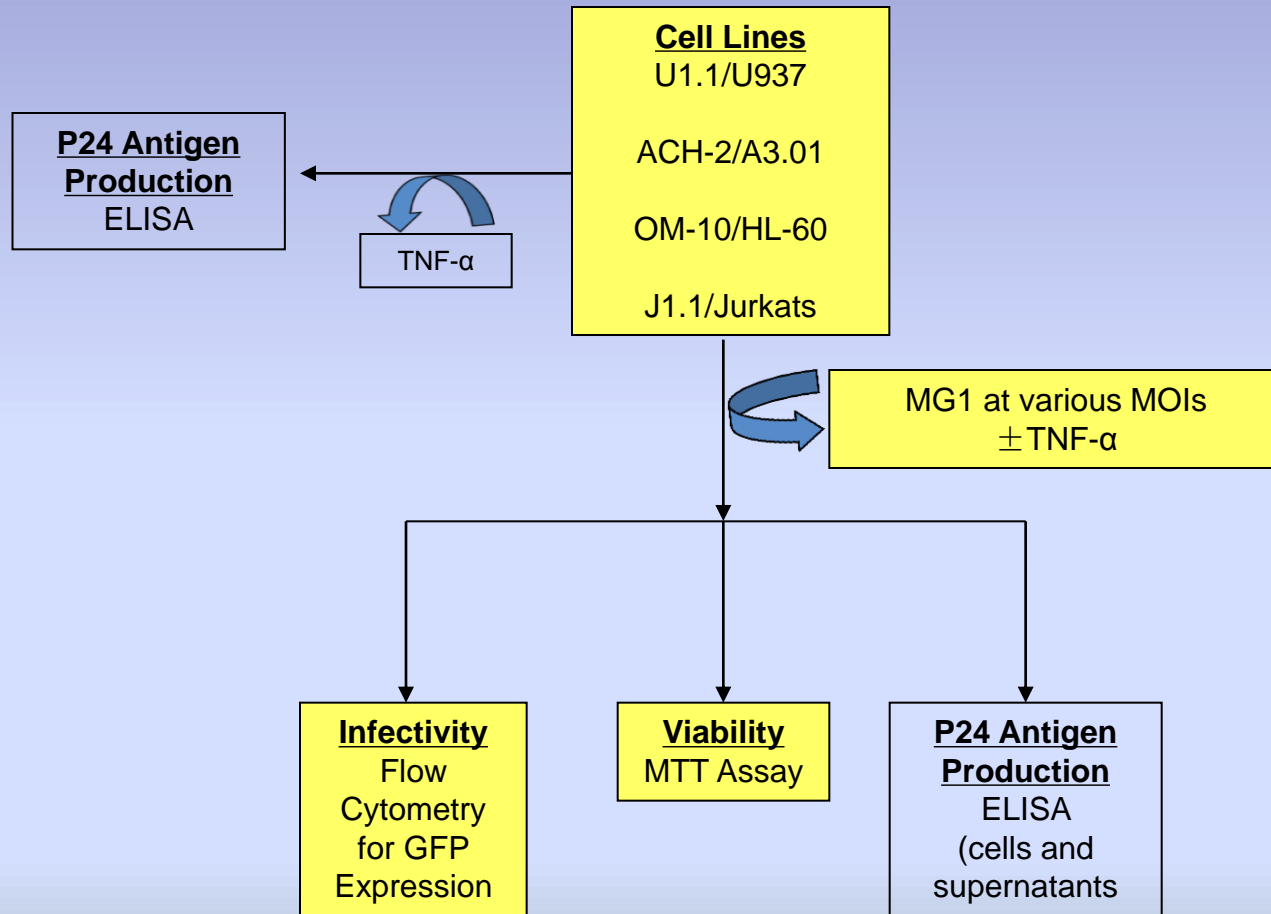
Objectives

- 1) To determine whether MG1 exerts greater killing in HIV-infected than non-HIV infected cells
- 2) To determine whether there is selective replication of MG1 in primary cells infected with HIV, with resultant cell death and decrease of HIV production

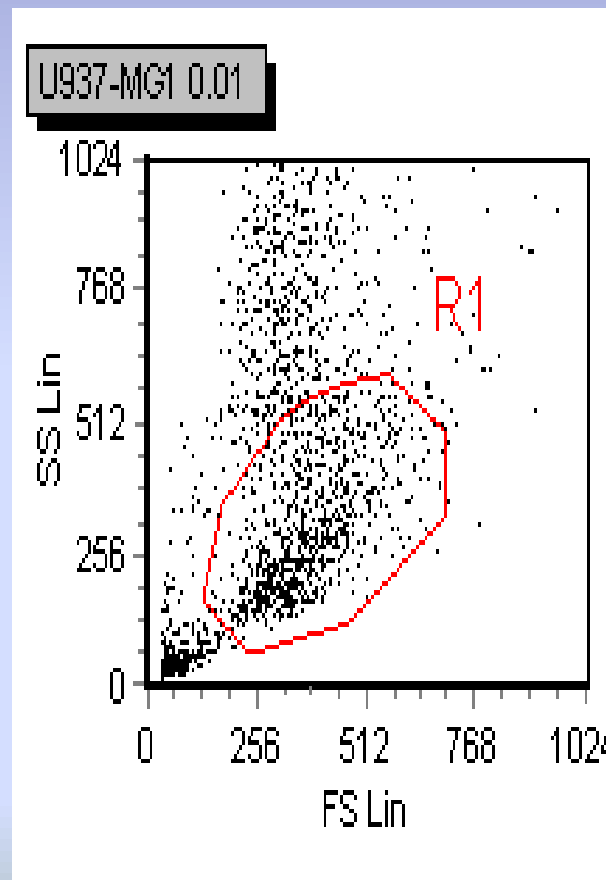
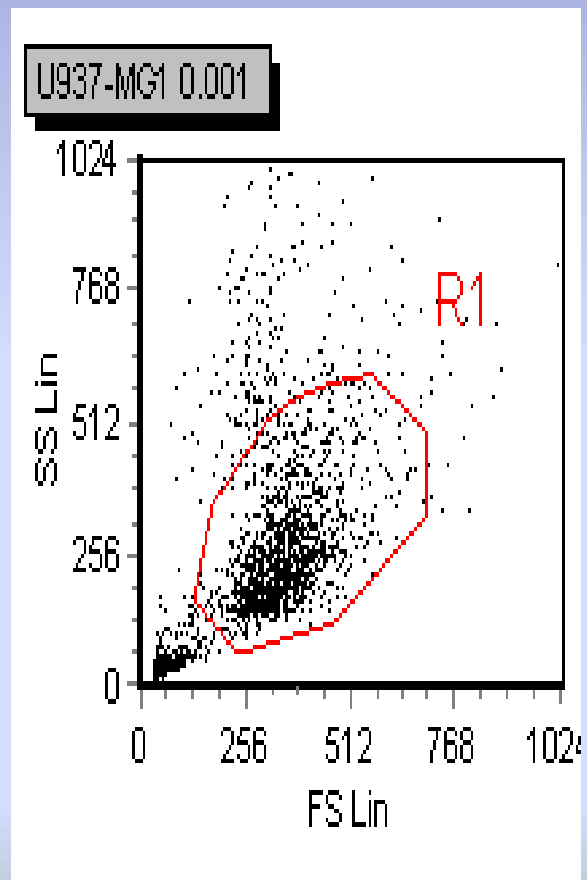
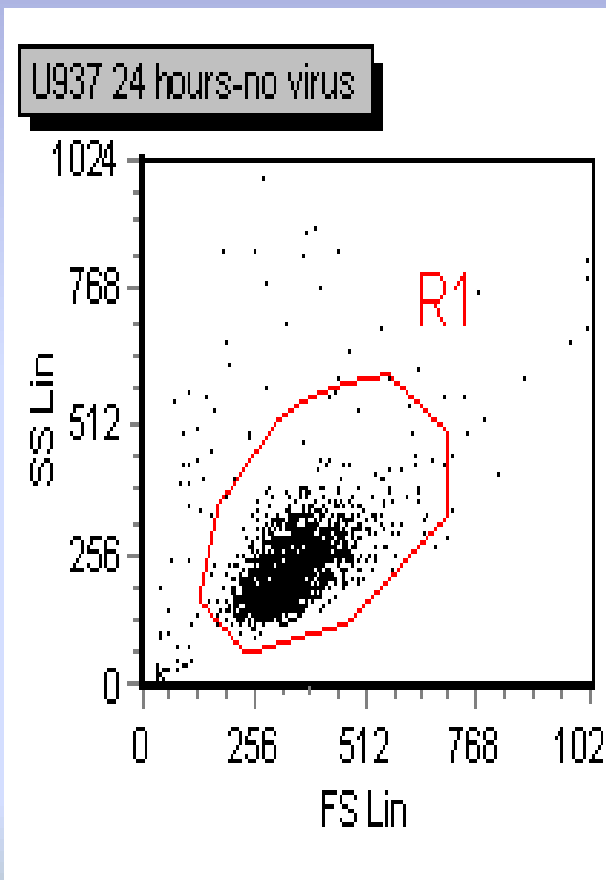
Cell Lines

Cell Lines	Lineage	Copies of HIV Proviral DNA	Mechanism of HIV Latency
U1 (U937)	Monocytic	2	Mutations in transcription activator Tat
ACH-2 (A3.01)	Lymphocytic	1	Mutations in Tat-associated gene region (TAR)
J1.1 (Jurkat)	Lymphocytic	1	Defective Ca ²⁺ mobilization and IL-2 secretion
OM-10 (HL-60)	Monocytic	1	Disruption in protein kinase 2 nd messenger pathway

Cell Lines: Experimental Outline

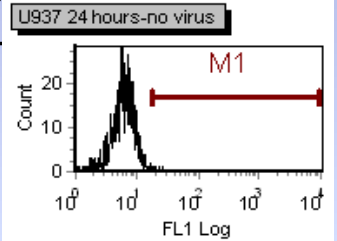
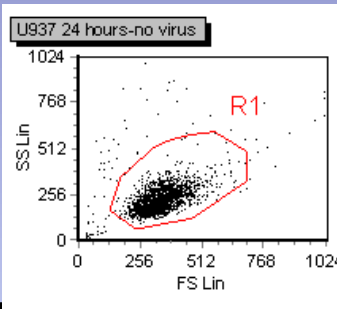


GFP Expression as Gated on Live Cells

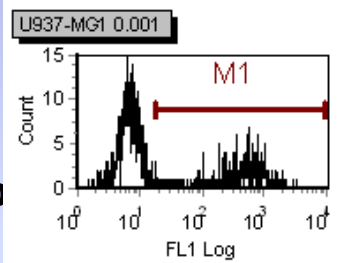
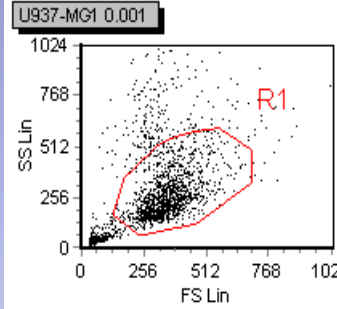


Percentage of GFP-Positive U937 and U1 Cells 24 hours Post-MG1 Infection (Gated on Live Cells)

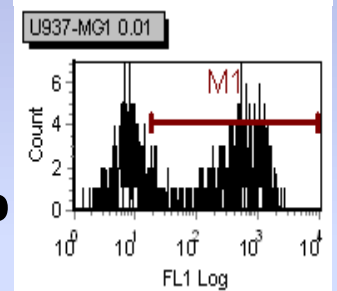
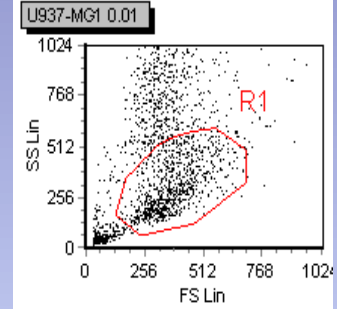
U937



<0.5%

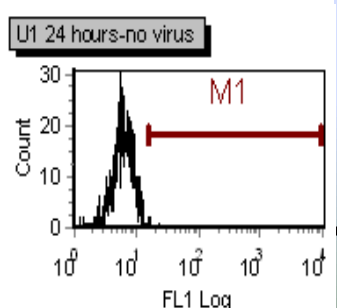
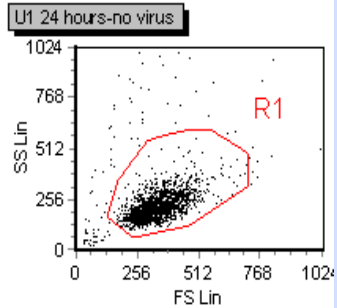


34%

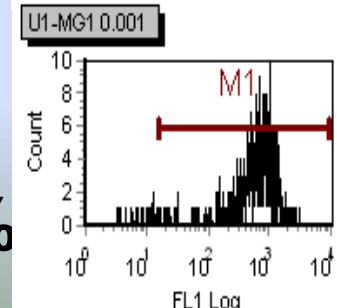
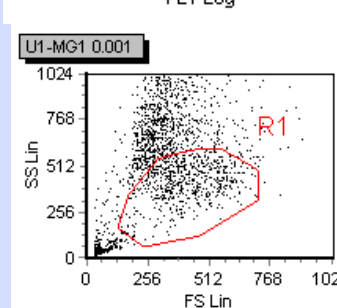


59%

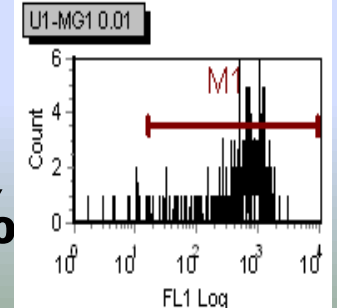
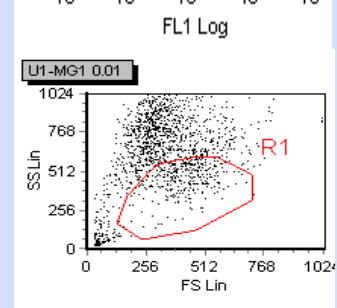
U1



<0.5%



97%

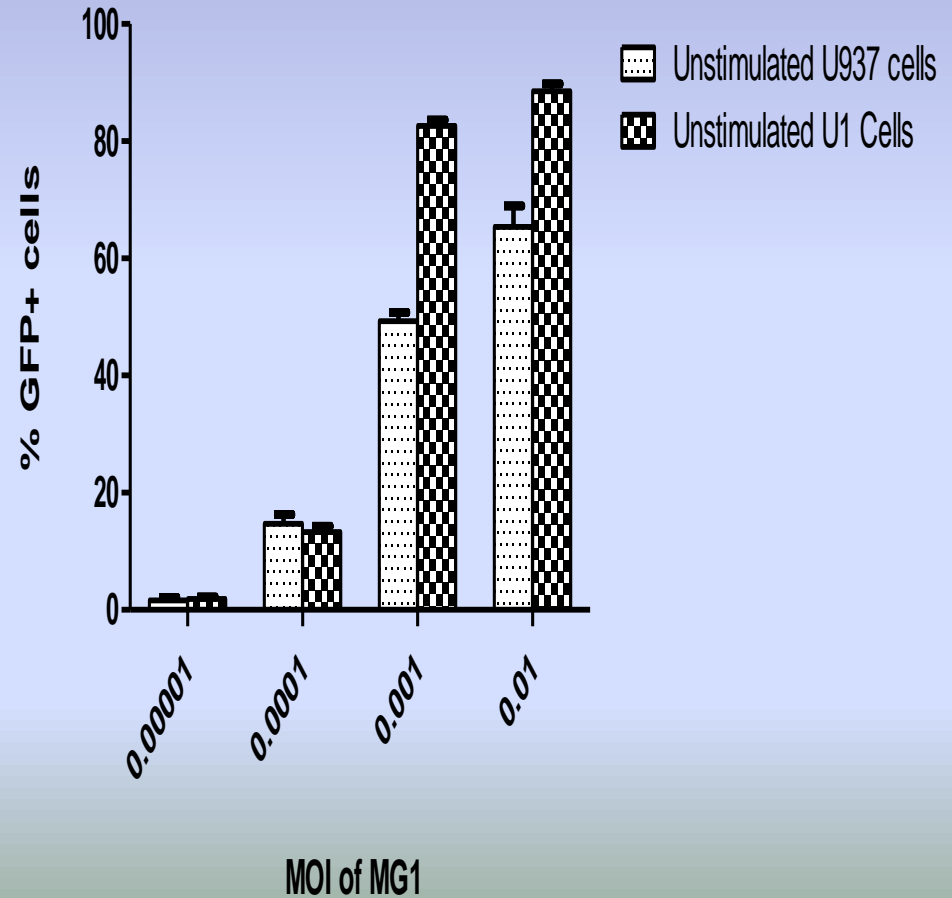
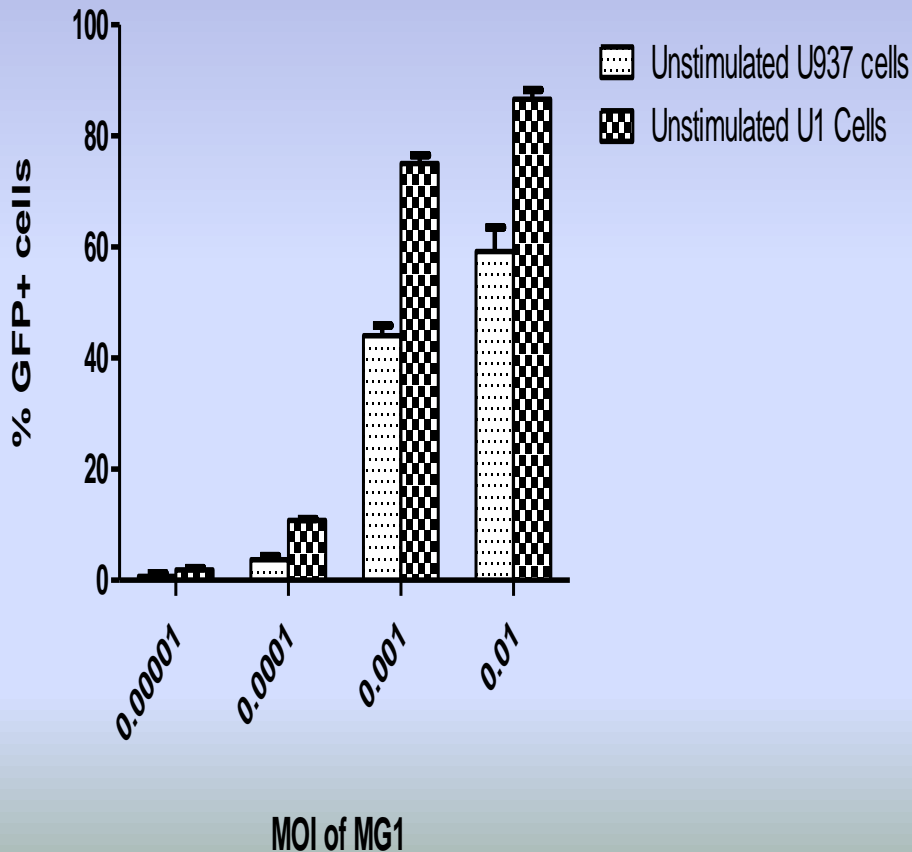


99%

Effect of HIV on MG1 Infectivity in U1 vs U937 Cells

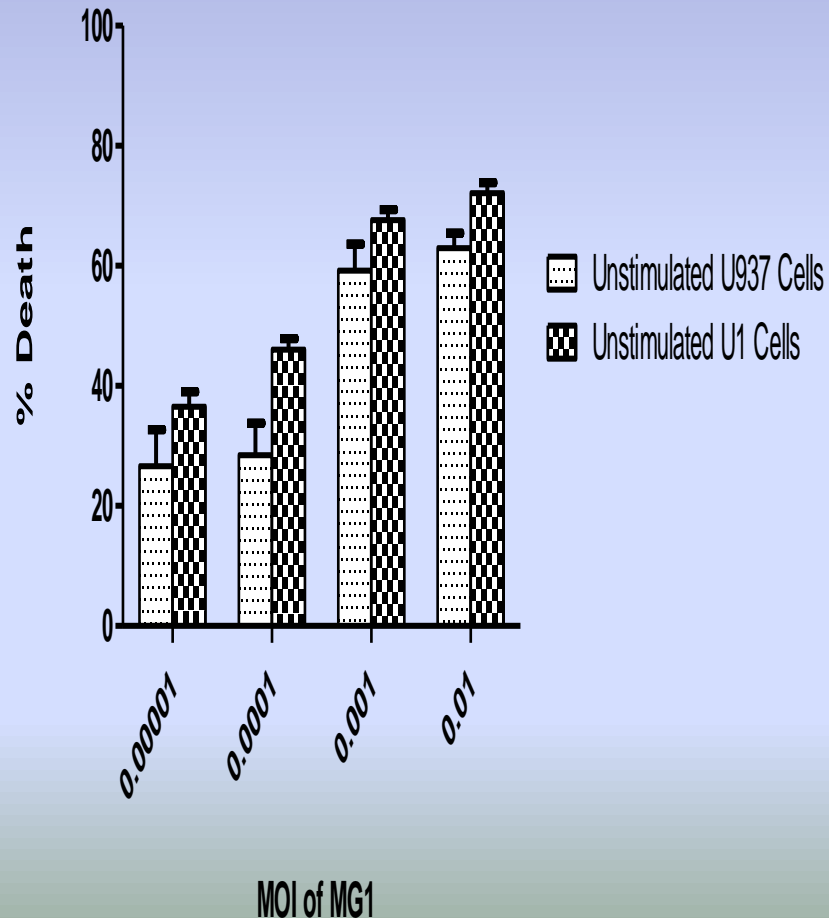
18 hours post MG1 Infection

24 hours post MG1 Infection

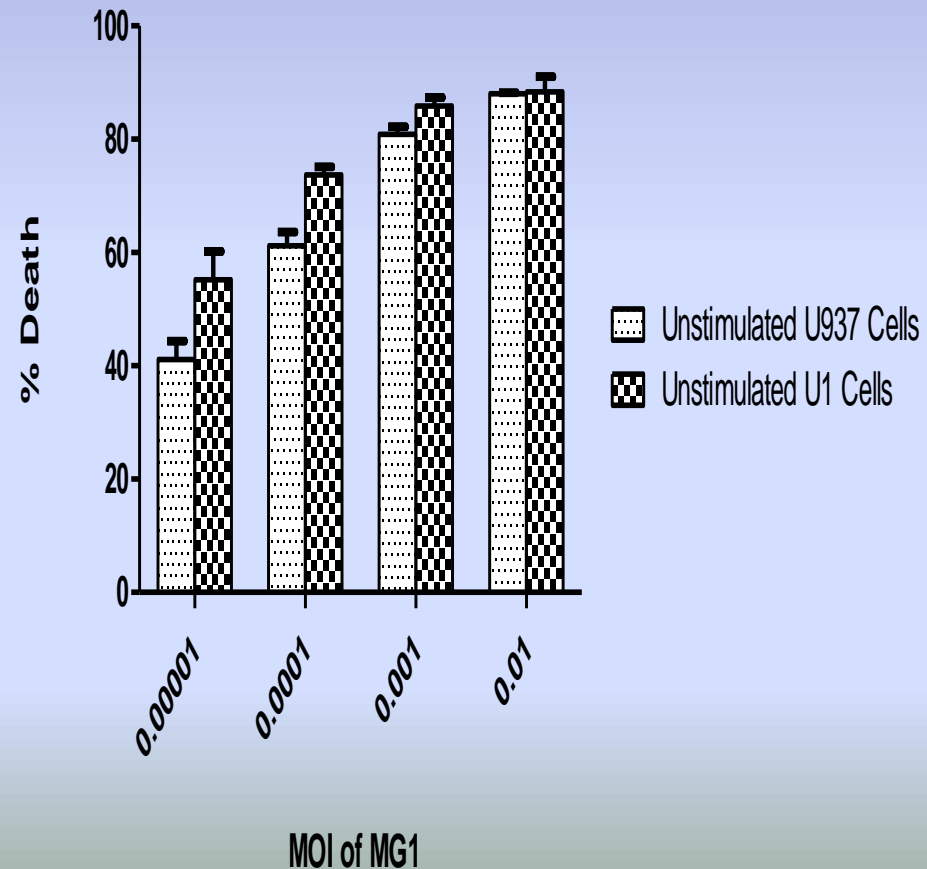


Effect of HIV on MG1-induced Cell Death in U1 vs U937 Cells

18 hours post MG1 Infection



24 hours post MG1 Infection



Other Cells Lines

- ACH-2 and A301 cells:
 - Infectivity and cell death profiles similar
- OM-10 and HL-60 cells:
 - Infectivity and cell death profiles similar
- J1.1 and Jurkat cells:
 - Infectivity profiles similar
 - J1.1 more resistant to MG1-mediated cell death

Objective 2

- To determine whether there is selective replication of MG1 in primary cells infected with HIV, with resultant cell death and decrease of HIV production

Primary Cell Experiments

PBMCs from HIV patients on HAART with VL \leq 40 copies/mL

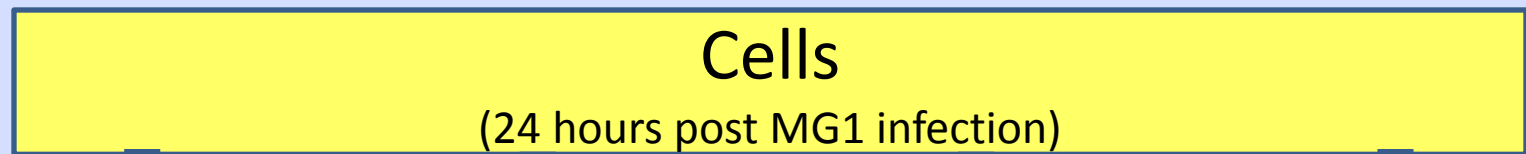


CD4+HLADR-CD25- cells by negative selection



MG1 Infection

(Uninfected control cells, MOI 0.01 \pm MOI 0.001)



Infectivity
(GFP by Flow)



Viability
(MTT Assay)



anti-CD3 + IL-2
Co-culture



p24 antigen
(ELISA)



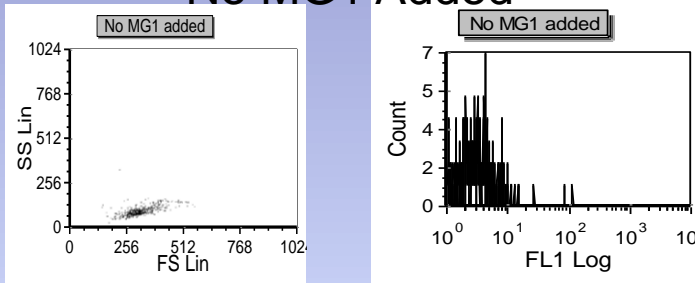
HIV RNA (RT-PCR)



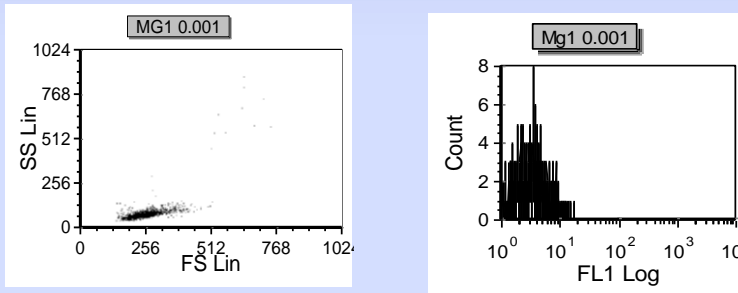
Total HIV
DNA (PCR)

Infectivity and Viability of CD4+CD25-HLADR- Cells from HIV+ Patients (N=20)

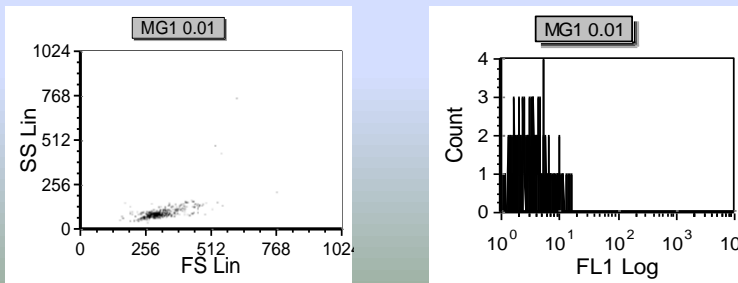
No MG1 Added



MG1 0.001



MG1 0.01



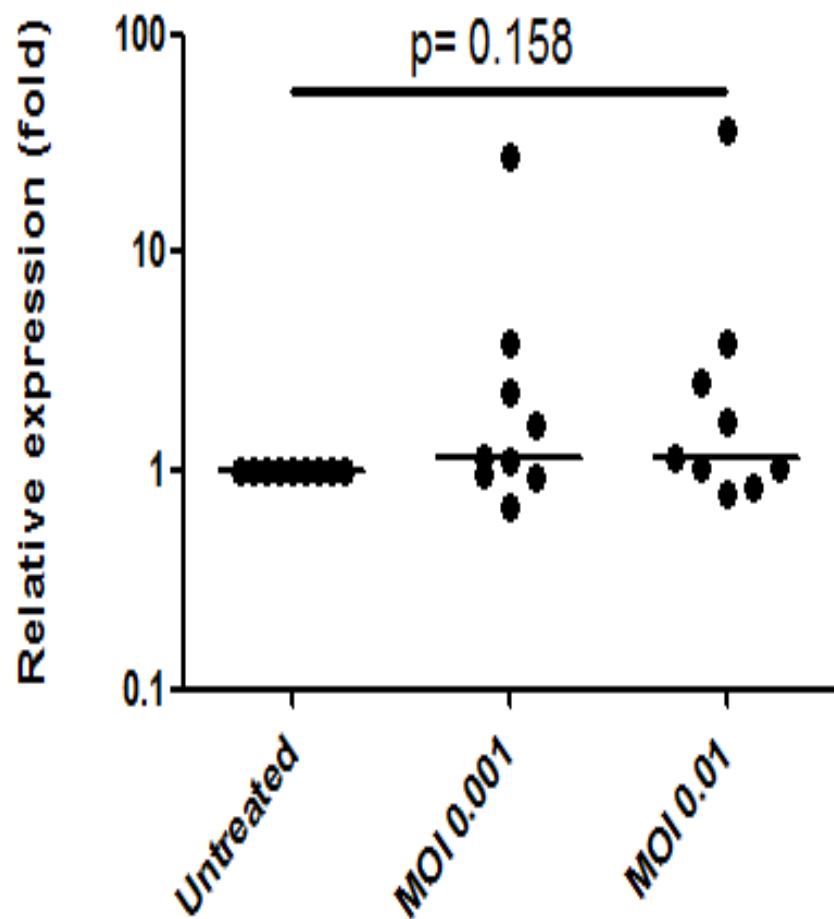
- Cells not infected by MG1
- Viability not reduced

p24 antigen concentrations in patient supernatants (N=20)

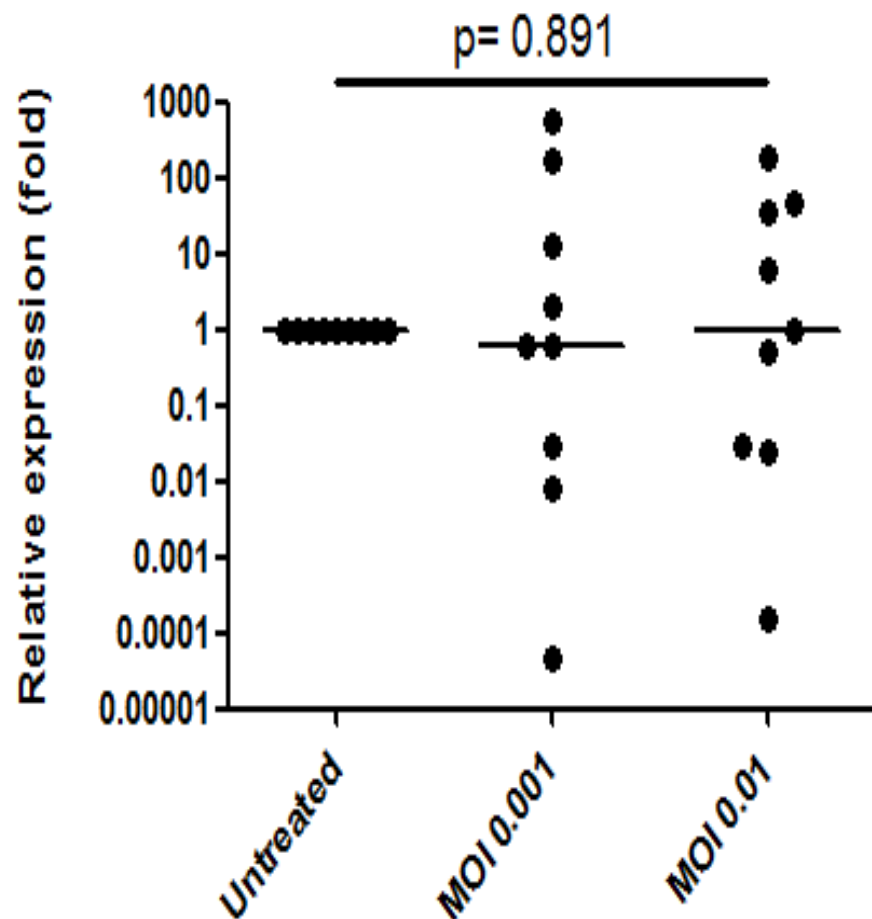
- below level of assay detection

PCR for Total HIV DNA on Patient Cells and Total HIV RNA on Supernatants (N=9)

Total DNA



Total RNA



Caution regarding PCR data



- Unclear whether our assay is sensitive enough to detect such low levels of events
- PCR-based assays do not differentiate between HIV that is replication-competent or not
- Unclear whether PCR is an effective method to monitor eradication strategies

Eisele E et al. 2012

Concluding Thoughts...*The Road Ahead*

- Results to date very preliminary
- Combination therapy will likely required



Acknowledgements

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