Oncolytic viruses as a potential approach to eliminate cells that constitute the latent HIV reservoir

Nischal Ranganath

Department of Biochemistry, Microbiology and Immunology
Faculty of Medicine
University of Ottawa

Supervisor: Dr. Jonathan Angel

Approved by:
Dr. Paul MacPherson
Dr. John C. Bell
Dr. Dave Stojdl

Thesis submitted to the Faculty of Graduate and Postdoctoral Studies in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in Microbiology and Immunology

Declaration

I, Nischal Ranganath, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Date: March 20, 2018

Abstract

HIV infection represents a major health and socioeconomic challenge worldwide. Despite significant advances in therapy, a cure for HIV continues to be elusive. The design of novel curative strategies will require targeting and elimination of cells that constitute the latent HIV-1 reservoir. However, such an approach is impeded by the inability to distinguish latently HIV-infected cells from uninfected cells.

The type-I interferon (IFN-I) response is an integral antiviral defense mechanism, but is impaired at multiple levels during productive HIV infection. Interestingly, similar global impairments in IFN-I signaling have been observed in various human cancers. This led to the development of IFN-sensitive oncolytic viruses, including the recombinant Vesicular Stomatitis Virus (VSVΔ51) and Maraba virus (MG1), as virotherapy designed to treat various cancers.

Based on this, it was hypothesized that IFN-I signaling is impaired in latently HIV-infected cells (as observed in productively infected cells) and that VSVΔ51 and MG1 may be able to exploit such intracellular defects to target and eliminate latently HIV-infected cells, while sparing healthy cells. First, using cell line models of HIV-1 latency, intracellular defects in IFN-I responses, including impaired IFNα/β production and expression of IFNAR1, MHC-I, ISG15, and PKR, were demonstrated to represent an important feature of latently HIV-infected cells. Consistent with this, the latently HIV-infected cell lines were observed to have a greater sensitivity to VSVΔ51 and MG1 infection, and MG1-mediated killing, than the HIV-uninfected parental cells.

Next, the ability of oncolytic viruses to kill latently HIV-infected human primary cells was demonstrated using an *in vitro* resting $CD4^+$ T cell model of latency. Interestingly, while both VSVΔ51 and MG1 infection resulted in a significant reduction in inducible p24 expression, a dose-dependent decrease in integrated HIV-1 DNA was only observed following MG1 infection. In keeping with this, MG1 infection of memory $CD4^+$ T cells from HIV-1 infected individuals on HAART also resulted in a significant decrease in inducible HIV-1 *gag* RNA expression.

By targeting an intracellular pathway that is impaired in latently HIV-infected cells, the findings presented in this dissertation highlight a novel, proof-of-concept approach to eliminate the latent HIV-1 reservoir. Given that VSVΔ51 and MG1 are currently being studied in cancer clinical trials, there is significant potential to translate this work to *in vivo* studies.

Acknowledgments

I want to begin by saying that my journey through PhD has been the most challenging, yet rewarding academic experience of my life. First and foremost, I would like to thank and dedicate this dissertation to my parents, Latha and Ranganath, for inspiring me to ask questions, to remain open-minded and humble, and for providing me with this opportunity to learn. I would like to thank my partner, Kritica Arora, for her unwavering support, optimism, and for continually challenging me to be the best version of myself. This thesis is as much yours, as it is mine.

The work presented here is a collaboration that would not be possible without the contributions and guidance of current and past members of the Angel Lab. I am grateful to Dr. Sandra Côté for her mentorship and unrelenting patience, for paving the way for the success of this project and my own, as well as instilling in me her commitment to the scientific process. I am also immensely thankful for the mentorship, friendship, intellectual conversations, and moral support provided to me by Maria Sanchez, Teslin Sandstrom, Stephanie Burke-Schinkel, Marko Cavar, Andrea Ibrahim, and Drs. Jason Fernandes and Elliot Faller.

I would like to sincerely thank my supervisor, Dr. Jonathan Angel, for placing his trust in me to undertake this proof-of-concept study, for his mentorship and guidance, for providing me with the independence to undertake scientific inquiry, and for pushing me to succeed as a prospective clinician-scientist. In this respect, I am also grateful for my thesis advisory committee including Drs. Macpherson, Bell, and Stojdl, for challenging my understanding, pushing me to inquire, and for helping guide me through the process of

undertaking sound, scientific work. I would also like to acknowledge Drs. Bell and Stojdl for providing me with access to the oncolytic viruses that form the foundation of this study.

I am thankful for the support of the Department of Biochemistry, Microbiology, and Immunology, Faculty of Medicine, and the MD/PhD program. In particular, I am grateful for Dr. Michael Scholassmacher who has been an exceptional mentor and advocate in helping me navigate the field of basic science and pushing me to thrive and succeed.

Last but certainly not least, I am grateful to all the volunteers and patients who donated samples for this study and for the nursing staff at the Ottawa General Hospital for their support in collecting these samples. I would also like to acknowledge funding agencies including Vanier Canada Graduate Scholarship Doctoral Award, Canadian Institute for Health Research, Canadian Foundation for AIDS Research, and the International AIDS Society for supporting this work.

Table of Contents

List of Abbreviations

AIDS	Acquired immunodeficiency syndrome
APC	Allophycocyanin
APOBEC	Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like
ARS	Antiretroviral syndrome
ARS	Acute retroviral syndrome
BLT	Bone marrow, liver, and thymus
BSA	Bovine serum albumin
CCL19	Chemokine (C-C motif) ligand 19
CCL20	Chemokine (C-C motif) ligand 20
CCR5	CC-chemokine receptor type 5
CD	Cluster of differentiation
CDC	Centers for Disease Control
CDK9	Cyclin-dependent kinase 9
cDNA	Complementary DNA
CFSE	Carboxyfluorescein succinimidyl ester
cGAS	Cyclic guanosine monophosphate-adenosine monophosphate synthase
CRV	Conditionally replicating viruses
CTL	Cytotoxic $CD8^+$ T lymphocytes
CTLA-4	Cytotoxic T-Lymphocyte Associated Protein 4
CXCL9	Chemokine (C-X-C motif) ligand 9
CXCR4	CXC-chemokine receptor type 4
DC	Dendritic cells
dCA	Didehydro-Cortistatin A
DMEM	Dulbecco's Modified Eagle's medium
DMSO	Dimethyl sulfoxide
dsDNA	Double-stranded DNA
dsRNA	Double-stranded RNA

EDTA	Ethylenediaminetetraacetic acid
eGFP	Enhanced green fluorescent protein
eIF2α	eukaryotic initiation factor 2
ELISA	Enzyme-linked immunosorbent assay
FBS	Fetal bovine serum
G	Glycoprotein
GALT	Gut-associated lymphoid tissue
HAART	Highly active antiretroviral therapy
HBSS	Hank's balanced salt solution
HDAC1	Histone deacetylase 1
HDACi	Histone deacetylase inhibitor
HIV	Human immunodeficiency virus
HLAC	Human lymphoid aggregate culture
HMT	Histone methyltransferases
HPCs	Hematopoietic progenitor cells
HSA	Heat-stable antigen
ICB	Immune checkpoint blockers
IFI16	IFN-inducible factor 16
IFN	Interferon
IFN-I	Type I interferon
IFNα	Interferon-α
IFNβ	Interferon-β
IFNAR1	IFNα/β receptor-1
IFNAR2	IFNα/β receptor-2
IL-2	Interleukin-2
IL-7	Interleukin-7
IMDM	Iscove's Modified Dulbecco's Medium
IRF	Interferon regulatory factor
ISG	Interferon stimulated gene

ISG15	Interferon stimulated gene 15
ISGF3	Interferon stimulated gene factor 3
ISRE	IFN-stimulated responsive elements
ISTI	Integrase strand transfer inhibitor
IT	Intratumoral
IV	Intravenous
JAK1	Janus kinase 1
L	Large protein of the RNA-dependent RNA polymerase
LAG-3	Lymphocyte activation gene 3
LDL-R	Low density lipoprotein receptor
LTR	Long terminal repeat
M	Matrix protein
MAVS	Mitochondrial antiviral-signaling
mDC	Myeloid dendritic cells
MG1	Recombinant maraba virus
MHC-I	Major histocompatibility complex I
MIP1β	Macrophage inflammatory Protein-1β
miRNA	microRNA
MOI	Multiplicity of infection
MS-RNA	Multiply-spliced RNA
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
N	Nucleoprotein
NF-κB	Nuclear factor-κB
NFAT	Nuclear factor of activated T cells
NGS	Normal goat serum
NHP	Non-human primate
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NSG	NOD/SCID-gamma chain null
OV	Oncolytic virus

P	Phosphoprotein
P-TEFb	Positive transcription elongation factor
PAMPs	Pathogen-associated molecular patterns
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
PD-1	Programmed cell death protein 1
pDC	Plasmacytoid dendritic cells
PE	Phycoerythrin
PE-cy5	Phycoerythrin-cyanin5
PE-cy7	Phycoerythrin-cyanin7
PFA	Paraformaldehyde
PHA	Phytohaemagglutinin
PKC	Protein kinase C
PKR	Protein kinase R
Poly(I:C)	Polyinosinic-polycytidylic acid
PRR	Pattern recognition receptor
PSG	Penicillin, streptomycin, and L-glutamine
PTB	Polypyrimidine tract binding
QVOA	Quantitative viral outgrowth assay
R4-tropic	CXCR4-tropic
R5-tropic	CCR5-tropic
R848	Resiquimod
RAL	Raltegravir
RIG-I	Retinoic acid-inducible gene I
RLR	RIG-I-like receptor
RNA Pol II	RNA polymerase II
RNP	Ribonucleoprotein
RP10	RPMI-1640 supplemented with 10% FBS and PSG

RPMI-1640	Roswell Park Memorial Institute 1640 medium
RT-qPCR	Quantitative reverse transcription PCR
SAHA	Suberoylanilide Hydroxamic Acid (also known as Vorinostat)
SAMHD1	SAM domain and HD domain-containing protein 1
SDS	Sodium dodecyl sulfate
SEM	Standard error of the mean
SIV	Simian Immunodeficiency Virus
SIV_{MAC251}	Simian Immunodeficiency Virus-MAC251
ssDNA	Single-stranded DNA
ssRNA	Single-stranded RNA
STAT1	Signal transducer and activator of transcription 1
STAT2	Signal transducer and activator of transcription 2
$t_{1/2}$	Half-life
TANK	TRAF family member-associated NF-κB activator
Tat	Trans-activator of transcription
TBK1	TANK-binding kinase 1
T_{CM}	Central memory $CD4^+$ T cells
T_{EM}	Effector memory $CD4^+$ T cells
TIGIT	T-cell immunoglobulin and ITIM domain
TILDA	Tat/rev Induced Limiting Dilution Assay
TLR	Toll-like receptor
TREX1	3'-repair exonuclease 1
T_{TM}	Transitional memory $CD4^+$ T cells
TYK2	Tyrosine kinase 2
UBE1L	E1-like ubiquitin-activating enzyme
UNAIDS	United Nations Programme on HIV/AIDS
US-RNA	Unspliced RNA
USP18	Ubiquitin specific peptidase 18
UV	Ultraviolet

VL	Viral load
VSV	Vesicular stomatitis virus
VSVΔ51	Recombinant vesicular stomatitis virus-Δ51
WHO	World Health Organization
WT	Wild-type

List of Figures and Illustrations

List of Tables

Chapter 1: HIV latency and novel strategies to target and eliminate cells that constitute the latent HIV reservoir

1.1. Latent HIV-1 infection and the search for a cure

1.1.1. HIV-1 infection: from the bedside to the bench

1.1.1.1. HIV-1 infection: evolution of a global pandemic

Between 1981 and 1982, the Centers for Disease Control (CDC) reported a significant increase in the incidence of uncommon diseases, including *Pneumocystis carinii* pneumonia, Kaposi's sarcoma, and mucosal candidiasis, in New York City, San Francisco, and Los Angeles (Brennan and Durack, 1981; Durack, 1981; Haverkos and Curran, 1982). These opportunistic infections, typically observed in immunocompromised individuals, were identified predominantly in previously healthy homosexual men (Gottlieb et al., 1981). This led to the first clinical description of Acquired Immunodeficiency Syndrome (AIDS) (Gallo and Montagnier, 2003). In 1983, two independent groups led by Dr. L. Montagnier (Institut Pasteur, France) and Dr. R. Gallo (National Institutes of Health, USA) identified Human Immunodeficiency Virus (HIV) as the etiologic agent of this clinical syndrome (Barré-Sinoussi et al., 1983; Gallo et al., 1984). HIV-1 infection has since reached pandemic proportions with an estimated 36.7 million people living with HIV/AIDS worldwide and 2.1 million new cases reported each year (UNAIDS, 2016). The devastating impacts of HIV-1 infection on the health and quality of life of individuals affected, as well as the significant healthcare and socioeconomic burden posed by the disease, urgently necessitate the search for a cure.

1.1.1.2. Pathogenesis of HIV-1

Soon after the first isolation of HIV-1 in 1983, studies characterizing the pathogenesis of HIV-1 infection were well under way. HIV was identified as an enveloped, positive-sense, single-stranded RNA virus (ssRNA) belonging to the family of *Retroviridae.*

Cell tropism: The CD4 cell surface protein was identified as the primary receptor that dictated the cellular tropism of HIV-1 (Dalgleish et al., 1984; Klatzmann et al., 1984b). Consistent with this, early clinical evidence and *in vitro* studies demonstrated significant selective depletion of $CD4^+$ T lymphocytes following HIV infection (Klatzmann et al., 1984a). In addition, the CC-chemokine receptor type 5 (CCR5) and the CXC-chemokine receptor type 4 (CXCR4) were identified as the main co-receptors that facilitated fusion and entry of HIV into cells (Cocchi et al., 1995; Dragic et al., 1996; Feng et al., 1996). Interestingly, CCR5 was demonstrated to be the most important co-receptor during early transmission and primary HIV-1 infection *in vivo* (Kahn and Walker, 1998; Moore and Klasse, 2007), while HIV-1 variants utilizing CXCR4 were identified later in the course of disease (Forsman and Weiss, 2008). In addition to the $CD4^+$ T cells, macrophages and other cells of myeloid lineage, including microglial cells and dendritic cells (DCs), expressing CD4 and the co-receptors have also been identified as potential targets for HIV-1 infection (Hladik and McElrath, 2008).

Lifecycle: Cellular entry of HIV-1 requires the binding of the surface viral glycoprotein, gp120, to CD4 and a chemokine co-receptor expressed on target cells. This results in a conformational change that allows the transmembrane subunit, gp41, to mediate fusion of the viral envelope with the target cell membrane (Chan and Kim, 1998).

Following uncoating of the HIV core, the ssRNA genome of the virus is reverse transcribed, by the HIV reverse transcriptase, into a double-stranded complementary DNA (cDNA) sequence. The pre-integration complex composed of this viral cDNA, HIV-1 proteins (Vpr, p17, and integrase) and host factors, is transported to the nucleus (Piller et al., 2003). Here, HIV-1 is stably integrated into the host chromosomal DNA through the activity of HIV-1 integrase (Coffin et al., 1997). Unintegrated forms of HIV-1 DNA, including circular, 1-long terminal repeat (LTR), and 2-LTR circles may also be present within the nucleus (Sloan and Wainberg, 2011) The HIV-1 provirus serves as the blueprint for the transcription of viral mRNA, resulting in both spliced and unspliced variants. The viral mRNA transcripts are then translated to produce Gag and Gag-Pol polyproteins, as well as the accessory proteins Tat, Rev, Nef, Vif, Vpu, and Vpr, which together with the full-length genomic RNA, begin to form the immature virion. Gag and Gag-Pol polyproteins are then processed by HIV-1 protease at the cell surface, or within a budding virion, to form the viral capsid. Finally, as the virus buds, the viral envelope glycoproteins and host membrane proteins are incorporated, resulting in the formation of a mature virus capable of further propagating infection (Figure 1) (Coffin et al., 1997; Frankel and Young, 1998; Simon et al., 2006).

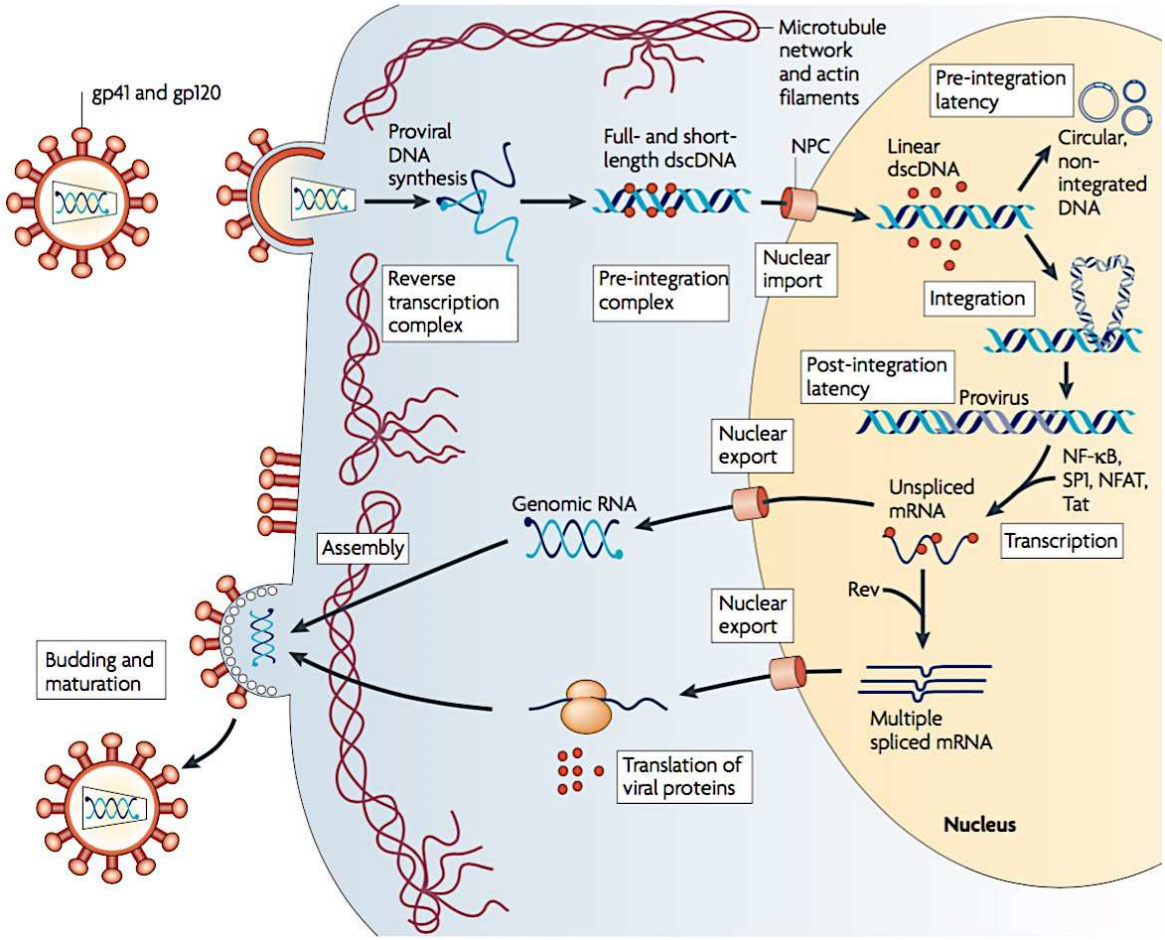

gp41 and gp120
Microtubule network and actin filaments
Proviral DNA synthesis
Full- and short-length dscDNA
NPC
Pre-integration latency
Linear dscDNA
Circular, non-integrated DNA
Reverse transcription complex
Pre-integration complex
Nuclear import
Integration
Post-integration latency
Provirus
Nuclear export
NF-κB, SP1, NFAT, Tat
Unspliced mRNA
Genomic RNA
Assembly
Transcription
Rev
Nuclear export
Budding and maturation
Multiple spliced mRNA
Translation of viral proteins
Nucleus

Figure 1: HIV-1 life cycle and viral latency. Viral fusion and entry requires the binding of glycoprotein gp120 to CD4 receptors at the cell surface as well as to CC chemokine receptor type 5 (CCR5) or CXC chemokine receptor type 4 (CXCR4). The viral nucleocapsid enters the cytoplasm and uses cytoplasmic dynein to move toward the nuclear pore complex (NPC). The viral RNA is retrotranscribed into proviral double-stranded cDNA (dscDNA), which can stay in the cytosol, where it is highly unstable and exists in a transient, reversible pre-integration latent state, or can form a pre-integration complex consisting of dscDNA, viral proteins and some host cell proteins. When ATP levels are adequate, the pre-integration complex is transported into the nucleus through the NPC, and the dscDNA either circularizes as one or two long terminal repeat-containing circles or is integrated into a host cell chromosome. After integration, the provirus remains quiescent, existing in a permanent post-integration latent state. On activation, the viral genome is transcribed by the synergic interaction of cellular transcription factors (nuclear factor-κB (NF-κB), nuclear factor of activated T cells (NFAT) and specificity protein 1 (SP1)) and the viral transactivator, Tat. Rev, a viral protein, regulates the splicing and cytosolic transport of some of the viral mRNAs, which are translated into regulatory and structural viral proteins. New virions assemble and bud through the cell membrane, maturing through the activity of the viral protease. Reprinted by permission from Macmillan Publishers Ltd: Nat. Rev. Microbiol., Coiras, M., López-Huertas, M.R., Pérez-Olmeda, M., and Alcamí, J. (2009). Understanding HIV-1 latency provides clues for the eradication of long-term reservoirs. *7*, 798–812. (Figure 1)., Copyright 2009

1.1.1.3. Pathogenesis of HIV-1 infection

HIV transmission occurs predominantly through the anogenital mucosa by direct sexual contact. However, the virus can be also transmitted through blood or blood products, and from an infected mother to infant either intrapartum or perinatally (Barré-Sinoussi et al., 1983; Coffin et al., 1997). Irrespective of the route of transmission, primary HIV-1 infection often involves the cells present within the local microenvironment including macrophages, DCs, and both activated and resting $CD4^+$ T lymphocytes (Haase, 2005; Hladik and McElrath, 2008). Subsequently, viral replication within target cells and the trafficking of the infected cells through the bloodstream to secondary lymphoid tissues facilitates the widespread dissemination of HIV-1 throughout the body. This acute phase of infection coincides with a dramatic decrease in $CD4^+$ T cells in peripheral blood and tissues (Figure 2) (Simon and Ho, 2003). The greatest depletion is observed within the gut-associated lymphoid tissue (GALT), with a dramatic 30-60% loss in $CCR5^+$ memory $CD4^+$ T cells observed within a few days of infection (Brenchley et al., 2006; 2004; Mattapallil et al., 2005). This massive loss of cells may be a consequence of direct viral infection and cytopathic effects (Brenchley et al., 2006; Mattapallil et al., 2005) and/or indirect death of bystander cells mediated by apoptotic (Finkel et al., 1995) or pyroptotic (Doitsh et al., 2013) pathways.

Typically, 2-4 weeks following initial inoculation, individuals may experience a constellation of flu-like symptoms, including fever, fatigue, headache, rash, myalgia, pharyngitis, cervical or axillary lymphadenopathy, arthralgias, weight loss, nausea, and diarrhea (Yerly and Hirschel, 2012). Together, these symptoms are termed acute

retroviral syndrome (ARS). Soon after, a rapid 2-3 log decline in HIV-1 plasma viral load, resolution of ARS, and a stabilization of $CD4^+$ T cell counts is observed (Forsman and Weiss, 2008) (Figure 2). This coincides with the appearance of HIV-specific immune responses including virus-specific cytotoxic $CD8^+$ T cells (Koup et al., 1994) and non-neutralizing antibodies (Cooper et al., 1987). In the absence of treatment, the viral load in peripheral blood reaches a 'set-point', which has been demonstrated to be predictive of the rate of progression to AIDS (Mellors et al., 1997; 1996). Next, a period of clinical latency of approximately 8 to 12 years ensues (Moss and Bacchetti, 1989). During this time, an individual may be asymptomatic, but ongoing viral replication and reciprocal immune response to HIV-1, mediated by virus-specific $CD8^+$ T cells and neutralizing antibodies, can still be observed. In the absence of appropriate interventions, the hallmark of the disease is a gradual destruction of naïve and memory $CD4^+$ T cells resulting in the eventual progression to AIDS (Figure 2) (Baltimore and Feinberg, 1989).

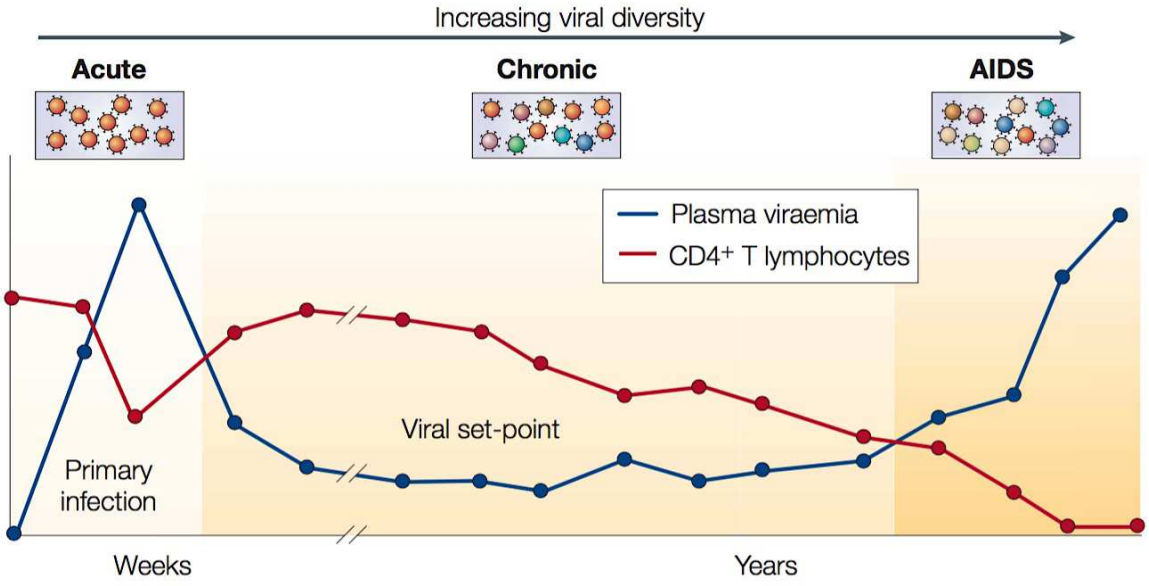

Increasing viral diversity
Acute
Chronic
AIDS
Plasma viraemia
CD4+ T lymphocytes
Viral set-point
Primary infection
Weeks
Years

Figure 2: The natural course of HIV-1 infection on the basis of the longitudinal evolution of the two key surrogate markers – plasma viremia and $CD4^+$ T lymphocyte count. The phase of primary HIV-1 infection is generally associated with clinical symptoms of the acute retroviral syndrome. After the first months of infection, plasma viremia stabilizes at an individual level and the $CD4^+$ T-lymphocyte number normalizes. The protracted chronic phase of infection is clinically silent even though HIV-1 replicates continuously at high levels. AIDS is defined by the occurrence of opportunistic infections or HIV-1-associated malignancies as defined by the Centers for Disease Control and Prevention (CDC) classification of 1993. The risk of opportunistic infections increases significantly when the number of $CD4^+$ T lymphocytes falls to below 200 per mm^3. Viral diversity increases over the course of infection in accordance with the continuously high level of viral replication and the error-prone nature of HIV-1 reverse transcription. Reprinted by permission from Macmillan Publishers Ltd: Nat. Rev. Microbiol., Simon, V., and Ho, D.D. (2003). HIV-1 dynamics in vivo: implications for therapy. 1, 181–190. (Figure 2)., Copyright 2003

1.1.2. Discovery of latent HIV-1 infection as the primary barrier to eradication

1.1.2.1. The advent of HAART

The introduction of highly active antiretroviral therapy (HAART) in 1995 had a profound impact on the management of HIV-1 infection. A significant decline in morbidity and mortality associated with AIDS, as well as a dramatic improvement in the health and quality of life of individuals living with HIV-1 was observed (Palella et al., 1998). In parallel, HAART enabled individuals with HIV-1 infection to have near-normal life expectancies (Antiretroviral Therapy Cohort Collaboration, 2008; van Sighem et al., 2010). However, HAART is not without limitations. Despite ongoing therapy, the immunologic profile of HIV-1 infected individuals fails to completely normalize (Corbeau and Reynes, 2011). HAART has also been associated with a range of adverse effects (Carr and Cooper, 2000) and an increase in the risk of metabolic dysfunction, cancer, liver, and cardiovascular disease (Deeks and Phillips, 2009; Friis-Møller et al., 2003). In addition, the global delivery of HAART, especially in resource-limited settings, continues to be a major challenge (Katlama et al., 2013; UNAIDS, 2016). Most importantly, a lifelong adherence to HAART is necessary (de Pee et al., 2012), as a rapid rebound of HIV-1 replication is consistently observed within 4-6 weeks following cessation of therapy (Davey et al., 1999).

1.1.2.2. Viral kinetics on HAART and the discovery of the latent HIV-1 reservoir

Following initiation of HAART, a logarithmic, multiphasic decay in plasma HIV-1 levels is observed (Figure 3). The first and most rapid phase of decay, with a half-life ($t_{1/2}$) of ~1-2 days, has been associated with the clearance of free plasma virions and the death of activated, HIV-infected $CD4^+$ T cells (Perelson et al., 1996). The second phase of viral

decay ($t_{1/2}$ = 2 weeks) has been postulated to reflect the death of HIV-1 infected cells with longer life spans including macrophages, dendritic cells, and partially activated $CD4^+$ T cells (Perelson et al., 1997; Shan and Siliciano, 2013). The third and final phase of decay however, represents an extremely stable cell population with minimal residual viremia and an estimated $t_{1/2}$ of 44 months (Finzi and Siliciano, 1998; Finzi et al., 1999; Siliciano et al., 2003). In 1997, several groups concurrently demonstrated that this third phase may be attributable to the presence of a long-lived, latent HIV-1 reservoir *in vivo* within resting memory $CD4^+$ T cells (Chun et al., 1997c; Finzi et al., 1997; Wong et al., 1997a). With a half-life of 44.2 months, eradication of this reservoir was estimated to require 73.4 years of HAART. In addition, this latent reservoir was demonstrated to contain provirus that was inducible upon appropriate stimulation and represented an important source of viral rebound in the absence of HAART (Joos et al., 2008; Wong et al., 1997a). Going forward, the elimination of this reservoir represents the critical step towards the eradication of HIV-1.

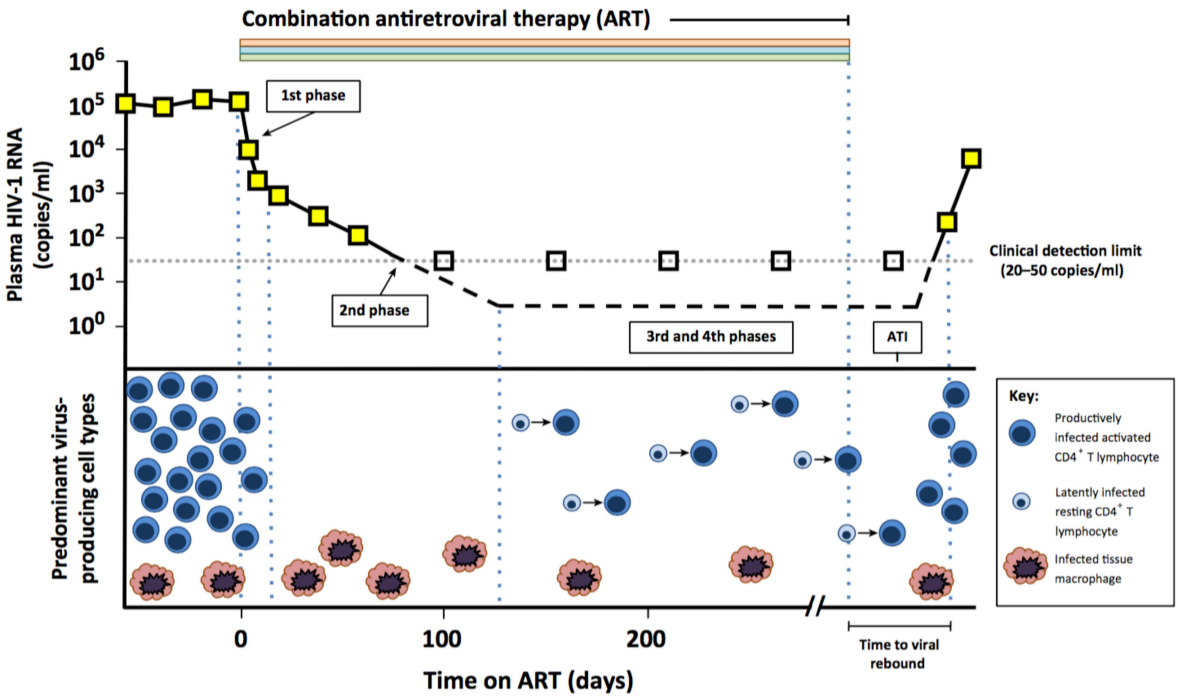

Combination antiretroviral therapy (ART)
Plasma HIV-1 RNA (copies/ml)
10^6
10^5
10^4
10^3
10^2
10^1
10^0
1st phase
2nd phase
3rd and 4th phases
ATI
Clinical detection limit (20–50 copies/ml)
Predominant virus-producing cell types
Key:
Productively infected activated CD4+ T lymphocyte
Latently infected resting CD4+ T lymphocyte
Infected tissue macrophage
0
100
200
Time to viral rebound
Time on ART (days)

Figure 3. Sources and kinetics of plasma viremia on HAART. The initiation of ART results in a biphasic decay in plasma viremia. The first phase reflects the death of productively infected $CD4^+$ T lymphocytes. Infected cells with a longer half-life, such as tissue macrophages, are thought to produce the second phase of viral decay, during which the viral load falls below the detection limit of commercial assays (20–50 copies/ml). Patients maintain low-level viremia during ART that likely arises from spontaneous reactivation of latently infected resting $CD4^+$ T cells. The contribution of non-T cell reservoirs including chronically infected tissue macrophages to residual viremia remains incompletely understood. Analytical treatment interruption (ATI) consists of study participants stopping ART with close monitoring for adverse effects of unchecked viral replication and quantifiable viral rebound. The time to viral rebound during ATI is thought to provide an estimate of the efficacy of the intervention in reducing reservoir size. Figure adapted from Durand et al. [22]. Reprinted from Trends in Molecular Medicine, 22, Spivak, A.M., and Planelles, V., HIV-1 Eradication: Early Trials (and Tribulations). 10-27, Copyright 2016, with permission from Elsevier.

1.1.3. HIV-1 latency and the cells that constitute the latent reservoir

1.1.3.1. Defining HIV-1 latency

HIV-1 latency is classicaly defined as the persistence of HIV-1 as integrated proviral DNA in a transcriptionally silent state, which upon appropriate stimulation can be induced to produce infectious virions (Archin et al., 2014). In accordance with this, it can be postulated that viral signatures including RNA and proteins may be absent within latently infected cells. In contrast to this definition however, HIV-1 has been demonstrated to integrate predominantly into transcriptionally active sites within the genome of resting $CD4^+$ T cells from patients on HAART (Han et al., 2004). In keeping with this, HIV-1 RNA, including unspliced (US-RNA) and multiply-spliced (MS-RNA) transcripts encoding Tat and Rev, were readily detectable, but strictly localized within the nucleus of latently HIV-infected resting $CD4^+$ T cells (Chun et al., 2003; Hermankova et al., 2003; Lassen et al., 2004; 2006). Despite the predominance of abortive viral transcripts, HIV-1 replication was inducible within resting $CD4^+$ T cells following overexpression of Tat or polypyrimidine tract binding protein (PTB), an RNA-binding protein known to enhance nuclear export (Lassen et al., 2004; 2006). These findings support the potential for multiple levels of transcriptional and post-transcriptional blocks to HIV-1 replication within resting $CD4^+$ T cells. Accordingly, whether low levels of RNA expression can lead to viral protein expression in latently HIV-1 infected cells is largely unknown at present. Expression of HIV-1 proteins, including Tat and Gag, has been reported within *in vitro* models of latent HIV-1 infection (Butera et al., 1994; Pace et al., 2012). Therefore, given the evidence,

latently HIV-1 infected cells may best be defined as cells that carry integrated DNA, but are unable to produce infectious virions unless appropriately stimulated.

1.1.3.2. Characteristics of the latent HIV-1 reservoir

Cells that constitute the latent HIV-1 reservoir: A reservoir may be defined as a stable, long-lived cell population that is able to sustain the presence of replication-competent HIV-1 despite effective HAART (Eisele and Siliciano, 2012). The presence of inducible virus is an important feature of this reservoir because the majority of detectable viruses in HIV-infected individuals on HAART contain defective genomes (Ho et al., 2013). Based on this definition, the resting memory $CD4^+$ T cells, specifically the central (T_{CM}) and transitional (T_{TM}) memory subsets, have been identified to be the major cellular reservoir for latent HIV-1 infection *in vivo* (Chomont et al., 2009; Chun et al., 1997c; Finzi et al., 1997; Wong et al., 1997a). Consistent with their function as stores of immunologic memory, these cells are extremely long-lived and represent the primary targets of HIV-1 infection within GALT and other lymphoid tissue (Siliciano and Greene, 2011).

The role of other cell types including monocytes/macrophages, naïve $CD4^+$ T cells, and hematopoietic progenitor cells (HPCs) as potential reservoirs remains controversial. Monocytes are relatively resistant to HIV-1 infection due to delays in reverse transcription and integration (Arfi et al., 2008), as well as the blockade of HIV-1 replication imparted by the restriction factor, SAM domain and HD domain-containing protein 1 (SAMHD1) (Laguette et al., 2011). In contrast, persistent HIV-1 infection of macrophages has been readily observed *in vivo* (Igarashi et al., 2001). Macrophages are resistant to viral cytopathic effects and have been shown to be important reservoirs for HIV-1, following

depletion of $CD4^+$ T cells during the later stages of chronic HIV-1 infection (Igarashi et al., 2001). However, whether macrophages can sustain replication-competent latent HIV-1 infection has yet to be determined. Naïve $CD4^+$ T cells from HIV-1 infected individuals have been shown to support latent HIV-1 infection, although the size of the reservoir is ~1-2 logs smaller than that of the memory $CD4^+$ T cell compartment (Wightman et al., 2010). Lastly, low levels of integrated HIV-1 DNA within HPCs was observed in a subset of patients on suppressive HAART (Carter et al., 2010). However, further investigation into the role of HPCs in HIV-1 latency will be necessary, as others have failed to replicate this finding *in vivo* (Durand et al., 2012). Therefore, memory $CD4^+$ T cells represent the primary reservoir of latent HIV-1 infection and an important target for HIV-1 eradication studies.

Time to establishment of the latent HIV-1 reservoir: The latent HIV-1 reservoir is established very early in the course of HIV-1 infection (Ananworanich et al., 2015). Chun and colleagues demonstrated that initiation of HAART within 10 days following onset of ARS was unable to prevent the reservoir establishment in HIV-1 infected individuals (Chun et al., 1998). Using non-human primate (NHP) models, the latent reservoir was demonstrated to be established as early as 3 days following mucosal infection with Simian Immunodeficiency Virus-MAC251 (SIV_{MAC251}) and resulted in significant viral rebound following treatment cessation (Whitney et al., 2014).

Size of the latent HIV-1 reservoir: The size of the latent HIV-1 reservoir varies dramatically depending on the assays utilized, the study populations, and effectiveness of HAART. PCR-based assays, which fail to differentiate between intact and defective HIV-1 provirus, estimate the size of the reservoir at 604 copies per million resting $CD4^+$ T cells

(Eriksson et al., 2013). In contrast, the quantitative viral outgrowth assay (QVOA), which represents the current gold-standard in measuring the replication-competent HIV-1 reservoir, estimates the size at 0.61 infectious units per million (IUPM) resting $CD4^+$ T cells (Eriksson et al., 2013). However, QVOA may underestimate the size of the reservoir due to the persistence of replication-competent, non-induced viruses within the assay (Ho et al., 2013). As a result, PCR and QVOA likely represent the upper and lower limits of the true reservoir size (Eriksson et al., 2013; Ho et al., 2013). Interestingly, novel assays that incorporate features of both PCR and QVOA estimate the frequency of $CD4^+$ T cells harboring integrated HIV DNA at ~300 per million cells (Vandergeeten et al., 2014). Despite significant variability, it is widely accepted that the size of the latent HIV-1 reservoir within the body is extremely small, with <10 million cells carrying replication-competent provirus (Chun et al., 1997a).

1.1.4. Mechanisms underlying establishment and maintenance of latent HIV-1 infection

1.1.4.1. Establishment of the latent HIV-1 reservoir

Although the mechanism by which the latent HIV-1 reservoir is established is currently unknown, there are two prevailing hypotheses (Siliciano and Greene, 2011). The first hypothesis suggests that latent HIV-1 infection is established by happenstance following infection of activated $CD4^+$ T cells that survive long enough to revert to a resting memory state. These resting $CD4^+$ T cells have been shown to be non-permissive to HIV-1 replication due to inefficient reverse transcription, impaired nuclear import, and

expression of the restriction factor, SAMHD1 (Baldauf et al., 2012; Pierson et al., 2002). This hypothesis is supported by the existence of latent HIV-1 infection predominantly within the resting memory $CD4^+$ T cell compartment (Chomont et al., 2009). Alternatively, it has been postulated that latent HIV-1 infection may be established through direct infection of resting $CD4^+$ T cells. Despite the lower permissiveness of resting $CD4^+$ T cells, several studies have reported the establishment of productive and latent HIV-1 infection within these cells (Agosto et al., 2007; Swiggard et al., 2005; Vatakis et al., 2009). In addition, resting $CD4^+$ T cells cultured within a lymphoid tissue microenvironment that mimics *in vivo* conditions, rather than in isolated culture, were demonstrated to be significantly more permissive to HIV-1 infection (Kinter et al., 2003). The early establishment of the latent HIV-1 reservoir, in parallel with the presence of latent HIV-1 within naïve and memory $CD4^+$ T cells directly support the second hypothesis. Ultimately, both models likely contribute to the establishment of HIV-1 latency and highlight features of resting $CD4^+$ T cells that facilitate latent HIV-1 infection.

1.1.4.2. Maintenance of the latent HIV-1 reservoir

The maintenance of HIV-1 in a state of latency within host cells is likely a multifactorial process that is regulated by transcriptional, epigenetic, and post-transcriptional blocks to viral replication (Figure 4). Initially, integration of HIV-1 within heterochromatic sites was postulated as a potential impediment to viral transcription. Several studies have since demonstrated that HIV-1 selectively integrates within actively transcribed genes in resting $CD4^+$ T cells from patients on HAART (Han et al., 2004; Schröder et al., 2002). Interestingly however, depending on the orientation of the

provirus relative to the host gene, viral transcription can be significantly inhibited through transcriptional interference mediated by promoter occlusion (Shan et al., 2011) or collision of elongating RNA polymerases (RNA Pol II) (Han et al., 2008) (Figure 4c). In addition, critical host transcription factors necessary for effective HIV-1 transcription, including nuclear factor-κB (NF-κB) and nuclear factor of activated T cells (NFAT), are sequestered within the cytoplasm of resting $CD4^+$ T cells (Duverger et al., 2009; Ganesh et al., 2003; Kinoshita et al., 1997) (Figure 4a). Availability of the positive transcription elongation factor (P-TEFb) complex, which is tightly regulated by Cyclin T1 and phosphorylated cyclin-dependent kinase 9 (CDK9), is also significantly reduced during the transition of activated $CD4^+$ T cells to central memory cells (Budhiraja et al., 2013) (Figure 4d). Absence of P-TEFb results in inefficient transcription of short, abortive viral mRNA, as previously reported in resting $CD4^+$ T cells (Lassen et al., 2004). HIV-1 Tat significantly enhances viral transcription and elongation by liberating the P-TEFb complex and recruiting transcription factors to the HIV-1 promoter (Siliciano and Greene, 2011). Consistent with this, significant defects or insufficient expression of Tat have been demonstrated both in primary cells and cell line models of HIV-1 latency (Emiliani et al., 1998; Tyagi et al., 2010) (Figure 4e).

Epigenetic modifications represent an additional barrier to HIV-1 replication (Figure 4b). Irrespective of the site of integration, the 5'-LTR of HIV-1 contains two nucleosomes, Nuc-0 and Nuc-1, which regulate basal virus transcription (Verdin et al., 1993). Activity of histone deacetylase 1 (HDAC1) at these sites was identified to have a repressive effect on recruitment of RNA Pol II and HIV-1 transcription (Williams et al.,

2006). In parallel, methylation at the 5'-LTR by histone methyltransferases (HMTs) such as EZH2 and Suv39H1 may play an important role in transcriptional silencing of HIV-1 (Chéné et al., 2007; Friedman et al., 2011). Lastly, the nuclear sequestration of *tat* and *rev* MS-RNA in resting $CD4^+$ T cells has led to the investigation of post-transcriptional mechanisms underlying latent HIV-1 infection (Lassen et al., 2006). Inhibition at this level may be mediated by absence of nuclear export mechanisms (Lassen et al., 2006), blocks to viral splicing (Donahue and Wainberg, 2013; Pace et al., 2012), and expression of microRNA (miRNA) targeting HIV-1 mRNA (Huang et al., 2007). All in all, a multitude of factors likely contribute to the ongoing maintenance of the latent HIV-1 reservoir.

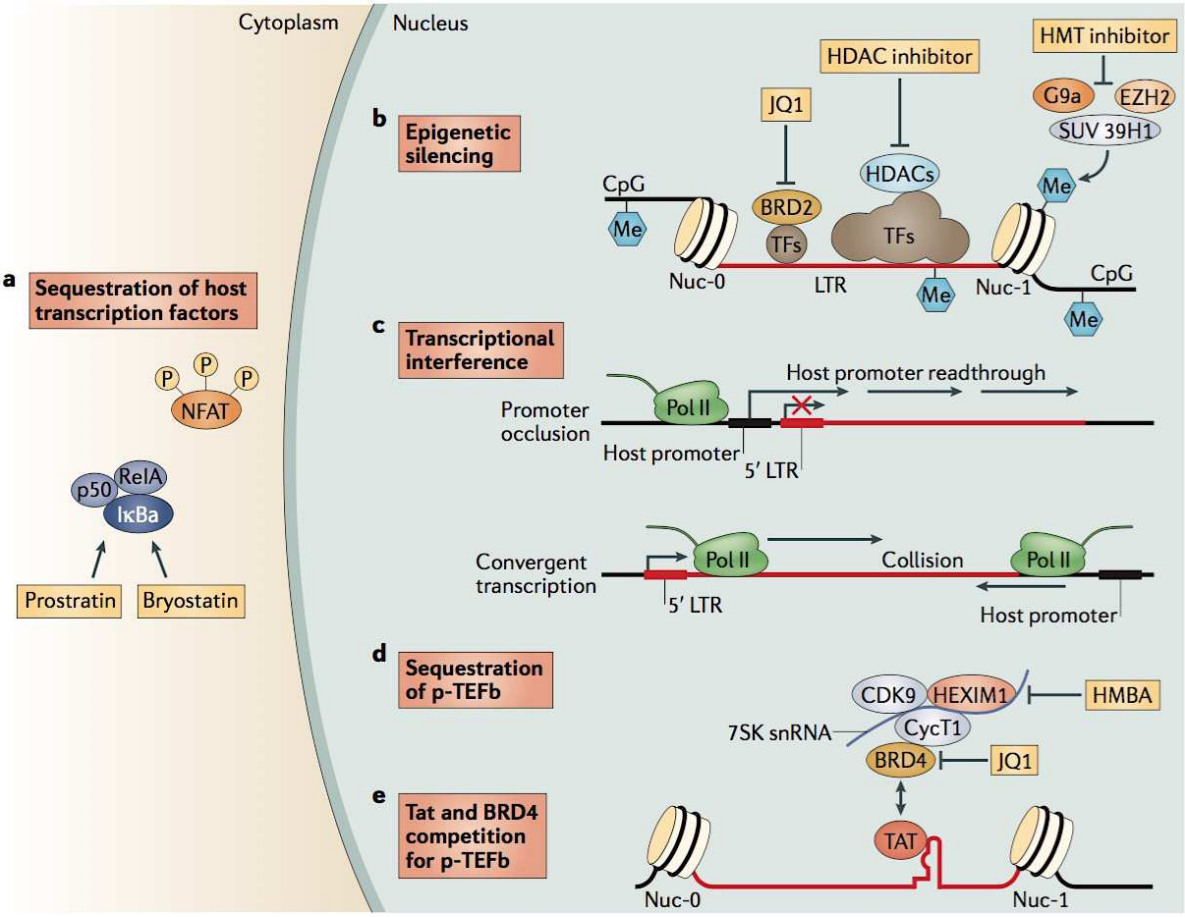

Cytoplasm
Nucleus
a
Sequestration of host transcription factors
P
P
P
NFAT
p50
RelA
IκBa
Prostratin
Bryostatin
b
Epigenetic silencing
HDAC inhibitor
HMT inhibitor
JQ1
G9a
EZH2
SUV 39H1
HDACs
CpG
Me
BRD2
TFs
TFs
Me
Nuc-0
LTR
Me
Nuc-1
CpG
Me
c
Transcriptional interference
Host promoter readthrough
Promoter occlusion
Pol II
Host promoter
5′ LTR
Convergent transcription
Pol II
Collision
Pol II
5′ LTR
Host promoter
d
Sequestration of p-TEFb
CDK9
HEXIM1
HMBA
7SK snRNA
CycT1
BRD4
JQ1
e
Tat and BRD4 competition for p-TEFb
TAT
Nuc-0
Nuc-1

Figure 4: Mechanisms involved in the maintenance of HIV-1 latency and strategies to disrupt latency. HIV-1 latency is maintained by several mechanisms. A. Transcription factors (TFs), including nuclear factor-κB (NF-κB) and nuclear factor of activated T cells (NFAT), are sequestered in the cytoplasm, which leads to transcriptional silencing. Bryostatin and prostratin induce activation of NF-κB, leading to its translocation to the nucleus where it activates HIV-1 transcription. B. The HIV-1 long terminal repeat (LTR) is flanked by the Nuc-0 and Nuc-1 nucleosomes that, when latent, can encode repressive post-translational histone modifications. Histone deacetylases (HDACs), which are recruited by transcription factors (such as YY1 and CBF-1), remove the acetyl groups from chromatin. Histone methyltransferases (HMTs), such as SUV39H1, G9a and EZH2, deposit methyl groups onto histones. HDACs and HMTs enforce the repressive state. Both HDAC inhibitors and HMT inhibitors can induce transcription from quiescent LTR promoters. HIV-1 DNA can also be methylated, although recent evidence suggests that DNA methylation is an epiphenomenon that does not play a part in HIV-1 latency. Bromodomain-containing (BRD) proteins have a complex role in HIV-1 transcription initiation and processivity. Recent evidence suggests that BRD2 has a unique role in enforcing HIV-1 latency, and therefore, BRD inhibitors such as JQ1 may be of use as latency-reversing agents. C. Transcriptional interference may contribute to the regulation of HIV-1 latency. If viral DNA is integrated within an intron of an upstream host gene, readthrough of RNA polymerase II (Pol II) displaces key transcription factors on the HIV-1 LTR (known as promoter occlusion). Conversely, if the viral genome is integrated in the opposite polarity relative to the host gene, host RNA Pol II complexes may induce premature termination of HIV-1 transcription (known as convergent transcription). D, E. The positive transcription elongation factor b (p-TEFb) complex (which comprises CDK9 and cyclin T1 (CycT1)) is sequestered in an inactive ribonucleoprotein complex with HEXIM1–7SK small nuclear RNA (snRNA). BRD4 may compete with the viral Tat activator for binding to p-TEFb. Hexamethylene bisacetamide (HMBA) releases p-TEFb from the HEXIM1–7SK snRNA inhibitory complex and the small-molecule inhibitor JQ1 may antagonize BRD4, both of which enable induction of latent HIV-1 expression. Reprinted by permission from Macmillan Publishers Ltd: Nat. Rev. Microbiol., Archin, N.M., Sung, J.M., Garrido, C., Soriano-Sarabia, N., and Margolis, D.M. (2014). Eradicating HIV-1 infection: seeking to clear a persistent pathogen. *12*, 750–764. (Figure 1)., Copyright 2014.

1.1.5. Current therapeutic approaches to eliminate the latent HIV reservoir

Improved understanding of the mechanisms behind establishment and maintenance of the latent HIV-1 reservoir have led to the investigation of several therapeutic strategies aimed at HIV-1 eradication.

1.1.5.1. Early initiation and treatment intensification

Initial attempts to eliminate the reservoir included the intensification of HAART regimens with the addition of protease, integrase, and/or cell entry inhibitors. However, this approach was shown to have minimal effects on the size of the latent HIV-1 reservoir (Dinoso et al., 2009; Gandhi et al., 2010; J Buzón et al., 2010). In contrast, early initiation of HAART during the primary phase of HIV-1 infection, has been shown to significantly reduce both cell-associated HIV DNA and US-RNA (Lewin and Rouzioux, 2011; Strain et al., 2005). Despite the reduction in the size of the reservoir however, viral rebound was consistently observed in all patients upon treatment interruption (Chun et al., 2010; Hocqueloux et al., 2010).

1.1.5.2. "Shock and kill" approach

Early trials hypothesized that the activation of $CD4^+$ T cells, in the context of ongoing HAART, may result in induction of HIV-1 replication, as well as depletion of the reservoir by viral cytopathic effects. Consistent with this, trials with interleukin-2 (IL-2) and anti-human CD3 antibody (OKT3) demonstrated a modest increase in viral RNA and significant decrease in size of the latent HIV-1 reservoir (Chun et al., 1999; Kulkosky et al., 2002; Prins et al., 1999). However, significant adverse effects and systemic immune activation were observed in these patients, resulting in the discontinuation of these

studies (Prins et al., 1999). Next, the use of IL-7, a cytokine that plays an integral role in T cell homeostasis and proliferation, was investigated both due to its safety profile *in vivo* and demonstrated efficacy in reactivating latent HIV-1 (Wang et al., 2005). However, treatment with IL-7 enhanced the proliferation of memory $CD4^+$ T cells and resulted in an overall increase in the absolute number of cells carrying integrated HIV-1 DNA (Vandergeeten et al., 2013). IL-7 therapy was therefore deemed unsuitable as it promoted the persistence of latent HIV-1 infection.

Next, strategies that selectively enhance HIV-1 replication, without altering T cell activation, were investigated. Given the significance of epigenetic modifications on the maintenance of latency, HDAC inhibitors (HDACi) including Suberoylanilide Hydroxamic Acid (SAHA; also known as Vorinostat) and Romidepsin were studied as potential HIV-1 reactivation strategies. Treatment with SAHA, an approved chemotherapeutic agent for cutaneous T-cell lymphoma, resulted in a significant and sustained increase in cell-associated US-RNA in resting $CD4^+$ T cells from patients on HAART (Archin et al., 2012; Elliott et al., 2014). Similarly, Romidepsin was also shown to induce HIV-1 transcription, with minimal adverse effects (Søgaard et al., 2015). In addition to HDACi, agents that target $CD4^+$ T cell signaling pathways and activation of transcription factors have been studied. Prostratin and Bryostatin have been shown to reactivate HIV-1 *in vitro* through the activation of the protein kinase C (PKC) and NF-κB (Chan et al., 2013; DeChristopher et al., 2012). Similarly, treatment with Disulfiram enhances Akt signaling resulting in a modest induction of viral replication in patient resting $CD4^+$ T cells (Doyon et al., 2013; Spivak et al., 2014).

However, reversal of HIV-1 latency alone may not be enough to purge the reservoir (Shan et al., 2012). In addition to the "shock" provided by latency reversal agents, "kill" strategies such as the generation of HIV-specific cytotoxic $CD8^+$ T lymphocytes (CTLs) may be necessary to eliminate the cells expressing viral signatures (Shan et al., 2013). Interestingly however, unless HAART is started early, a majority of latent proviruses within resting $CD4^+$ T cells carry escape mutations within HIV-1 *gag* epitopes (Deng et al., 2015). As a result, therapeutic strategies involving latency reversal, in combination with virus-specific CTL responses targeting a broad-spectrum of viral epitopes are currently being studied. Despite the promise of the "shock and kill" approach, a multi-pronged approach to eliminate latently infected cells will likely be necessary given the multitude of mechanisms underlying the maintenance of HIV-1 latency (Spina et al., 2013).

1.2. Type I IFN responses to HIV infection

1.2.1. The type I interferon system

Interferons (IFN) were discovered in 1957 by Isaacs and Lindenmnann and were termed as such due to their capacity to interfere with and inhibit viral replication (Isaacs and Lindenmann, 1987). Since then, three distinct families of IFN have been described. The emphasis of the present work will be on the type I interferon (IFN-I) family of cytokines, which include IFNα (13 subtypes) and IFNβ, as well as several poorly defined subsets (IFNε, IFNτ, IFNκ, IFNω, IFNδ and IFNζ) (Pestka et al., 2004). The IFN-I system is an integral component of the innate immune response capable of exerting antiviral, anti-

proliferative, and immunomodulatory functions (Müller et al., 1994; Platanias, 2005; Sadler and Williams, 2008).

The IFN-I response to viruses, such as HIV-1, begins with the sensing of viral pathogen-associated molecular patterns (PAMPs) by the host pattern recognition receptors (PRRs) (Figure 5). PRRs situated on the cell surface, as well as within the cytosolic and endosomal compartments recognize and respond to foreign nucleic acids, self-DNA, and other PAMPs (McNab et al., 2015). In the context of HIV-1 infection, several families of PRRs play an integral role in detecting viral signatures. The retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), which include RIG-I and melanoma differentiation-associated gene 5 (MDA5), are important cytosolic receptors that detect double-stranded RNA (dsRNA) and 5'-triphosphates on uncapped viral RNA (Hornung et al., 2006; Yoneyama et al., 2004). The toll-like receptors (TLR), including TLR3, TLR7 and TLR8, and TLR9, recognize dsRNA, ssRNA, and unmethylated CpG DNA, respectively (Alexopoulou et al., 2001; Heil et al., 2004; Hemmi et al., 2000). In addition, HIV-1 ssDNA and dsDNA intermediates are recognized by several cytosolic DNA-sensors including the cyclic guanosine monophosphate-adenosine monophosphate synthase (cGAS) (Gao et al., 2013) and IFN-inducible factor 16 (IFI16) (Jakobsen et al., 2013).

Following detection of viral factors, these PRRs signal through several distinct adaptor proteins and kinases (Figure 5). Despite the diverse signaling cascades however, the pathways converge on a family of transcription factors known as IFN-regulatory factors (IRFs), which play a critical role in activating the transcription of *IFNA/B* genes. The production of IFNα and IFNβ occurs in two phases (Sato et al., 2000). Most cells

constitutively express IRF-3 and the activation of PRRs results in the first wave of IRF3-mediated transcription and production of IFNβ and IFNα4 (Sato et al., 1998b). These cytokines then function in an autocrine and paracrine manner by binding to the cell-surface IFNα/β receptor-1 and -2 subunits (IFNAR1 and IFNAR2), which together form the primary IFN-I receptors. This establishes a positive feedback loop that results in a second wave of transcription of the *IRF7* gene and subsequently the IFNα subset of cytokines (Sato et al., 2000; 1998a).

During IFN-I signaling, IFNα or IFNβ binds to IFN-I receptors resulting in the activation of Janus kinase 1 (JAK1) and tyrosine kinase 2 (TYK2). These kinases phosphorylate signal transducer and activator of transcription 1 (STAT1) and STAT2, resulting in the dimerization, nuclear translocation, and binding of these factors to IRF9, which together form the IFN stimulated gene factor 3 (ISGF3) complex. ISGF3 then binds to the IFN-stimulated responsive elements (ISREs), resulting in the transcription of over 300 IFN-stimulated genes (ISGs) that exert antiviral and anti-proliferative functions (Der et al., 1998; Ivashkiv and Donlin, 2014). Through this process, the IFN-I system responds to viral infections by creating a potent antiviral microenvironment both within infected and uninfected, bystander cells.

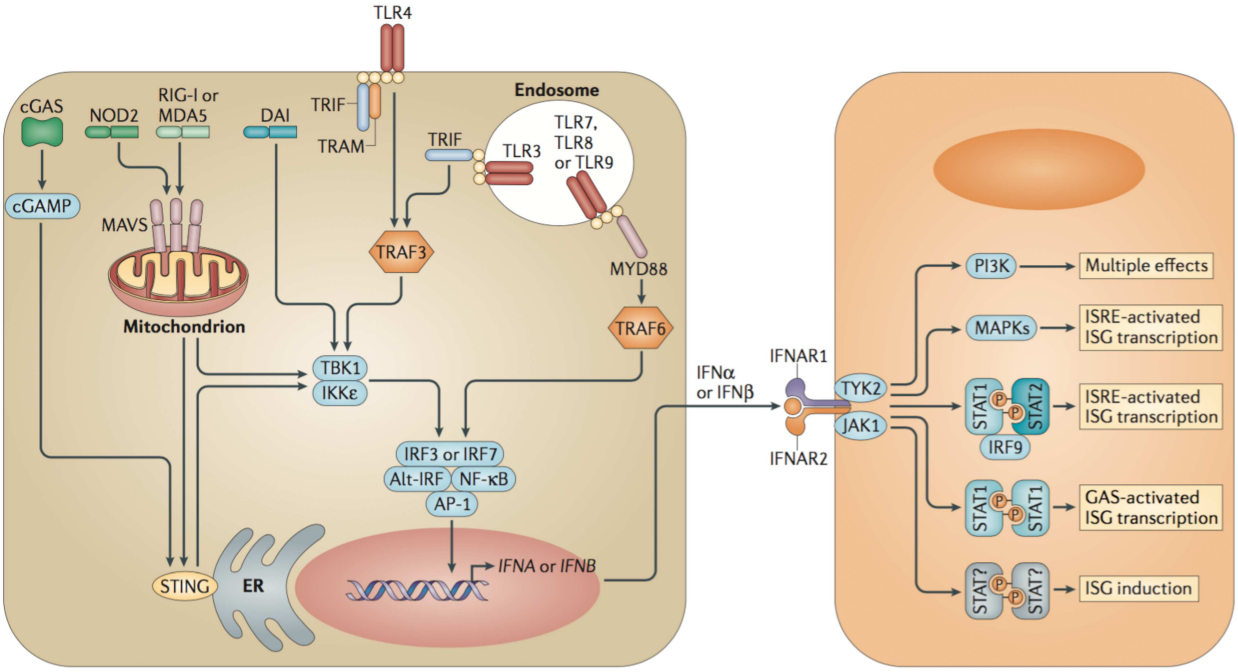

TLR4
cGAS
NOD2
RIG-I or
MDA5
DAI
TRIF
TRAM
TRIF
Endosome
TLR7,
TLR8
or TLR9
TLR3
cGAMP
MAVS
TRAF3
MYD88
Mitochondrion
TRAF6
TBK1
IKKε
IRF3 or IRF7
Alt-IRF
NF-κB
AP-1
STING
ER
IFNA or IFNB
IFNα
or IFNβ
IFNAR1
IFNAR2
TYK2
JAK1
PI3K
Multiple effects
MAPKs
ISRE-activated
ISG transcription
STAT1
STAT2
IRF9
ISRE-activated
ISG transcription
STAT1
STAT1
GAS-activated
ISG transcription
STAT?
STAT?
ISG induction

Figure 5: Pathways of type I interferon induction and receptor signaling. Recognition of microbial products by a range of cell-surface and intracellular pattern recognition receptors, including Toll-like receptors (TLRs) and retinoic acid-inducible gene I (RIG-I), can lead to induction of the genes encoding type I interferons (IFNs). On the binding of type I IFNs to their receptor (IFNAR), multiple downstream signalling pathways can be induced, leading to a diverse range of biological effects. The canonical signal transducer and activator of transcription 1 (STAT1)–STAT2–IFN-regulatory factor 9 (IRF9) signalling complex (also known as the IFN-stimulated gene factor 3 (ISGF3) complex) binds to IFN-stimulated response elements (ISREs) in gene promoters, leading to induction of a large number of IFN-stimulated genes (ISGs). Type I IFNs can also signal through STAT1 homodimers, which are more commonly associated with the IFNγ-mediated signalling pathway. Other STAT heterodimers and homodimers may also be activated downstream, including STAT3, STAT4 and STAT5. Other signalling pathways that do not rely on Janus kinase (JAK) and/or STAT activity may also be activated, including mitogen-activated protein kinases (MAPKs) and the phosphoinositide 3-kinase (PI3K) pathway, thereby leading to diverse effects on the cell. Alt-IRF, IRFs other than IRF3 or IRF7; AP 1, activator protein1; cGAMP, cyclic di-GMP-AMP; cGAS, cytosolic GAMP synthase; DAI, DNA-dependent activator of IRFs; ER, endoplasmic reticulum; GAS, γ-activated sequence; IKKε, IκB kinase-ε; MAVS, mitochondrial antiviral signalling protein; MDA5, melanoma differentiation-associated gene 5; MYD88, myeloid differentiation primary response protein 88; NF-κB, nuclear factor-κB; NOD2, NOD-containing protein 2; STING, stimulator of IFN genes; TBK1, TANK-binding kinase 1; TRAF, TNF receptor-associated factor; TRAM, TLR adaptor molecule (also known as TICAM2); TRIF, TIR domain-containing adaptor protein inducing IFNβ; TYK2, tyrosine kinase 2. Reprinted by permission from Macmillan Publishers Ltd: Nature Publishing Group., McNab, F., Mayer-Barber, K., Sher, A., Wack, A., and O'Garra, A. (2015). Type I interferons in infectious disease. 15, 87–103. (Figure 1)., Copyright 2015.

1.2.2. The interplay between HIV-1 and the IFN-I system: viral countermeasures that facilitate immune evasion

The IFN-I system represents a first-line host defense against HIV-1 infection (McNab et al., 2015). However, the interplay between HIV-1 and IFN-I responses *in vivo* is complex. Primary HIV-1 infection is characterized by a significant, but transient, increase in plasma IFNα levels (Stacey et al., 2009), as well as upregulation of various ISGs and host retroviral restriction factors (Li et al., 2009). This can have a significant influence on systemic infection, viral replication, and disease pathogenesis (Rotger et al., 2010). However, the increase in ISG expression observed early in infection fails to effectively control viral replication and the extent of ISG increase correlates poorly with viral loads and fails to predict $CD4^+$ T cell decline (Bosinger and Utay, 2015; Rotger et al., 2010). This may be because HIV-1, like many other viruses, has evolved numerous countermeasures that allow the virus to effectively evade the IFN-I response and establish chronic infection *in vivo* (Altfeld and Gale, 2015; Gale and Sen, 2009; Rustagi and Gale, 2014). The mechanisms by which HIV-1 counteracts the IFN-I system are described below.

1.2.2.1. Reduced production and responsiveness to IFN-I

Although all cells are equipped to produce and respond to IFN-I cytokines, the plasmacytoid DCs (pDC), and to a lesser extent macrophages and myeloid DCs (mDC), are the primary source of IFNα/β cytokines *in vivo* (Liu, 2005; Siegal et al., 1999). However, during primary HIV-1 infection, a significant reduction has been observed in the number of circulating and lymphoid pDCs and mDCs (Pacanowski et al., 2001; Soumelis et al., 2001) and the amount of IFNα produced per cell (Kamga et al., 2005). This may be

mediated by several potential mechanisms including an increase in apoptosis of pDCs due to enhanced activation and maturation, as well as an impaired responsiveness of pDCs and mDCs to viral factors (Fitzgerald-Bocarsly and Jacobs, 2010; Martinson et al., 2007). Following initiation of HAART, a normalization in the production of IFNα has been reported (Siegal et al., 2001). However, absolute pDC numbers fail to fully recover despite significant immune reconstitution following therapy (Chehimi et al., 2002). Systemic impairments in the production of IFN-I therefore represent an underlying feature of HIV-1 infection.

1.2.2.2. Defects in viral sensing

On a cellular level, defects in the detection of and response to viral PAMPs are observed during HIV-1 infection. Protein kinase R (PKR) functions both as a PRR capable of detecting viral dsRNA, as well as an antiviral ISG that inhibits protein synthesis via phosphorylation of the α subunit of the eukaryotic initiation factor 2 (eIF2α) (Hershey, 1991). HIV-1 Tat has been shown to counteract this process by enhancing the degradation of PKR, preventing PKR autophosphorylation and activation, and by impairing eIF2α phosphorylation through direct competition for the binding site on PKR (Brand et al., 1997; Cai et al., 2000; Clerzius et al., 2010). Similarly, RIG-I, a critical sensor of HIV-1 dsRNA, is targeted for lysosomal degradation in HIV-1 infected cells by the viral protease (Solis et al., 2011). pDCs recognize viral PAMPs via TLR7 and TLR9, resulting in a rapid and robust induction of IFNα and other proinflammatory cytokines. In pDCs from HIV-infected individuals, however, significantly lower levels of IFNα production have been reported following TLR7 and TLR9 stimulation (Kaushik et al., 2013). Although the exact mechanism

behind TLR7 impairment is presently unclear, HIV-1 gp120 has been shown to suppress IFN-I production downstream of the TLR9 pathway through its interaction with CD303, a negative regulator of IFN (Martinelli et al., 2007). Lastly, cGAS and IFI16 have been shown to be important cytoplasmic DNA sensors that trigger an IFN-I response following activation (Gao et al., 2013; Jakobsen et al., 2015). However, HIV-1 may evade the effects of these PRRs through recruitment of the host 3'-repair exonuclease 1 (TREX1) protein, which digests excess HIV-1 DNA that would otherwise be sensed by cGAS or IFI16 (Hasan and Yan, 2014; Yan et al., 2010).

1.2.2.3. Impairments in IFN signaling

HIV-1 also targets non-redundant adaptor proteins, kinases, and transcription factors critical to IFN-I signaling (Figure 5). Following detection of viral PAMPs, RIG-I signals through the mitochondrial antiviral-signaling (MAVS) protein, resulting in the phosphorylation and nuclear translocation of IRF3. HIV-1 Vpu and Nef proteins are capable of degrading MAVS and thereby prevent the transcription of antiviral ISGs (Sanchez et al., 2015). In addition, multiple PRR signaling pathways converge on the TANK-binding kinase 1 (TBK1), which phosphorylates IRF3 and triggers IFN-I gene expression. The inhibition of TBK1 autophosphorylation and a subsequent block to IFN-I production has been identified in the context of HIV-1 infection to be mediated by the Vpr and Vif proteins (Harman et al., 2015). Furthermore, impaired phosphorylation and/or expression of IRF3 and IRF7 has been observed, both in the context of *in vitro* HIV-1 infection, and within $CD4^+$ T cells or pDCs from HIV-1 infected patients (Doehle et al., 2009). HIV-1 Vpu may play and important role in promoting lysosomal or caspase-

mediated degradation of IRF3 (Doehle et al., 2012; Park et al., 2014). However, the mechanism by which IRF7 is impaired during HIV-1 infection remains to be elucidated.

1.2.2.4. Downregulation of antiviral ISGs and HIV-1 restriction factors

Downstream of the IFN-I signaling cascade are hundreds of antiviral ISGs that play crucial roles in curtailing HIV-1 infection. The major histocompatibility complex-I (MHC-I), which is responsible for the presentation of viral antigens to $CD8^+$ T cells, has been shown to be internalized and sequestered away from the cell surface through the actions of HIV-1 Nef (Blagoveshchenskaya et al., 2002; Cohen et al., 1999). Similarly, ISG15, which is one of the most prominent ISGs induced following IFNα stimulation, can significantly impair the budding and release of HIV-1 through a process known as ISGylation (Pincetic et al., 2010). However, HIV-1 may block the effects of ISGylation through the recruitment of the host ISG15 deconjugating enzyme known as ubiquitin specific peptidase 18 (USP18) (Okumura et al., 2006). In addition, an array of IFN-inducible antiretroviral restriction factors such as SAMHD1 (Laguette et al., 2011), apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) cytidine deaminases (Mangeat et al., 2003), and Tetherin (Neil et al., 2008), which target different stages of the viral lifecycle are induced following HIV-1 infection. These restriction factors represent antiretroviral proteins that are effectively counteracted by various HIV-1 or HIV-2 accessory proteins (Simon et al., 2015).

In summary, significant impairments at multiple levels of the IFN-I signaling pathway have been identified in the setting of HIV-1 infection. These defects are mediated both through the direct effect of HIV-1 proteins, as well as through the indirect

interplay between virus and host cellular factors. The ability of HIV-1 to evade the innate immune response and establish a chronic infection *in vivo* therefore represents a hallmark of HIV-1 infection.

1.2.3. State of IFN-I system during latent HIV-1 infection

Although the state of the IFN-I system is well described during productive HIV-1 infection, very little is known at present regarding IFN-I signaling and responsiveness in the context of HIV-1 latency. Therefore, characterization of IFN-I responses within latently HIV-1 infected cells warrants further investigation and represents an important objective of the present work.

1.3. Bridging the gap between cancer and HIV infection: oncolytic virotherapy as a strategy to target and eliminate the latent HIV reservoir

1.3.1. Defects in IFN-I signaling represent a fundamental feature of several cancers

IFN-I has long been known to exert significant anti-neoplastic pressures against various cancers and has therefore been utilized in the clinical setting to treat a range of malignancies including melanoma, renal cell carcinoma, and hairy cell leukemia (Belardelli, 1995; Gresser et al., 1983). The anti-tumor effects exerted by IFN-I function both at the level of tumor cells via regulation of growth, apoptosis, and differentiation, as well as on a systemic level through the modulation of innate and adaptive immune responses (Dunn et al., 2005a; Parker et al., 2016; Smyth, 2005). Driven by the selective pressure exerted by the IFN-I system, tumor cells undergo a process of immunoediting

that results in the development of resistance to IFN-I (Dunn et al., 2005a; 2006; Kloke and Niederle, 1990). Mirroring the impairments observed during HIV-1 infection, defects at multiple levels of IFN-I signaling have been identified to be a common mechanism of escape from anti-tumor immunity within various human cancers (Critchley-Thorne et al., 2009). Deletion or loss of function of IFNα/β genes and downregulation of the IFNAR has been identified in several IFN-resistant tumors (Colamonici et al., 1992; Heyman et al., 1994; Pfeffer and Donner, 1990). In addition, the expression and activation of critical signaling proteins including JAK1, STAT1, and STAT2, have been shown to be impaired in human melanoma, prostate cancer, and cutaneous T-cell lymphoma (Dunn et al., 2005b; Sun et al., 1998; Wong et al., 1997b).

Several PRRs including RIG-I, MDA5, and PKR can effectively repress tumor cells through the regulation of protein translation, cell proliferation, and apoptosis. However, cancers driven by mutations within the *ras* oncogene undergo uncontrolled proliferation and protein synthesis (Bos, 1989). Within these tumors, activated Ras significantly blocks PKR-mediated inhibition of protein translation (Battcock et al., 2006; Noser et al., 2007). The Ras/Raf pathway has also been shown to counteract the ability of MDA5 to induce differentiation and apoptosis of tumor cells (Lin et al., 2006). RIG-I, which is induced by the p53 tumor suppressor protein, enhances apoptosis within tumor cells (Hsu et al., 2012). However, in human breast and pancreatic cancer both the level of expression and responsiveness of RIG-I are significantly lower (Ilkow et al., 2015; Shyu et al., 2005).

The transcription factors downstream of these PRRs have also been demonstrated to be impaired. The presence of an alternative spliced isoform of IRF3 in hepatocellular

carcinoma (HCC) leads to impaired IFNβ production (Marozin et al., 2008). As well, significant downregulation of IRF7 has been identified as the primary mechanism by which breast cancer cells escape IFN-I signaling and become capable of metastasizing to the bone (Bidwell et al., 2012). Several downstream IFN-induced effector proteins such as MHC-I and the antigen processing machinery have been shown to be substantially impaired *in vivo* within various human tumors (Marincola et al., 2000; Seliger et al., 2000). In addition, the E1-like ubiquitin-activating enzyme (UBE1L), which together with ISG15 enhances tumor suppression, is deleted in almost all cases of small cell lung cancer (Feng et al., 2008). In summary, the defects in IFN-I signaling and responsiveness discussed here represent just a small snapshot of the global impairments in the IFN-I system within various cancers.

1.3.2. Exploiting IFN-I defects in cancers using oncolytic virotherapy

Oncolytic virotherapy or the clinical application of viruses as a strategy to kill tumor cells is not a new concept (Martuza et al., 1991; Wheelock and Dingle, 1964). It was derived from early observations wherein malignancies such as Burkitt's lymphoma, Hodgkin's disease, and melanoma went into remission following natural viral infections or the administration of live-attenuated viral vaccines (Bluming and Ziegler, 1971; Kelly and Russell, 2007; Zygiert, 1971). Since then, numerous oncolytic viruses (OV), defined as viruses that selectively infect or replicate in tumor cells but spare normal cells, have been identified and characterized (Antonio Chiocca, 2002). The selectivity of these viruses for tumor cells occurs during infection, mediated by viral tropism and cellular receptor expression, and/or at the level of viral replication. Replication selective OV exploit specific

impairments in homeostatic pathways present within tumor cells, but not healthy cells, in order to efficiently replicate and induce oncolysis (Antonio Chiocca, 2002).

Resistance to IFN-I represents an important immune evasion strategy that facilitates growth and survival of various tumor cells. Although this promotes tumor establishment, it comes at the cost of a compromised antiviral defense system, which increases the susceptibility of tumor cells to viral infections. Two naturally occurring *Rhabdoviruses*, the vesicular stomatitis virus (VSV) and maraba virus, have been identified as potent oncolytic agents that selectively replicate in tumor cells with defects in IFN-I signaling and responsiveness, but are unable to infect normal cells capable of mounting an IFN-I response (Brun et al., 2010; Stojdl et al., 2000b). Thus far, wild-type (WT) and genetically engineered forms of VSV and maraba virus have been shown to be both safe and effective in reducing the tumor burden within various models of malignant glioma, melanoma, ovarian cancer, HCC, breast adenocarcinoma, and leukemia (Balachandran et al., 2000; Brun et al., 2010; Fernandez et al., 2002; Obuchi et al., 2003; Stojdl et al., 2000b; 2003).

1.3.3. Recombinant vesicular stomatitis virus and maraba virus selectively target and eliminate tumors with IFN-I defects

1.3.3.1. VSV and maraba virus lifecycle

VSV and maraba virus are closely related *Rhabdoviruses* that share significant similarities in structure, lifecycle, virulence, and pathogenesis (Brun et al., 2010; Stojdl et al., 2000b). Both viruses are enveloped, non-segmented, single-stranded, negative-sense

RNA viruses. The ~11kb ssRNA genome encodes 5 viral proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and the large protein of the RNA-dependent RNA polymerase (L) (Figure 6) (Barber, 2004; Lichty et al., 2004a). VSV has a wide tropism and can infect most mammalian cells. Recently, the low density lipoprotein receptor (LDL-R) was identified to be the putative cell surface receptor for VSV and maraba virus (Finkelshtein et al., 2013; Tong et al., 2015). Following attachment of the viral G protein to the LDL-R, VSV undergoes clathrin-mediated endocytosis (Harrison, 2008; Sun et al., 2005). Acidification of the endosomal compartment then causes fusion and release of the viral ribonucleoprotein (RNP) into the cytoplasm. The RNA-dependent-RNA polymerase complex, composed of N, P, and L proteins, then transcribes the viral genome in a 3' to 5' direction producing the positive-sense RNA. This strand is then used to sequentially produce N, P, M, G, and L mRNA and proteins, as well as additional negative-strand genomic RNA. Finally, the viral assembly occurs at the cell surface and new virions are released (Figure 6) (Barber, 2004; Lichty et al., 2004a). Although the maraba virus has not been extensively characterized, it is thought to undergo a similar lifecycle as VSV.

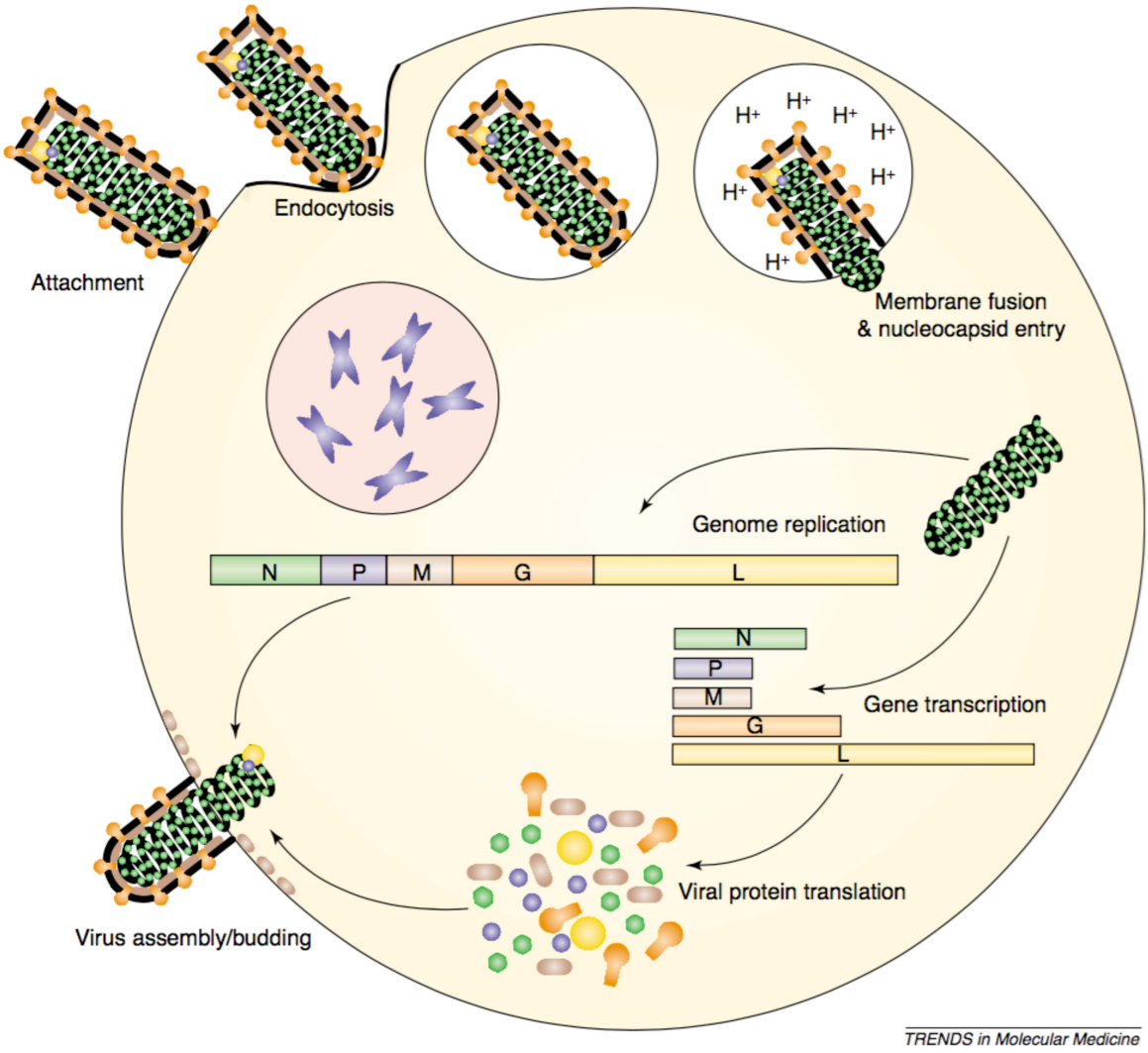

Attachment
Endocytosis
H+
Membrane fusion
& nucleocapsid entry
Genome replication
N
P
M
G
L
Gene transcription
Viral protein translation
Virus assembly/budding
TRENDS in Molecular Medicine

Figure 6: The vesicular stomatitis virus (VSV) replication cycle. Attachment of viral particles to the host cell is mediated by the glycoprotein of VSV. Attached particles are engulfed by endocytosis and enter the cellular endosomal trafficking pathway. As late endosomes are acidified, the drop in endosomal pH triggers a conformational change in the glycoprotein that mediates fusion between the viral envelope and the endosomal membrane. The viral nucleocapsid is then able to escape into the cytoplasm and initiate viral replication. The viral polymerase first transcribes the individual mRNAs for each viral gene, which are then translated by host ribosomes to yield functional viral proteins. At later stages of infection, the viral polymerase switches from transcription to replication and synthesizes copies of the negative-sense VSV genome through positive-strand intermediates. Finally, viral proteins and genomic RNA are assembled into complete virus particles and the virus exits the cell by budding through the plasma membrane. Reprinted from Trends in Molecular Medicine, 10, Lichty, B.D., Power, A.T., Stojdl, D.F., and Bell, J.C., Vesicular stomatitis virus: re-inventing the bullet, 210-216, Copyright 2004, with permission from Elsevier.

1.3.3.2. Recombinant VSVΔ51 and MG1: interplay between host IFN-I response and viral replication

During the course of VSV and maraba infection, the M protein plays an integral role in facilitating viral escape from IFN-I responses by inhibiting host transcription and nuclear export of cellular mRNA, including those encoding IFN-I and ISGs (Black and Lyles, 1992; Petersen et al., 2001). This blockade results in the induction of apoptosis within OV-infected cells (Kopecky et al., 2001). To improve the therapeutic index of VSV and maraba virus, genetically engineered strains that are impaired in their ability to disable host IFN-I response were developed. Two attenuated VSV and maraba strains, hereafter referred to as VSVΔ51 and MG1, not only induce the production of IFN following infection, but are also exceptionally sensitive to its effects (Brun et al., 2010; Stojdl et al., 2003).

VSVΔ51 contains a deletion of methionine 51 in the M protein. As a result, the virus is unable to block the transcription and export of mRNA encoding antiviral ISGs in normal cells (Furió et al., 2012; Stojdl et al., 2003). Similarly, MG1 was developed through the introduction of two substitution mutations within the M (leucine at position 123 to tryptophan) and G (glutamine at position 242 to arginine) proteins (Brun et al., 2010). In addition to being unable to block IFN-I production, MG1 has been reported to replicate more rapidly and reach higher titers in the early phases of infection than the WT virus (Brun et al., 2010). Furthermore, the double mutation in maraba virus confers greater cytopathic capacity to MG1, when compared to the Δ51 mutation in VSV (Furió et al., 2012). Lastly, both recombinant viruses have been engineered to encode enhanced green

fluorescent protein (eGFP), thereby facilitating rapid quantification and visualization of OV infection.

1.3.3.3. The application of VSVΔ51 and MG1 as oncolytic virotherapy

The integrity of the IFN-I system has been shown to be the primary factor that dictates the capacity of VSVΔ51 and MG1 to replicate in and lyse tumor cells. Consistent with this, both viruses are hypervirulent in several IFN-resistant models of melanoma, leukemia, breast, prostate, renal, and colon cancer. Specifically, VSVΔ51 and MG1 have been shown to induce durable cures and significantly enhance survival when administered in xenograft and syngeneic mice models of human melanoma, ovarian cancer, and metastatic colon cancer (Brun et al., 2010; Pol et al., 2014a; Stojdl et al., 2000b; 2003).

The mechanism by which these OV eliminate tumor cells is likely a multifactorial process. Infection of tumor cells with VSV and maraba virus can directly cause cell death both through the lytic effects of viral replication and the induction of apoptosis (Oliere et al., 2008). In parallel, the pro-inflammatory tumor microenvironment, created as a result of ongoing OV infection, can significantly compromise the tumor vasculature, and lead to the death of OV-infected and uninfected tumor cells (Breitbach et al., 2011; 2007). Lastly, the recruitment of both innate and adaptive immune cells to the site of OV infection, in combination with recognition of viral PAMPs and tumor cell antigens, has been reported to substantially enhance anti-tumor immunity (Marchini et al., 2016). These pathways play a complementary role in effectively reducing the tumor burden.

1.3.3.4. Advantages of VSVΔ51 and MG1 as therapeutic viruses

Several characteristics of VSVΔ51 and MG1 may help to facilitate the translation of these oncolytic agents into the clinical setting as cancer therapies. First, human infection is infrequent, as both viruses primarily target rodents, cattle, pigs, and horses. Pre-existing antibodies to these viruses are therefore likely to be rare in most populations (Roberts et al., 1999). This is critical given that humoral immunity represents an important barrier to OV infection (Power et al., 2007). In addition, infection of humans with VSV is usually clinically asymptomatic or characterized by mild flu-like symptoms, highlighting the safety profile of these viruses (Hanson et al., 1950).

Second, VSVΔ51 and MG1 have short replication cycles, which accelerates the dissemination of the OV within the tumor microenvironment. Consequently, the oncolytic potential of these viruses and the immune response to OV infection can be evaluated rapidly using preclinical cancer models. On a biological level, the lifecycle of both viruses is restricted to the cytoplasm, thereby avoiding any risk of genotoxicity. Lastly, the viral genomes can accommodate ~4.5kb of foreign RNA, and can thus be re-engineered to enhance OV specificity and antitumor immunity (Lichty et al., 2014).

Both VSVΔ51 and MG1 are not only effective at eliminating IFN-unresponsive tumors, but have also been shown to have an exceptional safety profile in several preclinical cancer models (Brun et al., 2010; Stojdl et al., 2003). While VSVΔ51 is approaching clinical trials, a phase I/II clinical trial investigating the safety and efficacy of MG1 in advanced melanoma is currently underway (ClinicalTrials.gov Identifier: NCT02285816). Therefore,

VSVΔ51 and MG1 represent ideal therapeutic candidates with significant clinical potential.

1.4. Rationale

Global impairments in IFN-I signaling represent the fundamental bridge that links HIV-1 infected cells and tumor cells. Although defects at multiple levels of IFN-I signaling have been identified during productive HIV-1 infection, the state of the IFN-I system in latently HIV-1 infected cells remain to be elucidated. Characterization of the IFN-I response within these cells therefore represents an important objective of the current study. The oncolytic viruses, VSVΔ51 and MG1, have been shown to be effective therapeutic candidates capable of eliminating tumors *in vivo* by exploiting global impairments within the IFN-I system. Therefore, as a proof-of-concept, the capacity of VSVΔ51 and MG1 to selectively target and eliminate latently HIV-1 infected cells by exploiting potential defects in IFN-I pathways will be investigated.

1.5. Hypothesis

The oncolytic viruses, VSVΔ51 and MG1, can selectively target and eliminate latently HIV-1 infected cells, including cells from HIV-infected individuals on effective HAART.

1.6. Project Aims

This work is intended to further our understanding of HIV-1 latency, evaluate a novel therapeutic strategy to target the latent HIV-1 reservoir, and to create a logical and systematic approach to investigate the therapeutic efficacy of novel HIV-1 eradication strategies.

Chapter 3: To characterize IFN-I responses in the context of HIV-1 latency utilizing cell line models of latent HIV-1 infection.

Chapter 4: To evaluate the susceptibility of latently HIV-1 infected and parental HIV-uninfected cell lines to VSVΔ51 and MG1 infection and killing.

Chapter 5: To investigate the capacity of VSVΔ51 and MG1 to selectively target and eliminate latently HIV-1 infected cells using an *in vitro* primary cell model of HIV-1 latency and *ex vivo* memory $CD4^+$ T cells isolated from HIV-1 infected individuals on HAART.

Chapter 2: Materials and methods

2.1. Reagents

2.1.1. Cell culture

Gibco® Roswell Park Memorial Institute 1640 medium (RPMI-1640) with and without phenol red indicator, Gibco® Dulbecco's Modified Eagle's medium (DMEM), and Gibco® Iscove's Modified Dulbecco's Medium (IMDM) were purchased from Life Technologies (Carlsbad, CA). Reagents for supplementation of media included heat-inactivated fetal bovine serum (FBS), penicillin and streptomycin, and L-glutamine (all from Life Technologies), as well as zeocin and blasticidin (InvivoGen, San Diego, USA).

2.1.2. Cell stimulation

Universal type I interferon-α (IFNα) was purchased from PBL Assay Science (Piscataway, NJ) and low molecular weight polyinosinic-polycytidylic acid (poly(I:C)) was purchased from InvivoGen. Suberoylanilide hydroxamic acid (SAHA or Vorinostat) was obtained from Sigma Aldrich (Oakville, ON), recombinant human Chemokine (C-C motif) ligand 19 (CCL19) was obtained from R&D Systems (Minneapolis, MN) and raltegravir (RAL) was obtained from Santa Cruz Biotechnology (Dallas, TX). Cell activation reagents used included phytohaemagglutinin (PHA) (Sigma-Aldrich), recombinant human Interleukin-2 (IL-2) (Cell Sciences, Canton, MA), LEAF^{TM} purified anti-human CD3 (OKT3 clone) and anti-human CD28 (CD28.2 clone) (both from BioLegend, San Diego, CA).

2.1.3. Transfection

ProFection® Mammalian Calcium Phosphate Transfection System was purchased from Promega (Madison, WI) and Lipofectamine® 2000 Transfection Reagent was purchased from Invitrogen (Burlington, ON).

2.2. Ethics statement and study participants

This study was approved by The Ottawa Health Science Network Research Ethics Board. HIV infected individuals followed at the Immunodeficiency Clinic of the Ottawa Hospital were selected for the study based on sustained plasma viral load suppression (viral load <40 copies/mL for >6 months) on HAART and CD4 counts >400 cells/uL. Information regarding demographics of HIV infected study participants, duration of viral load suppression, CD4 counts, and therapy are provided in Table 1. Both HIV infected and uninfected volunteers provided written informed consent to partake in the study.

Table 1: Baseline characteristics of study participants

Characteristics	Mean (±SEM)
Age (Years)	45.1 (3.9)
Sex	
Male	8
Female (Male, Female)	0
Time from documented seroconversion (years)	8.3 (2.7)
Time from initiation of HAART (years)	7 (2.3)
Duration of suppressed peripheral blood VL (years)	5.6 (1.4)
CD4 count (cells/μL)	561.1 (43.3)
Antiretroviral regimen (% patients)	
NNRTI-based	25
ISI-based	75

HAART = highly active antiretroviral therapy; VL = viral load; NNRTI = non-nucleoside reverse transcriptase inhibitor; ISI = integrase strand inhibitor; SEM = standard error of the mean

2.3. Cell culture

2.3.1. Cell lines

U937 (CRL-1593.2™), HL60 (CCL-240™), Vero (CCL-81™) and 293T cells (CRL-3216™) were obtained through American Type Culture Collection (ATCC, Manassas, VA). The following cell lines were obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: U1 (Folks et al., 1987) from Dr. Thomas Folks and OM-10.1 cells (Butera et al., 1994; 1991b) from Dr. Salvatore Butera. HEK-blue™ IFNα/β cells were purchased from InvivoGen.

Latently HIV-1 infected U1 and OM10.1 cells, as well as the respective HIV-uninfected U937 and HL60 parental cells were cultured in RPMI-1640 medium supplemented with 10% FBS and PSG (penicillin [100units(U)/mL], streptomycin [100μg/mL], and L-glutamine [2mM]) (RP10 medium). Cell lines were seeded and maintained at $0.2x10^5$ to $1x10^6$ cells/mL and passaged every 2-3 days. Vero and 293T cells were cultured in DMEM with 10% FBS and PSG (complete DMEM medium). HEK-blue™ IFNα/β cells were maintained in complete DMEM medium supplemented with 100 mg/mL zeocin and 10 mg/mL blasticidin. Cells were maintained in a monolayer and trypsinized and reseeded at a density of $0.1\text{-}0.2x10^6$ cells/mL every 2-3 days. Cell lines were cultured in a humidified atmosphere at 37°C and 5% CO_2.

2.3.2. Isolation of peripheral blood mononuclear cells

Peripheral blood from all participants was collected in sterile syringes containing 100U/mL filter-sterilized Heparin Sodium (LEO Pharma Inc., Thornhill, ON). Peripheral

blood mononuclear cells (PBMC) were isolated by density gradient separation as per manufacturer's protocol. Briefly, 30mL of blood was layered at a ratio of 2:1 on Lymphoprep™ density gradient medium (Stemcell Technologies, Vancouver, BC) and centrifuged at 470 *g* for 30 minutes (Megafuge 1.0, Heraeus Instruments, Germany). The buffy coat layer was collected and washed twice with Gibco® 1x Hank's Balanced Salt Solution (HBSS) (Life Technologies). The isolated PBMC were counted and resuspended at $5x10^7$ cells/mL in lymphocyte sort buffer (phosphate buffered saline (PBS), 0.5% FBS, and 2mM ethylenediaminetetraacetic acid (EDTA, pH 8; Sigma Aldrich) in preparation for resting or memory $CD4^+$ T cell sort.

2.3.3. Enrichment of resting and memory $CD4^+$ T cells

Isolation of $CD4^+$ T cells from PBMC of HIV uninfected individuals was performed by negative selection using the EasySep™ Human $CD4^+$ T cell Enrichment Kit (StemCell Technologies) following the manufacturer's protocol. The negative fraction containing $CD4^+$ T cells was counted and resuspended at $1x10^8$ cells/mL in lymphocyte sort buffer. Resting $CD4^+$ T were defined as cells with low expression of CD69 and HLA-DR, the respective early and late markers of T cell activation (Rea et al., 1999). Resting $CD4^+$ T cells were enriched from $CD4^+$ T cells using mouse IgG_1 monoclonal anti-CD69 (clone FN50; BD Pharmingen, San Jose, CA) and anti-HLA-DR (Clone L203; R&D Systems) antibodies with the EasySep™ Human "Do-It-Yourself" positive selection kit (Stemcell), according to the manufacturer's protocol. Memory $CD4^+$ T cells were isolated from PBMC of virally suppressed HIV infected subjects by negative selection using the EasySep™ Human

Memory $CD4^+$ T cell enrichment kit (Stemcell). The purity of resting and memory $CD4^+$ T cells was assessed by flow cytometry and was routinely >95%. Following isolation, resting and memory $CD4^+$ T cells were counted, resuspended in RP10 medium at $2x10^6$ cells/mL, and rested overnight in a humidified atmosphere at 37°C and 5% CO_2.

2.4. Production of HIV-1 stocks

2.4.1. Production of HIV-$1_{NL4.3}$ on 293T cells

The pNL4-3 plasmid (Adachi et al., 1986) encoding the CXCR4-tropic (X4-tropic) virus was obtained from Dr. Malcolm Martin through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH. Viral stocks were produced using the ProFection® Mammalian Transfection System (Promega). $2x10^6$ 293T cells were plated in T75 flasks in complete DMEM medium and cultured for 24 hours. The cells were then transfected with 20μg of pNL4-3 by calcium phosphate transfection as per manufacturer's protocol. After 48 hours of transfection, HIV-1_{NL4-3} viruses were harvested, clarified by centrifugation (1200 *g* for 10 minutes), filtered sequentially through 0.45μm and 0.2μm polyvinylidene fluoride filters (UltiDent Scientific, St. Laurent, QC), aliquoted, and stored at -80°C. Viral stocks were quantified for p24 protein expression by ELISA.

2.4.2. Propagation of viral stocks on CD8-depleted PBMC

HIV_{NL4-3} stocks were amplified on CD8-depleted PBMC. Briefly, CD8-depleted PBMC were isolated from HIV uninfected donors using the EasySep™ Human CD8 Positive Selection Kit (Stemcell). The negative fraction containing CD8-depleted PBMC were

resuspended at $2x10^6$ cells/mL in RP10 medium supplemented with PHA (5μg/mL) and IL-2 (30U/mL) and cultured for 3 days. On day 3 of stimulation, $20x10^6$ CD8-depleted PBMC blasts were centrifuged and resuspended at $20x10^6$/mL in RP10 medium (mock infection) or HIV_{NL4-3} supernatant and infected at 37°C for 2 hours. HIV-1 virus was left in culture medium, media volume was increased to maintain cells at a concentration $2x10^6$ cells/mL, and cells were left in culture for 14 days. On days 4, 7, and 11, $20x10^6$ fresh, activated CD8-depleted PBMC were added as "feeder" cells at a concentration of $2x10^6$ cells/mL in RP10 medium with IL-2 (30U/mL). After 14 days of virus amplification, mock and $HIV\text{-}1_{NL4-3}$ viruses were harvested as described above.

2.5. Production of VSVΔ51 and MG1 stocks

2.5.1. Propagation of VSVΔ51 and MG1 stocks

The eGFP-expressing recombinant VSVΔ51 and MG1 were obtained from Dr. John Bell and Dr. David Stojdl. Both viruses were propagated using Vero cells as described previously (Brun et al., 2010; Stojdl et al., 2003). Following 24 hours of amplification on Vero cells, mock, VSVΔ51, and MG1-infected cell culture supernatants were harvested, clarified by centrifugation (300 *g* for 10 minutes) and filtered with a 0.2μm pore Nalgene filter (Nalge Nunc, Rochester, NY). Viral supernatant was concentrated by centrifugation at 30000 *g* (Optima L-100 XP, Beckman Coulter, Brea, CA) for 90 minutes at 4°C, resuspended in PBS, aliquoted, and stored at -80°C.

2.5.2. VSVΔ51 and MG1 titration by standard plaque assay

Viral titre of VSVΔ51 and MG1 stocks used in experiments was determined by standard plaque assay on Vero cells with 1% Agarose in DMEM containing 10% FBS (Baer and Kehn-Hall, 2014; Stojdl et al., 2000b). Mock, VSVΔ51, and MG1 stocks were titred in duplicates by 10-fold serial dilution.

2.5.3. Ultraviolet inactivation of VSVΔ51 and MG1

Ultraviolet-inactivation of virus was performed as previously described (Zhang et al., 2014). Briefly, OV stock was diluted to a concentration of $1x10^9$ PFU/mL in PBS and was UV irradiated at 120mJ/cm^2 for 2 minutes using the Spectrolinker XL-1000 UV crosslinker (Spectornics, Westbury, NY). Confirmation of virus inactivation was assessed by infection of Vero cells with 10-fold dilutions of UV-inactivated OV up to 72 hours. Absence of infection and cell viability were confirmed by flow cytometry.

2.6. Characterization of IFN-I responses in cell line models of HIV-1 latency

2.6.1. IFNα stimulation

Prior to IFNα stimulation, cell lines were passaged at $0.5x10^6$ cells/mL for 16-18 hours to ensure that cells were in the exponential growth phase. Next, cell lines were counted, centrifuged (300 *g* for 5 minutes), resuspended in RP10 medium at $1x10^6$cells/mL, and $1x10^6$ cells per condition were seeded in a 24-well plate. Cell lines were then left unstimulated or stimulated with increasing 10-fold dilutions of IFNα (10-1000U/mL) for 24 hours. After 24 hours in culture, cells were collected and surface

expression of interferon IFNα/β-receptor subunit-1 (IFNAR1) and major histocompatibility complex-I (MHC-I), as well as intracellular expression of interferon-stimulated gene 15 (ISG15) and protein kinase R (PKR) were evaluated by flow cytometry.

2.6.2. Poly(I:C) transfection

Cell lines were passaged at $0.5x10^6$ cells/mL for 16-18 hours. Cell lines were then counted, centrifuged (300 *g* for 5 minutes), resuspended in RP10 medium at $1.25x10^6$cells/mL, and $1x10^6$ cells per condition were seeded in a 24-well plate. Cell lines were treated with Opti-MEM® reduced serum media (Invitrogen) alone, transfected with Opti-Mem®, or transfected with increasing 10-fold concentrations of poly(I:C) (0.1-10 μg/mL) for 48 hours using the Lipofectamine® 2000 Transfection Reagent according to the manufacturer's instructions. Transfection was confirmed by flow cytometry in all cell lines using the Rhodamine conjugated poly(I:C) (InvivoGen). After 48 hours, cell culture supernatant was collected for quantification of IFNα/β secretion using the HEK-Blue™ IFNα/β (InvivoGen) detection assay and cells were collected for evaluation of intracellular expression of ISG15 and PKR by flow cytometry.

2.7. Investigation of oncolytic virus infection in latently HIV-1 infected and uninfected cell lines

2.7.1. VSVΔ51 and MG1 infection

Prior to OV infection, cell lines were passaged at $0.5x10^6$ cells/mL for 16-18 hours. Next, $1x10^6$ cells per condition were seeded in 24-well plate at $5x10^6$ cells/mL in RP10

medium without phenol red indicator. Cell lines were then mock-infected or infected with VSVΔ51 or MG1 at multiplicity of infection (MOI) 0.00001-0.1 for 2 hours at 37°C. Media volume was increased to maintain cells at a concentration $1x10^6$ cells/mL. Between 12 to 28 hours following infection, kinetics of OV infection was quantified by measuring eGFP expression by flow cytometry and cell viability was evaluated by MTT and AlamarBlue® assays as described below.

2.7.2. Reactivation of latent HIV-1 infection

Reactivation of latent HIV-1 in cell lines was carried out by stimulating cell lines with SAHA. SAHA was prepared at a stock concentration of 10mM in dimethyl sulfoxide (DMSO; Corning Inc., Corning, NY) as per manufacturer's instructions. Prior to SAHA stimulation, both HIV-uninfected and latently HIV-1 infected cell lines were passaged at $0.5x10^6$ cells/mL for 16-18 hours. Cells were then resuspended at $0.5x10^6$ cells/mL in RP10 medium alone (unstimulated) or RP10 medium containing SAHA (1μM) (SAHA stimulated), and incubated at 37°C for 24 hours. Reactivation of latent HIV-1 was confirmed by measuring intracellular p24 expression by flow cytometry.

Next, unstimulated and SAHA stimulated cell lines were washed and prepared for OV infection as described above. Cells were infected with VSVΔ51 or MG1 at MOI 0.001-0.1 for 24 hours. After 24 hours, OV infection and cell viability was evaluated. Additionally, total media, containing cells and culture medium, was collected for the evaluation of p24 protein expression by ELISA.

2.8. Evaluation of oncolytic virus infection in latently HIV-1 infected primary cells

2.8.1. Establishment of *in vitro* resting CD4$^+$ T cell model of HIV-1 latency

Resting CD4$^+$ T cells isolated from HIV-uninfected individuals were plated at 4x10^6 cells/mL in RP10 medium and treated with 100nM CCL19 for 2 hours. Cells were then centrifuged and resuspended at 10x10^6 cells/mL in RP10 medium. HIV_{NL4-3} (100ng p24 per 1x10^6 cells) was added and infection by spinoculation was performed at 1200 *g* for 120 minutes at room temperature. Cells were then washed three times with PBS and resuspended at 2x10^6 cells/mL in RP10 medium with IL-2 (30U/mL) and left in culture for 3 days. Establishment of HIV-1 latency was confirmed by evaluating integrated HIV-1 DNA by qPCR as described (Vandergeeten et al., 2014), as well as HIV gag RNA by RT-qPCR (Palmer et al., 2003) and p24 production by ELISA over 6 days post-HIV-1 infection.

2.8.2. VSVΔ51 and MG1 infection

In vitro HIV-1 infected resting CD4$^+$ T cells and memory CD4$^+$ T cells from patients were washed with PBS and plated in 24-well plates at 5x10^6 cells/mL in RP10 medium with IL-2 (30U/mL) and RAL (10uM). Cells were then mock-infected or infected with VSVΔ51 or MG1 at 10-fold serial dilutions (MOI 0.1-10) for 2 hours at 37^{o}C. Viruses were left in culture medium and media volume was increased to maintain cells at a concentration of 1x10^6 cells/mL. At 24 and 48 hours post-OV infection, OV infection and cell death was quantified by flow cytometry as described above. After 48 hours of VSVΔ51 or MG1 infection, 5x10^5 to 1x10^6 cells were washed twice in PBS, pelleted, and stored at

-80°C before quantification of integrated HIV-1 DNA. In parallel, an appropriate number of cells were collected for viral outgrowth assay (VOA) described below.

2.8.3. Viral outgrowth assay

VOA was performed to evaluate effect of VSVΔ51 or MG1 infection on HIV-1 latency in *in vitro* primary cell and *ex vivo* models of latency by adapting previously established protocols (Laird et al., 2013; Siliciano and Siliciano, 2005).

2.8.3.1. *In vitro* HIV-1 infected resting $CD4^+$ T cells

For the *in vitro* model of latency (IVML), after 48 hours of VSVΔ51 or MG1 infection, $2.5x10^5$ resting $CD4^+$ T cells were collected for each infection condition and washed five times in PBS to remove any residual OV. Cells were resuspended in RP10 medium with PHA (5ug/mL) and IL-2 (30U/mL) at $0.5x10^6$ cells/mL and incubated at 37°C for 24 hours. 3-day PHA/IL-2 activated, CD8-depleted PBMC were then added to the activated $CD4^+$ T cells in 10-fold excess at $5x10^6$ cells/mL in RP10 medium with IL-2 (30U/mL) and incubated for 14 days. Culture supernatants were collected at day 0, 4, 7, 10, and 14 and viral outgrowth was evaluated by p24 ELISA.

2.8.3.2. Memory $CD4^+$ T cells from HAART-treated HIV infected subjects

Memory $CD4^+$ T cells from HAART-treated subjects were infected with OV for 48 hours, after which $1x10^6$ cells were collected and washed five times in PBS. Cells were resuspended at $1x10^6$ cells/mL in RP10 medium with IL-2 (30U/mL), plated in 24-well plates pre-coated with 1ug/mL anti-CD3 and anti-CD28, and incubated at 37°C for 24 hours. 3-day PHA/IL-2 activated, $CD4^+$ T cells were added as feeder cells in 5-fold excess

at $5x10^6$ cells/mL in RP10 medium with IL-2 (30U/mL). A second round of feeder cells were added in a similar manner at day 7 of viral outgrowth and cells were incubated for a total of 21 days. Culture supernatant was collected at day 0, 4, 7, 10, 14, and 21 and viral outgrowth was evaluated by p24 ELISA and HIV-1 gag RNA RT-qPCR.

2.9. Flow cytometry

Antibodies utilized are highlighted in Table 2 and antibody volumes indicated below were determined by titration on appropriate cells.

2.9.1. Intracellular p24

To evaluate intracellular HIV-1 p24 expression, $1x10^5$ cells were washed with PBS, resuspended in 100μl of pre-warmed 4% paraformaldehyde (PFA; Sigma Aldrich) and incubated at room temperature for 15 minutes. Cells were then washed in 1mL of PBS containing 1% BSA (PBS/1% BSA) and stained with 1μL KC57-RD1 antibody (Beckman Coulter) in 100μL of 0.5% Saponin (Sigma Aldrich) and 10% normal goat serum (NGS; ThermoFisher Scientific). Cells were incubated in the dark at room temperature for 30 minutes, after which cells were washed with 1mL PBS/1% BSA, resuspended in PBS, and analyzed using the FC500 Beckman Coulter Flow Cytometer (Beckman Coulter).

2.9.2. IFN-I inducible factor expression

2.9.2.1. Cell surface expression: IFNAR1 and MHC-I

Following IFNα stimulation of cell lines, $1x10^5$ cells were collected for IFNAR1 and MHC-I staining and washed with PBS/1% BSA. Cells were stained with 1μL of anti-human

IFNAR1-phycoerythrin (PE) (R&D Systems) or 0.5μL anti-human HLA-ABC-phycoerythrin-cyanin5 (PE-cy5) (Affymetrix eBioscience, San Diego, CA) in 100μL PBS/1% BSA and incubated in the dark at room temperature for 30 minutes. Cells were then washed and fixed in 1% PFA for 15 minutes and analyzed by flow cytometry.

2.9.2.2. Intracellular expression: ISG15 and PKR

Following IFNα and poly(I:C) stimulation of cell lines, $1x10^5$ cells were collected for PKR and ISG15 staining. Cells were washed in PBS/1% BSA, fixed with 100μl of pre-warmed 4% PFA for 15 minutes, and washed again to remove PFA. Intracellular ISG15 expression was evaluated by staining cells with 2.5μL anti-human ISG15-Alexa Fluor®488 antibody (R&D Systems) or 1μL of the isotype-matched control antibody (R&D Systems) in 100μL of 0.5% Saponin for 30 minutes in the dark at room temperature. Cells were then washed with PBS/1% BSA. In parallel, intracellular PKR expression was evaluated by adding 1μL unconjugated mouse anti-human PKR antibody (Abcam, Cambridge, United Kingdom) to cells in 100μL of 0.5% Saponin for 30 minutes at room temperature. Cells were then washed and stained with 4μL of goat anti-mouse IgG-allophycocyanin (APC) (Abcam) or 2μL isotype-matched control antibody (1mg/mL; clone 1F8; Abcam) in 100μL of 0.5% Saponin for 30 minutes in the dark. Cells were washed three times in PBS/1% BSA and analysis was performed by flow cytometry.

2.9.3. Oncolytic virus infection and cell death

VSVΔ51 and MG1 were engineered to express eGFP during cellular infection (Brun et al., 2010; Stojdl et al., 2000b). Consequently, OV infection in cell lines and primary cells

was quantified by evaluating GFP expression by flow cytometry. In parallel, cell death was assessed by Propidium Iodide (PI; BioLegend) staining. $1x10^5$ cells from OV infection were collected and stained with 1μL PI (0.5mg/mL), fixed with 1% PFA for 15 minutes, and analyzed by flow cytometry.

2.9.4. LDL-R

For evaluation of low density lipoprotein receptor (LDL-R) expression in cell lines, $1x10^5$ cells were collected and washed with PBS/ 1% BSA. Cells were stained with 1μL of anti-human LDL-R-PE antibody (R&D Systems) in 100μL PBS/1% BSA and incubated in the dark for 30 minutes at room temperature. Cells were then washed and fixed in 1% PFA for 15 minutes and analyzed by flow cytometry.

2.9.5. Resting and memory $CD4^+$ T cell purity

To evaluate purity, $1x10^5$ resting and memory $CD4^+$ T cells were washed with PBS containing 0.1% BSA (PBS/0.1% BSA) and stained with 5μL anti-CD4-phycoerythrin-cyanin7 (PE-cy7) (BioLegend), 1μL anti-CD69-PE (R&D systems), 5μL anti-HLA-DR-APC (BioLegend), and 2.5μL anti-CD45RO-PE (BioLegend) antibodies in 100μL PBS/0.1% BSA for 30 minutes at room temperature. Cells were washed and fixed in 1% paraformaldehyde for 15 minutes and analyzed by flow cytometry.

Table 2: Antibodies used for flow cytometry

Target	React.	Species	Product Number	Company	Clone	Conc. (μg/mL)	Conj.
KC57 (p24)	Human	Mouse	6604667	Beckman Coulter	FH190-1-1	20	PE
IFNAR1	Human	Mouse	FAB245P	R&D Systems	85228	25	PE
HLA-ABC	Human	Mouse	15-9983	Affymetrix eBioscience	W6/32	50	PE-Cy5
ISG15/UCRP	Human	Rat	IC8044G	R&D Systems	851701	20	Alexa Fluor® 488
ISG15/UCRP isotype control	Human	Rat	IC006G	R&D Systems	54447	50	Alexa Fluor® 488
PKR (1° Antibody)	Human, Rat	Mouse	Ab202136	Abcam	6H3A10	1000	N/A
PKR (2° Antibody)	Mouse	Goat	Ab120782	Abcam		500	APC
PKR isotype control	Human	Mouse	Ab91358	Abcam	1F8	1000	APC
LDL-R	Human	Mouse	FAB2148P	R&D Systems	472413	25	PE
CD4	Human	Mouse	344612	BioLegend	SK3	100	PE/Cy7
CD69	Human	Mouse	FAB23591P	R&D Systems	298614	25	PE
HLA-DR	Human	Mouse	307610	BioLegend	L243	24	APC
CD45RO	Human	Mouse	304206	BioLegend	UCHL1	50	PE

IFNAR1 = IFNα receptor 1; PE = phycoerythrin; HLA = human leukocyte antigen; ISG = interferon stimulated gene; PKR = protein kinase R; APC = allophycocyanin; LDL-R = low density lipoprotein receptor; CD = cluster of differentiation;

2.10. Cell viability assays

2.10.1. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay

Vybrant® MTT Cell Proliferation Assay kit (Invitrogen) was used to evaluate cell viability following OV infection in cell lines. 12mM stock solution of MTT was prepared as per manufacturer's instructions. At each time point of OV infection, $1x10^5$ cells from each condition (MOI 0.00001-0.1) were plated in 96-well plates in quadruplicate at $1x10^6$ cells/mL. MTT stock solution was diluted 1 in 5 in RP10 medium without phenol red indicator, 50μL was added to each well, and incubated at 37°C for 4 hours. MTT reaction was stopped and any remnant viral particles were lysed with a 10% sodium dodecyl sulfate (SDS; Fisher Scientific) solution containing 0.01M HCl (Fisher Scientific) and incubated at 37°C for 1 hour. Each sample was mixed and absorbance was read at 570nm using the Multiskan Ascent 96 Plate Reader (MTX Lab Systems Inc., Bradenton, FL).

2.10.2. AlamarBlue® cell viability assay

At each time point of OV infection in cell lines, $1x10^5$ cells from each condition (MOI 0.00001-0.1) was plated in 96-well plates in quadruplicate. AlamarBlue® Cell Viability Reagent (ThermoFisher Scientific, Waltham, MA) was diluted 1 in 5 in RP10 medium without phenol red indicator, 50μL was added to each well, and incubated at 37°C for 4 hours. To ensure complete lysis of any remnant viral particles, cells were treated with a 10% SDS solution containing 0.01M HCl and incubated at 37°C for 1 hour. Fluorescence was read at an excitation wavelength of 530 and emission wavelength of 590nm using the Fluoroskan Ascent™ Microplate Fluorometer (ThermoFisher Scientific).

2.11. CellTrace™ CFSE Cell Proliferation Assay

CellTrace™ Carboxyfluorescein succinimidyl ester (CFSE) Cell Proliferation assay (Life Technologies) was used to assess rate of cell proliferation between cell lines. 5mM stock concentration of CFSE was prepared in DMSO. Cell lines were plated at $0.5x10^6$ cells/mL in RP10 medium for 16-18 hours. Cells were then counted, washed, and $1x10^6$ cells per condition were stained with 5uM CFSE as indicated in manufacturer's instructions. Following CFSE staining, cells were plated at $1x10^6$ cells/mL in a 24-well plate in complete RP10 medium, serum free medium (RPMI-1640 with PSG and no FCS), or RP10 medium with 0.25uM colchicine (Sigma Aldrich). CFSE staining was evaluated at 0, 24, 48, and 72 hours by flow cytometry.

2.12. Enzyme linked immunosorbent assay (ELISA)

2.12.1. p24 ELISA

Quantification of p24 antigen in $HIV_{NL4.3}$ virus stocks and evaluation of virus expression in culture supernatant of viral outgrowth assay was performed by ELISA. Samples were lysed with 1% Triton-X and p24 antigen expression was quantified by HIV-1 p24 Antigen Capture Kit (Frederick National Laboratory for Cancer Research, Frederick, MD; NIH AIDS Reagent Program) following the manufacturer's protocol. Absorbance was read at 450nm wavelength with a reference wavelength of 540nm using the Multiskan Ascent 96 Plate Reader.

2.12.2. IFNα ELISA

Cell lines were left uninfected or infected with VSVΔ51 and MG1 (MOI 0.001, 0.01, and 0.1) for 24 hours, after which culture medium was collected. Total IFNα protein expression was measured using the VeriKine human IFN-α ELISA kit (PBL Assay Science, Piscataway, NJ) following the manufacturer's instructions. Absorbance was read at 450nm wavelength with a reference wavelength of 540nm using the Multiskan Ascent 96 Plate Reader.

2.13. HEK-Blue™ IFNα/β detection assay

Quantification of IFNα production in response to poly(I:C) stimulation in cell lines was performed using the HEK-blue™ IFNα/β cell detection assay (InvivoGen). 50μL of cell-free culture supernatant from poly(I:C) stimulation, as well as 2-fold dilutions of IFNα standard (PBL Assay Science) (5 units/mL to 0.078 units/mL) were added to a 96-well plate. HEK-blue™ IFNα/β cells were trypsinized and resuspended at $0.5x10^6$ cells/mL ($7.5x10^4$ cells/well) in complete DMEM medium and incubated at 37°C for 24 hours. Next, 100μL of culture supernatant from HEK-blue™ IFNα/β cells was transferred to a new 96-well plate and lysed with 1% triton-X for 1 hour at 37°C to eliminate any remnant HIV-1. 20μL of lysed culture supernatant and 180μL of QUANTI-Blue™ Secreted Embryonic Alkaline Phosphatase Detection reagent (InvivoGen), prepared as per manufacturer protocol, were added to a 96-well plate. The plate was incubated at 37°C and monitored for colour change for up to 48 hours. Absorbance was read at 655nm wavelength using the Multiskan Ascent 96 Plate Reader.

2.14. Molecular biology

2.14.1. RNA and DNA Extraction

Cell associated-RNA was extracted using the Illustra RNAspin mini kit (GE Healthcare Life Sciences, Mississauga, ON) per manufacturer's instructions. Cell-free RNA was extracted from 140uL of supernatant using the QIAmp viral RNA mini kit (Qiagen, Venlo, Limburg, Netherlands). Genomic DNA (gDNA) extraction for the quantification of integrated HIV-1 proviral DNA was performed as described (Vandergeeten et al., 2014). Briefly, cells were digested in lysis buffer (10mM Tris-HCl, pH 8.0, 50nM KCl, 400ug/mL Proteinase K; Invitrogen) for 12-16 hours at 55^{o}C in a heating shaker. RNA and DNA integrity was monitored by agarose gel electrophoresis. RNA and DNA concentrations were measured using ND-1000 Spectrophotometer (NanoDrop, Wilmington, DE). All samples were stored at -80^{o}C until further use.

2.14.2. HIV-1 RNA quantitative reverse transcription PCR (RT-qPCR)

Reverse transcription (RT) reactions were performed using the iScript™ cDNA Synthesis Kit (BioRad, Hercules, CA) per manufacturer's protocol. Quantification of HIV-1 genomic RNA was performed using a previously described primer-probe set targeting the conserved region of *gag* (Palmer et al., 2003; 2008). Ribosomal protein s18 (*RPS18*) gene was used as the reference gene for cell-associated *Gag* RNA measurements. RT-qPCR reaction for *Gag* and *RPS18* was performed using SsoAdvanced™ Universal Probes Supermix and SsoAdvanced™ Universal SYBR Green Supermix (BioRad), respectively, with the indicated primer concentrations (Table 3). Following thermal activation of polymerase

(95°C for 30 seconds), 45 cycles of PCR amplification (with each cycle consisting of 95°C for 15 seconds and 60°C for 1 minute) were performed using the CFX Connect™ Real-Time PCR Detection System (BioRad).

2.14.3. Integrated HIV-1 DNA PCR

Two-step nested PCR targeting CD3 and integrated HIV-1 DNA was performed with previously described primer-probe sets (Vandergeeten et al., 2014). A pre-amplification reaction of integrated HIV DNA and CD3 was carried out in a 50μL reaction mix containing 15μL of gDNA lysate, 25ul of iQ™ Supermix (BioRad) and, 300nM each of the 4 primers (ULF1, UR1, HCD3OUT5', and HCD3OUT2'), and 150nM of ULF1 primer. To maintain amplification in an exponential phase, 12-cycle first-round amplification was carried out as described (Vandergeeten et al., 2014) using a T100™ Thermal Cycler (BioRad).

The second round of PCR was performed in a final volume of 20ul containing 6.4uL of the first PCR product for integrated HIV-1 DNA amplification, 6.4uL of a 1/1000 dilution of the first PCR product for CD3 amplification, as well as the corresponding second-step primer-probe sets at indicated concentrations (Table 3) (Vandergeeten et al., 2014). PCR reactions were then carried out using the CFX Connect™ Real-Time PCR Detection System (BioRad) using the following amplification steps for all reactions: a denaturation step (95°C for 10 minutes), followed by 40 cycles of amplification (95°C for 30s, 57°C for 1 minute), and a final droplet stabilization step (4°C for 5 minutes and 90°C for 5 minutes). Relative quantification was performed between samples using CFX Manager 3.1 software (BioRad)

Table 3: Primers and probes used for PCR reactions

Target	Primer or probe name	Sequence (5′ to 3′)	Conc. (nM)
RPS18 (Côté et al., 2013)	RPS18 Forward	TAGAGGGACAAGTGGCGTTC	300
	RPS18 Reverse	CGCTGAGCCAGTCAGTGTZ	300
Gag (Palmer et al., 2003)	Gag 6F	CATGTTTTCAGCATTATCAGAAGGA	300
	Gag 84R	TGCTTGATGTCCCCCCACT	300
	Gag Probe	FAM-CCACCCCACAAGATTTAAACACCATGCTAA-Q	100
CD3 (Vandergeeten et al., 2014)	HCD3OUT5′	ACTGACATGGAACAGGGGAAG	300
	HCD3OUT3′	CCAGCTCTGAAGTAGGGAACATAT	300
	HCD3IN5′	GGCTATCATTCTTCTTCAAGGT	1250
	HCD3IN3′	CCTCTCTTCAGCCATTTAAGTA	1250
	CD3 Probe	FAM-AGCAGAGAA/ZEN/CAGTTAAGAG CCTCCAT/3IABkFQ	200
Integrated HIV DNA (Vandergeeten et al., 2014)	ULF1	ATGCCACGTAAGCGAAACTCTGGGTCTCTCTDGTTAG AC	150
	Alu1	TCCCAGCTACTGGGGAGGCTGAGG	300
	Alu2	GCCTCCCAAAGTGCTGGGATTACA	300
	LambdaT	ATGCCACGTAAGCGAAACT	1250
	UR2	CTGAGGGATCTCTAGTTACC	1250
	Integrated DNA Probe	FAM-CACTCAAGG/ZEN/CAAGCTTTATTGAGGC-3IABkFQ	200

FAM: 6-carboxyfluorescein group reporter; Q: 6-carboxytetramethylrhodamine group quencher; ZEN: ZEN™ internal quencher; 3IABkFQ: 3′-Iowa Black® FQ

2.15. Statistical analysis

Flow cytometry was performed using the FC500 Beckman Coulter Flow Cytometer and analysis of data was conducted using FCS Express Research Edition 4.0 (De Novo Software, Los Angeles, CA). Analysis of qPCR was completed using the BioRad CFX Manager Software. Graphs and statistical analysis was generated using GraphPad Prism 5.0 Software (San Diego, CA), and P values ≤ 0.05 were considered significant for all comparisons. As determined *a priori*, statistical analysis used included Student's t-test, one-way analysis of variance (ANOVA) with Dunnett's multiple comparisons test for *post hoc* analysis, two-way ANOVA with Bonferroni correction for *post hoc* analysis, and linear regression analysis.

Chapter 3: IFN-I responses are impaired in latently HIV-1 infected cell lines

3.1. Introduction

3.1.1. Rationale

Widespread disruption of the IFN-I signaling cascade has been reported during productive HIV-1 infection. As described in the introduction, these include significant defects in IFN-I signaling, disruption of viral sensing through degradation of pattern recognition receptors (PRRs) (Britto et al., 2013; Clerzius et al., 2010; Li et al., 2016a; Solis et al., 2011), inhibition of interferon regulatory factors (IRFs) (Doehle et al., 2012), and significant impairment of various interferon stimulated genes (ISGs), antiretroviral effectors, and restriction factors (Lubben et al., 2007; Malim and Emerman, 2008; Neil et al., 2008; Sheehy et al., 2002). However, the antagonism of IFN-I signaling within latently HIV-1 infected cells remains to be characterized. The objective of this chapter is therefore to investigate IFN-I responses in the context of HIV-1 latency.

3.1.2. Characterization of the IFN-I system in the context of HIV-1 latency using cell line models

Cell line models of latent HIV-1 infection present several advantages that have led to their widespread use in studies delineating HIV-1 pathogenesis and mechanisms underlying latent HIV infection (la Fuente et al., 2002; Mousseau et al., 2015; Taura et al., 2015). An important feature of cell lines is that they represent a homogenous, clonal population of latently infected cells, which were derived from their respective HIV-uninfected parental cell lines (Figure 7A and 7B). Additionally, reactivation of latent HIV-

1 infection and induction of viral replication can be triggered within these models through appropriate stimulation (Cassol et al., 2006). However, these models are not without their limitations. In contrast to the resting memory $CD4^+$ T cells that represent the primary latent HIV-1 reservoir, the transformed cell line models of HIV-1 latency are constantly replicating and therefore lack the capacity for quiescence. Additionally, cell lines may contain genotypic and phenotypic modifications driven by the malignant nature of these cells. Ultimately, however, due to the complex mechanisms underlying the establishment of HIV latency (Donahue and Wainberg, 2013) none of the current *in vitro* models truly recapitulate features of latency as they occur *in vivo*. Cell line models therefore offer an important platform to delineate and understand features of latent HIV-1 infection. Within this study, two independent, latently HIV-1 infected cell lines, U1 and OM10.1, as well as their respective parental HIV-uninfected cell lines, U937 and HL60, were utilized to characterize IFN-I pathways.

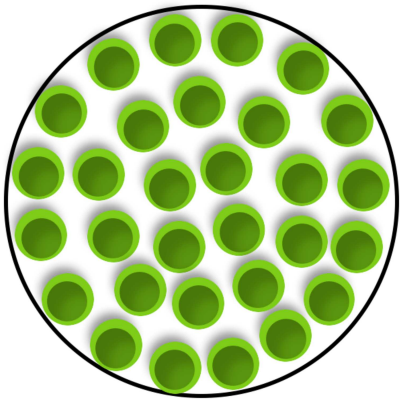
3.1.2.1. U937 and U1 cells

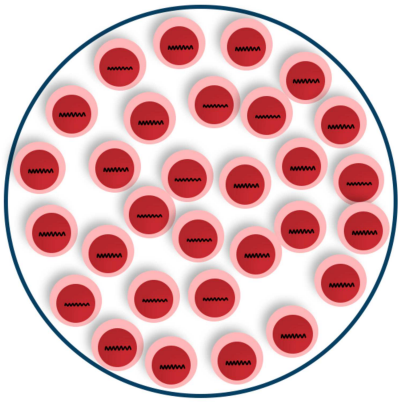
U937 cells are a promonocytic cell line obtained from a patient with histiocytic lymphoma (Sundström and Nilsson, 1976). U1 cells were derived from chronic infection of U937 cells with the HIV-$1_{LAI/LAV}$ strain, resulting in 2 integrated copies of HIV-1 proviral DNA per cell (Folks et al., 1987; 1988). The state of post-integration latency is maintained in U1 cells through two mutations in the HIV-1 trans-activator of transcription (Tat), rendering Tat defective in its ability to regulate and enhance HIV-1 transcriptional activation (Emiliani et al., 1998). Under basal conditions, HIV-1 latency is maintained in U1 cells at a post-transcriptional level, with multiply spliced HIV-1 RNA expressed at a

relatively high frequency (Butera et al., 1994). However, minimal expression of unspliced HIV-1 mRNA and corresponding regulatory proteins have been observed (Folks et al., 1987; Pomerantz et al., 1990).

3.1.2.2. HL60 and OM10.1 cells

HL60 cells are a promyelocytic cell line obtained from a patient with acute promyelocytic leukemia (Collins et al., 1977). The OM10.1 cells were clonally derived from HL60 cells that were co-cultured with γ-irradiated, HIV-$1_{LAI/LAV}$-infected A3.01 T lymphocytes (Butera et al., 1991b; 1991a). OM10.1 cells carry a single copy of integrated HIV-1 proviral DNA in a state of viral latency (Butera et al., 1991b). Post-integration latency is maintained in OM10.1 cells on a transcriptional level due to the deficiency of cellular regulatory factors necessary for proviral transcription (Butera et al., 1994). Impairments in protein kinase C (PKC) activity and downstream transcription factors such as NF-κB have been identified to be critical for modulating HIV latency in OM10.1 cells (Butera et al., 1991b). At basal levels, <2% of OM10.1 cells constitutively express full-length HIV-1 RNA and proteins, including Tat and the p24 capsid protein (Butera et al., 1994).





HIV-uninfected cells	Latently HIV-1 infected cells
U937	U1
HL60	OM10.1

Figure 7: Latently HIV-1 infected cell lines represent a homogenous population of cells carrying integrated proviral DNA. Despite the malignant origin of cell line models of HIV-1 latency, these models present several important advantages. Latently HIV-1 infected cell lines uniformly contain inducible integrated proviral DNA, which is maintained in a state of latency by various transcriptional or post-transcriptional mechanisms. In addition, the latently HIV-1 infected cells can be studied in comparison to the parental HIV-1 uninfected cell lines, from which these cells were derived. This has led to the extensive use of cell line models of latent HIV-1 infection, including the U937 and U1 and the HL60 and OM10.1 cell lines.

3.1.2.3. State of the IFN-I system within cell line models of HIV-1 latency

Few studies have investigated the state of IFN-I pathways in latently HIV-1 infected cells. Utilizing the above cell line models of latent HIV-1 infection, Taura and colleagues evaluated the constitutive mRNA expression of various ISGs and antiretroviral restriction factors, including ISG15, PKR, SAMHD1, APOBEC3G. Although minimal differences in the mRNA profiles of U937 and U1 cells were observed, higher constitutive ISG15 and PKR mRNA expression was reported in OM10.1 cells, relative to HIV-uninfected HL60 cells (Taura et al., 2015). Interestingly however, significant impairments in the production of pro-inflammatory cytokines, including TNFα, IL-6, and IL-1β, by the latently HIV-1 infected cell lines, relative to HIV-uninfected cells, was observed in response to treatment with various PRR agonists including poly(I:C) and Resiquimod (R848) (Taura et al., 2015). These observations lend support to the potential presence of defects in IFN-I signaling within latently infected cell lines and support characterization of IFN-I pathways.

3.2. Hypothesis

IFN-I responses are impaired in the latently HIV-1 infected U1 and OM10.1 cells in comparison to the respective HIV-uninfected U937 and HL60 parental cells.

3.3. Results

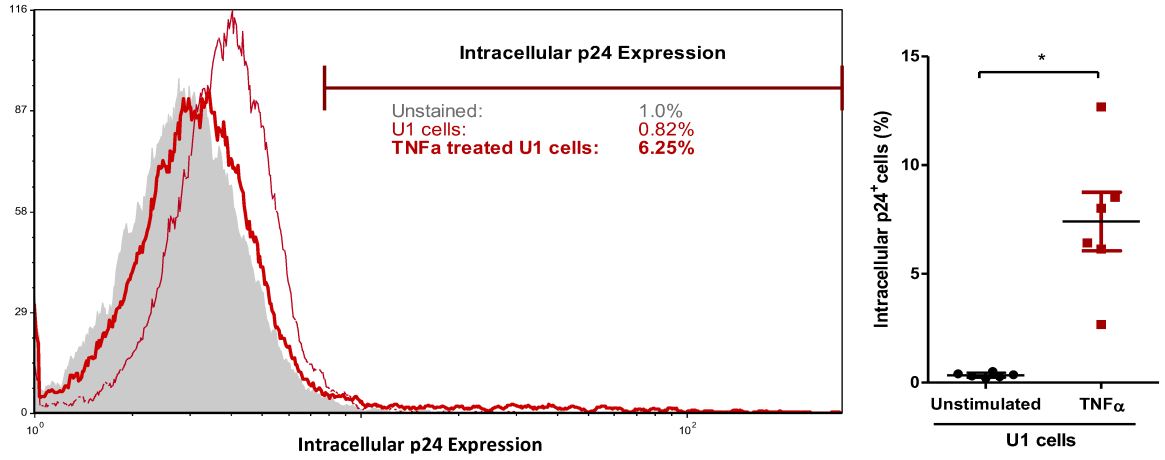
Aspects of the IFN-I pathway, which are crucial to the antiviral response against HIV-1 infection, including the production of IFNα/β cytokines, the expression of IFNAR1, and the induction of antiviral ISGs, including MHC-I, ISG15, and PKR, were investigated.

HIV-1 latency defined as the absence of ongoing viral replication, as well as inducible HIV-1 replication following stimulation with TNFα, was demonstrated in the U1 and OM10.1 cells by measuring intracellular p24 protein expression (Figure 8A and 8B), as well as p24 secretion by ELISA (data not shown).

3.3.1. Constitutive expression of IFNAR1, MHC-I, and PKR is lower in latently HIV-1 infected cells

First, the state of the IFN-I system within latently HIV-1 infected and uninfected cell lines was characterized by evaluating the basal expression of IFNAR1, MHC-I, ISG15, and PKR by flow cytometry. Within the U1 and OM10.1 cells, the constitutive expression of IFNAR1 was ~2-fold lower when compared to the HIV-uninfected controls (Figure 9A). Additionally, surface expression of MHC-I was demonstrated to be significantly lower in both U1 and OM10.1 cells, than in the respective parental cells (Figure 9B). There was minimal constitutive expression of ISG15, an IFN-inducible, ubiquitin-like antiviral protein, in all cell lines (Figure 9C). Similarly, there was no difference in the basal expression of PKR, a dsRNA-sensing PRR, between U1 and HIV-uninfected U937 cells. In contrast, OM10.1 cells had higher expression of PKR in comparison to the HL60 cells (Figure 9D). Therefore, differences in constitutive expression of IFNAR1, MHC-I, and PKR were observed in two independent latently HIV-1 infected cell lines compared to the respective uninfected parental controls.

A


Intracellular p24 Expression
Unstained: 1.0%
U1 cells: 0.82%
TNFa treated U1 cells: 6.25%
Intracellular p24 Expression
Intracellular p24+cells (%)
Unstimulated
TNFα
U1 cells

B

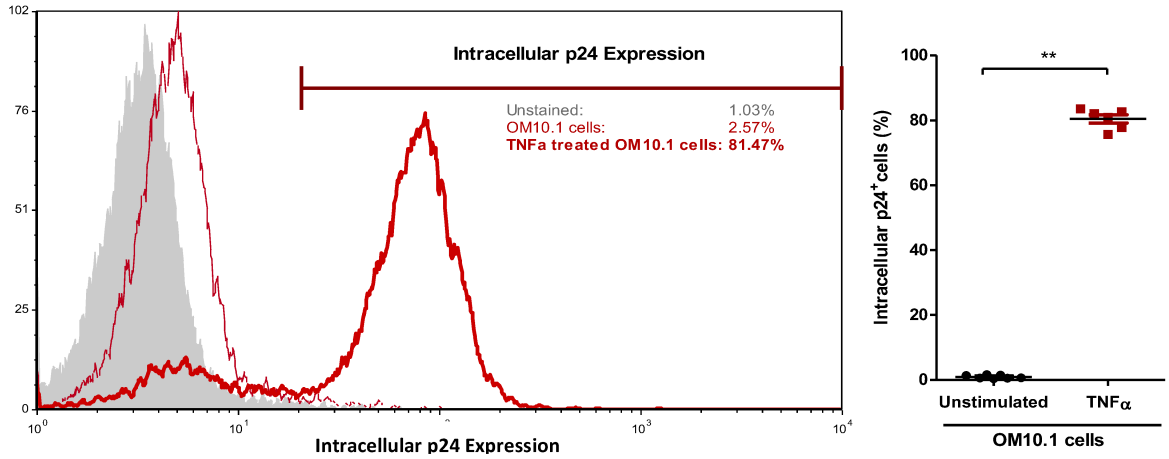

Intracellular p24 Expression
Unstained: 1.03%
OM10.1 cells: 2.57%
TNFa treated OM10.1 cells: 81.47%
Intracellular p24 Expression
Intracellular p24+cells (%)
Unstimulated
TNFα
OM10.1 cells

Figure 8. U1 and OM10.1 cells have minimal constitutive p24 expression, but HIV-1 replication is inducible following stimulation. U1 and OM10.1 cell lines were left unstimulated in complete medium or stimulated with 1ηg/mL of TNFα for 24 hours. Intracellular p24 protein expression was quantified in cell lines using flow cytometry. Representative histogram and cumulative data demonstrating minimal basal expression, but inducible expression of intracellular p24 in **A.** Latently HIV-1 infected U1 cells and HIV-uninfected U937 parental cells (n=6) and **B.** Latently HIV-1 infected OM10.1 cells and HIV-uninfected HL60 parental cells (n=6). n values represent separate biological replicates. *p=0.0004 and **p<0.0001 by unpaired t-test; Data represent mean ± SEM.

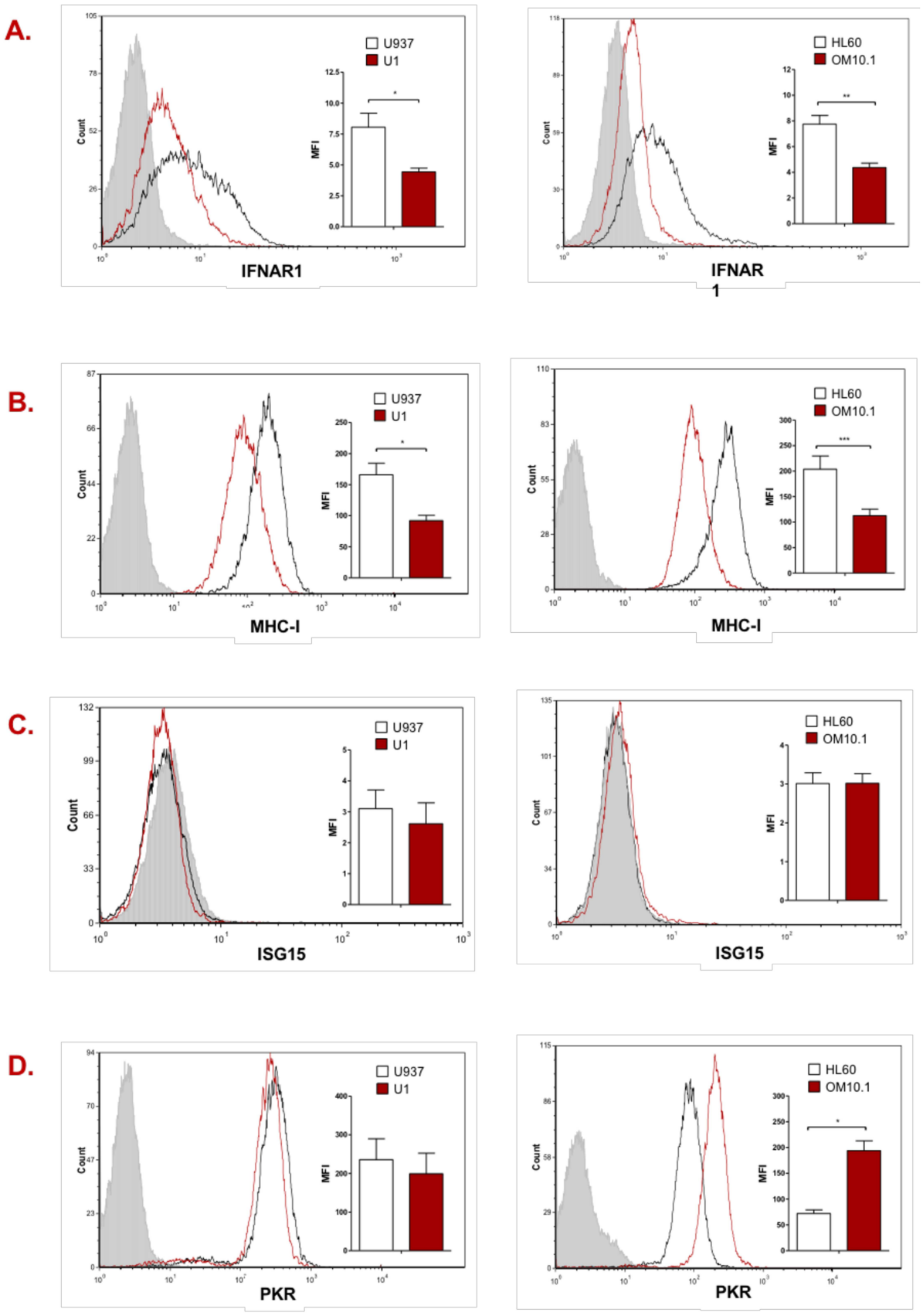
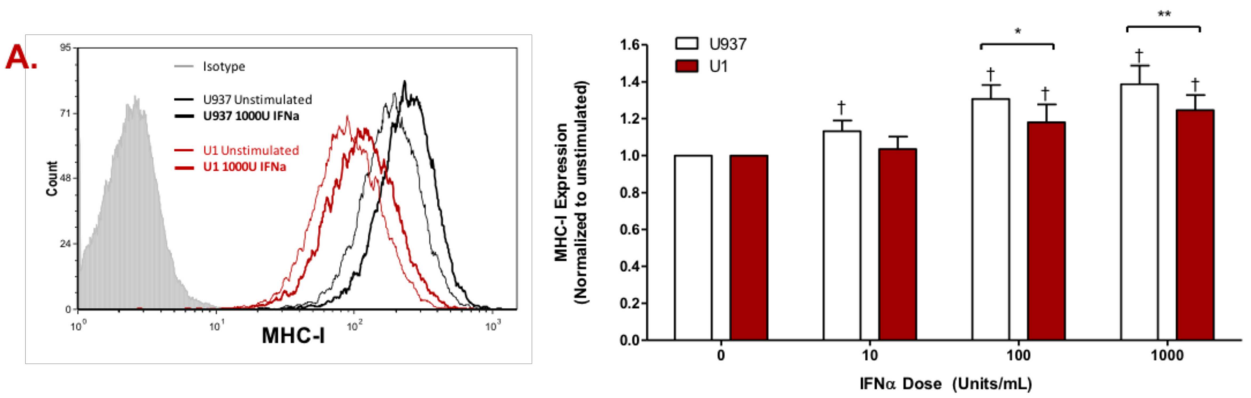

A.
U937
U1
Count
MFI
IFNAR1
HL60
OM10.1
IFNAR 1
B.
MHC-I
MHC-I
C.
ISG15
ISG15
D.
PKR
PKR

Figure 9. Expression of IFNAR1, MHC-I, ISG15, and PKR in HIV-uninfected and latently HIV-infected cells. Constitutive expression of several ISGs was quantified by flow cytometry in latently HIV-infected U1 and OM10.1 cells. Representative histogram and cumulative data for each ISG is shown. Surface expression of **A.** IFNAR1 (n=5) and **B.** MHC-I (n=6), as well as constitutive intracellular expression of **C.** ISG15 (n=6) and **D.** PKR (n=6) was assessed between the latently HIV-infected U1 and OM10.1 cells and respective HIV-uninfected U937 and HL60 controls. n values represent separate biological replicates. * $p<0.0001$, ** $p=0.002$, *** $p<0.0017$ by unpaired T-test; Data represent mean ± SEM.

3.3.2. Responsiveness to exogenous IFNα is impaired in latently HIV-infected cells

The IFN-I response *in vivo* is driven by the autocrine and paracrine effects of IFNα, which result in the robust induction of intracellular signaling cascades and the production of ISGs (Sadler and Williams, 2008). Consistent with this, the responsiveness of latently HIV-infected cells to stimulation with exogenous IFNα (10-1000U/mL) was evaluated by quantifying the induction of downstream ISGs, including MHC-I, ISG15, and PKR. MHC-I was upregulated in response to IFNα in all cell lines, but the level of MHC-I expression was significantly lower in the latently HIV-infected U1 (Figure 10A) and OM10.1 cells (Figure 10B) when compared to their respective controls. Similarly, IFNα enhanced the expression of ISG15 in a dose-dependent manner in all cell lines, but the level of ISG15 expression was lower in the latently HIV-infected U1 (Figure 11A) and OM10.1 cells (Figure 11B) than the controls. Finally, despite higher basal PKR expression, the induction of PKR following IFNα treatment was found to be impaired in OM10.1 cells relative to HL60 cells (Figure 12B), but did not differ between U1 and U937 cells (Figure 12A).


A.
Isotype
U937 Unstimulated
U937 1000U IFNa
U1 Unstimulated
U1 1000U IFNa
Count
MHC-I
U937
U1
MHC-I Expression
(Normalized to unstimulated)
IFNα Dose (Units/mL)
0
10
100
1000
*
**
†

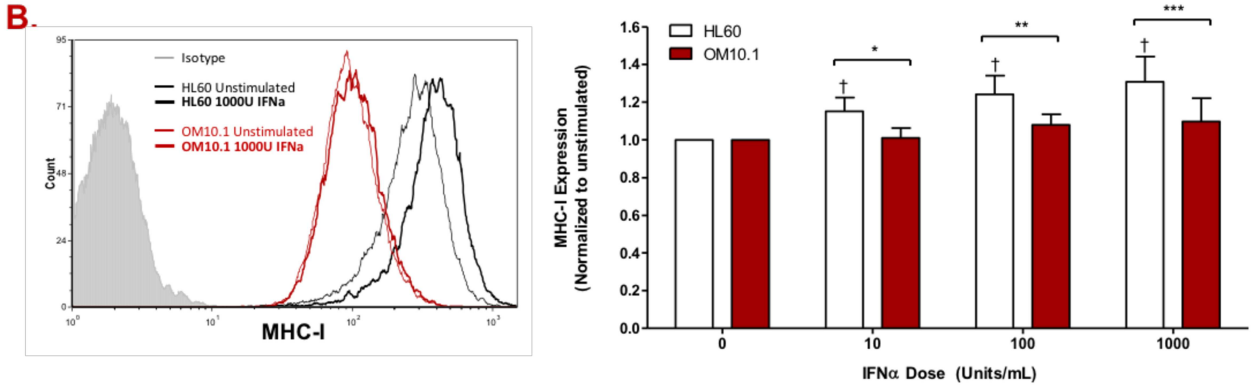
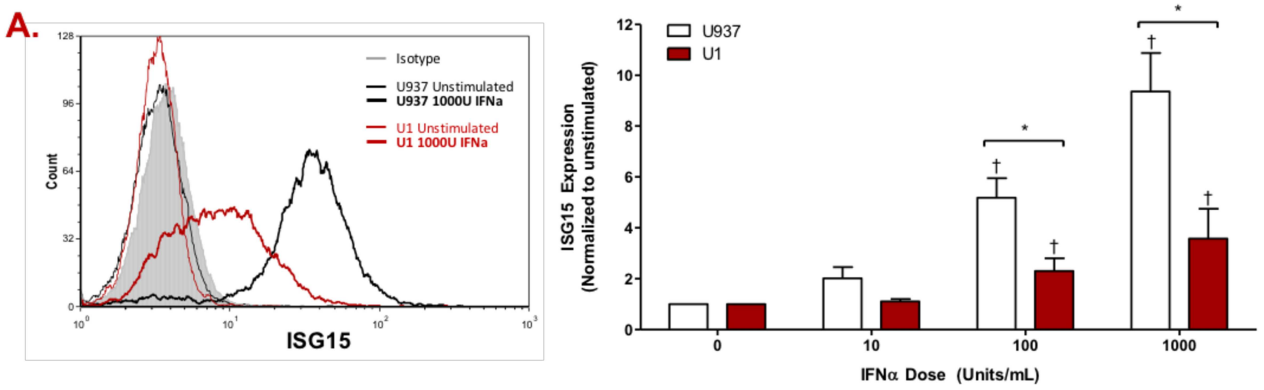

B.
Isotype
HL60 Unstimulated
HL60 1000U IFNa
OM10.1 Unstimulated
OM10.1 1000U IFNa
Count
MHC-I
HL60
OM10.1
MHC-I Expression
(Normalized to unstimulated)
IFNα Dose (Units/mL)
0
10
100
1000
*
**
***
†

Figure 10. IFNα-induced expression of MHC-I is impaired in latently HIV-infected U1 and OM10.1 cells. Cell lines were stimulated with 10, 100, or 1000 IU/mL of exogenous IFNα for 24 hours. Following stimulation, cells were collected and surface expression of MHC-I was assessed by flow cytometry. Representative histogram and summary data of IFNα-induced MHC-I expression normalized to unstimulated controls is shown for **A.** U937 and U1 cells (n=6) and **B.** HL60 and OM10.1 cells (n=6). n values represent separate biological replicates. † $p<0.0001$ by one-way ANOVA and $p<0.05$ by pairwise Dunnett's test compared to unstimulated cells. * $p<0.05$, ** $p<0.01$, and *** $p<0.001$ by Two-way ANOVA with Bonferroni post-test for multiple comparisons; Data represent mean ± SEM.


A.
Isotype
U937 Unstimulated
U937 1000U IFNa
U1 Unstimulated
U1 1000U IFNa
Count
ISG15
U937
U1
ISG15 Expression
(Normalized to unstimulated)
IFNα Dose (Units/mL)
0
10
100
1000

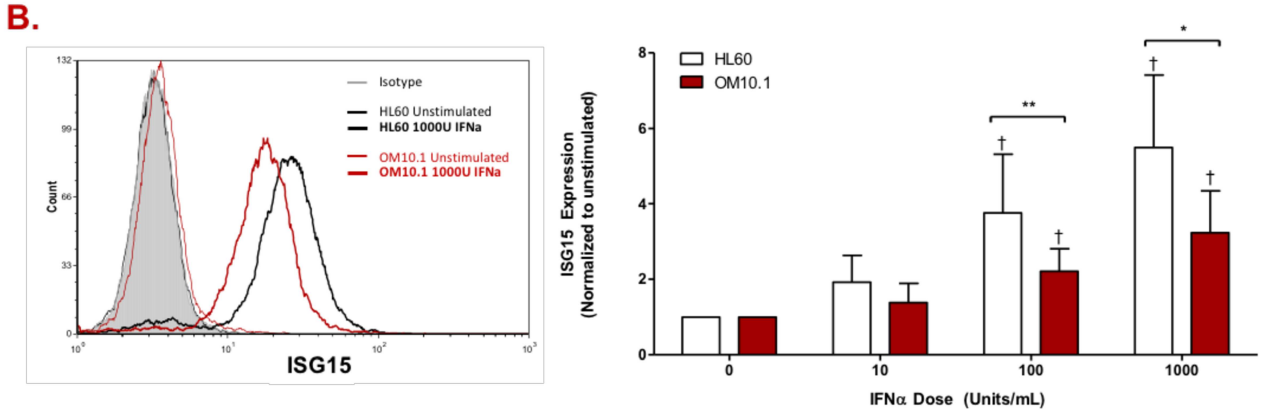
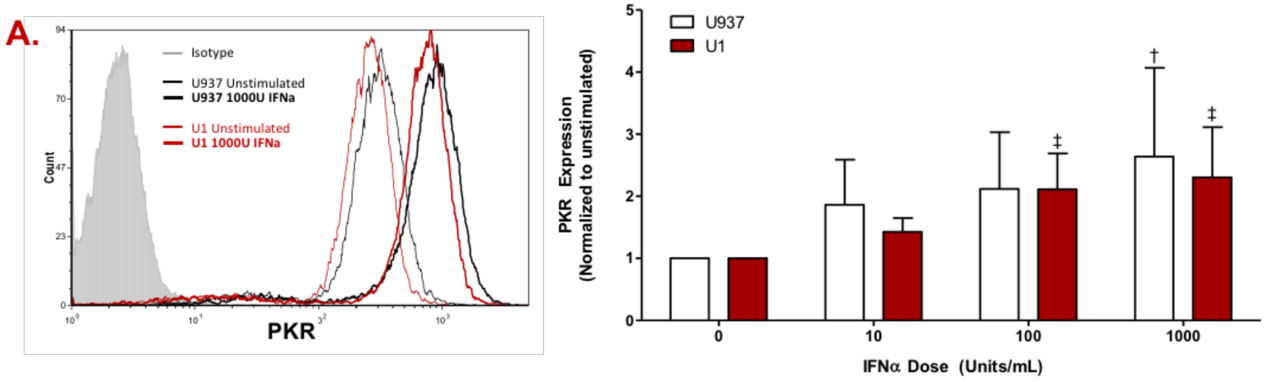

B.
Isotype
HL60 Unstimulated
HL60 1000U IFNa
OM10.1 Unstimulated
OM10.1 1000U IFNa
Count
ISG15
HL60
OM10.1
ISG15 Expression
(Normalized to unstimulated)
IFNα Dose (Units/mL)
0
10
100
1000

Figure 11. IFNα-induced expression of ISG15 is impaired in latently HIV-infected U1 and OM10.1 cells. Cell lines were treated with increasing concentrations of exogenous IFNα for 24 hours. Following stimulation, cells were fixed and permeabilized, after which intracellular ISG15 expression was measured by flow cytometry. Representative histogram and cumulative data of IFNα-induced ISG15 expression normalized to unstimulated controls is shown for **A.** U937 and U1 cells (n=6) and **B.** HL60 and OM10.1 cells (n=8). n values represent separate biological replicates. † $p<0.0001$ by one-way ANOVA and $p<0.05$ by pairwise Dunnett's Test compared to unstimulated cells. * $p<0.001$, ** $p<0.05$ by Two-way ANOVA with Bonferroni post-test for multiple comparisons; Data represent mean ± SEM.


A.
Isotype
U937 Unstimulated
U937 1000U IFNa
U1 Unstimulated
U1 1000U IFNa
Count
PKR
U937
U1
PKR Expression
(Normalized to unstimulated)
IFNα Dose (Units/mL)
0
10
100
1000

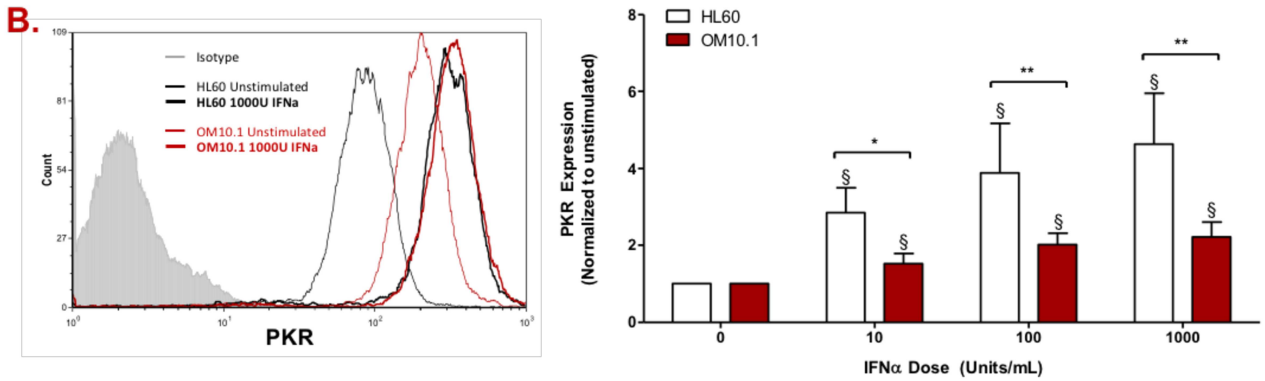

B.
Isotype
HL60 Unstimulated
HL60 1000U IFNa
OM10.1 Unstimulated
OM10.1 1000U IFNa
Count
PKR
HL60
OM10.1
PKR Expression
(Normalized to unstimulated)
IFNα Dose (Units/mL)
0
10
100
1000

Figure 12. IFNα-induced PKR expression was impaired in latently HIV-infected OM10.1 cells. Cell lines were left unstimulated or treated with increasing concentrations of exogenous IFNα for 24 hours. Following stimulation, cells were fixed and permeabilized, after which intracellular PKR expression was measured by flow cytometry. Representative histogram and cumulative summary of IFNα-induced PKR expression normalized to unstimulated controls is shown for **A.** U937 and U1 cells (n=6) and **B.** HL60 and OM10.1 cells (n=6). n values represent separate biological replicates. † p=0.04, ‡ p=0.0008, § p<0.0001 by one-way ANOVA and p<0.05 by pairwise Dunnett's Test compared to unstimulated cells. * p<0.05, ** p<0.001 by Two-way ANOVA with Bonferroni post-test for multiple comparisons; Data represent mean ± SEM.

3.3.3. Poly(I:C)-induced activation of IFN-I pathways is defective in latently HIV-infected cells

Next, to emulate exposure and cellular responses to viral PAMPs, cell lines were transfected with the synthetic dsRNA analog poly(I:C) (0.1-10ug/mL). Similar levels of transfection were confirmed in all cell lines using Rhodamine labelled poly(I:C) (data not shown). Following transfection, IFNα/β secretion was assessed using the HEK-Blue^{TM} IFNα/β biologic assay (Imbeault et al., 2009b), and intracellular ISG15 and PKR expression were assessed by flow cytometry as before.

Low levels of endogenous IFNα/β were detectable in culture supernatant of unstimulated U937 and U1 cells (Figure 13A). However, upregulation of IFNα/β production in response to poly(I:C) transfection was observed in U937 cells, but not U1 cells (Figure 13B). While poly(I:C) caused a dose-dependent increase in intracellular ISG15 expression in U937 cells, no change was observed in the latently HIV-infected U1 cells (Figure 13C). Similarly, poly(I:C)-induced PKR expression was impaired in the U1 cells, when compared to U937 cells (Figure 13D). Defects in the poly(I:C)-mediated induction of ISG15 were also observed in OM10.1 cells, when compared to the dose-dependent increase seen in the HIV-uninfected HL60 cells (Figure 14A). However, no significant difference in poly(I:C)-induced PKR expression was observed between HL60 and OM10.1 cells (Figure 14B)

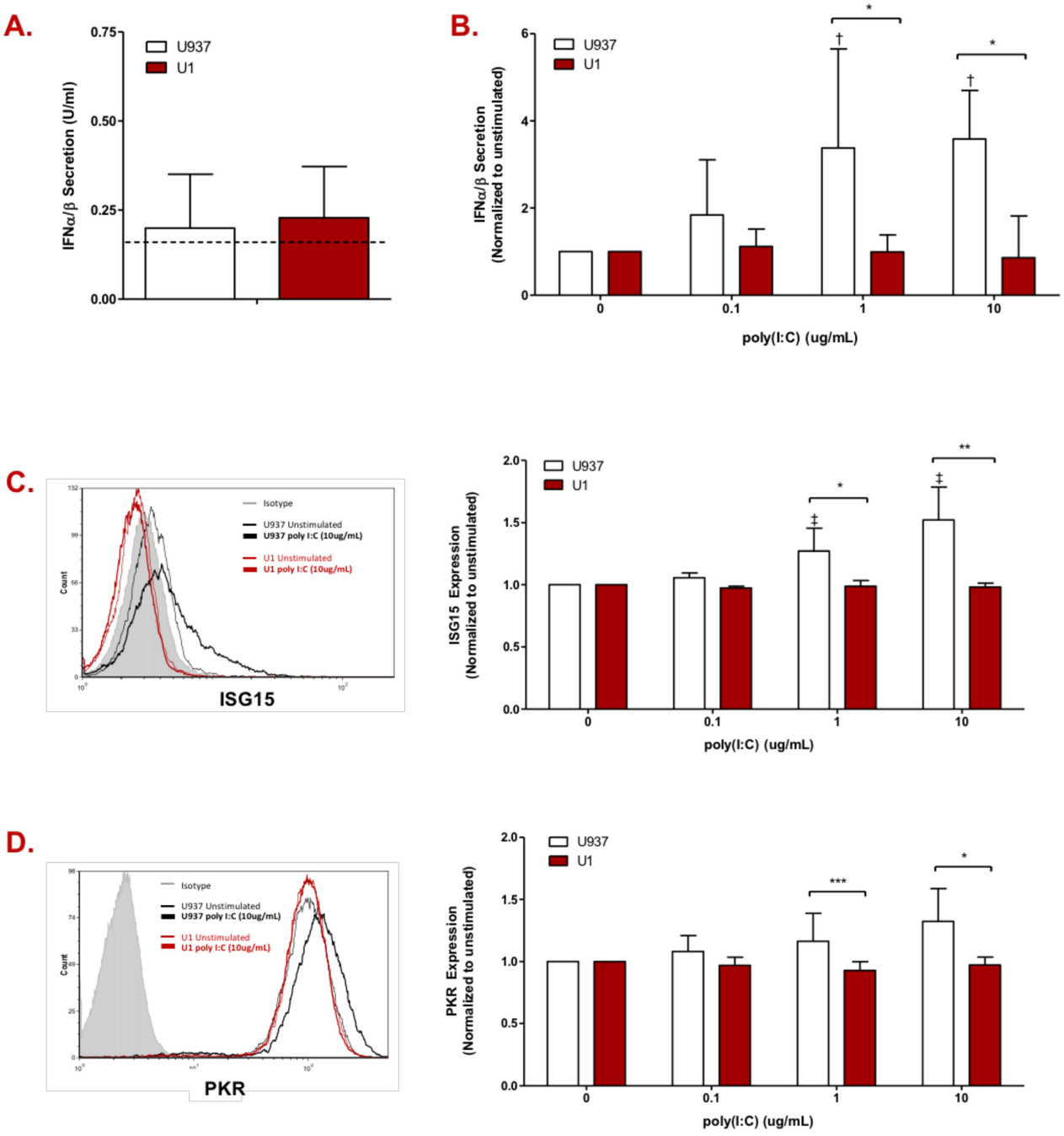
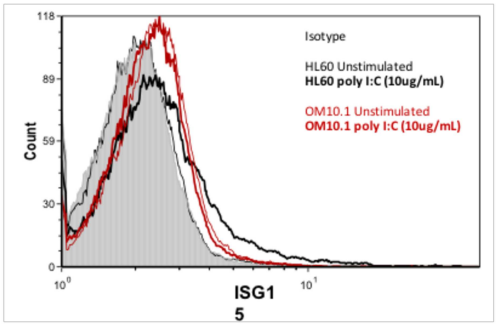
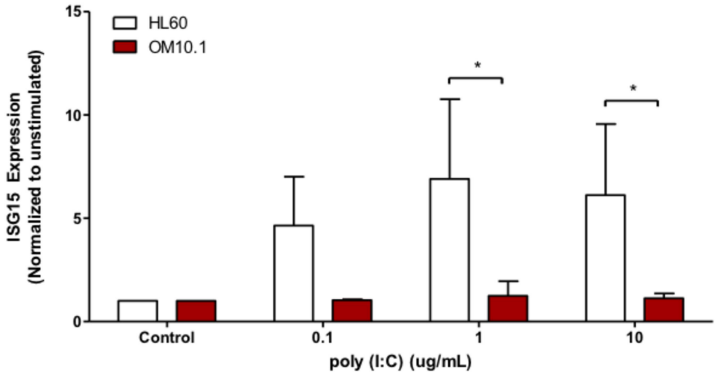

A.
U937
U1
IFNα/β Secretion (U/ml)
0.75
0.50
0.25
0.00
B.
U937
U1
IFNα/β Secretion
(Normalized to unstimulated)
poly(I:C) (ug/mL)
0
0.1
1
10
C.
Isotype
U937 Unstimulated
U937 poly I:C (10ug/mL)
U1 Unstimulated
U1 poly I:C (10ug/mL)
Count
ISG15
ISG15 Expression
(Normalized to unstimulated)
D.
PKR
PKR Expression
(Normalized to unstimulated)

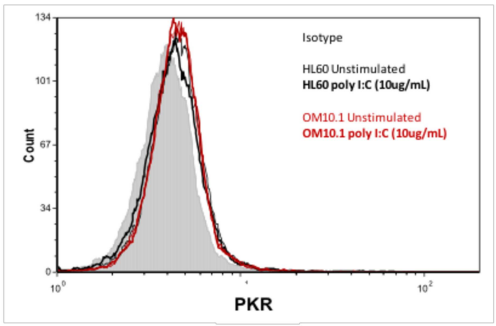
Figure 13. Responsiveness to poly(I:C) was defective in U1 cells, when compared to U937 cells. U937 and U1 cells were transfected (Lipofectamine®2000) with media alone or 0.1, 1, or 10ug/mL of poly(I:C) for 48 hours. **A.** Constitutive secretion of IFNα/β (n=5) was quantified in U937 and U1 cell-free supernatant using the HEK-Blue™ IFNα/β biologic assay (dashed line denotes lower limit of detection of assay = 0.15U/mL). **B.** Induction of IFNα/β secretion by U937 and U1 cells was quantified following poly(I:C) transfection (n=5). Poly(I:C) induced expression of **C.** ISG15 (n=5) and **D.** PKR (n=5), as measured by flow cytometry, was quantified in U937 and U1 cells. Representative histogram and cumulative summary of poly(I:C)-induced ISG expression is shown. n values represent separate biological replicates. † p=0.03, ‡ p=0.0004 as measured by one-way ANOVA and p<0.05 by pairwise Dunnett's Test compared to unstimulated cells. * p<0.01, ** p<0.001, *** p<0.05 by Two-way ANOVA with Bonferroni post-test for multiple comparisons; Data represent mean ± SEM.

A.


Isotype
HL60 Unstimulated
HL60 poly I:C (10ug/mL)
OM10.1 Unstimulated
OM10.1 poly I:C (10ug/mL)
Count
ISG15


HL60
OM10.1
ISG15 Expression (Normalized to unstimulated)
Control
0.1
1
10
poly (I:C) (ug/mL)

B.


Isotype
HL60 Unstimulated
HL60 poly I:C (10ug/mL)
OM10.1 Unstimulated
OM10.1 poly I:C (10ug/mL)
Count
PKR

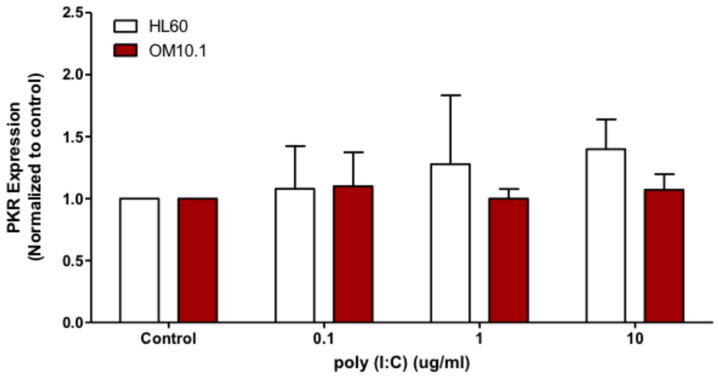

HL60
OM10.1
PKR Expression (Normalized to control)
Control
0.1
1
10
poly (I:C) (ug/ml)

Figure 14. Poly(I:C)-induced ISG15 expression is impaired in OM10.1 cells relative to HL60 cells. HL60 and OM10.1 cells were transfected (Lipofectamine®2000) with media alone or 0.1, 1, or 10ug/mL of poly(I:C) for 48 hours. Representative histogram and cumulative summary of poly(I:C)-induced ISG expression is shown. Poly(I:C) induced expression of **A.** ISG15 (n=4) and **B.** PKR (n=5), as measured by flow cytometry, was quantified in HL60 and OM10.1 cells. n values represent separate biological replicates. Non-significant induction in ISG15 or PKR was observed in HL60 and OM10.1 cells as measured by one-way ANOVA. * $p<0.01$ by Two-way ANOVA with Bonferroni post-test for multiple comparisons; Data represent mean ± SEM.

3.4. Discussion

Utilizing two independent cell line models of HIV-latency, widespread defects in IFN-I responses, including impaired secretion of IFNα/β cytokines and expression of IFNAR1, MHC-I, ISG15, and PKR, were demonstrated following exogenous IFNα or poly(I:C) treatment. These impairments in IFN-I responsiveness within latently HIV-1 infected cells represent a novel finding with potential implications in the context of latent HIV-1 infection.

3.4.1. Implications of impaired IFN-I responses in establishment and/or maintenance of latent HIV-1 infection

Significant reduction in the constitutive expression of IFNAR1 was observed in both the U1 and OM10.1 cells. As reported by Sandler and colleagues, blockade of IFNAR1 in SIV-infected rhesus macaques (RM) resulted in a substantial impairment of ISG expression *in vivo*, particularly in the pathways associated with PRRs (Sandler et al., 2014). Consistent with this, lower expression of IFNAR1 within latently HIV-1 infected cells may represent a unique mechanism by which HIV-1 escapes the early IFN-I response and establishes/maintains the latent reservoir. Similarly, lower constitutive expression and induction of MHC-I was reported in the latently HIV-1 infected cell lines. The downregulation of MHC-I by the HIV-1 Nef protein is frequently observed during productive HIV-1 infection, leading to impaired viral antigen presentation and escape from cytotoxic T lymphocyte (CTL) responses (Foster et al., 2011). Defects in MHC-I induction in latently infected cells may, in a similar manner, promote immune evasion. In

accordance with this, Ostrowski and colleagues are investigating the use of Nef inhibitors as a strategy to enhance the recognition and CTL-mediated killing of latently HIV-1 infected cells (Smithgall and Thomas, 2013).

Significant impairments in the induction of ISG15 and PKR were also observed in response to exogenous IFNα and poly(I:C) treatment. ISG15 plays critical antiviral roles both through the regulation of IFN-I pathways, as well as through direct inhibition of HIV-1 proteins by a process termed ISGylation (Pincetic et al., 2010; Zhao et al., 2013). Impaired expression of ISG15 in latently HIV-infected U1 and OM10.1 cells may accentuate the downstream defects in the IFN-I pathways crucial for antiviral defense. Similarly, PKR is an important RNA-sensing PRR that is able to elicit numerous antiviral effects, including the inhibition of viral protein translation and activation of downstream IFN-I signalling cascades (Clerzius et al., 2010; Stetson and Medzhitov, 2006). Lower levels of PKR within latently HIV-1 infected cells may contribute to abnormalities in viral sensing and innate immune responses to HIV-1 infection.

The observed impairment in IFNα-induced ISG expression in latently HIV-infected U1 and OM10.1 cells compared to healthy parental controls can potentially be explained by differences in constitutive IFNAR1 expression (Figure 9A). To address this possibility, cell lines were transfected with the synthetic dsRNA analog, poly(I:C). Recognition of poly(I:C) by various intracellular RNA-sensing PRRs, including TLR3, RIG-I, MDA5, and PKR, has been shown to directly induce ISG expression (Thompson and Locarnini, 2007). Interestingly, significant defects in IFNα/β production, as well as ISG15 and PKR induction were observed in the latently HIV-1 infected U1 and OM10.1 cells, but not in U937 and

HL60 controls. This decrease in IFN-I induction is consistent with previously reported impairments in production of proinflammatory cytokines by U1 cells, but not U937 cells, in response to stimulation with poly(I:C) and R848 (Taura et al., 2015). Importantly, these defects in induction of ISGs following poly(I:C) stimulation highlight the disruption in the capacity of latently HIV-1 infected cell lines to sense viral PAMPs and induce an appropriate antiviral response. Interestingly, a similar dampening of responsiveness to viral PAMPs has also been demonstrated in pDCs and mDCs during acute HIV-1 infection (Fitzgerald-Bocarsly and Jacobs, 2010; Martinson et al., 2007). The potential presence of such defects *in vivo* within latently HIV-1 infected cells may contribute to the maintenance and expansion of the reservoir by creating an environment permissive to low level residual viral replication (Siliciano and Greene, 2011).

3.4.2. Potential mechanisms underlying the observed defects in IFN-I responsiveness

The underlying mechanism(s) for the defective IFN-I response observed within the latently HIV-infected cell line models employed have yet to be elucidated. Interestingly, similar defects in IFN-I pathways have been reported during productive HIV infection to be mediated by viral regulatory and accessory proteins including Tat, Vpu, Vif, and Nef. However, whether HIV-1 proteins are expressed in latently HIV-1 infected cells is at present controversial. Although latent HIV-1 infection in U1 and OM10.1 cells is characterized by minimal p24 antigen expression (Figure 8), low-level gene transcription (Butera et al., 1994) and expression of viral proteins, including those known to interfere with IFN-I signaling, may be present (Butera et al., 1994; Mousseau et al., 2015). A recent

report demonstrated that a Tat inhibitor could further suppress OM10.1 cells into a state of 'deep latency' marked by transcriptional silence, thereby suggesting that within models of HIV latency, viral proteins such as Tat may be present and functional (Mousseau et al., 2012). In addition, basal expression of Tat has been previously reported in several cell line models of latent infection (la Fuente et al., 2002).

The presence of viral transcripts in HIV-1 infected cells has also been demonstrated in the context of HIV-1 latency. In a primary $CD4^+$ T cell model of HIV latency, Pace and colleagues demonstrated the presence of low level Gag protein expression, as well as viral transcripts encoding *gag*, *env*, *vif*, *and tat/rev*, despite the absence of ongoing HIV-1 replication (Pace et al., 2012). Similarly, Siliciano and colleagues demonstrated that *tat* and *rev* transcripts were present, but strictly localized to the nucleus in resting $CD4^+$ T cells from HAART-treated patients (Lassen et al., 2006). Given the importance of post-transcriptional and translational blocks to latency, the presence of viral proteins in latently HIV-1 infected cells and the extent of impact on IFN-I pathways needs to be investigated further. Nevertheless, the observed impairment of IFN-I pathways reported in U1 and OM10.1 cells may in part be mediated by ongoing interactions between host and viral factors.

Alternatively, impairment in the IFN-I system may represent an early finding that contributes to the establishment of HIV-1 latency. If IFN-I defects are present at baseline in HIV-susceptible cells, these cells may be permissive to HIV-1 infection and establishment of a latent HIV-1 infection. On the other hand, HIV-1 infection may introduce permanent changes to the infected cell, resulting in IFN-I defects that persist

during latency. Consistent with this, Seu and colleagues utilized a kinome-based approach to demonstrate stable changes in signal transduction and transcription factor expression in T cells from HIV-infected individuals (Seu et al., 2015). They reported that the permanent alterations in cell signaling secondary to HIV-1 infection results in an anergic, unresponsive T cell phenotype, which may be critical to the establishment and maintenance of latent HIV-1 infection. Similar mechanisms may underlie the deficiency in IFN-I responsiveness reported here.

3.4.3. Importance of characterizing the IFN-I system using multiple models of HIV-1 latency

Despite similarities in defective IFN-I responses in both U1 and OM10.1 cell lines, the observed defects were not identical. A reduction in PKR production was observed in OM10.1 cells relative to HL60 cells following IFNα treatment. However, similar levels of PKR induction were identified between the U1 and U937 cells. This variability between latently infected cell lines may be attributable to the differences in the mechanisms underlying establishment and maintenance of latency.

Impaired expression and activation of PKR during productive HIV-1 infection has been previously shown to be mediated by HIV-1 Tat (Clerzius et al., 2010; McMillan et al., 1995). Mutation in the Tat protein represents the primary mechanism underlying the post-integration latency in U1 cells (Clerzius et al., 2010; Emiliani et al., 1998). As a result, defects in PKR induction in OM10.1 cells, but not in U1 cells, may be a direct consequence of the defective Tat protein within U1 cells. Basal expression of Tat has been observed in

both cell lines (la Fuente et al., 2002). Going forward, the activation and functionality of PKR within these cells should be investigated further. Nevertheless, this difference between cell lines highlights the importance of evaluating IFN-I pathways using more than one model of HIV latency.

3.5. Future Directions

Defects in IFN-I responses were observed in latently HIV-1 infected U1 and OM10.1 cell lines, when compared to their respective HIV-uninfected U937 and HL60 parental cell lines. To our knowledge, these impairments represent a novel finding, which has not been previously reported in the context of HIV-1 latency.

Delineation of the mechanisms underlying the defects in IFN-I responses represent the next step in understanding features of HIV-1 latency. Potential mechanisms we propose include the ongoing suppression of IFN-I pathways by HIV-1 factors, the presence of inherent IFN-I defects in HIV-susceptible cells that permit establishment of latent HIV-1 infection, and/or permanent cellular changes that occur during HIV-1 infection and persist during latency. Within the cell line models, further characterization of the IFN-I signaling cascade and downstream ISGs on a transcriptional and translational level could provide insight into the extent and source of IFN-I defects. In addition, the potential presence of HIV-1 proteins, as well as the interplay between host and viral factors that may lead to IFN-I impairments will require further investigation. In parallel, the presence of permanent cellular changes in latent HIV-1 infection can be evaluated. One potential approach to test this hypothesis would be the use of CRISPR/cas9

technology to excise HIV-1 proviral DNA from the latently infected cell. Investigation of IFN-I defects before and after knockout of integrated HIV-1 DNA could provide insight into the role that integrated HIV-1 DNA and viral factors play in mediating IFN-I pathway impairments.

Given that U1 and OM10.1 cells are cell lines of myeloid origin, the use of a similar framework to delineate aspects of IFN-I pathways within lymphocytic cell line models of HIV-1 latency may be valuable (Spina et al., 2013). Additionally, a critical future direction will be the translation of the current work to *in vitro* and *ex vivo* primary cell models of HIV-1 latency, as very few studies have directly characterized IFN-I pathways and signaling in this context. The major challenge to such work is the rarity of latently HIV-1 infected cells *in vivo* (~0.1-1 IUPM per million resting $CD4^+$ T cells) (Siliciano et al., 2003) and the lack of phenotypic markers that facilitate selective enrichment of latently infected cells (Brooks and Zack, 2002). Consequently, characterization of IFN-I pathways and ISGs in HIV-1 infection has been restricted to investigating genome wide mRNA expression on a population level (Rotger et al., 2010).

Delineation of IFN-I responses in primary cell models of latency, using the methods described in this chapter, is limited by assay sensitivity, as well as the masking of potential defects by uninfected bystander cells that represent the majority of the HIV-infected PBMC population. Novel innovations such as dual reporter HIV-1 virus constructs may be useful tools in further understanding IFN-I pathways in primary cell models of HIV latency (Calvanese et al., 2013; Lassen et al., 2012). The dual reporter HIV-1 viruses constitutively express a stable, fluorescent protein such as mCherry upon integration into the human

genome, as well as a second fluorescent protein such as luciferase upon initiation of HIV-1 replication. This allows for identification of cells that are latently infected, as well as quantification of ongoing viral replication and/or reactivation of HIV-1 from latency. The use of such viral constructs may allow for enrichment of latently infected cells, as well as facilitate characterization of differences in IFN-I signaling between latently HIV-1 infected cells and the uninfected bystander cells (Lassen et al., 2012) .

3.6. Conclusion

This is the first report to describe significant impairments in IFN-I responses within the latently HIV-1 infected U1 and OM10.1 cells, relative to the uninfected parental U937 and HL60 cells, respectively. The defects in IFN-I signaling and responsiveness reported here may not only facilitate further understanding of HIV-1 latency, but also support the identification of potential therapeutic targets to selectively eliminate the latent HIV-1 reservoir *in vivo*.

Chapter 4: Latently HIV-1 infected cell lines are sensitive to VSVΔ51 and MG1 infection and cytopathic effects

4.1. Introduction

4.1.1. Exploiting defects in IFN-I responses using oncolytic viruses, VSVΔ51 and MG1, to target and kill latently HIV-1 infected cells

Extensive defects in IFN-I signaling due to viral countermeasures have been reported during ongoing HIV-1 replication. Similar impairments in IFN-I responsiveness, including IFNα/β production and expression of IFNAR1, MHC-I, ISG15, and PKR, were observed in the latently HIV-1 infected U1 and OM10.1 cells. Defects in IFN-I pathways may therefore represent a viable target for therapeutic strategies designed to eliminate the latent HIV-1 reservoir.

Impairments similar to those observed in HIV-1 infected cells have also been reported in numerous human cancers (Critchley-Thorne et al., 2009), including global defects in IFN-I signaling and ISG expression. This led to the identification of a number of *Rhabdoviridae*, including the vesicular stomatitis virus (VSV) and maraba virus, which function as potent oncolytic agents capable of selectively infecting and replicating within tumors with defective IFN-I responses (Stojdl et al., 2003). The genetically engineered forms of the two viruses, VSVΔ51 and MG1, have been shown to effectively clear IFN-I defective tumors *in vivo* with minimal toxicity (Brun et al., 2010; Lichty et al., 2014; Pol et al., 2014b; Stojdl et al., 2000b), supporting their potential use as anti-cancer therapies (Brun et al., 2010; Stojdl et al., 2000b).

These impairments in IFN-I pathways form the crucial link between latent HIV-1 infection and cancers. Therefore, we investigated the capacity of VSVΔ51 and MG1 to selectively target and kill the latently HIV-1 infected U1 and OM10.1 cells.

4.1.2. Combinatorial approaches to potentiate OV infection in latently HIV-1 infected cells

As previously described, a significant number of defects within IFN-I pathways are mediated by HIV-1 proteins and their interaction with host machinery (Britto et al., 2013; Clerzius et al., 2010; Doehle et al., 2012; Li et al., 2016a; Solis et al., 2011). In line with this, it was postulated that HIV-1 reactivation and the subsequent expression of viral factors may result in further dampening of the IFN-I defects observed within latently infected cells (see Chapter 3). HIV-1 reactivation may therefore enhance OV targeting and cytopathic effects.

Within the HIV-1 cure agenda, the current focus on the "shock and kill" approach has led to the investigation of several HIV-1 reactivation strategies that can be evaluated in combination with OV infection (Laird et al., 2015). Histone deacetylase inhibitors (HDACi), such as suberoylanilide hydroxamic acid (SAHA or Vorinostat), represent intriguing candidates as they have been reported to be safe (Archin et al., 2014) and effective in reactivating viral replication within latently HIV-1 infected cells *in vitro* and *in vivo* (Archin et al., 2012; Rasmussen et al., 2014; Wei et al., 2014). Interestingly, HDACi are also capable of modulating ISG expression and IFN-I responses (Chang et al., 2004). SAHA has been show to selectively potentiate VSVΔ51 infection and killing in tumor cells

by dampening pre-existing impairments in IFN-I induction and ISG expression (Nguyên et al., 2008a; Shulak et al., 2014). As a result, the use of SAHA as a strategy to selectively induce HIV-1 reactivation and augment OV infection in U1 and OM10.1 cells was explored.

4.2. Hypothesis

4.2.1. Latently HIV-1 infected U1 and OM10.1 cells are more susceptible to VSVΔ51 and MG1 infection and cytopathic effects than the HIV-uninfected parental U937 and HL60 cells.

4.2.2. SAHA enhances the infection and cytopathic effects of VSVΔ51 and MG1 in U1 and OM10.1 cells, but not in HIV-uninfected U937 and HL60 cells.

4.3. Results

4.3.1. Significantly higher levels of OV infection and cytopathic effects are observed in latently HIV-1 infected cell lines

First, the capacity of VSVΔ51 and MG1 to infect and kill latently HIV-1 infected cell lines was assessed. U1 and OM10.1 cells, as well as the respective parental HIV-uninfected U937 and HL60 cells were infected with VSVΔ51 or MG1 at various multiplicity of infection (MOI: 0.00001-0.1) for 12 to 28 hours. Oncolytic virus infection was assessed by measuring GFP expression by flow cytometry and cell survival was evaluated by AlamarBlue® and MTT assays.

Significantly greater VSVΔ51 infection was observed in the latently HIV-infected U1 (Figure 15A and 15B) and OM10.1 cells (Figure 16A and 16B) in a dose- and time-

dependent manner, when compared to their uninfected U937 and HL60 parental controls. However, no significant difference in VSVΔ51-induced cell death was observed in either the U937/U1 (Figure 15C and 15D) or the HL60/OM10.1 cell line pairs (Figure 16C and 16D).

Similarly, U1 (Figure 17A and 17B) and OM10.1 cells (Figure 18A and 18B) were significantly more susceptible than the respective U937 and HL60 controls to MG1 infection. In contrast to VSVΔ51, however, MG1 infection resulted in significant viral cytopathic effects in U1 (Figure 17 C and 17D) and OM10.1 cells (Figure 18C and 18D), with minimal cell death observed in the HIV-uninfected U937 and HL60 cells.

In summary, the latently HIV-1 infected U1 and OM10.1 cells were significantly more sensitive to VSVΔ51 and MG1 infection, as well as MG1-induced cytopathic effects than the HIV-uninfected U937 and HL60 parental cells.

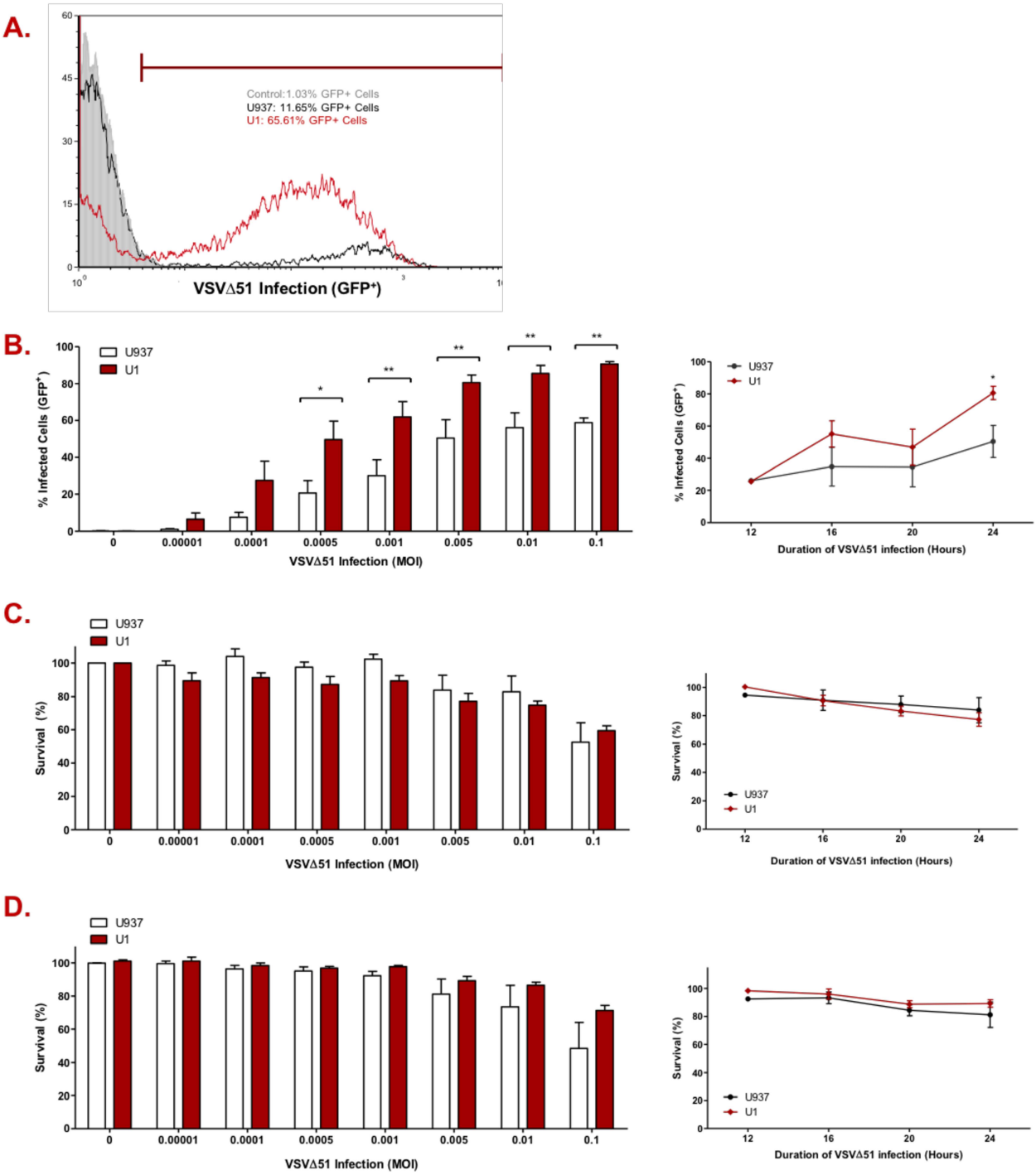

A.
Control:1.03% GFP+ Cells
U937: 11.65% GFP+ Cells
U1: 65.61% GFP+ Cells
VSVΔ51 Infection (GFP+)
B.
U937
U1
% Infected Cells (GFP+)
VSVΔ51 Infection (MOI)
Duration of VSVΔ51 infection (Hours)
C.
Survival (%)
D.

Figure 15. Latently HIV-infected U1 cells are significantly more susceptible than the HIV-uninfected U937 cells to infection by VSVΔ51. U1 and U937 cells were infected with VSVΔ51 at indicated MOI and time points. OV infection was quantified by measuring GFP expression by flow cytometry. Cell survival was assessed by Alamar Blue (Panel C) and MTT (Panel D) assays. **A.** Representative histogram demonstrating VSVΔ51 infection of U937 and U1 cells at MOI 0.005 at 20 hours post-infection. **B.** VSVΔ51 infection (GFP^+ cells) of U937 and U1 cells was performed at indicated MOI following 20 hours of infection (n=6; left panel), as well as at indicated time points following infection at MOI 0.005 (n=8; right panel). Cell death was quantified in both U937 and U1 cells following 20 hours of VSVΔ51 infection at indicated MOI (n=4; left panel) and at indicated time points following infection at MOI 0.005 (n=4; right panel) by **C.** Alamar Blue assay and **D.** MTT assay. n values represent separate biological replicates. *p<0.05; **p<0.01, ***p<0.001 by Two-way ANOVA with Bonferroni post-test for multiple comparisons between U937 and U1 cells; Data represent mean ± SEM.

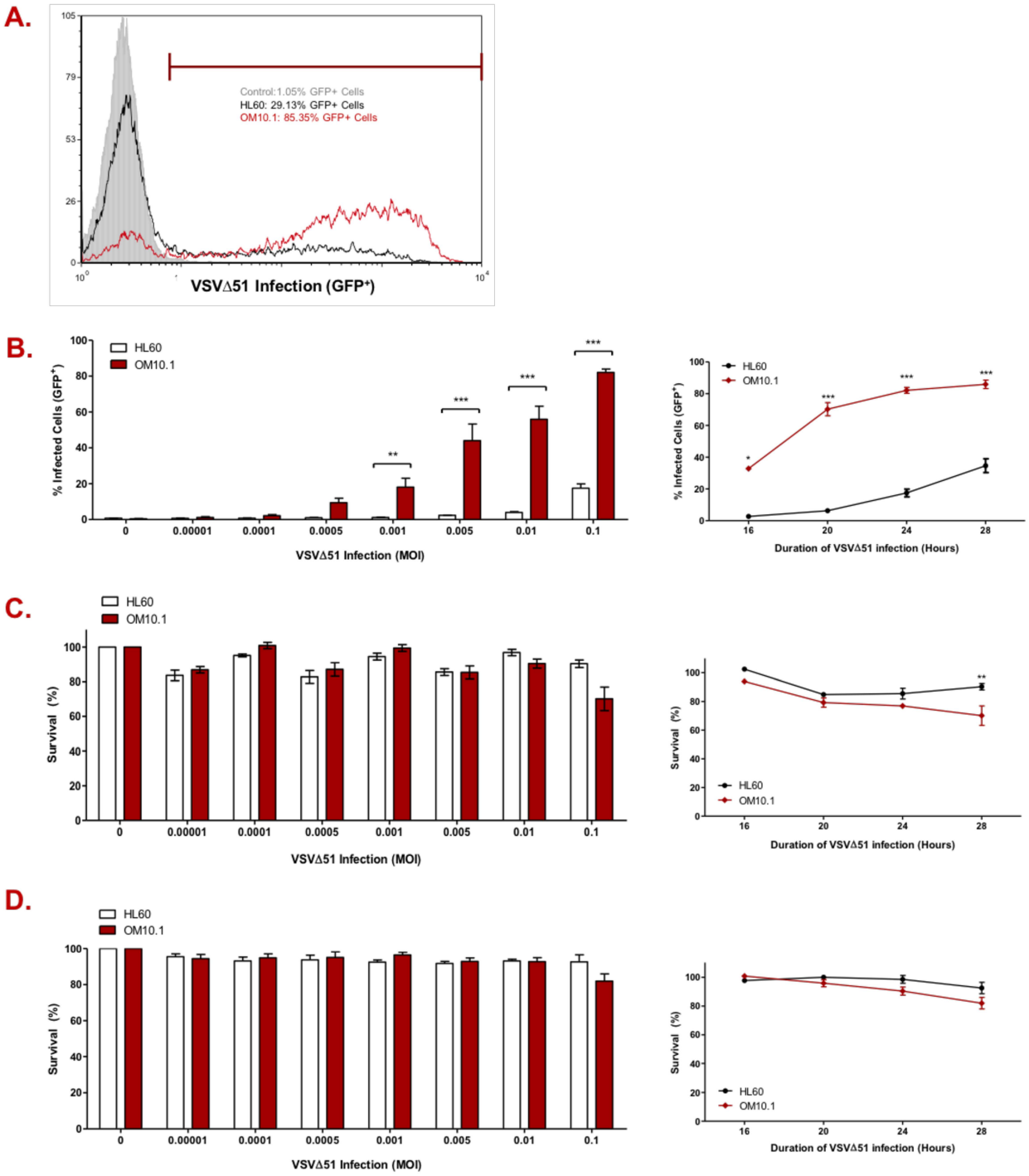

A.
Control:1.05% GFP+ Cells
HL60: 29.13% GFP+ Cells
OM10.1: 85.35% GFP+ Cells
VSVΔ51 Infection (GFP+)
B.
HL60
OM10.1
% Infected Cells (GFP+)
VSVΔ51 Infection (MOI)
Duration of VSVΔ51 infection (Hours)
C.
Survival (%)
D.

Figure 16. Significantly higher levels of VSVΔ51 infection were observed in the latently HIV-infected OM10.1 cells relative to HIV-uninfected HL60 cells. HL60 and OM10.1 cells were infected with VSVΔ51 at indicated MOI and time points. OV infection was quantified by measuring GFP expression by flow cytometry. Cell survival was assessed by Alamar Blue (Panel C) and MTT (Panel D) assays. **A.** Representative histogram demonstrating VSVΔ51 infection of HL60 and OM10.1 cells at MOI 0.1 at 24 hours post-infection. **B.** VSVΔ51 infection (GFP^+ cells) in HL60 and OM10.1 cells was quantified at indicated MOI following 24 hours of infection (n=7; left figure), as well as at indicated time points following infection at MOI 0.1 (n=7; right figure). Cell death was measured in HL60 and OM10.1 cells following 28 hours of VSVΔ51 infection at indicated MOI (n=5; left figure) and at indicated time points following infection at MOI 0.1 (n=5; right figure) by **C.** Alamar Blue assay and **D.** MTT assay. n values represent separate biological replicates. *p<0.05; **p<0.01, ***p<0.001 by Two-way ANOVA with Bonferroni post-test for multiple comparisons between HL60 and OM10.1 cells; Data represent mean ± SEM.

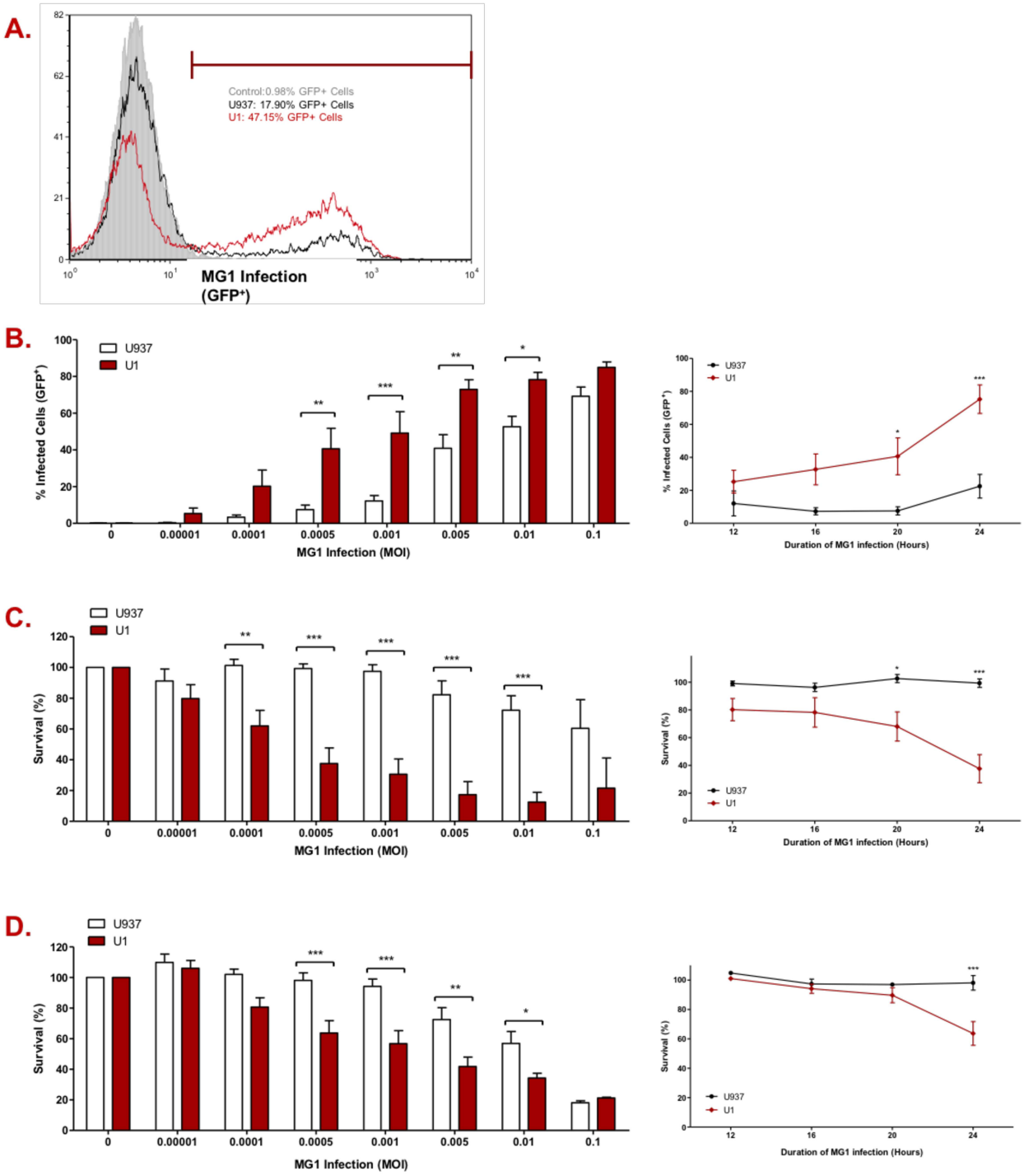

A.
Control:0.98% GFP+ Cells
U937: 17.90% GFP+ Cells
U1: 47.15% GFP+ Cells
MG1 Infection (GFP+)
B.
U937
U1
% Infected Cells (GFP+)
MG1 Infection (MOI)
Duration of MG1 infection (Hours)
C.
Survival (%)
MG1 Infection (MOI)
Duration of MG1 infection (Hours)
D.
Survival (%)
MG1 Infection (MOI)
Duration of MG1 infection (Hours)

Figure 17. Latently HIV-infected U1 cells are significantly more susceptible than HIV-uninfected U937 cells to MG1 infection and cytopathic effects. U1 and U937 cells were infected with MG1 at indicated MOI and time points. OV infection was quantified by measuring GFP expression by flow cytometry. Cell survival was assessed by Alamar Blue (Panel C) and MTT (Panel D) assays. **A.** Representative histogram demonstrating MG1 infection of U937 and U1 cells at MOI 0.0005 at 20 hours post-infection. **B.** MG1 infection was quantified in U937 and U1 at indicated MOI following 20 hours of infection (n=6; left figure), as well as at indicated time points following infection at MOI 0.0005 (n=9; right figure). Cell death was measured in U937 and U1 cells following 20 hours of MG1 infection at indicated MOI (n=6; left figure) and at indicated time points following infection at MOI 0.0005 (n=7; right figure) by **C.** Alamar Blue assay and **D.** MTT assay. n values represent separate biological replicates. *p<0.05; **p<0.01, ***p<0.001 by Two-way ANOVA with Bonferroni post-test for multiple comparisons between U937 and U1 cells; Data represent mean ± SEM.

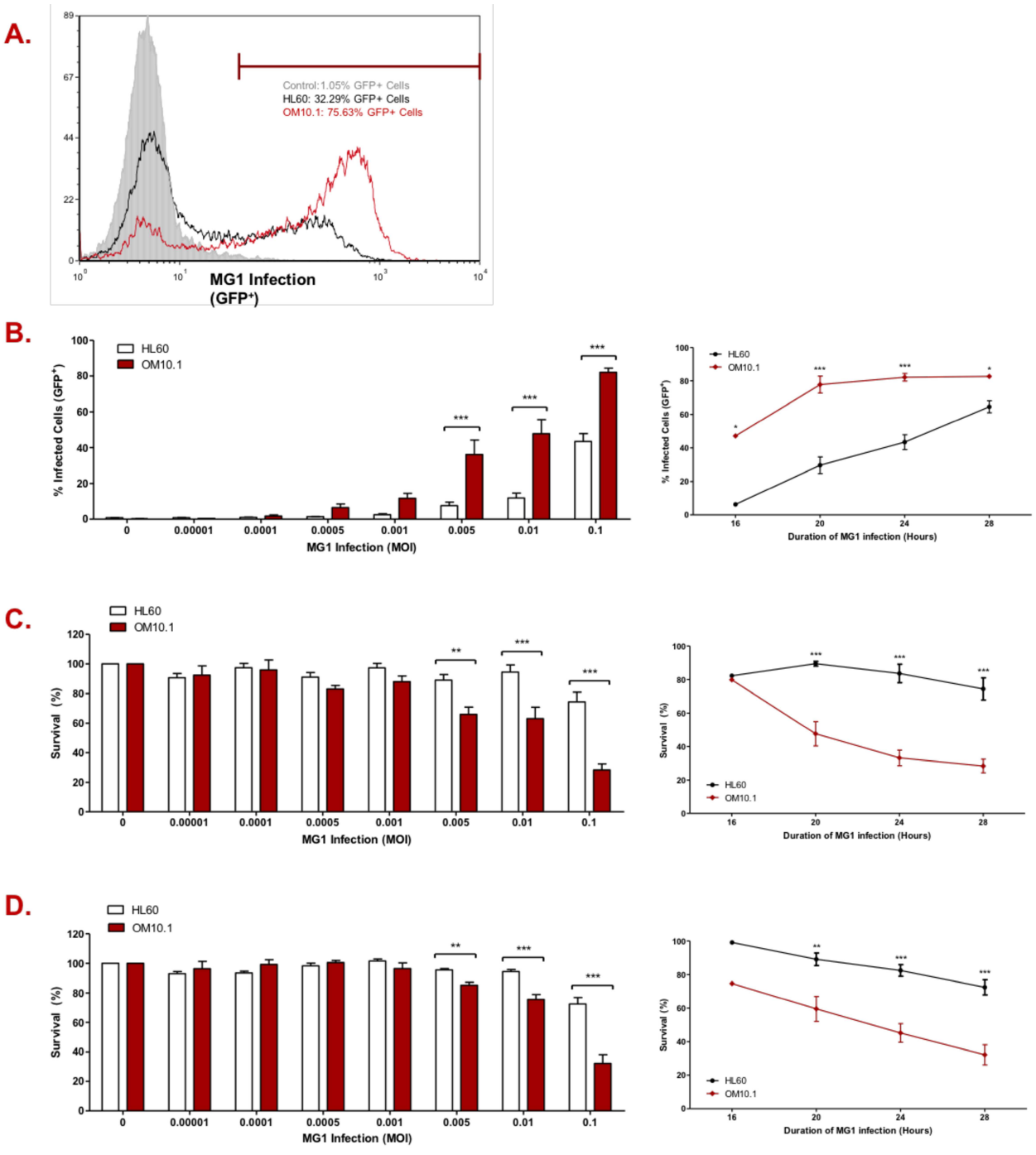
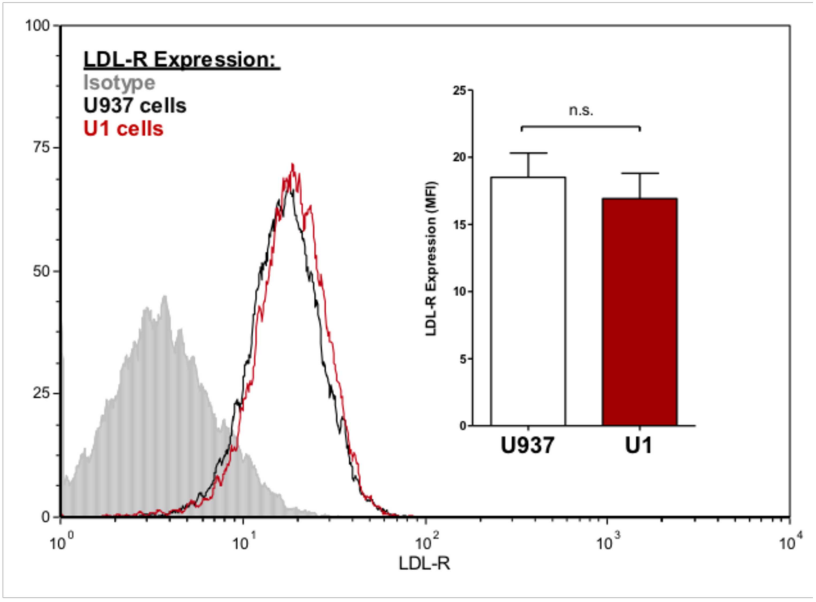

A.
Control:1.05% GFP+ Cells
HL60: 32.29% GFP+ Cells
OM10.1: 75.63% GFP+ Cells
MG1 Infection (GFP+)
B.
HL60
OM10.1
% Infected Cells (GFP+)
MG1 Infection (MOI)
Duration of MG1 infection (Hours)
C.
Survival (%)
MG1 Infection (MOI)
Duration of MG1 infection (Hours)
D.
Survival (%)
MG1 Infection (MOI)
Duration of MG1 infection (Hours)

Figure 18. Significantly higher levels of MG1 infection and cell death are observed in the latently HIV-infected OM10.1 cells relative to HIV-uninfected HL60 cells. HL60 and OM10.1 cells were infected with MG1 at indicated MOI and time points. OV infection was quantified by measuring GFP expression by flow cytometry. Cell survival was assessed by Alamar Blue (Panel C) and MTT (Panel D) assays. **A.** Representative histogram demonstrating MG1 infection of HL60 and OM10.1 cells at MOI 0.1 at 24 hours post-infection. **B.** MG1 infection was quantified in HL60 and OM10.1 cells at indicated MOI following 24 hours of infection (left figure), as well as at indicated time points following infection at MOI 0.1 (right figure) (n=6). Cell death was assessed following 28 hours of MG1 infection at indicated MOI (n=5; left figure) and at indicated time points following infection at MOI 0.1 (n=5; right figure) by **C.** Alamar blue and **D.** MTT assays. n values represent separate biological replicates. *p<0.05; **p<0.01, ***p<0.001 by Two-way ANOVA with Bonferroni post-test for multiple comparisons between HL60 and OM10.1 cells; Data represent mean ± SEM.

4.3.2. Impaired secretion of IFNα is observed in U1 and OM10.1 cells infected with VSVΔ51 or MG1

To identify the mechanism(s) by which OV infection was enhanced in latently HIV-1 infected cell lines, pathways known to influence OV infectivity and virulence were characterized. Expression of low density lipoprotein receptor (LDL-R), identified to be the primary cellular receptor for VSVΔ51 and MG1 (Finkelshtein et al., 2013), was observed to be similar between U1 and U937 cells (Figure 19A), as well as between HL60 and OM10.1 cells (Figure 19B). Entry into cell cycle and rate of cellular proliferation have also been identified as critical factors that influence OV replication (Oliere et al., 2008). Proliferation after 24 hours in culture, as assessed by CFSE dilution assay, was not significantly different between latently HIV-1 infected and uninfected cell lines (Figure 20A and 20B). Additionally, synchronization of cell cycle by serum starvation (Figure 21A and 21B) (Rosner et al., 2013) was shown to have minimal effect on the qualitative pattern of VSVΔ51 or MG1 infection, with significantly greater infection observed in the U1 (Figure 21C) and OM10.1 cells (Figure 21D), than in the respective U937 and HL60 cells. Therefore, several cell-intrinsic factors known to influence OV infection were excluded as potential mechanisms mediating the enhanced sensitivity of U1 and OM10.1 cells to OV infection.

A.


LDL-R Expression:
Isotype
U937 cells
U1 cells
100
75
50
25
0
10^0
10^1
10^2
10^3
10^4
LDL-R
n.s.
LDL-R Expression (MFI)
25
20
15
10
5
0
U937
U1

B.

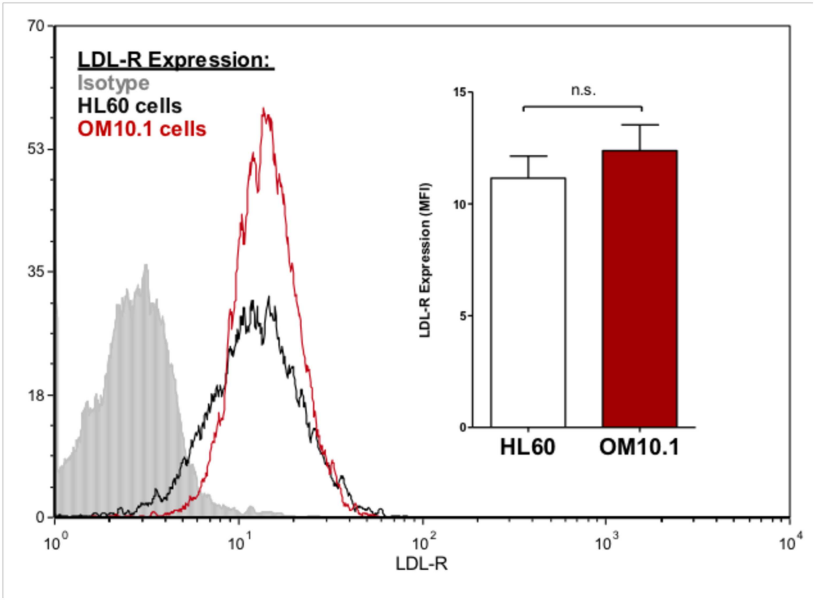
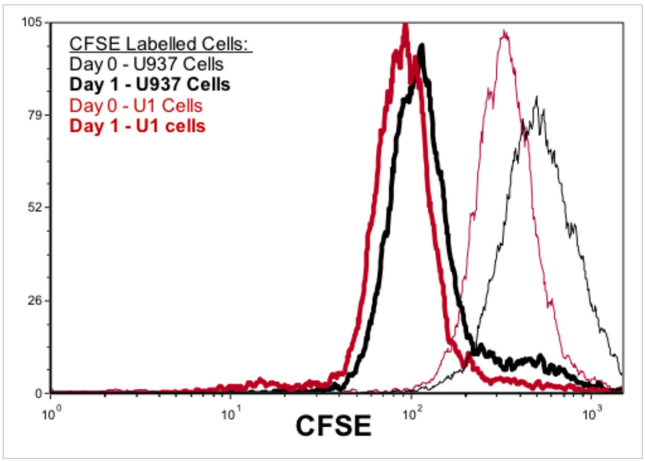
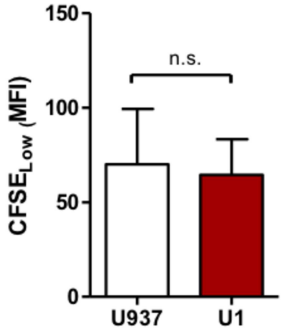

LDL-R Expression:
Isotype
HL60 cells
OM10.1 cells
70
53
35
18
0
10^0
10^1
10^2
10^3
10^4
LDL-R
n.s.
LDL-R Expression (MFI)
15
10
5
0
HL60
OM10.1

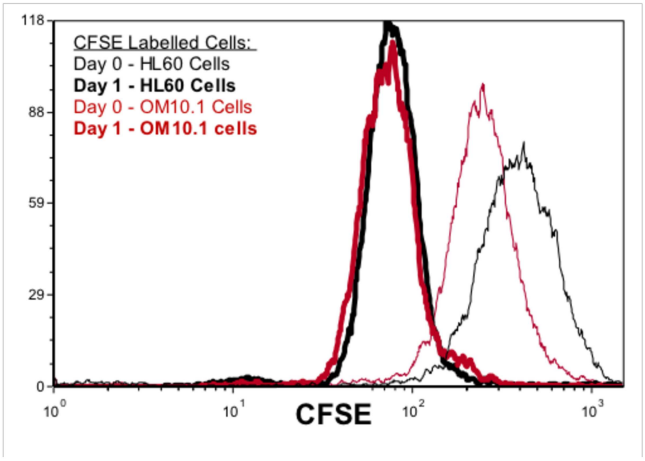
Figure 19. Level of LDL-R expression was similar between latently HIV-infected and HIV-uninfected cell lines. Surface expression of LDL-R was measured by flow cytometry in **A.** U937 and U1 cells (n=5) and **B.** HL60 and OM10.1 cells (n=5). n values represent separate biological replicates. n.s.: no significant difference in LDL-R expression between cell line pairs by unpaired T-test; Data represent mean ± SEM.

A.


CFSE Labelled Cells:
Day 0 - U937 Cells
Day 1 - U937 Cells
Day 0 - U1 Cells
Day 1 - U1 cells
105
79
52
26
0
10^0
10^1
10^2
10^3
CFSE


150
100
50
0
n.s.
$CFSE_{Low}$ (MFI)
U937
U1

B.


CFSE Labelled Cells:
Day 0 - HL60 Cells
Day 1 - HL60 Cells
Day 0 - OM10.1 Cells
Day 1 - OM10.1 cells
118
88
59
29
0
10^0
10^1
10^2
10^3
CFSE

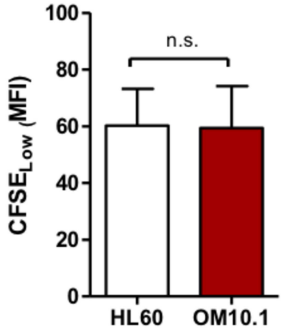

100
80
60
40
20
0
n.s.
$CFSE_{Low}$ (MFI)
HL60
OM10.1

Figure 20. The rate of cellular proliferation over 24 hours was similar between latently HIV-infected and uninfected cell lines. Cell lines were stained with CFSE and left in culture with complete medium for 24 hours. Rate of cell proliferation was assessed by quantifying the decrease in CFSE intensity ($CFSE_{low}$) by flow cytometry in **A.** U937 and U1 cells (n=3) and **B.** HL60 and OM10.1 cells (n=3). Representative histogram demonstrating CFSE intensity at 0 and 24 hours (left) and cumulative CFSE intensity at 24 hours (right) is shown. n values represent separate biological replicates. n.s.: no significant difference in CFSE intensity between cell line pairs by unpaired T-test; Data represent mean ± SEM.

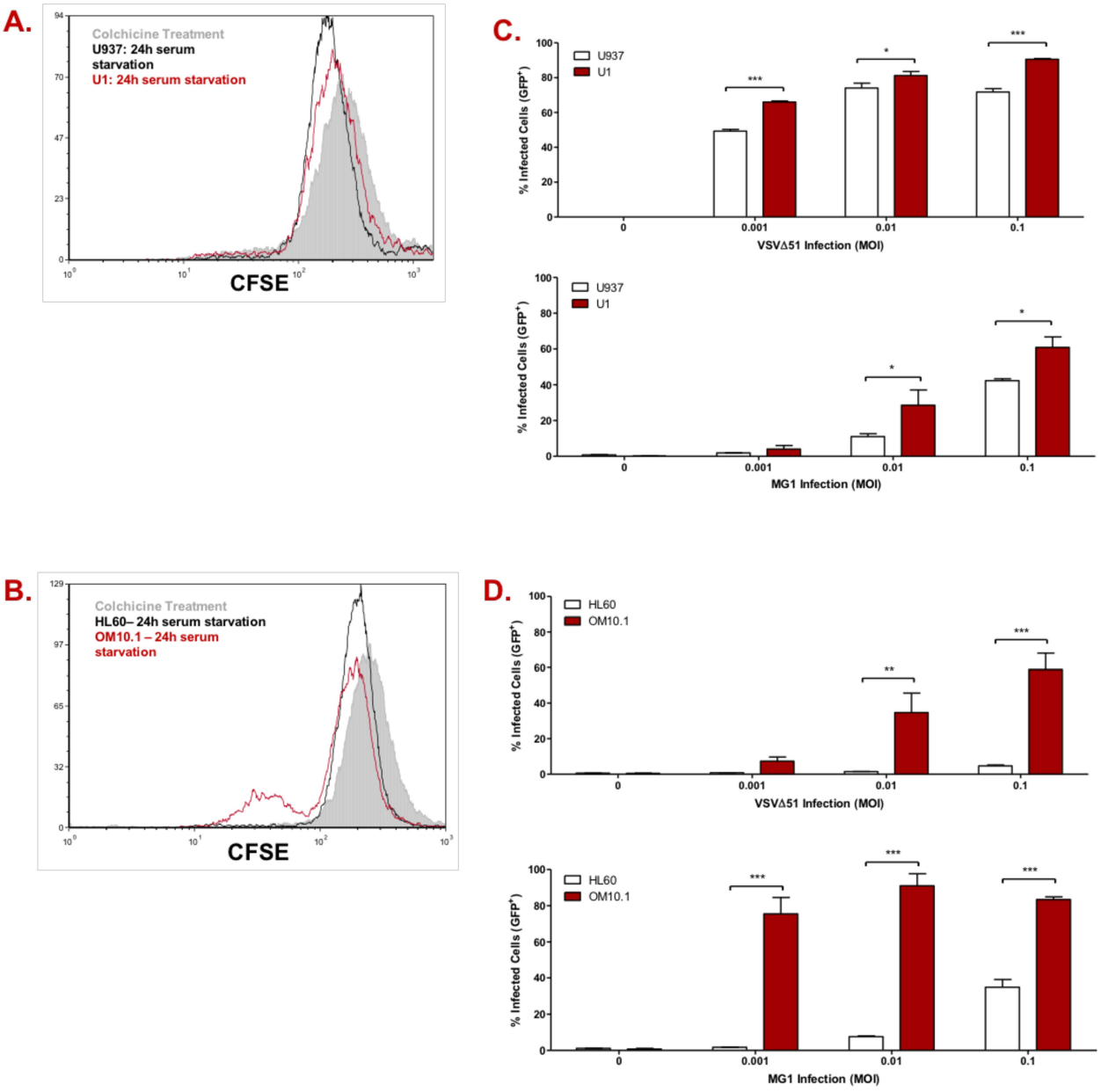
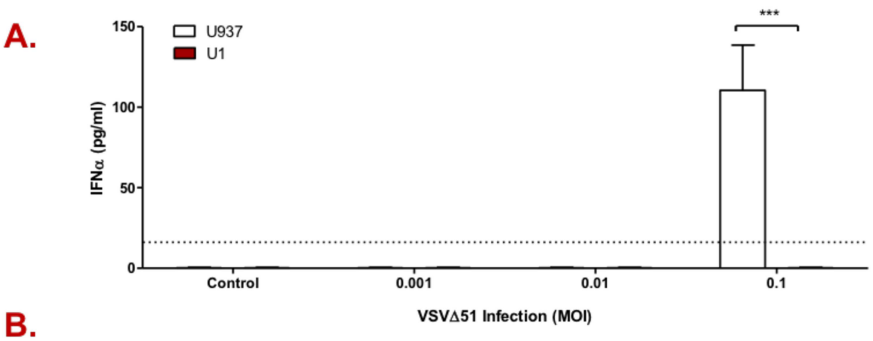
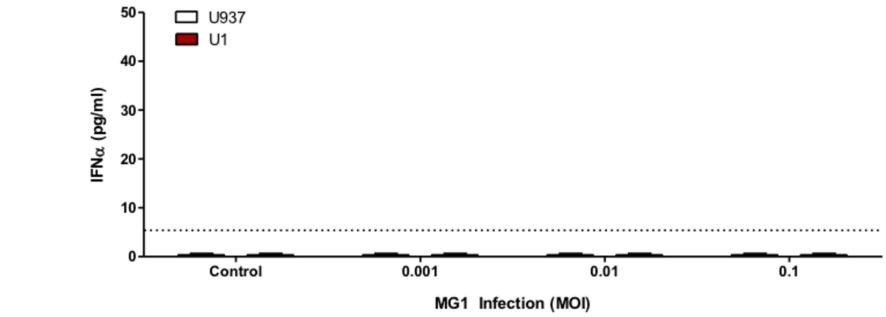
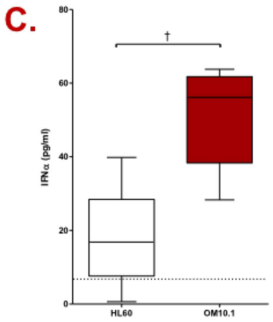

A.
Colchicine Treatment
U937: 24h serum starvation
U1: 24h serum starvation
CFSE
B.
Colchicine Treatment
HL60– 24h serum starvation
OM10.1 – 24h serum starvation
CFSE
C.
U937
U1
% Infected Cells (GFP+)
VSVΔ51 Infection (MOI)
MG1 Infection (MOI)
D.
HL60
OM10.1
% Infected Cells (GFP+)
VSVΔ51 Infection (MOI)
MG1 Infection (MOI)

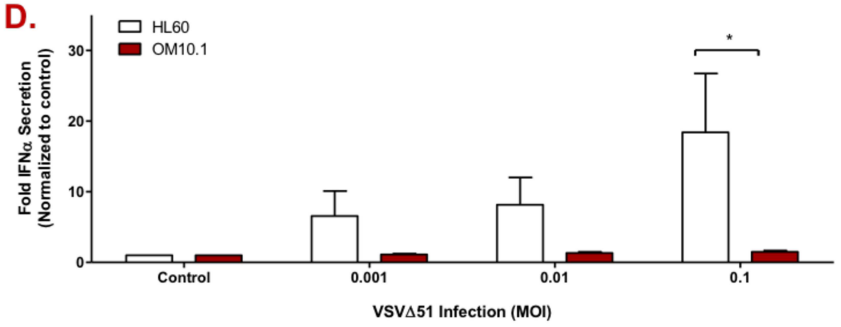
Figure 21. Cell cycle synchronization by serum starvation has no effect on the qualitative pattern of enhanced OV infection in latently HIV-infected cell lines. Synchronization of cell cycle was performed by incubating cell lines in serum-free medium (SFM) for 24 hours. Cell cycle synchronization of **A.** U937 and U1 cells (n=3) and **B.** HL60 and OM10.1 cells (n=3) was confirmed by CFSE assay; colchicine-treated cells were used as negative controls. **C.** U937 and U1 cells (n=3) and **D.** HL60 and OM10.1 (n=3) cells were then infected with VSVΔ51 (top panel) or MG1 (bottom panel) at the indicated MOI for 24 hours. n values represent separate biological replicates. *p<0.05; **p<0.01, ***p<0.001 by Two-way ANOVA with Bonferroni post-test for multiple comparisons between HL60 and OM10.1 cells; Data represent mean ± SEM.

Next, the IFN-I response to OV infection was evaluated by quantifying IFNα secretion in the culture supernatant of OV-infected cells. VSVΔ51 infection resulted in a detectable increase in IFNα secretion in U937 cells at MOI of 0.1, but not in U1 cells (Figure 22A). No IFNα was detected in either U937 or U1 culture supernatant in response to MG1 infection (Figure 22B). Higher basal level of IFNα was observed in OM10.1 culture supernatants relative to HL60 cells. Interestingly, however, both VSVΔ51 (Figure 22C) and MG1 infection (Figure 22D) resulted in a significant increase in IFNα secretion by the HIV-uninfected HL60 cells, but not by the OM10.1 cells. Therefore, impairments in IFNα secretion in response to VSVΔ51 and/or MG1 infection were observed in the latently HIV-1 infected U1 and OM10.1 cells, relative to the respective parental cell lines.


A.
U937
U1
IFNα (pg/ml)
150
100
50
0
***
Control
0.001
0.01
0.1
VSVΔ51 Infection (MOI)


B.
U937
U1
IFNα (pg/ml)
50
40
30
20
10
0
Control
0.001
0.01
0.1
MG1 Infection (MOI)


C.
†
IFNα (pg/ml)
HL60
OM10.1


D.
HL60
OM10.1
Fold IFNα Secretion
(Normalized to control)
30
20
10
0
*
Control
0.001
0.01
0.1
VSVΔ51 Infection (MOI)

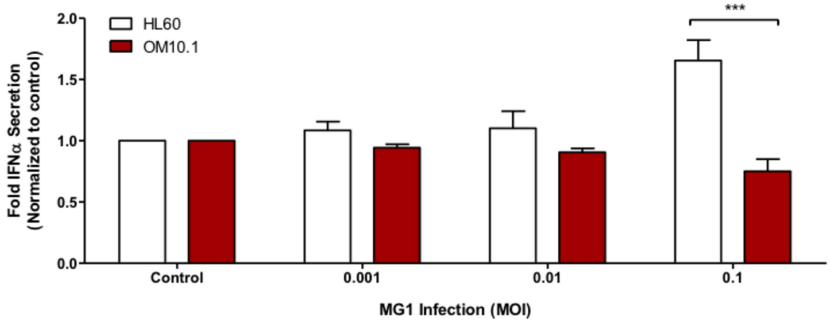

HL60
OM10.1
Fold IFNα Secretion
(Normalized to control)
2.0
1.5
1.0
0.5
0.0
***
Control
0.001
0.01
0.1
MG1 Infection (MOI)

Figure 22. Latently HIV-infected U1 and OM10.1 cells are impaired in their ability to secrete IFNα in response to VSVΔ51 or MG1 infection. Cell lines were infected with VSVΔ51 or MG1 at indicated MOI for 24 hours. Cell-free supernatants were collected and IFNα secretion was quantified by IFNα ELISA. Secretion of IFNα was quantified following **A.** VSVΔ51 infection (n=4) or **B.** MG1 infection (n=4) of U937 and U1 cells. **C.** Basal expression of IFNα was quantified using HL60 and OM10.1 cells (n=5). Finally, IFNα secretion following **D.** VSVΔ51 infection (n=3; top panel) or MG1 infection (n=3; bottom panel) of HL60 and OM10.1 cells was quantified. Dotted lines indicate the lower limit of detection of the IFNα ELISA (5pg/mL). † $p = 0.0055$ by unpaired T-test. *$p<0.05$; **$p<0.01$, ***$p<0.001$ by Two-way ANOVA with Bonferroni post-test for multiple comparisons between latently HIV-infected and uninfected cell lines; Data represent mean ± SEM.

4.3.3. SAHA stimulation of HIV-1 infected cell lines selectively enhanced OV infection and cytopathic effects, with a corresponding decrease in HIV-1 p24 production

To characterize the effect of HIV-1 reactivation by SAHA on OV infection and killing, both latently HIV-1 infected and uninfected cell lines were left unstimulated or stimulated with SAHA for 24 hours. HIV-1 reactivation was confirmed in both the U1 and OM10.1 cells by quantifying intracellular p24 protein expression by flow cytometry (Figure 23A and 23B).

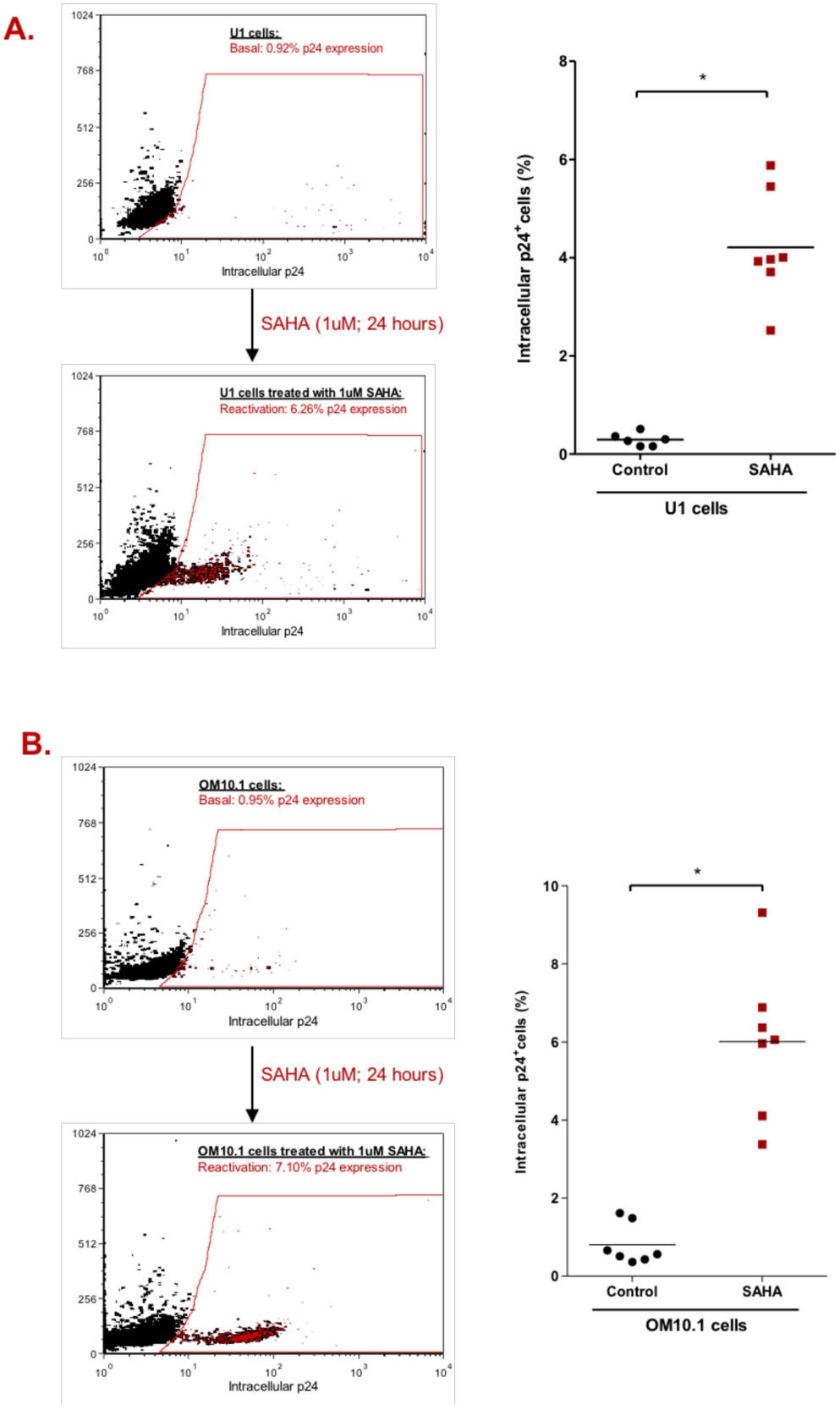

A.
U1 cells:
Basal: 0.92% p24 expression
Intracellular p24
SAHA (1uM; 24 hours)
U1 cells treated with 1uM SAHA:
Reactivation: 6.26% p24 expression
Intracellular p24
Intracellular p24+cells (%)
Control
SAHA
U1 cells
B.
OM10.1 cells:
Basal: 0.95% p24 expression
Intracellular p24
SAHA (1uM; 24 hours)
OM10.1 cells treated with 1uM SAHA:
Reactivation: 7.10% p24 expression
Intracellular p24
Intracellular p24+cells (%)
Control
SAHA
OM10.1 cells

Figure 23. Treatment of U1 and OM10.1 cells with SAHA (Vorinostat) results in reactivation of HIV-1 replication. Latently HIV-infected U1 and OM10.1 cells were treated with SAHA (1uM) for 24 hours and intracellular p24 expression was quantified by flow cytometry. Representative histogram and cumulative p24 expression following SAHA treatment is shown in **A.** U1 cells (n=7) and **B.** OM10.1 cells (n=7) relative to the untreated latently HIV-1 infected cell lines. n values represent separate biological replicates. *p<0.001 by unpaired T-test. Data represent mean ± SEM.

Unstimulated and SAHA stimulated U1 and OM10.1 cells, as well U937 and HL60 cells, were then infected with VSVΔ51 or MG1 (MOI 0.001-0.1) for 24 hours. Infection and cell survival were quantified as described above. Relative to unstimulated cells, SAHA stimulation resulted in a significant increase in VSVΔ51 infection in the latently infected U1 (Figure 24A and 24B) and OM10.1 (Figure 25A and 25B) cells, but not in the uninfected U937 and HL60 cells. Consistent with previous results, no significant VSVΔ51-induced cytopathic effects were observed in either the latently HIV-1 infected or uninfected cells, with or without SAHA stimulation (Figure 24C and Figure 25C).

SAHA stimulation also resulted in a significant enhancement of MG1 infection in both the U1 (Figure 26A and 26B) and OM10.1 cells (Figure 27A and 27B), but had no effect on the U937 and HL60 cells. In addition, compared to unstimulated controls, SAHA stimulation resulted in enhanced MG1-induced cytopathic effects in OM10.1 cells (Figure 27C), but not in U1 cells (Figure 26C). As with VSVΔ51 infection, no significant cell death was observed in either the unstimulated or SAHA stimulated U937 and HL60 cells infected with MG1 (Figure 26C and Figure 27C).

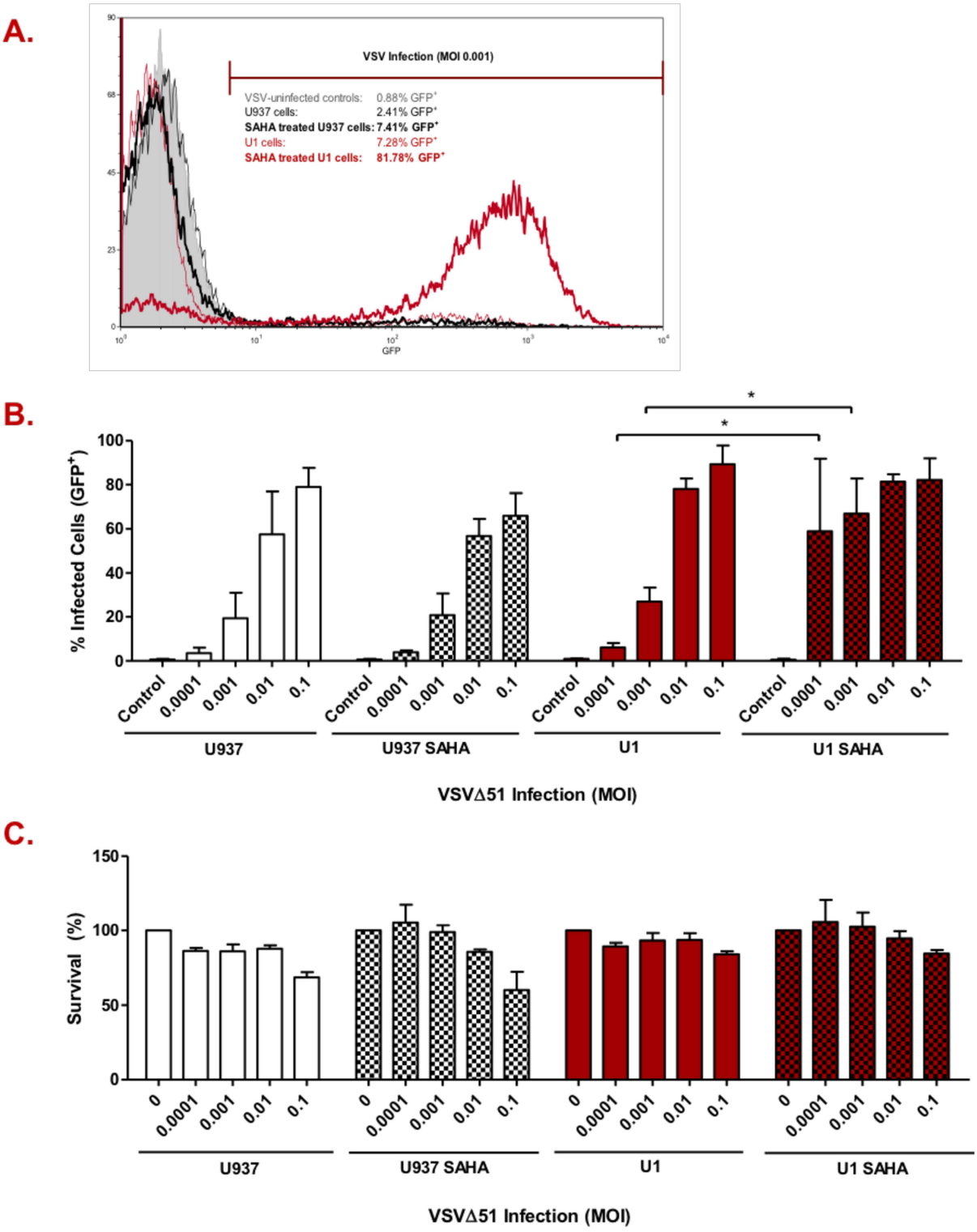
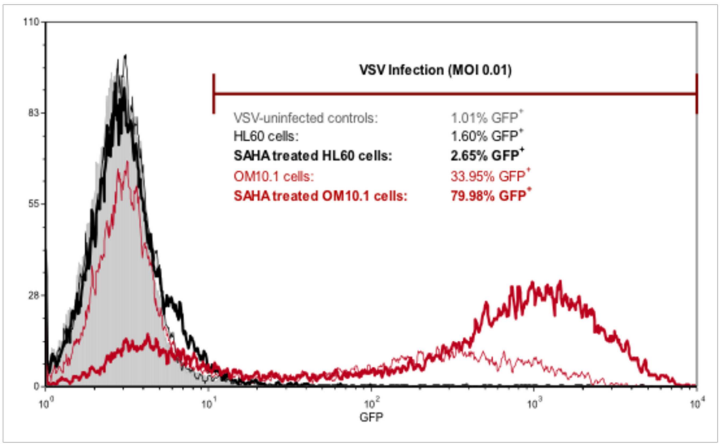
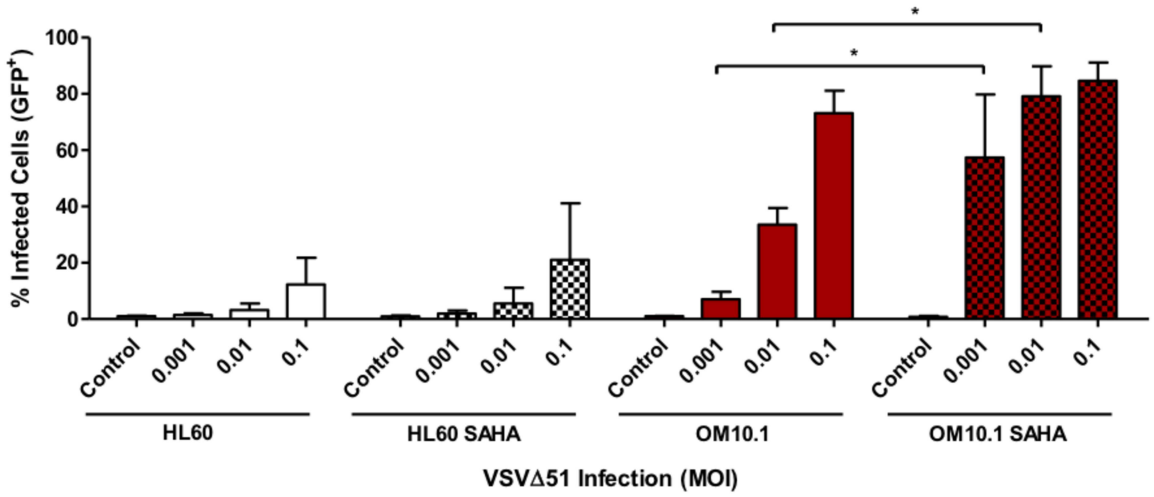

A.
VSV Infection (MOI 0.001)
VSV-uninfected controls: 0.88% GFP+
U937 cells: 2.41% GFP+
SAHA treated U937 cells: 7.41% GFP+
U1 cells: 7.28% GFP+
SAHA treated U1 cells: 81.78% GFP+
GFP
B.
% Infected Cells (GFP+)
Control
0.0001
0.001
0.01
0.1
U937
U937 SAHA
U1
U1 SAHA
VSVΔ51 Infection (MOI)
C.
Survival (%)
0
VSVΔ51 Infection (MOI)

Figure 24. SAHA stimulation results in a selective enhancement of VSVΔ51 infection in latently HIV-infected U1 cells, but not U937 cells. U937 and U1 cells were left untreated, or treated with 1uM SAHA for 24 hours prior to infection with VSVΔ51. Infection and cell survival were quantified as before following 24 hours of infection. **A.** Representative histogram demonstrating VSVΔ51 infection of U937 and U1 cells with and without SAHA stimulation. **B.** VSVΔ51 infection (n=4) and **C.** cell survival (n=4) in U937 and U1 cells with or without SAHA stimulation is shown. n values represent separate biological replicates. *p<0.001 by Two-way ANOVA with Bonferroni post-test for multiple comparisons U937 or U1 cells with or without SAHA treatment; Data represent mean ± SEM.

A.


VSV Infection (MOI 0.01)
VSV-uninfected controls: 1.01% GFP+
HL60 cells: 1.60% GFP+
SAHA treated HL60 cells: 2.65% GFP+
OM10.1 cells: 33.95% GFP+
SAHA treated OM10.1 cells: 79.98% GFP+
GFP

B.


% Infected Cells (GFP+)
Control
0.001
0.01
0.1
HL60
HL60 SAHA
OM10.1
OM10.1 SAHA
VSVΔ51 Infection (MOI)

C.

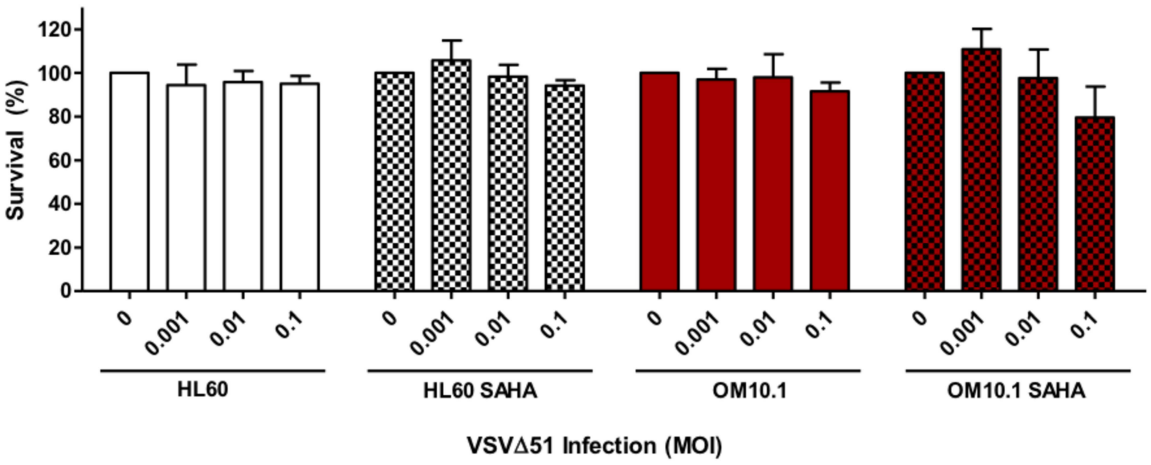
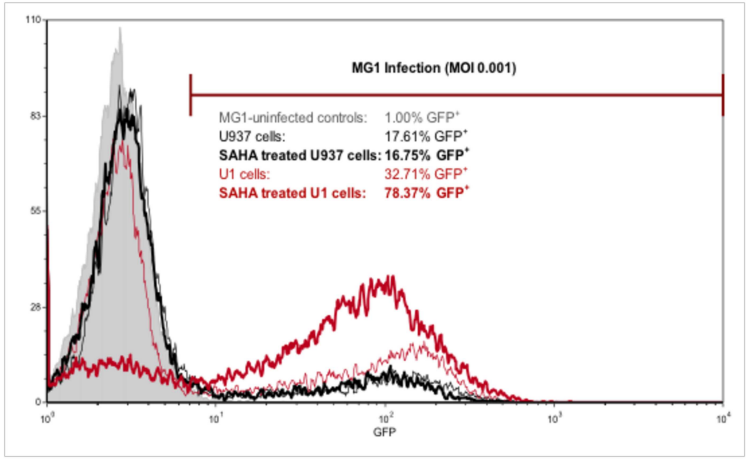
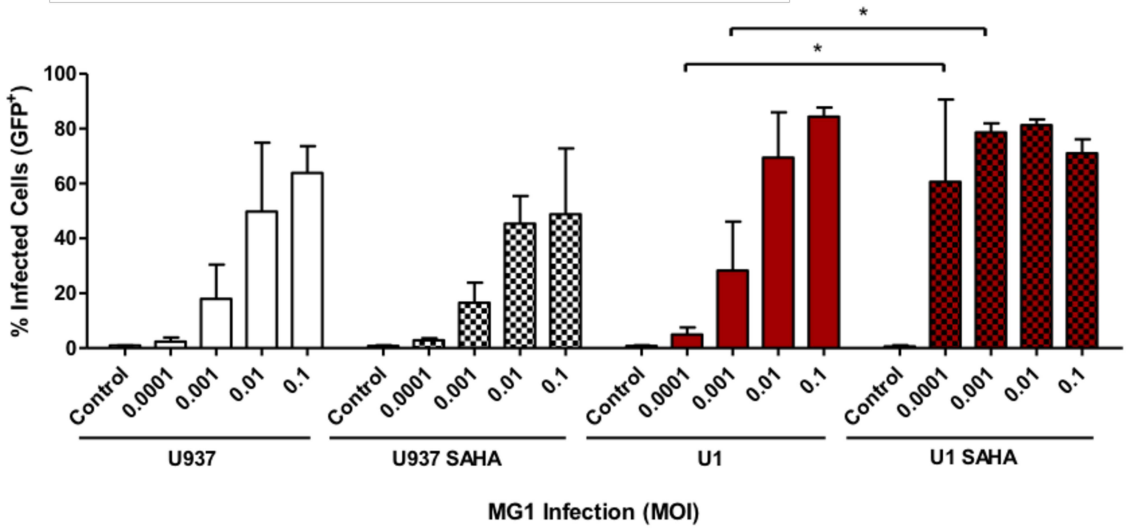

Survival (%)
0
0.001
0.01
0.1
HL60
HL60 SAHA
OM10.1
OM10.1 SAHA
VSVΔ51 Infection (MOI)

Figure 25. SAHA stimulation results in a selective enhancement of VSVΔ51 infection in latently HIV-infected OM10.1 cells, but not HL60 cells. HL60 and OM10.1 cells were left untreated, or treated with 1uM SAHA for 24 hours prior to infection with VSVΔ51. Infection and cell survival were quantified as before following 24 hours of infection. **A.** Representative histogram demonstrating VSVΔ51 infection of HL60 and OM10.1 cells with and without SAHA stimulation. **B.** VSVΔ51 infection (n=6) and **C.** cell survival (n=6) in HL60 and OM10.1 cells with or without SAHA stimulation is shown. n values represent separate biological replicates. *p<0.001 by Two-way ANOVA with Bonferroni post-test for multiple comparisons HL60 or OM10.1 cells with or without SAHA treatment; Data represent mean ± SEM.

A.


MG1 Infection (MOI 0.001)
MG1-uninfected controls: 1.00% GFP+
U937 cells: 17.61% GFP+
SAHA treated U937 cells: 16.75% GFP+
U1 cells: 32.71% GFP+
SAHA treated U1 cells: 78.37% GFP+
GFP

B.


% Infected Cells (GFP+)
Control
0.0001
0.001
0.01
0.1
U937
U937 SAHA
U1
U1 SAHA
MG1 Infection (MOI)

C.

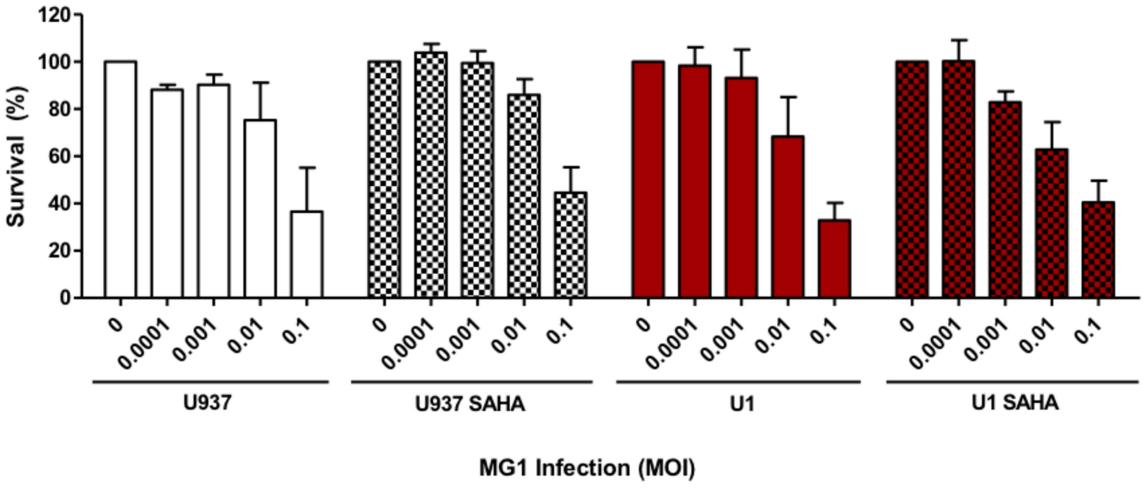

Survival (%)
0
0.0001
0.001
0.01
0.1
U937
U937 SAHA
U1
U1 SAHA
MG1 Infection (MOI)

Figure 26. SAHA stimulation increases the susceptibility of U1 cells, but not U937 cells, to MG1 infection. U937 and U1 cells were left untreated, or treated with 1uM SAHA for 24 hours prior to infection with MG1. Infection and cell survival were quantified as before following 24 hours of infection. **A.** Representative histogram demonstrating MG1 infection of U937 and U1 cells with and without SAHA stimulation. **B.** MG1 infection (n=5) and **C.** cell survival (n=5) in U937 and U1 cells with or without SAHA stimulation is shown. n values represent separate biological replicates. *$p<0.001$ by Two-way ANOVA with Bonferroni post-test for multiple comparisons U937 or U1 cells with or without SAHA treatment; Data represent mean ± SEM.

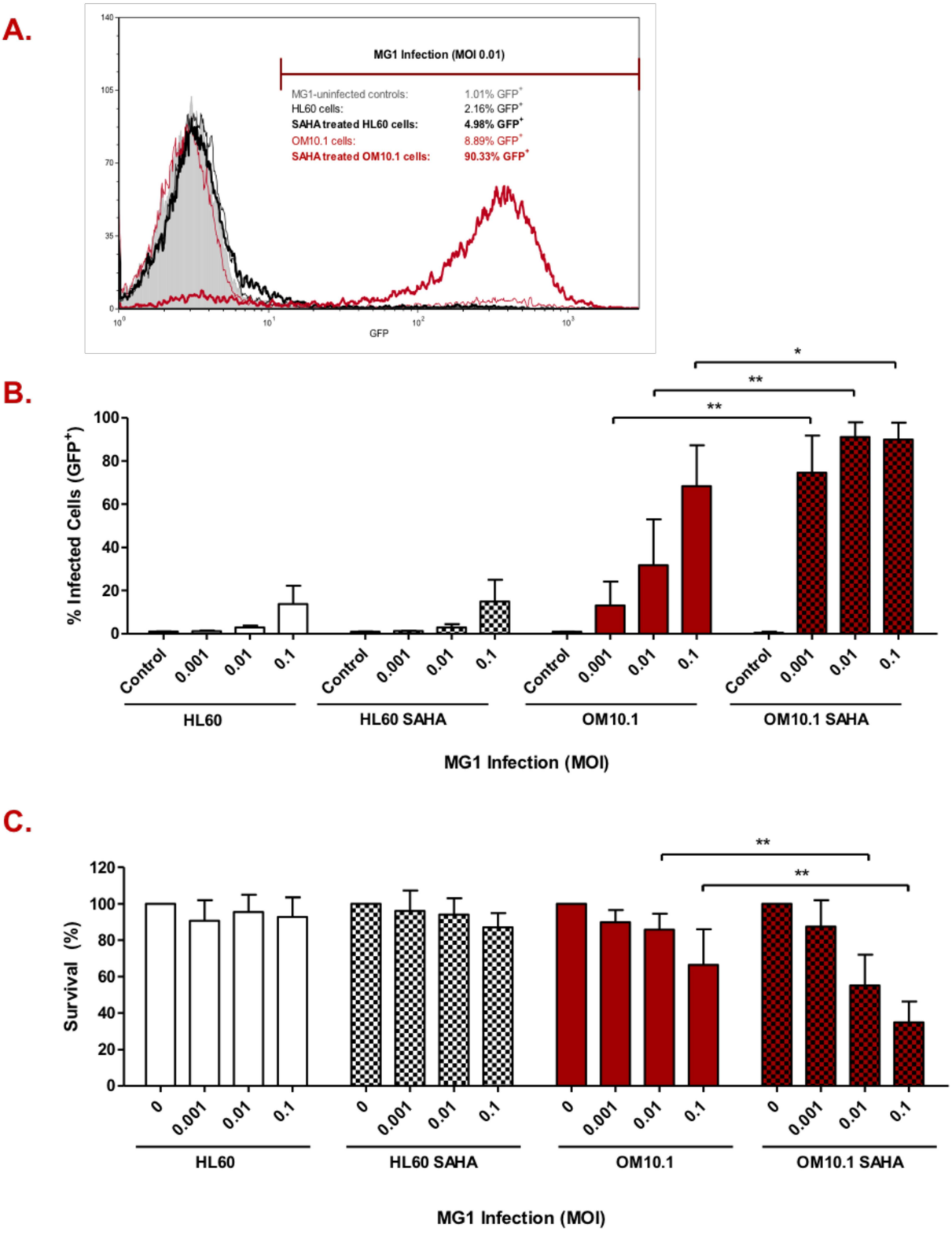

A.
MG1 Infection (MOI 0.01)
MG1-uninfected controls: 1.01% GFP+
HL60 cells: 2.16% GFP+
SAHA treated HL60 cells: 4.98% GFP+
OM10.1 cells: 8.89% GFP+
SAHA treated OM10.1 cells: 90.33% GFP+
GFP
B.
% Infected Cells (GFP+)
Control
0.001
0.01
0.1
HL60
HL60 SAHA
OM10.1
OM10.1 SAHA
MG1 Infection (MOI)
C.
Survival (%)
0
MG1 Infection (MOI)

Figure 27. SAHA stimulation results in a selective enhancement of MG1 infection and cytopathic effects in OM10.1 cells, but not in HL60 cells. HL60 and OM10.1 cells were left untreated, or treated with 1uM SAHA for 24 hours prior to infection with MG1. Infection and cell survival were quantified as before following 24 hours of infection. **A.** Representative histogram demonstrating MG1 infection of HL60 and OM10.1 cells with and without SAHA stimulation. **B.** MG1 infection (n=5) and **C.** cell survival (n=5) in HL60 and OM10.1 cells with or without SAHA stimulation is shown. n values represent separate biological replicates. *p<0.01, **p<0.001 by Two-way ANOVA with Bonferroni post-test for multiple comparisons HL60 or OM10.1 cells with or without SAHA treatment; Data represent mean ± SEM.

Finally, the effect of OV infection on HIV-1 production in SAHA stimulated U1 and OM10.1 cells was evaluated by measuring total p24 protein expression in cells and culture supernatant. Although VSVΔ51 infection of SAHA-treated U1 cells had minimal effect on HIV-1 p24 expression (Figure 28A), MG1 infection resulted in a significant decrease in p24 expression (Figure 28B). In SAHA stimulated OM10.1 cells however, a dose-dependent decrease in p24 expression was observed following both VSVΔ51 (Figure 28C) and MG1 infection (Figure 28D), albeit to a greater degree in MG1 infected cells.

In summary, SAHA stimulation resulted in a robust and selective enhancement of VSVΔ51 and MG1 infection within latently infected U1 and OM10.1 cell lines, but not HIV-uninfected parental controls. Additionally, OV infection effectively decreased ongoing HIV-1 replication in SAHA-stimulated U1 and OM10.1 cells.

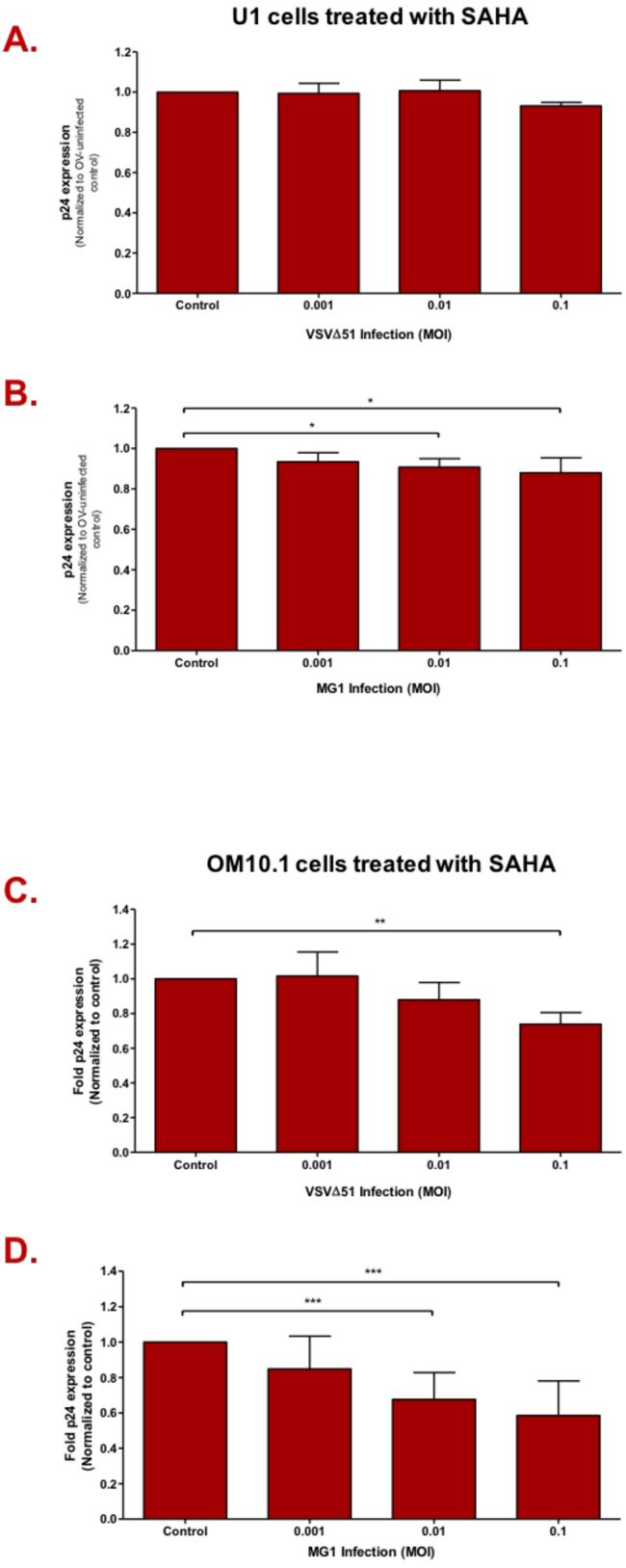

A.
U1 cells treated with SAHA
p24 expression
(Normalized to OV-uninfected control)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Control
0.001
0.01
0.1
VSVΔ51 Infection (MOI)
B.
p24 expression
(Normalized to OV-uninfected control)
*
*
MG1 Infection (MOI)
C.
OM10.1 cells treated with SAHA
Fold p24 expression
(Normalized to control)
1.4
**
VSVΔ51 Infection (MOI)
D.
Fold p24 expression
(Normalized to control)
***
***
MG1 Infection (MOI)

Figure 28. Oncolytic virus infection significantly abrogates HIV-1 production in SAHA treated U1 and/or OM10.1 cells. U1 and OM10.1 cells were treated with SAHA for 24 hours to induce active HIV-1 replication. These cells were then infected with VSVΔ51 or MG1 at the indicated MOI for 24 hours and cells with culture supernatant were collected to quantify total p24 expression by ELISA. Total HIV-p24 antigen expression (cells and supernatant) following **A.** VSVΔ51 infection (n=4) or **B.** MG1 infection (n=5) of SAHA-treated U1 cells, as well as **C.** VSVΔ51 infection (n=5) or **D.** MG1 infection (n=5) of SAHA-treated OM10.1 cells is shown. n values represent separate biological replicates. *p=0.0117, **p=0.0036, ***p=0.003 by one-way ANOVA and $p<0.05$ by pairwise Dunnett's test compared to OV-uninfected control cells; Data represent mean ± SEM.

4.4. Discussion

4.4.1. Potential mechanisms underlying enhanced OV infection and killing of latently infected U1 and OM10.1 cells

The novel findings presented here support the premise that defective IFN-I pathways in latently HIV-1-infected cells can serve as a target for IFN-sensitive oncolytic viruses, including VSVΔ51 and MG1. Significantly greater VSVΔ51 and MG1 infection, as well as MG1-mediated viral cytopathic effects, were observed in the two independent, latently HIV-1 infected cell lines, when compared to their respective parental HIV-uninfected cells. These results are consistent with the defects in IFN-I responsiveness and impaired ISG induction in latently HIV-1 infected cell lines demonstrated in the previous chapter.

Interestingly, the observed defects in expression of PKR, ISG15, IFNAR1, and IFN-I cytokines in latently HIV-1 infected U1 and OM10.1 cells may contribute directly to the extent and pattern of OV infection observed. In mouse models of VSV infection, PKR has been identified to be a critical factor in curtailing viral replication and spread (Baltzis et al., 2004; Stojdl et al., 2000a; 2003). Likewise, deletion of ubiquitin specific peptidase 18 (USP18), a protease that counteracts ISGylation of viral proteins by ISG15, has been shown to confer protection against intracerebral VSV infection, highlighting the antiviral activity of ISG15 (Ritchie et al., 2004; Sadler and Williams, 2008). Downregulation of IFNAR expression has also been demonstrated in bladder cancer cells as a mechanism by which tumor cells become susceptible to VSV-mediated oncolysis (Zhang et al., 2010).

As such, IFNα secretion was noted in culture supernatant following VSVΔ51 and MG1 infection of HIV-uninfected U937 and HL60 cells, but minimal to no induction of IFNα production was seen in the U1 or OM10.1 cells. The pattern of impaired IFNα production observed here in latently HIV-1 infected cells was consistent with the previously demonstrated defects in IFN-I cytokines secretion following poly(I:C) stimulation. This lends further credence to the idea that defects in IFN-I response in the latently infected U1 and OM10.1 cell lines may underlie the enhanced OV-mediated targeting and killing seen.

Alternate mechanisms, unrelated to IFN-I defects, that may contribute to enhanced OV infection were investigated in parallel. Given that levels of LDL-R expression were similar between HIV-uninfected and latently infected cell line pairs, enhanced viral entry was ruled out as a potential contributory factor to the enhanced sensitivity of U1 and OM10.1 cells to OV. In addition, differences in OV infection as a consequence of varying rates of cellular proliferation and entry into cell cycle (Oliere et al., 2008) were excluded by performing OV infection on cells whose proliferation had been previously synchronized by serum starvation.

Going forward, mechanistic understanding of the host-virus interactions that contribute to impairments in IFN-I responses may provide further insights into the higher sensitivity of the latently infected U1 and OM10.1 cells to OV infection, when compared to U937 and HL60 cells.

4.4.2. Understanding the selective enhancement of OV infection in latently HIV-1 infected cells following treatment with a histone deacetylase inhibitor

HIV-1 reactivation using SAHA was associated with a selective increase in VSVΔ51 and MG1 infection in both U1 and OM10.1 cells, but not in HIV-uninfected U937 and HL60 cells. It was hypothesized that the induction of HIV-1 replication and subsequent viral protein expression following SAHA treatment may further attenuate the IFN-I responses in latently infected cells, thereby potentiating OV effects. In accordance with this, a robust 10-20-fold increase in both VSVΔ51 and MG1 infection, even at a MOI as low as 10^{-4}, was observed in U1 and OM10.1 cells following SAHA treatment. Interestingly however, only 5-10% of SAHA-treated U1 and OM10.1 cells expressed the intracellular p24 protein (Figure 23), which increases during the later stages of HIV-1 replication (Figure 22A and 22B) (Krishnan and Zeichner, 2004). This suggests that although HIV reactivation may contribute to the enhancement of OV infection in latently infected cell lines, alternate effects of HDACi unrelated to HIV-1 reactivation, such as the direct modulation of ISG transcription and IFN-I responses, may also be implicated.

SAHA has been previously reported to enhance VSV infection in oncolysis-refractory tumors through selective dampening of IFN-I responses and augmentation of virus-induced apoptosis of tumor cells, but not healthy cells (Nguyên et al., 2008a; Shulak et al., 2014). Additionally, Valproic acid, another HDACi, has been shown to enhance antitumor efficacy of OV by suppressing the transcription of critical IFN-stimulated antiviral genes such as STAT1 and PKR, as well as decreasing cellular responsiveness to exogenous IFNβ (Otsuki et al., 2008). It is therefore possible that by modulating the

expression of key ISGs, in parallel with promoting HIV-1 transcription, SAHA contributes to the enhanced infection and killing of latently HIV-1 infected cells by OV. However, the mechanism(s) underlying this selectivity is yet to be elucidated.

4.4.3. Effects of VSVΔ51 and MG1 infection on HIV-1 replication following reactivation

Given that the entire population of latently HIV-1 infected U1 and OM10.1 cells contain integrated proviral DNA, OV-mediated cell death should directly diminish the amount of p24 expressed. In both cell line pairs, MG1 infection resulted in significant cell death, but VSVΔ51 infection had minimal cytopathic effects. Consistent with this, in SAHA treated U1 cells, a significant dose-dependent decrease in HIV-1 p24 expression was observed following MG1 infection, but not VSVΔ51 infection. In contrast, both VSVΔ51 and MG1 infection resulted in a dose-dependent decrease in HIV-1 p24 expression in SAHA treated OM10.1 cells. This decrease in p24 following VSVΔ51 infection was observed despite the absence of detectable differences in cell death. Therefore, in addition to direct viral cytopathic effects, OV may be able to impair HIV-1 replication in SAHA treated U1 and OM10.1 cells through alternate mechanisms.

For instance, VSVΔ51 and MG1 may directly interfere with HIV-1 replication by competing for the same cellular translational machinery (Oliere et al., 2008), thereby resulting in the observed decrease in p24 production. Interestingly, VSV has been shown to suppress replication of other competitor RNA viruses in a similar manner when co-infection was performed in tumor cells (Furió et al., 2012). Alternatively, both the wild-type VSV and maraba virus have the capacity to decrease host cell transcription (Black

and Lyles, 1992) and impair nuclear-cytoplasmic transport of host mRNA (Petersen et al., 2001). However, VSVΔ51 and MG1 strains, because of the engineered mutations, are attenuated in their ability to manipulate these aspects of host functions. Nevertheless, the potential contribution of lower HIV-1 transcription and/or impaired export of HIV-1 mRNA into the cytoplasm in the context of OV infection cannot be excluded (Brun et al., 2010). Therefore, in parallel to delineating effects of SAHA stimulation on OV infection, the mechanism(s) by which OV can influence ongoing HIV-1 replication both during productive HIV infection and following reactivation of latent cells warrants further investigation.

SAHA represents an interesting candidate that effectively complements OV infection in latently HIV-1 infected cells through its dual effects on HIV-1 reactivation, as well as regulation of IFN-I responses. Therefore, a combinatorial approach using SAHA to "shock" and reactivate latent HIV-1, as well as enhance OV-mediated killing of latently infected cells may represent a novel therapeutic approach to target and eliminate the latent HIV-1 reservoir.

4.5. Future Directions

Consistent with the defects in IFN-I pathways previously demonstrated, the latently HIV-1 infected U1 and OM10.1 cells were observed to be more sensitive to VSVΔ51 and MG1 than the respective HIV-uninfected U937 and HL60 cells. Further characterization of the IFN-I system in the context of VSVΔ51 and MG1 infection may provide insight into the potential mechanisms behind the observed selectivity of OV towards latently HIV-1

infected cells. IFN-I signaling and expression/functionality of antiviral ISGs known to influence OV virulence, should be characterized in the latently HIV-1 infected and uninfected cell lines following VSVΔ51 or MG1 infection.

The present study was focused on identifying differences in sensitivity of latently HIV-1 infected and uninfected cell lines to OV infection. Going forward, the specificity of OV towards latently HIV-infected cells should be investigated, as has been performed *in vitro* in various tumor models. For instance, leukemic cells were spiked into cultures with increasing quantities of normal peripheral blood stem cells to assess the ability of OV to selectively purge the tumor cells, without affecting healthy cells (Lichty et al., 2004b). Co-culture studies in which labelled, latently HIV-1 infected cell lines are diluted with HIV uninfected cells and then infected with OV may provide further insight into the ability of OV to specifically target and kill the latently HIV-1 infected cells.

SAHA treatment resulted in a highly specific augmentation of OV infection in latently infected cell lines. As described, SAHA may increase the virulence of OV through the enhancement of HIV-1 transcription, protein expression, and/or through selective regulation of IFN-I and downstream ISG expression. To further delineate the contribution of HIV-1 replication in enhancing OV infection, characterization of SAHA-mediated induction of viral transcription and production of regulatory and accessory viral proteins known to counteract IFN-I pathways may be of value. At present, the mechanism behind ability of SAHA to selectively dampen IFN-I responses in cells with pre-existing defects in IFN-I pathways is unknown. Therefore, the effect of SAHA on the IFN-I responses in both the latently HIV-1 infected and uninfected cell lines warrants further investigation.

Moving forward, the combination of OV with other latency reversal strategies could be evaluated. As mentioned, determining the underlying mechanisms behind SAHA-mediated amplification of OV infection is challenging due to its multiple intracellular targets. As a result, use of other latency reversal agents such as protein kinase C agonists (Prostratin or Bryostatin) or bromodomain inhibitors (JQ1), which selectively reactivate latent HIV-1 without cellular activation or IFN-I pathways, can be investigated in combination with OV (Laird et al., 2015; Spina et al., 2013).

4.6. Conclusion

The work presented here represents the first proof-of-concept study to highlight a potential use for VSVΔ51 and MG1 as therapeutic agents to target and kill latently HIV-1 infected cells. Latently HIV-1 infected U1 and OM10.1 cells were demonstrated to be significantly more sensitive to VSVΔ51 and MG1 infection and cytopathic effects, when compared to their respective parental HIV-uninfected U937 and HL60 cells. This supports the hypothesis that defects in IFN-I pathways within latently HIV-1 infected cells may allow the HIV reservoir to be eradicated in a targeted manner using OV. In addition, reactivation of HIV-1 by inhibiting HDACs with SAHA resulted in a selective enhancement of OV infection in U1 and OM10.1 cells, thereby highlighting a plausible approach for use of OV in combination with current latency reversal agents.

Chapter 5: VSVΔ51 and MG1 can target and eliminate latently HIV-infected cells in primary cell models of HIV-1 latency

5.1. Introduction

Thus far, impaired IFN-I responses and a corresponding increase in sensitivity to VSVΔ51 and MG1 were identified in the latently HIV-1 infected U1 and OM10.1 cells, when compared to the respective HIV-uninfected parental U937 and HL60 cells. Despite the previously described limitations, cell lines provided a functional model that allowed us to characterize IFN-I pathways, as well as evaluate the therapeutic potential of OV in the context of HIV-1 latency. The objective of this chapter was to translate the current study to physiologically relevant *in vitro* and *ex vivo* primary cell models of latent HIV-1 infection.

5.1.1. Evaluating the capacity of OV to target and eliminate latently HIV-1 infected cells using an *in vitro* resting $CD4^+$ T cell model of latency

As defined in the introduction, latent HIV-1 infection is characterized by the persistence of HIV-1 as integrated proviral DNA within long-lived reservoirs composed primarily of resting memory $CD4^+$ T cells (Chun et al., 1997c; Finzi et al., 1997). Within this population, replication-competent HIV-1 has been detected at very low frequency, typically in the range of 0.1-1 infectious unit per million (IUPM) resting $CD4^+$ T cells, in the blood and lymph nodes of infected individuals (Finzi et al., 1999). The early establishment of this reservoir is postulated to occur through infection of activated $CD4^+$ T cells that

revert back to a resting memory state (Siliciano and Greene, 2011) and/or through the direct infection of resting memory $CD4^+$ T cells (Cameron et al., 2010; Chavez et al., 2015; Pace et al., 2012). In addition, HIV-1 latency has been shown to be established and maintained by several distinct cellular and molecular mechanisms (Donahue and Wainberg, 2013; Ruelas and Greene, 2013; Siliciano and Greene, 2011).

Reflecting the complexities underlying latent HIV-1 infection *in vivo*, numerous *in vitro* models of HIV-1 latency have been developed to further delineate features of the latent reservoir. Within this study, a resting $CD4^+$ T cell model of HIV-1 latency developed by Lewin and colleagues was utilized to evaluate the capacity of OV to eliminate latently HIV-1 infected cells (Cameron et al., 2010; Saleh et al., 2007; 2011). The model is established through the *in vitro* HIV-1 infection of resting $CD4^+$ T cells treated with chemokine (C-C motif) ligand 19 (CCL19), resulting in an enhanced nuclear localization and integration of HIV-1 in the absence of ongoing viral replication (Cameron et al., 2010).

The model presents several practical and conceptual advantages that support its use within the current work. First, in studies investigating efficacy of latency reversal agents (LRA) on several *in vitro* models of latent HIV-1 infection, this model was shown to most closely reflect the responsiveness observed in resting $CD4^+$ T cells from HAART-treated individuals (Spina et al., 2013). Additionally, the time to establishment of latent HIV-1 infection within the model is short, thereby facilitating rapid evaluation of the ability of OV to kill latently HIV-1 infected cells. Lastly, 1% of resting $CD4^+$ T cells within the model have been reported to contain integrated proviral DNA, which is ~30-fold higher than the prevalence of proviral DNA in resting $CD4^+$ T cells *in vivo* (Finzi et al., 1997)

(Figure 29A). Therefore, this model was employed to assess the ability of VSVΔ51 and MG1 to selectively target and kill latently HIV-1 infected resting $CD4^+$ T cells.

5.1.2. Assessing the effects of OV infection on the latent HIV-1 reservoir *ex vivo* using memory $CD4^+$ T cells from HAART-treated individuals

In addition to the *in vitro* model, the potential capacity of OV to purge latently infected cells isolated from HIV-infected individuals effectively managed on HAART was investigated. In these patients, the latent provirus has been demonstrated to be differentially distributed within the central (T_{CM}), transitional (T_{TM}), and effector (T_{EM}) memory $CD4^+$ T cell compartments (Chomont et al., 2009). Due to the exceptional stability of memory $CD4^+$ T cells *in vivo*, with a half-life of ~44.2 months, eradication of the latent HIV-1 reservoir with antiretroviral therapy alone has previously been estimated to take approximately 73.4 years (Finzi et al., 1999; Siliciano et al., 2003). In addition, the reservoir may be replenished over time through ongoing homeostatic proliferation and residual antigen-driven immune activation (Chomont et al., 2009). Consequently, elimination of latent HIV-1 infection within the memory $CD4^+$ T cell compartment represents a critical step towards the eradication of HIV-1. Therefore, the capacity of VSVΔ51 and MG1 to eliminate memory $CD4^+$ T cells from HAART-treated individuals was investigated.

5.1.3. Quantifying the effects of OV infection on latently HIV-1 infected cells

Cell line models of HIV-1 latency represent a homogenous population of latently HIV-infected cells that could be compared to the HIV-uninfected parental cells. Consequently, we could quantify the capacity of OV to infect and kill these cells by using methods such as flow cytometry and cell viability assays. In contrast, latently HIV-1 infected cells represent only a small proportion of the total population of cells within the *in vitro* resting $CD4^+$ T cell model of latent HIV-1 infection, and more so in the memory $CD4^+$ T cells isolated from HIV-infected individuals (Figure 29A). In addition, there is presently a significant deficit in phenotypic markers that can specifically identify latently HIV-1 infected cells, thereby permitting their enrichment. This presents a major barrier to assessing the ability of OV to target and eliminate latently HIV-1 infected cells.

As such, the previously utilized assays lack the sensitivity to detect infection or killing of these rare latently HIV-1 infected cells. Therefore, we postulated that killing of latently HIV-1 infected cells would result in both a decrease in levels of integrated HIV-1 DNA within the population and a reduction of inducible HIV-1 outgrowth (Figure 29B). In the absence of non-specific cell death within the population, this approach provides an indirect, but validated measure of specific elimination of latently HIV-1 infected cells (Li et al., 2016b).

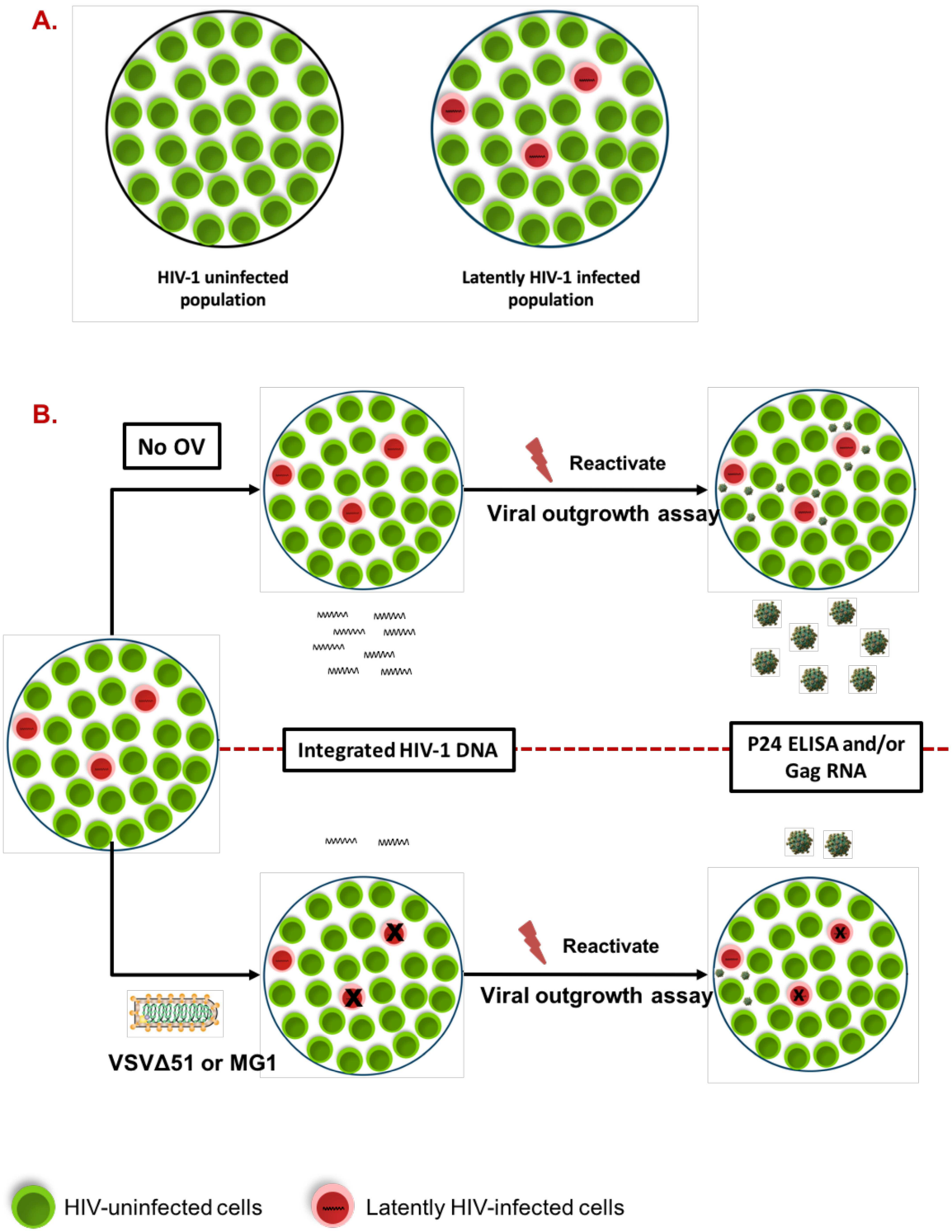

A.
HIV-1 uninfected population
Latently HIV-1 infected population
B.
No OV
Reactivate
Viral outgrowth assay
Integrated HIV-1 DNA
P24 ELISA and/or Gag RNA
Reactivate
Viral outgrowth assay
VSVΔ51 or MG1
HIV-uninfected cells
Latently HIV-infected cells

Figure 29. Challenges to using primary cell models of HIV-1 latency and approaches to quantifying elimination of latently HIV-1 infected cells in experimental systems. A. Latently HIV-1 infected cells represent only a minor proportion of the total population of cells and there is currently a deficit in phenotypic markers that distinguish these cells from surrounding HIV-uninfected cells. **B.** Elimination of latently HIV-1 infected cells following OV infection should result in a decrease in both levels of integrated HIV-1 DNA and inducible HIV-1 replication following reactivation and viral outgrowth. This in the context of an absence in non-specific killing is highly supportive of selective elimination of the latent HIV-1 reservoir.

5.2. Hypothesis

5.2.1. VSVΔ51 and MG1 can selectively target and eliminate the latently HIV-1 infected cells present within the *in vitro* resting $CD4^+$ T cell model of latent HIV-1 infection.

5.2.2. *Ex vivo* VSVΔ51 and MG1 infection can eliminate latently HIV-1 infected memory $CD4^+$ T cells isolated from HIV-1 infected individuals effectively managed on HAART.

5.3. Results

5.3.1. Infection with VSVΔ51 or MG1 results in a selective elimination of latently HIV-1 infected cells within the *in vitro* HIV-1 infected resting $CD4^+$ T cell population.

To investigate the capacity of OV to target and kill latently HIV-1 infected primary cells, the resting $CD4^+$ T cell model of latency developed by Lewin and colleagues was established and validated (Figure 30A and 30B) (Saleh et al., 2007; 2011). In contrast to the homogeneity of latently infected cell lines, only a small proportion of the population of resting $CD4^+$ T cells were identified to carry integrated proviral DNA (~5000 copies per $1x10^6$ resting $CD4^+$ T cells) (Figure 30C). HIV-1 latency within the model was defined by minimal ongoing viral replication, as quantified by the absence of increase in p24 protein production (data not shown) and cell-associated gag RNA over 6 days (Figure 30D), as well as a measurable HIV-1 reactivation following cell stimulation with PHA and IL-2 (Figure 30E).

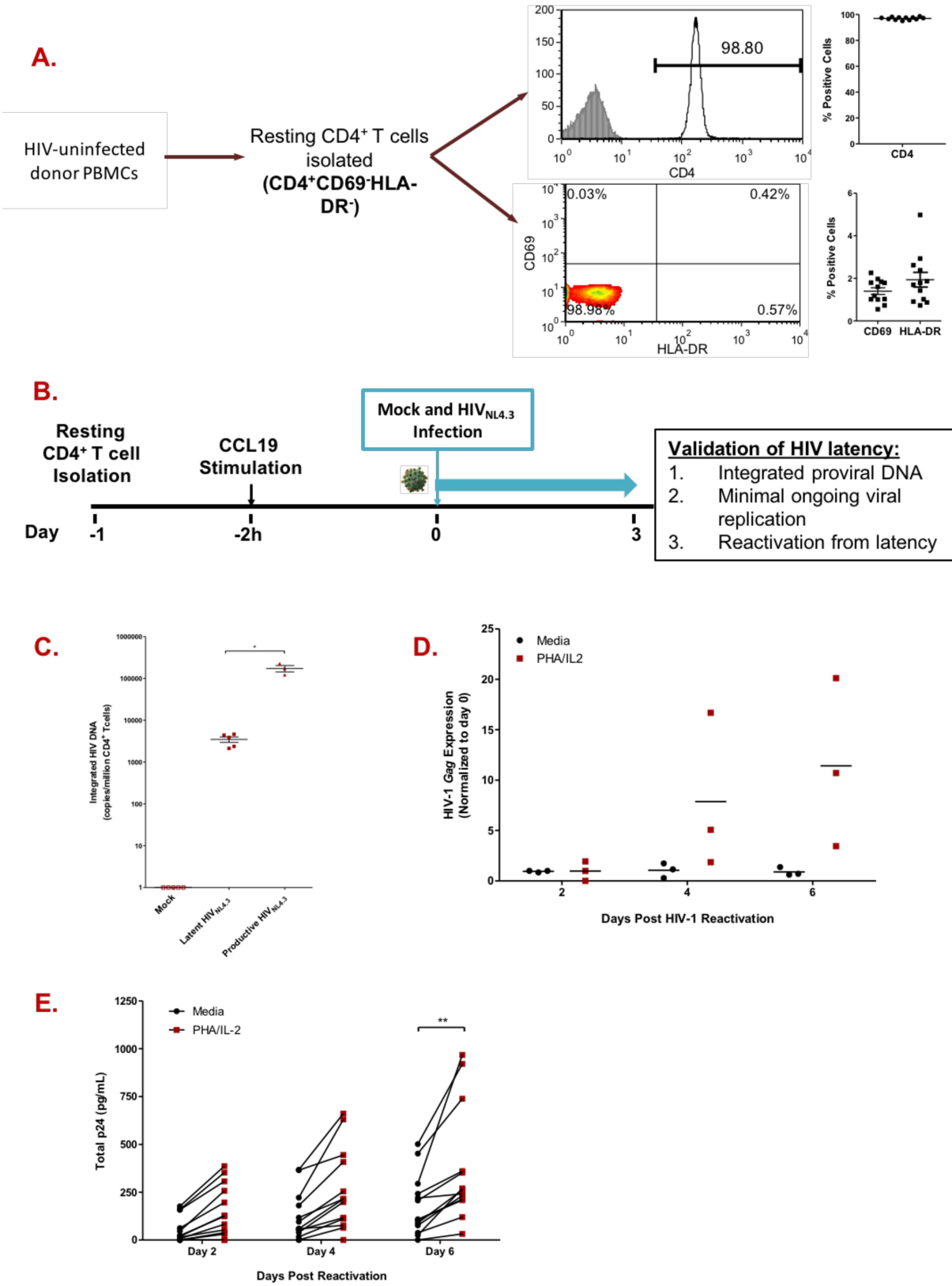
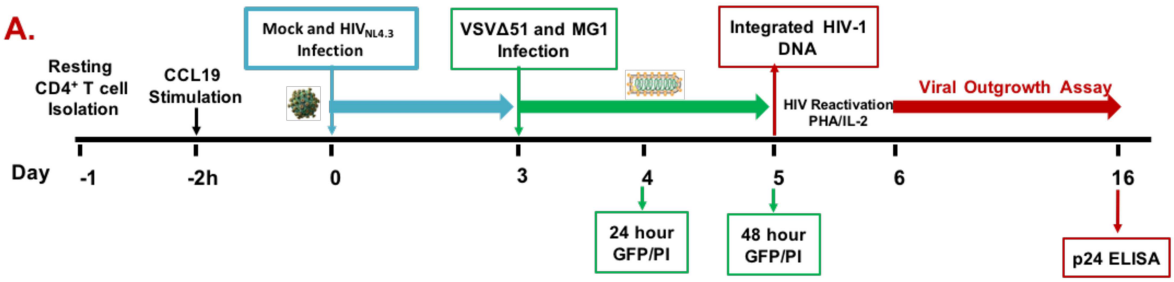

A.
HIV-uninfected donor PBMCs
Resting CD4+ T cells isolated (CD4+CD69-HLA-DR-)
98.80
CD4
% Positive Cells
CD69
HLA-DR
0.03%
0.42%
98.98%
0.57%
B.
Resting CD4+ T cell Isolation
CCL19 Stimulation
Mock and HIVNL4.3 Infection
Day
-1
-2h
0
3
Validation of HIV latency:
1. Integrated proviral DNA
2. Minimal ongoing viral replication
3. Reactivation from latency
C.
Integrated HIV DNA (copies/million CD4+ Tcells)
Mock
Latent HIVNL4.3
Productive HIVNL4.3
D.
Media
PHA/IL2
HIV-1 Gag Expression (Normalized to day 0)
Days Post HIV-1 Reactivation
E.
Media
PHA/IL-2
Total p24 (pg/mL)
Day 2
Day 4
Day 6
Days Post Reactivation

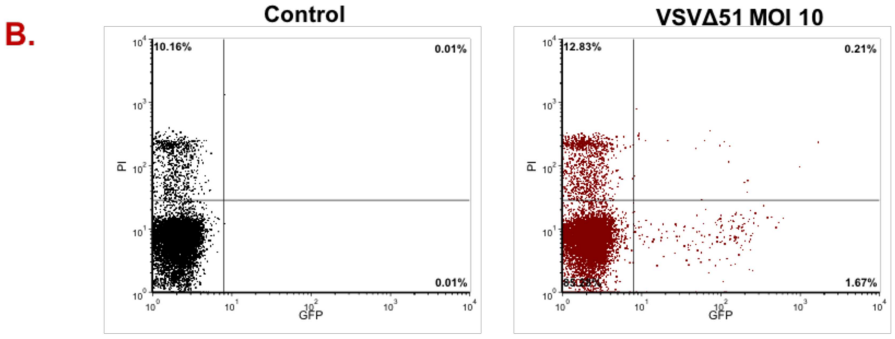
Figure 30. HIV-1 latency in the *in vitro* resting CD4$^+$ T cell model of HIV-1 latency is defined by the presence of integrated proviral DNA, absence of ongoing HIV-1 replication, and inducibility following stimulation. A. Resting CD4$^+$ T cells, characterized as CD4$^+$, CD69$^-$, and HLA-DR$^-$ cells, were isolated from healthy HIV-uninfected individuals and purity was confirmed by flow cytometry (n=13). **B.** Experimental design showing the timeline of $HIV_{NL4.3}$ infection and approach to establishment/validation of the *in vitro* model of HIV-1 latency. **C.** Level of integrated HIV-1 DNA was quantified by ddPCR following mock HIV-infection, latent HIV-infection of resting CD4$^+$ T cells, and productive infection of activated CD4$^+$ T cells (n=3). **D.** HIV-1 *gag* RNA expression (n=3) quantified by qPCR and **E.** p24-antigen expression (n=9) measured by ELISA was assessed in latently HIV-1 infected cells unstimulated or stimulated with PHA/IL-2 and left in culture for 6 days. n values represent separate biological replicates. * p=0.0002 by unpaired t-test and **p = 0.0008 by Two-way ANOVA with Bonferroni post-test for multiple comparisons between latently HIV-1 infected CD4+ T cells left in media alone or stimulated with PHA/IL-2. Data represent mean ± SEM.

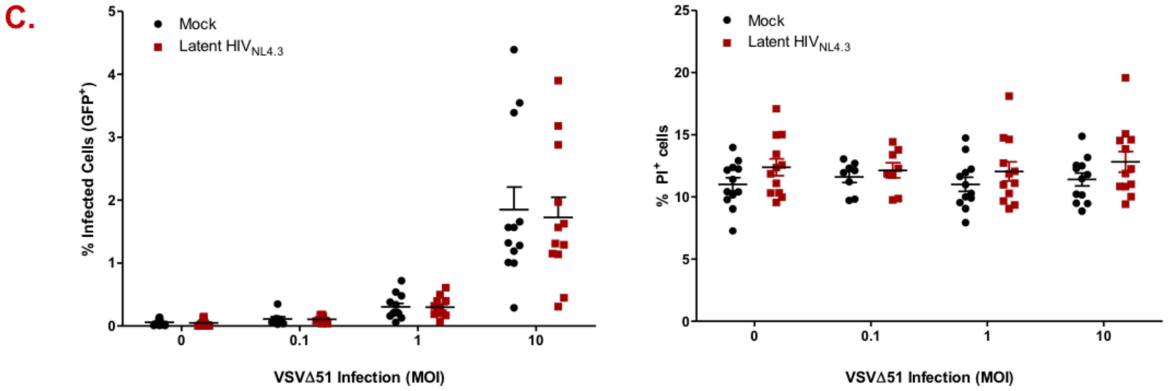
Using this model, mock and latently HIV-1 infected resting $CD4^+$ T cells were infected with VSVΔ51 or MG1 at indicated MOI for 48 hours (Figure 31A). OV infection and cell death was quantified by flow cytometry to assess the susceptibility of primary resting $CD4^+$ T cells to OV. As expected, minimal OV infection and cytopathic effects were observed following VSVΔ51 or MG1 infection, in either the mock or latently HIV-1 infected resting $CD4^+$ T cells (Figure 31C and 31D). In addition, no difference in OV infection or killing was observed between the mock and HIV-infected resting $CD4^+$ T cells.

Next, the ability of VSVΔ51 or MG1 to target and kill the latently HIV-1 infected resting $CD4^+$ T cells was evaluated as described above. Elimination of latently HIV-1 infected cells was quantified directly by measuring level of integrated HIV-1 DNA and degree of inducible HIV-1 replication following reactivation and outgrowth (Figure 29B).

Integrated proviral DNA was quantified in the latently HIV-1 infected resting $CD4^+$ T cells following 48 hours of VSVΔ51 or MG1 infection (Figure 31A). Importantly, OV infection of both mock and latently infected cells was performed in the presence of Raltegravir to prevent new cycles of HIV-1 infection. VSVΔ51 infection of resting $CD4^+$ T cells had minimal quantifiable effect on the level of integrated HIV-1 DNA (Figure 32A). In contrast, a significant dose-dependent decrease in integrated HIV-1 DNA, with ~37% mean reduction at MOI of 10, was observed following MG1 infection of the latently HIV-1 infected resting $CD4^+$ T cells (Figure 32B and C).


A.
Resting CD4+ T cell Isolation
CCL19 Stimulation
Mock and HIVNL4.3 Infection
VSVΔ51 and MG1 Infection
Integrated HIV-1 DNA
HIV Reactivation PHA/IL-2
Viral Outgrowth Assay
Day
-1
-2h
0
3
4
5
6
16
24 hour GFP/PI
48 hour GFP/PI
p24 ELISA


B.
Control
VSVΔ51 MOI 10
10.16%
0.01%
0.01%
12.83%
0.21%
1.67%
PI
GFP


C.
Mock
Latent HIVNL4.3
% Infected Cells (GFP+)
% PI+ cells
VSVΔ51 Infection (MOI)
VSVΔ51 Infection (MOI)

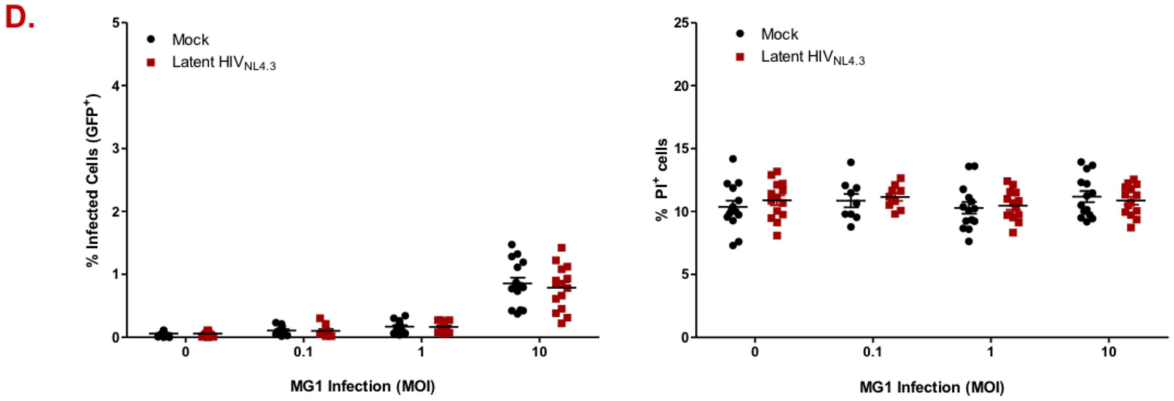

D.
Mock
Latent HIVNL4.3
% Infected Cells (GFP+)
% PI+ cells
MG1 Infection (MOI)
MG1 Infection (MOI)

Figure 31. Both mock and latently HIV-infected resting CD4$^+$ T cells demonstrate minimal susceptibility to OV infection and killing. A. Experimental design for VSVΔ51 or MG1 infection of an *in vitro* primary cell model of latent HIV-1 infection in resting CD4$^+$ T cells. Mock and latently HIV-1 infected resting CD4$^+$ T cells were infected with VSVΔ51 or MG1 at indicated MOI for 48 hours. **B.** Representative histogram demonstrating OV infection (GFP$^+$ cells) and cell death (PI$^+$ cells) is shown. **C.** VSVΔ51 infection (left) and cell death (right), as well as **D.** MG1 infection (left) and cell death (right) following infection of mock or latently HIV-1 infected CD4$^+$ T cells is shown (n=13). n values represent separate biological replicates (individual healthy donors). No significant difference in OV infection or cell death between mock and latently HIV-1 infected cells by Two-way ANOVA; Data represent mean ± SEM.

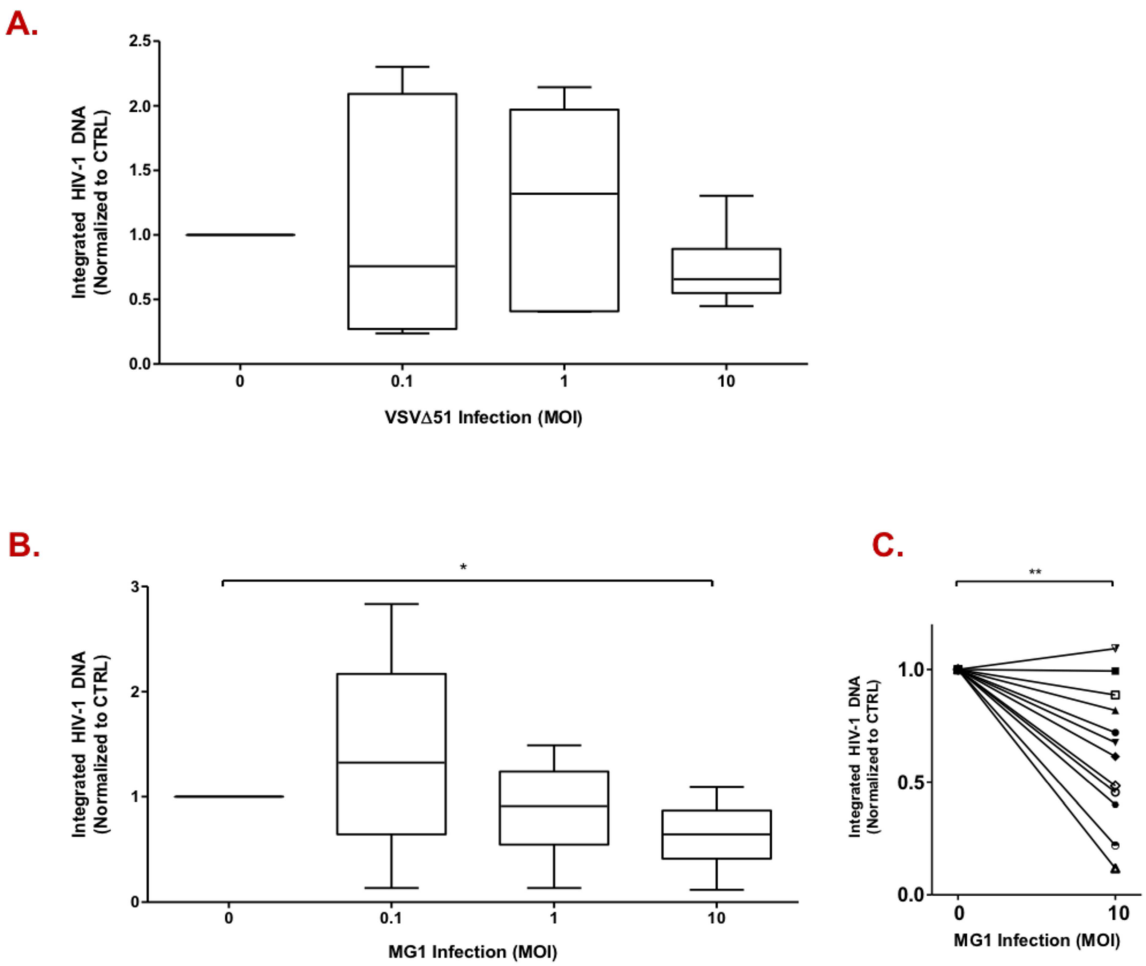

A.
Integrated HIV-1 DNA (Normalized to CTRL)
2.5
2.0
1.5
1.0
0.5
0.0
0
0.1
1
10
VSVΔ51 Infection (MOI)
B.
*
Integrated HIV-1 DNA (Normalized to CTRL)
3
2
1
0
0
0.1
1
10
MG1 Infection (MOI)
C.
**
Integrated HIV-1 DNA (Normalized to CTRL)
1.0
0.5
0.0
0
10
MG1 Infection (MOI)

Figure 32. MG1, but not VSVΔ51 infection, results in a dose-dependent reduction in the level of integrated HIV-1 DNA. Latently HIV-1 infected resting $CD4^+$ T cells were infected with VSVΔ51 or MG1 at indicated MOI for 48 hours. Cell pellets were then collected, DNA isolation was performed, and integrated HIV-1 DNA was quantified by a validated two-step nested PCR. Integrated HIV-1 DNA expression in resting $CD4^+$ T cells following **A.** VSVΔ51 infection (n=6) and **B.** MG1 infection (n=12) is shown. **C.** Relative number of integration events in each independent experiment (individual donors) is demonstrated in MG1-uninfected and MG1 MOI 10 conditions (n=12). n values represent separate biological replicates (individual healthy donors). * $p = 0.0086$ by One-way ANOVA on ranks and $p<0.05$ by Dunn's multiple comparison test and ** $p = 0.0012$ by paired t-test; Data represent mean ± SEM.

Note: Integrated HIV-1 DNA PCR following VSVΔ51 (n=6) and MG1 infection (n=8) was performed by N Ranganath. An additional n=4 of integrated HIV-1 DNA PCR for MG1 infection was performed by S.C. Côté.

Next, the effect of OV infection on replication-competent latent HIV-1 infection was evaluated. Following 48 hours of infection, residual VSVΔ51 or MG1 was removed from cell culture by performing multiple washes. The latently infected resting $CD4^+$ T cells were then treated with PHA and IL-2 to induce HIV-1 reactivation, and activated, CD8-depleted PBMCs were added to facilitate HIV-1 amplification (Figure 31A). The absence of ongoing OV replication within the cell culture during the 10 days of viral outgrowth was confirmed by flow cytometry (data not shown). After 10 days, HIV-1 p24 expression in culture supernatant was consistently observed in latently HIV-1 infected resting $CD4^+$ T cell controls that were not infected with OV. Interestingly however, VSVΔ51 infection resulted in a dramatic decrease in p24 expression with a 90-95% reduction observed at MOIs of 0.1, 1, and 10 (Figure 33A). MG1 infection also resulted in a significant dose-dependent decrease in p24 expression, which closely mirrored the observed decrease in integrated HIV-1 DNA (Figure 33B).

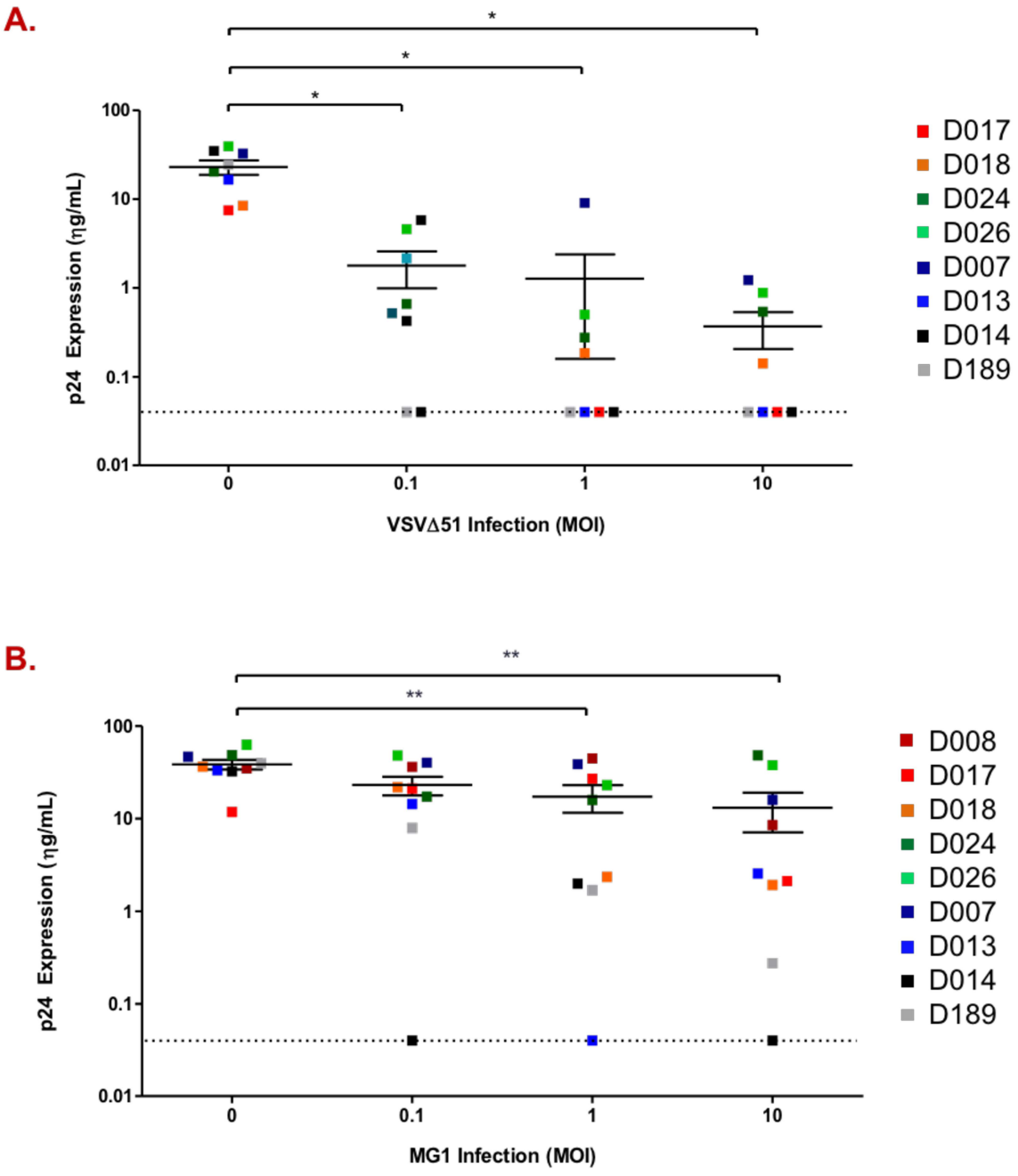

A.
*
*
*
100
10
1
0.1
0.01
p24 Expression (ηg/mL)
0
0.1
1
10
VSVΔ51 Infection (MOI)
D017
D018
D024
D026
D007
D013
D014
D189
B.
**
**
100
10
1
0.1
0.01
p24 Expression (ηg/mL)
0
0.1
1
10
MG1 Infection (MOI)
D008
D017
D018
D024
D026
D007
D013
D014
D189

Figure 33. A significant dose-dependent reduction in replication-competent HIV-1 is observed after both VSVΔ51 and MG1 infection. Latently HIV-1 infected resting $CD4^+$ T cells were infected with VSVΔ51 or MG1 at indicated MOI for 48 hours. Cells were reactivated with PHA and IL-2 and maintained in culture with feeder cells for 10 days, after which induced HIV-1 replication was quantified by measuring p24 expression in cell-free supernatant by ELISA. Inducible p24-antigen expression following 48 hours of **A.** VSVΔ51 infection (n=8) and **B.** MG1 infection (n=9) of latently HIV-infected resting $CD4^+$ T cells is shown. Figure legends represent separate biological replicates (individual healthy donors). *$p<0.0001$, **$p=0.0128$ by One-way ANOVA and $p<0.05$ by Dunnett's multiple comparison test compared to MOI 0; Data represent mean ± SEM.

To investigate if this observed reduction in HIV-1 p24 expression was dependent on the capacity of OV to infect and sustain ongoing replication, VSVΔ51 and MG1 were first UV-inactivated. Complete UV-inactivation of OV was confirmed by the absence of infection and cytopathic effects in Vero cells that are highly sensitive to OV infection (data not shown). Resting $CD4^+$ T cells were then infected with UV-inactivated VSVΔ51 or MG1 at the highest OV MOI. Interestingly, levels of integrated HIV-1 DNA were comparable between UV-inactivated MG1 and MG1-uninfected controls (Figure 34A), while MG1 (MOI 10) infection consistently resulted in a decrease in integrated HIV-1 DNA. In addition, UV-inactivation significantly abrogated the impact of VSVΔ51 (Figure 34B) or MG1 infection (Figure 34C) on HIV-1 outgrowth. Nevertheless, a modest, but non-significant reduction in p24 expression was still observed when cells were infected with UV-inactivated OVs.

In summary, MG1, but not VSVΔ51 infection, resulted in a decrease in the level of integrated HIV-1 DNA in latently HIV-1 infected resting $CD4^+$ T cells. Interestingly however, a significant decrease in the p24 expression was observed following reactivation of latently HIV-1 infected cells that were infected with either VSVΔ51 or MG1. This reduction in the integrated HIV-1 DNA and p24 expression following OV infection was significantly diminished by first UV-inactivating the OV, thereby highlighting the importance of OV replication.

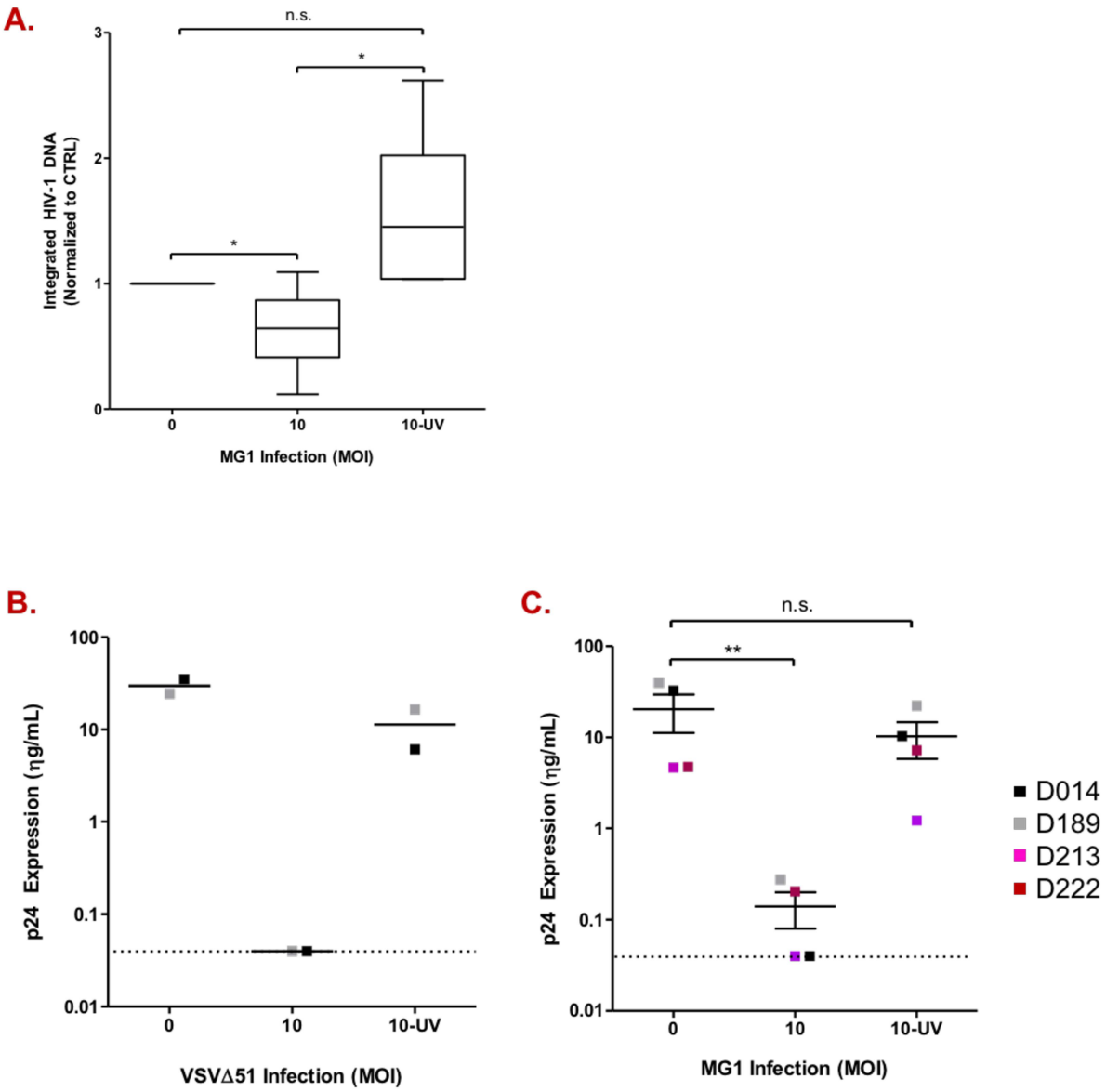

A.
n.s.
*
*
Integrated HIV-1 DNA (Normalized to CTRL)
3
2
1
0
0
10
10-UV
MG1 Infection (MOI)
B.
p24 Expression (ηg/mL)
100
10
1
0.1
0.01
0
10
10-UV
VSVΔ51 Infection (MOI)
C.
n.s.
**
p24 Expression (ηg/mL)
100
10
1
0.1
0.01
0
10
10-UV
MG1 Infection (MOI)
D014
D189
D213
D222

Figure 34. UV-inactivation of VSVΔ51 or MG1 significantly impairs the ability of OV to eliminate latently HIV-1 infected resting CD4$^+$ T cells. As before latently HIV-1 infected cells were infected with VSVΔ51 or MG1, as well as the UV-inactivated OV for 48 hours, after which integrated HIV-1 DNA and inducible HIV-1 replication by outgrowth were quantified. **A.** Integrated HIV-1 DNA was quantified in latently HIV-infected resting CD4$^+$ T cells following infection with MG1 or UV-inactivated MG1 at indicated MOI (n=6). Inducible HIV-1 p24 expression was quantified following infection of latently HIV-infected resting CD4$^+$ T cells with replication-capable or UV-inactivated **B.** VSVΔ51 (n=2) and **C.** MG1 (n=4). Figure legend represents separate biological replicates (individual healthy donors). *p<0.001, **p=0.0228 by One-way ANOVA on ranks and p<0.05 by Dunn's multiple comparison test; Data represent mean ± SEM.

Note: OV infection, DNA isolation, and HIV-1 outgrowth assay was prepared by N Ranganath. Integrated HIV-1 DNA PCR for n=6 of MG1 infection was performed was performed by S.C. Côté. N=2 for infection with UV-inactivated VSVΔ51 and MG1 was performed by N Ranganath. An additional N=2 for UV-inactivated MG1 experiment was performed by T.S. Sandstrom.

5.3.2. MG1 infection results in the elimination of latently HIV-1 infected memory CD4$^+$ T cells isolated from HIV-1 infected individuals on HAART

To evaluate the capacity of OV to selectively target and eliminate the latent HIV-1 reservoir *ex vivo*, memory CD4$^+$ T cells were isolated from HIV-infected individuals (Figure 35). Baseline characteristics of HIV-infected patients studied are summarized in Table 1. All individuals had sustained plasma viral load suppression on HAART and CD4$^+$ T cell counts >400 cells/μL.

Following isolation, memory CD4$^+$ T cells were infected with MG1 at the indicated MOI for 48 hours (Figure 36A). MG1 was chosen as a candidate for further evaluation *ex vivo* due to its ability to significantly reduce both integrated HIV-1 DNA and inducible HIV-1 following outgrowth. In keeping with OV infection of *in vitro* model, minimal infection and OV-mediated cytopathic effects were observed following or MG1 infection (Figure 36C) of memory CD4$^+$ T cells isolated from patients.

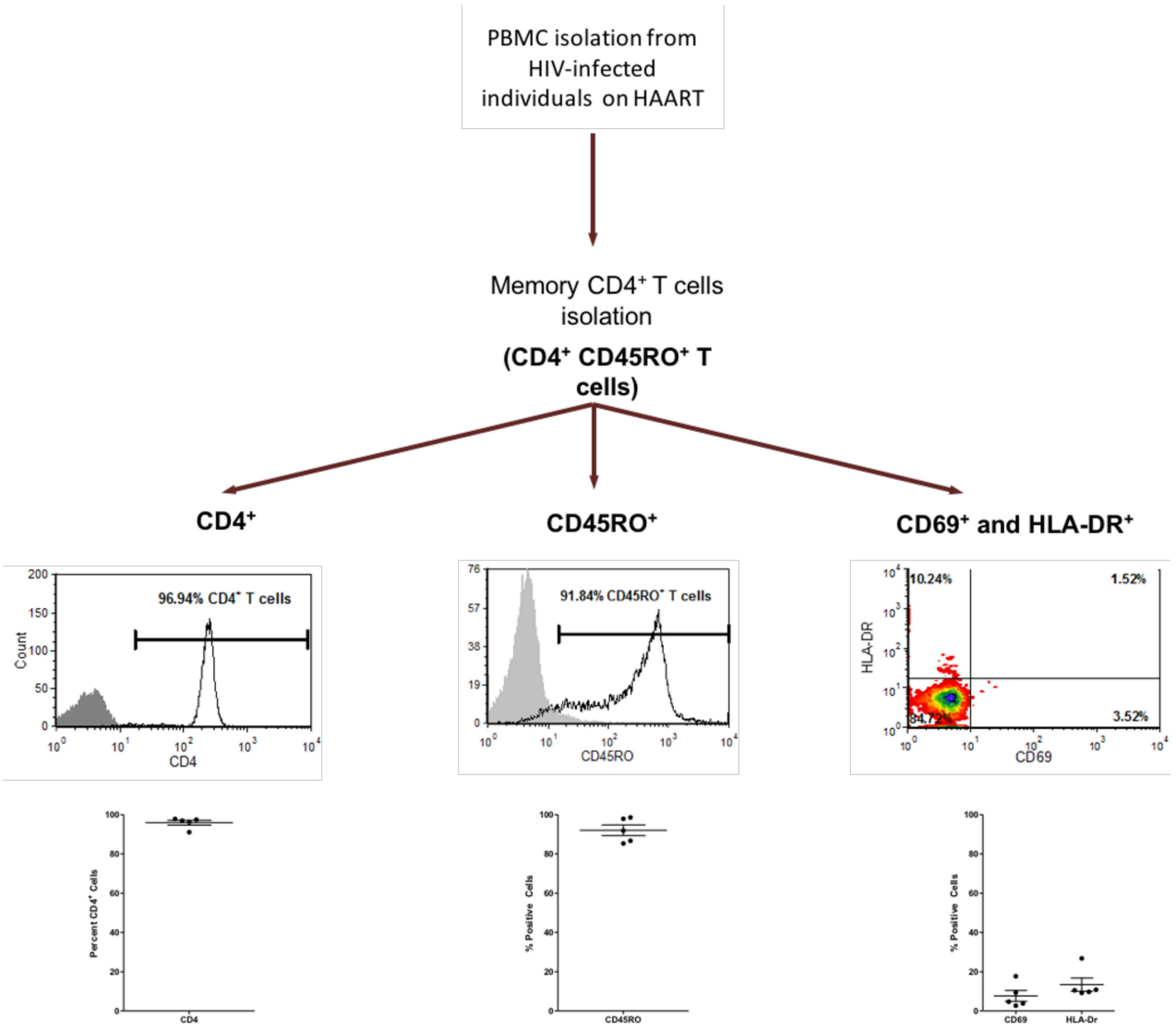
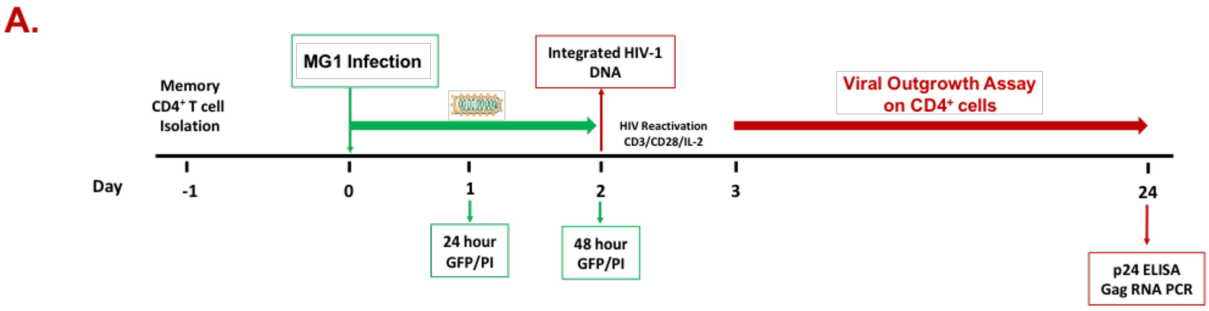
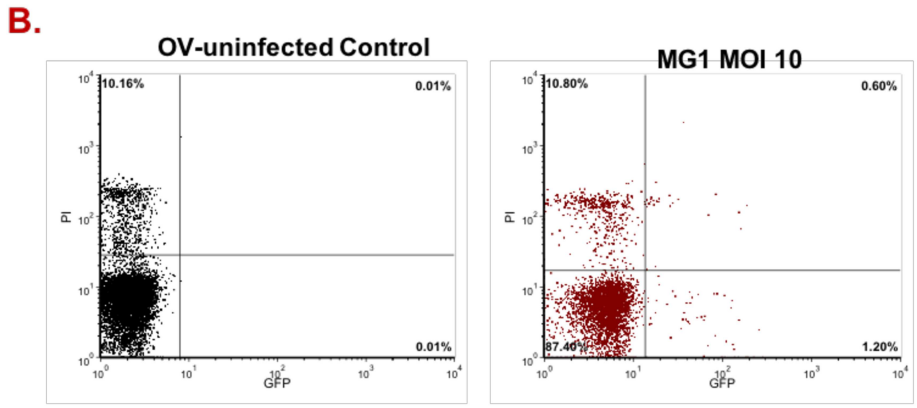

PBMC isolation from HIV-infected individuals on HAART
Memory CD4+ T cells isolation
(CD4+ CD45RO+ T cells)
CD4+
CD45RO+
CD69+ and HLA-DR+
96.94% CD4+ T cells
Count
CD4
91.84% CD45RO+ T cells
CD45RO
10.24%
1.52%
3.52%
HLA-DR
CD69
Percent CD4+ Cells
CD4
% Positive Cells
CD45RO
% Positive Cells
CD69
HLA-Dr

Figure 35. Memory $CD4^+$ T cells were isolated from HIV-1 infected individuals effectively managed on HAART. Memory $CD4^+$ T cells, characterized as $CD4^+$ and $CD45RO^+$ cells, were isolated from HIV-1 infected individuals on HAART. Purity and degree of cellular activation (CD69 and HLA-DR) were confirmed by flow cytometry (n=13).


A.
MG1 Infection
Integrated HIV-1 DNA
Viral Outgrowth Assay on CD4+ cells
Memory CD4+ T cell Isolation
HIV Reactivation CD3/CD28/IL-2
Day
-1
0
1
2
3
24
24 hour GFP/PI
48 hour GFP/PI
p24 ELISA Gag RNA PCR


B.
OV-uninfected Control
MG1 MOI 10
10.16%
0.01%
0.01%
10.80%
0.60%
87.40%
1.20%
PI
GFP

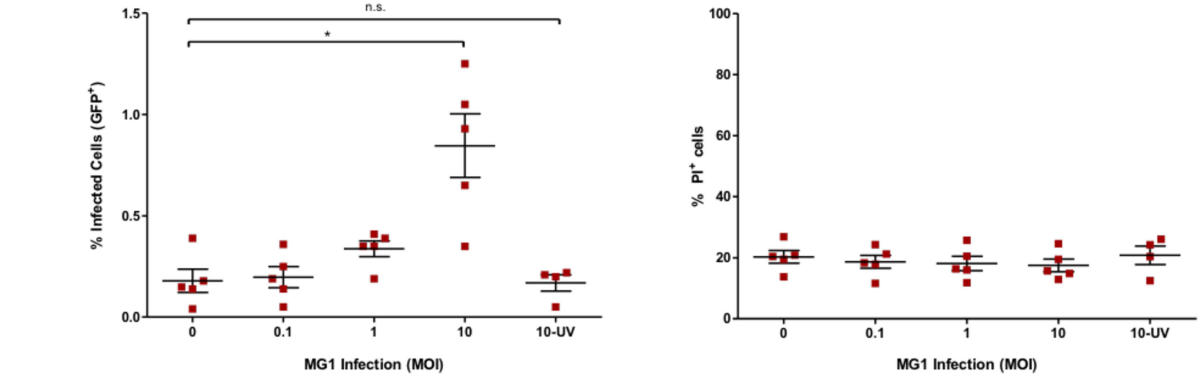

C.
n.s.
*
% Infected Cells (GFP+)
MG1 Infection (MOI)
0
0.1
1
10
10-UV
% PI+ cells
MG1 Infection (MOI)

Figure 36. Minimal MG1 infection and cytopathic effects are observed in memory CD4$^+$ T cells isolated from HIV-1 infected individuals on HAART. A. Experimental design for MG1 infection of memory CD4$^+$ T cells isolated from HIV-1 infected individuals. **B.** Representative histogram demonstrating MG1 infection (GFP$^+$ cells) and cell death (PI$^+$ cells). **C.** MG1 infection (left) and MG1-mediated cell death (right) was quantified following 48 hours of MG1 or UV-inactivated MG1 infection of memory CD4$^+$ T cells from HIV-1 infected individuals (n=5). n values represent separate biological replicates (individual HIV-1 infected donors); n.s. = not significant, *$p<0.0001$ by One-way ANOVA and $p<0.05$ by Tukey's multiple comparison test compared to MG1 MOI 0. No significant cell death (PI$^+$ cells) was observed following MG1 infection at any MOI by One-way ANOVA. Data represent mean ± SEM.

Next, the capacity of OV to eliminate the latent HIV-1 reservoir was evaluated by viral outgrowth after 48 hours of OV infection as previously described. To stimulate maximal HIV-1 reactivation, OV-infected and uninfected memory $CD4^+$ T cells were treated with anti-CD3 and anti-CD28 antibodies. Activated $CD4^+$ T cells were then added to the culture for 21 days to facilitate HIV-1 outgrowth. Inducible HIV-1 replication was quantified by measuring HIV-1 *gag* RNA expression in culture supernatant on day 21 of outgrowth. Interestingly, MG1 infection of memory $CD4^+$ T cells resulted in a dose-dependent reduction in HIV-1 *gag* RNA in cell-free supernatant (Figure 37). Consistent with prior findings, UV-inactivation of MG1 significantly abrogated the capacity of MG1 to reduce HIV-1 replication (Figure 37).

These findings highlight the capacity of MG1 infection to significantly reduce the inducible HIV-1 reservoir within memory $CD4^+$ T cells isolated from aviremic HIV-1 infected individuals. Going forward, the effect of MG1 infection on total and integrated HIV-1 DNA within memory $CD4^+$ T cells will be quantified.

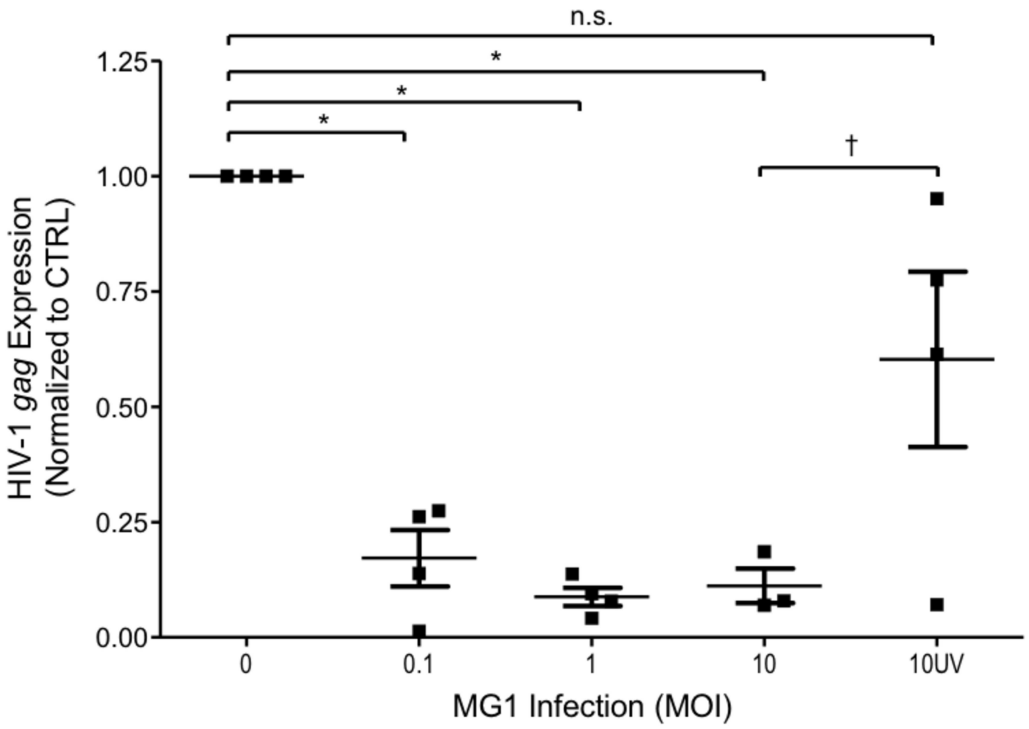

n.s.
*
*
*
†
1.25
1.00
0.75
0.50
0.25
0.00
HIV-1 gag Expression
(Normalized to CTRL)
0
0.1
1
10
10UV
MG1 Infection (MOI)

Figure 37. MG1 infection results in a significant reduction in inducible HIV-1 *gag* expression in memory CD4$^+$ T cells isolated from HIV-1 infected individuals. Memory CD4$^+$ T cells were infected with MG1 at indicated MOI for 48 hours, after which the cells were reactivated with CD3 and CD28 stimulation. Cells were then maintained in culture for 21 days with the addition of feeder cells. Cell-free supernatant was collected and HIV-1 expression was quantified by measuring HIV-1 *gag* RNA expression by RT-q-PCR (n=4). n values represent separate biological replicates (individual HIV-1 infected donors). n.s. = not significant, *$p<0.0001$ by One-way ANOVA and $p<0.05$ by Dunnett's multiple comparison test compared to MOI 0 and $^{\dagger}p=0.0354$ by paired t-test; Data represent mean ± SEM.

5.4. Discussion

The findings presented here provide a proof-of-concept that the latent HIV-1 reservoir can be targeted and purged using the IFN-sensitive oncolytic viruses, VSVΔ51 and MG1. The efficacy and therapeutic potential of OV as a strategy to eliminate latently HIV-1

infected cells were evaluated using an *in vitro* resting $CD4^+$ T cell model of HIV-1 latency and *ex vivo* memory $CD4^+$ T cells isolated from HIV-infected individuals on HAART.

As described previously, two important barriers to the use of primary cell models of HIV-1 latency are the rarity of latently HIV-1 infected cells and the lack of phenotypic markers to enrich these cells (Eisele and Siliciano, 2012; Siliciano et al., 2003) (Brooks and Zack, 2002) (Figure 29A). In the *in vitro* model of latency, ~0.5-1% of cells within the total population were identified to be latently HIV-1 infected. *Ex vivo*, ~0.1-1 IUPM $CD4^+$ T cells carry inducible integrated HIV-1 DNA (Siliciano et al., 2003). As anticipated, given the low frequency of latently HIV-1 infected cells, no significant difference in OV infection or killing was observed using flow cytometry between the mock and latently HIV-1 infected populations.

Most importantly, however, low levels of VSVΔ51 and MG1 infection (2-4% GFP^+ cells), as well as minimal to no OV-mediated cytopathic effects were observed, even at the highest OV doses (MOI 10). Consistent with the demonstrated safety profile of VSVΔ51 and MG1 in cancer models (Lichty et al., 2004b), these results highlight the intrinsic resistance of $CD4^+$ T cells to OV infection and introduce the potential for safe systemic administration of OV in individuals with HIV-1 infection.

The capacity of OV to target and eliminate latently HIV-1 infected cells *in vitro* and *ex vivo* was evaluated by measuring integrated proviral DNA, as well as p24 expression or HIV *gag* RNA following HIV-1 reactivation and outgrowth. In the *in vitro* latently HIV-1 infected resting $CD4^+$ T cells, VSVΔ51 infection had no significant effect on the level of integrated DNA. However, a significant decrease in p24 expression, relative to VSVΔ51-

uninfected controls, was observed following outgrowth. In contrast, MG1 infection resulted in a significant dose-dependent reduction of both integrated HIV-1 DNA and p24 expression following HIV-1 outgrowth. In the memory $CD4^+$ T cells isolated from HIV-1 infected individuals on HAART, MG1 infection resulted in a significant reduction in inducible HIV-1 replication as measured by HIV *gag* RNA expression. Albeit preliminary, further investigation into the effects of MG1 infection on integrated/total HIV-1 DNA in memory $CD4^+$ T cells is warranted.

These novel findings highlight the ability of OV to target and eliminate latently HIV-1 infected $CD4^+$ T cells in primary cell models of HIV-1 infection. Importantly however, the differences observed between VSVΔ51 and MG1 infection also introduce the potential for multiple mechanisms by which OV may interfere with the latent HIV-1 reservoir.

5.4.1. Direct OV-mediated cytopathic effects may underlie the effects of MG1 infection on latently HIV-1 infected $CD4^+$ T cells

We hypothesized that if impairment in IFN-I pathways are present within latently HIV-1 infected cells, VSVΔ51 or MG1 may be able to infect and selectively replicate within these cells. The subsequent cytopathic effects of OV infection should therefore significantly reduce the number of cells carrying integrated HIV-1 DNA and the potential for HIV-1 reactivation.

In keeping with this, MG1 infection, but not VSVΔ51, resulted in a reduction in both levels of integrated DNA and inducible HIV-1 replication following outgrowth. Interestingly, MG1 has been shown to infect cells more rapidly and exert greater

cytopathic effects than VSVΔ51 in several tumor models (Brun et al., 2010; Furió et al., 2012). In addition, introduction of the analogous MG1 double mutation in M and G protein within the VSV genome confers VSV with enhanced apoptotic potential (Furió et al., 2012). Therefore, despite similar quantitative levels of VSVΔ51 and MG1 infection, the greater cytopathic potential of MG1 may directly contribute to the observed dose-dependent decrease in integrated DNA and HIV-1 replication. Most importantly, given the lack of non-specific cell death observed in the context of MG1 infection, the ~30-40% reduction in levels of integrated HIV-1 DNA (at MOI 10) strongly supports the potential for elimination of latently HIV-1 infected resting $CD4^+$ T cells by MG1.

5.4.2. VSVΔ51 may reduce HIV-1 outgrowth by inducing an IFN-I response and/or competing with HIV-1 for cellular machinery

VSVΔ51 infection resulted in a substantial decrease in HIV-1 outgrowth from the *in vitro* latently infected resting $CD4^+$ T cells, despite the lack of change in integrated HIV-1 DNA. This finding was unexpected given that integrated HIV-1 DNA has been demonstrated to closely predict the size of the replication-competent viral pool (Kiselinova et al., 2016). However, VSVΔ51 may be able to impact the reactivation and/or HIV-1 replication through mechanisms independent of virus-mediated killing.

Infection of healthy cells with VSVΔ51 triggers the production of various cellular and soluble factors including IFN-I cytokines and effector ISGs (Stojdl et al., 2003). Although the wild-type VSV is able to effectively block IFN-I responses, the mutations engineered within VSVΔ51 impair the ability of the virus to do so (Brun et al., 2010; Stojdl

et al., 2003). Thus, VSVΔ51 infection can induce an IFN-I response, but is unable to protect itself against the antiviral effects of this system.

In the *in vitro* model of latent HIV-1 infection, the majority of the population is comprised of HIV-uninfected, bystander cells (Figure 29A). Although the latently HIV-1 infected cells may have impairments in IFN-I signaling, the bystander cells can still elicit an antiviral response to VSVΔ51 infection. The subsequent activation of the IFN-I system may create an antiviral microenvironment that not only affects the maintenance of HIV-1 latency, but also inhibits HIV-1 reactivation and/or ongoing HIV-1 replication. The potent capacity of IFN-I to suppress HIV-1 replication has been previously reported (Doyle et al., 2015; Goujon and Malim, 2010; Malim and Emerman, 2008; Neil et al., 2008), and may in part explain the robust VSVΔ51-mediated decrease in viral outgrowth, in the absence of changes to integrated HIV-1 DNA.

Alternatively, the presence of OV within the latently HIV-1 infected cells may also significantly diminish HIV-1 reactivation and/or replication through direct competition between OV and HIV-1 for host transcription factors and cellular translational machinery, as described in chapter 4 (Furió et al., 2012). Similar mechanisms may also contribute to the observed reduction in HIV-1 DNA and p24 expression following MG1 infection.

5.4.3. UV-inactivation of OV highlights the multiple mechanisms by which OV may interfere with HIV-1 latency

VSVΔ51 and MG1 were inactivated by UV-irradiation in order to discern if OV replication and killing was necessary, or if the presence of viral components were sufficient to induce

the observed decrease in integrated DNA and inducible HIV-1 replication. As shown by Zhang and colleagues, UV-inactivation results in OV that retain genomic and structural integrity, as well as the capacity for cellular entry, but are largely deficient in their ability to replicate in and kill tumor cells (Zhang et al., 2014). This loss of OV replication and cytopathic effects were confirmed on Vero cells following UV-inactivation.

Interestingly, UV-inactivation of MG1 significantly diminishes the effect of MG1 infection on integrated HIV-1 DNA and p24 expression following outgrowth in both *in vitro* resting $CD4^+$ T cells and *ex vivo* memory $CD4^+$ T cells. Similar reduction in the effect of VSVΔ51 on viral outgrowth was also observed following UV-inactivation. The greater potency of replication-competent VSVΔ51 and MG1 in reducing latent HIV-1 infection underscores the importance of OV replication and cytopathic effects in mediating these effects.

On the other hand, a modest, albeit non-significant reduction in p24 or *gag* RNA expression, but not integrated HIV-1 DNA, is still observed following infection of $CD4^+$ T cells with UV-inactivated OV. UV-inactivated MG1 has been previously reported to activate natural killer cells and dendritic cells *in vivo*, through the production of IFN-I and other proinflammatory cytokines (Zhang et al., 2014). Similar induction of a soluble cytokine response may be responsible for the reduction in p24 or *gag* RNA following infection with UV-inactivated OV. These findings further reinforce the potential for multiple levels of OV-mediated interference of latent HIV-1 infection.

5.5. Future Directions

The current study sought to translate the work investigating the effects of VSVΔ51 and MG1 infection on HIV-1 latency to *in vitro* and *ex vivo* primary cell models of latent HIV-1 infection. A significant reduction in the size of the latent HIV-1 reservoir as quantified by a decrease in integrated proviral DNA and replication of HIV-1 following reactivation, were observed following VSVΔ51 or MG1 infection. This effect of OV infection on latently infected cells was attenuated by UV-inactivation of VSVΔ51 and MG1.

Ex vivo MG1 infection of memory $CD4^+$ T cells from HIV-1 infected individuals resulted in an observable reduction in HIV-1 *gag* RNA expression following 21 days of viral outgrowth. Quantifying the ability of MG1 to eliminate integrated HIV-1 DNA represents the next critical step to deciphering the potential mechanisms by which OV interfere with latent HIV-1 infection. Most importantly, the *ex vivo* study, albeit preliminary, is promising and warrants further investigation in a larger cohort of patients.

OV may interfere with latent HIV-1 infection on multiple levels. Selective OV replication and direct cytopathic effects within latently HIV-1 infected cells, as well as indirect OV-mediated activation of the antiviral IFN-I system in bystander cells were hypothesized as two plausible explanations for the differences in the impact of VSVΔ51 or MG1 infection on the latent HIV-1 reservoir. Various strains of VSV and maraba virus with differences in fitness, replication rates, cytopathic capacity, and the ability to block IFN-I responses have been identified or engineered (Brun et al., 2010; Stojdl et al., 2000b). Going forward, wild-type and recombinant strains of the OV can be utilized in parallel

with VSVΔ51 or MG1 to further delineate the impact of viral fitness and cytopathic potential on latent HIV-1 infection.

In parallel, characterization of the IFN-I cytokines, as well as effector ISGs within the latently HIV-1 infected resting $CD4^+$ T cell population during the 48 hours of VSVΔ51 or MG1 infection may provide insight into the state of IFN-I responses just prior to HIV-1 reactivation. Consistent with this, IFNβ and IFNα expression, as well as activation of various antiviral ISGs, have been temporally observed following infection with several strains of VSV (Stojdl et al., 2003). The rarity of latently HIV-1 infected cells and the low assay sensitivity, however, represent an important barrier to such an experiment. Nevertheless, use of neutralizing anti-IFNβ and/or anti-IFNα antibodies during OV infection, just prior to HIV-1 reactivation in resting $CD4^+$ T cells, may be useful in delineating the impact of IFN-I responses, if any, on latent HIV-1 infection.

5.6. Conclusion

To our knowledge, the work presented in this chapter represents the first study investigating the therapeutic application of the oncolytic viruses, VSVΔ51 and MG1, towards the goal of targeting and eliminating the latent HIV-1 reservoir. Building on previous work in cell line models of latent HIV-1 infection, the ability of VSVΔ51 and MG1 to purge latently HIV-1 infected cells was evaluated using a resting $CD4^+$ T cell model of latency. Interestingly, a significant dose-dependent reduction in levels of integrated DNA and outgrowth of HIV-1 was observed following MG1 infection. While VSVΔ51 infection significantly interfered with HIV-1 outgrowth, it did not appear to influence levels of

integrated DNA. The differences between the viruses highlight the potential for multiple mechanisms by which OV may interfere with latent HIV-1 infection.

Ex vivo MG1 infection of memory $CD4^+$ T cells isolated from HIV-1 infected individuals on HAART resulted in a significant reduction in HIV *gag* RNA expression following outgrowth. This effect was significantly abrogated through UV-inactivation of MG1. The work presented here represents a significant step towards the development of a therapeutic strategy aimed at targeting and eliminating the latent HIV-1 reservoir. Most importantly, this represents a novel approach that utilizes potential intracellular defects within latently HIV-1 infected cells to selectively purge the latent HIV-1 reservoir.

Chapter 6: Discussion and future directions

6.1. Oncolytic viruses as a potential approach to eliminate cells that constitute the latent HIV-1 reservoir

The objective of the proof-of-concept study presented in this dissertation was to investigate the capacity of two oncolytic viruses, VSVΔ51 and MG1, to selectively target and kill latently HIV-1 infected cells by exploiting potential defects in IFN-I pathways.

In chapter 3, impairments in IFN-I responsiveness were demonstrated within two independent, latently HIV-1 infected cell lines, U1 and OM10.1, when compared to their respective parental HIV-uninfected cell lines, U937 and HL60. This included IFNα/β production and expression of IFNAR1, MHC-I, ISG15, and PKR. Interaction between viral factors and host proteins, and/or permanent cellular modifications induced during the establishment of latent HIV-1 infection were hypothesized to be two plausible mechanisms underlying these defects.

In chapter 4, the IFN-defective U1 and OM10.1 cells were shown to be significantly more sensitive to VSVΔ51 and MG1 infection and MG1-mediated cytopathic effects, when compared to the parental controls. In addition, reactivation of HIV-1 replication using the HDAC inhibitor (HDACi), SAHA, resulted in a selective enhancement of OV infection in the latently HIV-1 infected cell lines, but not HIV-uninfected parental cells. A reciprocal decrease in HIV-1 p24 expression was observed following VSVΔ51 and/or MG1 infection of SAHA-treated cell lines. Although the exact mechanism remains to be established, impairments in IFN-I responses were postulated to be the likely mechanism underlying enhanced susceptibility of U1 and OM10.1 cells to OV.

In chapter 5, VSVΔ51 and MG1 infection were demonstrated to significantly reduce the number of latently HIV-1 infected cells using both an *in vitro* resting $CD4^+$ T cell model of HIV-1 latency and *ex vivo* memory $CD4^+$ T cells isolated from HIV-infected individuals on HAART. Key differences were observed between the effects of VSVΔ51 and MG1 on levels of integrated proviral DNA and HIV-1 outgrowth, supporting the hypothesis that OV may interfere with latent HIV-1 infection on multiple levels. The *ex vivo* study, albeit preliminary, provides support for the assessment of oncolytic viruses as a strategy to target and eliminate the latent HIV-1 reservoir *in vivo*.

6.2. Understanding the differences in the impact of VSVΔ51 and MG1 infection on latently HIV-1 infected cells

VSVΔ51 and MG1 share numerous similarities in tropism, lifecycle, virulence, and pathogenesis. In the present study, however, differences in cytopathic capacity, induction of IFN-I responses, and effect on latent HIV-1 infection were observed between VSVΔ51 and MG1.

The latently HIV-1 infected cell lines, relative to parental controls, were uniformly more sensitive to both VSVΔ51 and MG1 infection. However, the extent of cell death was significantly greater following MG1 infection, than VSVΔ51 infection. Previous studies characterizing the mutational profiles of recombinant VSV and maraba viruses have reported similar differences in the cytopathic potential of both viruses. The double mutation in the M (L123W) and G (Q242R) proteins of maraba virus, in contrast to the Δ51 mutation in the M protein of VSV, has been shown to significantly enhance early viral

replication and improve cell killing (Brun et al., 2010). Interestingly, introduction of the same double mutation to VSV also results in higher levels of apoptosis when compared to the WT-virus (Furió et al., 2012). Therefore, improved cell killing may represent an important mechanism for MG1-mediated effects on latent HIV-1 infection. Consistent with this, infection of latently HIV-1 infected resting $CD4^+$ T cells with MG1, but not VSVΔ51, resulted in a dose-dependent decrease in integrated proviral DNA.

As previously described, VSVΔ51 and MG1 are significantly attenuated in their ability to block normal cellular IFN-I responses (Stojdl et al., 2003). Consequently, IFN-I production is observed in response to OV infection of healthy cells (Brun et al., 2010; Stojdl et al., 2003). Consistent with this, infection with both OV resulted in induction of IFNα secretion in cell lines. Interestingly however, a more robust IFN-I response was observed following VSVΔ51 infection, than MG1 infection, especially in the HIV-uninfected U937 and HL60 cells. This difference may highlight potential variabilities between VSVΔ51 and MG1 in their ability to induce an IFN-I response or their ability to block endogenous IFN-I production. Most importantly, the differences observed between the viruses underscores the importance of investigating both OV as potential therapeutic candidates to target the latent HIV-1 reservoir.

6.3. Developing models to study mechanisms underlying IFN-I signaling impairments and OV infection of latently HIV-1 infected primary cells

The characterization of IFN-I defects using U1 and OM10.1 cell lines represents a novel set of intracellular impairments present in latently HIV-1 infected cells, but not

uninfected parental cells. Although these cell lines provided an invaluable tool to evaluate the IFN-I system, applying these findings to physiological models of latent HIV-1 infection is limited by the transformed nature of these cells.

Going forward, delineation of IFN-I signaling within latently HIV-1 infected primary cells will be necessary. In addition, the demonstration of selective replication and killing of latently HIV-1 infected cells by OV, as well as characterization of possible mechanisms underlying enhanced susceptibility of HIV-infected cells to VSVΔ51 and MG1 infection represent key future directions. Presently, two major challenges preclude the undertaking of such studies using primary cell models of HIV-1 latency. First, latently HIV-1 infected cells are a rare population both *in vitro* and *in vivo*, representing a very small fraction of the $CD4^+$ T cell population (Chun et al., 1997b). Second, there is a lack of phenotypic markers that can distinguish latently HIV-infected cells from HIV-uninfected cells (Descours et al., 2017).

To overcome these obstacles, novel HIV-1 constructs such as the murine heat-stable antigen (HSA) expressing HIV-1 (HIV-1_{HSA}) that allow for the amplification and/or enrichment of latently HIV-1 infected cells may be of value (Imbeault et al., 2009a). During productive infection with HIV-1_{HSA}, the HSA protein is expressed on the cell surface. As a result, productively HIV-1 infected cells can then be enriched by selective magnetic-bead or FACS-based isolation. These cells can then be used to establish post-activation models of HIV-1 latency as reported in several studies (Bosque and Planelles, 2009; Yang et al., 2009).

In the search for potential markers, Fromentin and colleagues have demonstrated that a majority of latently HIV-1 infected cells carrying inducible genomes may express a combination of cell-surface, immune checkpoint molecules including cell death protein 1 (PD-1), T-cell immunoglobulin and ITIM domain (TIGIT), and lymphocyte activation gene 3 (LAG3) (Fromentin et al., 2016). More recently, CD32a was identified as a potential marker that can be utilized to enrich 26.8% to 86.3% of the inducible latent HIV-1 reservoir *in vivo* (Descours et al., 2017). Despite the significance of these findings, practical application in clinical studies continues to be a challenge due to the rarity of latently HIV-infected cells. $CD32a^+$ $CD4^+$ T cells represent only 0.012% of the entire population of $CD4^+$ T cells (Descours et al., 2017). Going forward, use of such markers for selective enrichment of latently HIV-1 infected cells may represent a major tool to further understand features of HIV-1 latency.

Ultimately, such approaches represent an important and necessary step in characterizing the IFN-I system in HIV-1 latency, as well as in gaining a mechanistic insight into the host-virus interactions that may underlie the enhanced susceptibility of latently HIV-1 infected cells to OV infection.

6.4. Translation of current work to *ex vivo* and *in vivo* models of HIV-1 latency

The capacity of VSVΔ51 and MG1 to target and eliminate latently HIV-1 infected cells was evaluated in a step-wise approach using cell lines, an *in vitro* resting $CD4^+$ T cell model of HIV-1 latency, and *ex vivo* in memory $CD4^+$ T cells isolated from HIV-infected individuals (Figure 38A-C). The current study was focused on evaluating the effects of OV

infection in $CD4^+$ T cells, as they represent the primary latent HIV-1 reservoir (Chun et al., 1995). However, VSVΔ51 and MG1 have a broad tropism and are capable of infecting other primary cells including monocytes, macrophages, and B cells (Lichty et al., 2004b). Therefore, evaluation of the impact of OV infection on HIV-1 latency within a microenvironment that mimics *in vivo* settings, may represent a logical next step in translation of this work into animal models.

The human lymphoid aggregate culture (HLAC) developed using tonsillar tissue represents such a system (Glushakova et al., 1997). The HLAC model closely emulates the lymphoid architecture including the presence of various immune cell subsets, the potential for cell-to-cell interactions, and secretion of soluble factors and cytokines (Glushakova et al., 1995; Homann et al., 2009). Importantly, Chavez and colleagues have demonstrated the ability to establish latent HIV-1 infection using this system (Chavez et al., 2015). Therefore, the influence of the lymphoid milieu on OV infection, as well as the reciprocal impact of OV infection on latent HIV-1 reservoirs present within this model can be evaluated using HLAC.

Going forward, the use of preclinical models to characterize the therapeutic efficacy, safety, and mechanisms by which OV interfere with the latent HIV-1 reservoir will be integral in translating this work to clinical studies. As such, several humanized mouse and primate models of HIV-1 infection have been developed to understand viral immunopathogenesis and evaluate therapeutic strategies (Garcia, 2016; Zhang and Su, 2012) (Figure 38D-F). The NOD/SCID-gamma chain null (NSG) mouse model engrafted with human thymus, liver, and autologous $CD34^+$ hematopoietic stem cells (BLT)

represents an interesting system for the evaluation of OV infection. The BLT mouse model contains virtually all human hematopoietic cell types and is able to sustain a systemic HIV-1 infection and immune response that mimics human infection (Garcia, 2016). Importantly, in HAART-treated, humanized BLT mice, the establishment of an inducible, latent HIV-1 reservoir within resting $CD4^+$ T cells has been demonstrated (Denton et al., 2012; Marsden et al., 2012). The capacity of VSVΔ51 or MG1 to target and eliminate the latent HIV-1 reservoir, as well as the safety profile of OV *in vivo* can therefore be evaluated effectively using HIV-1 infected BLT mice.

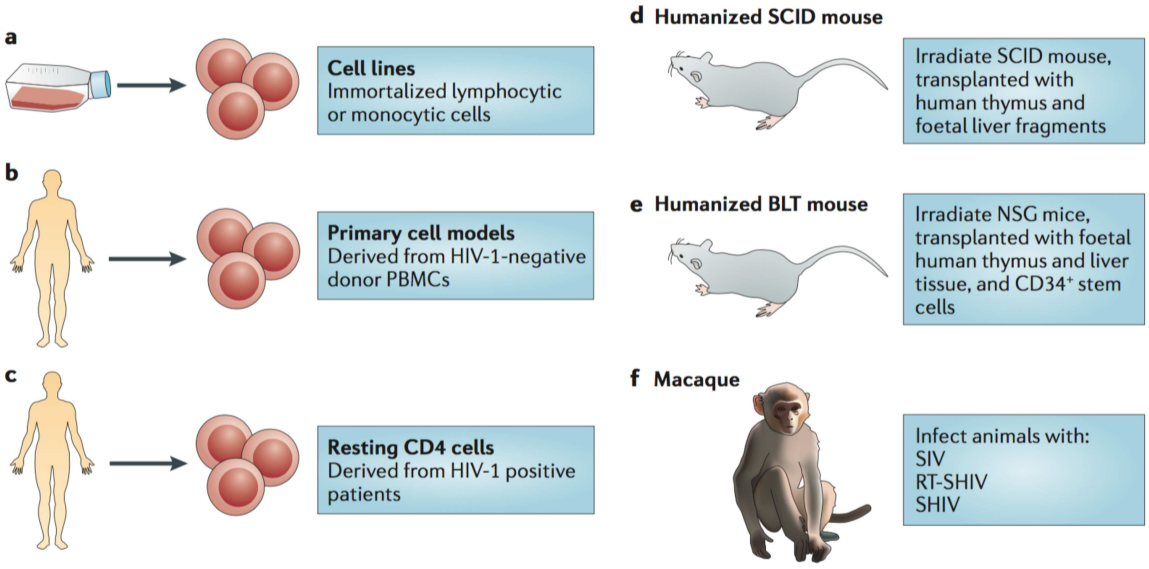

a
Cell lines
Immortalized lymphocytic or monocytic cells
b
Primary cell models
Derived from HIV-1-negative donor PBMCs
c
Resting CD4 cells
Derived from HIV-1 positive patients
d Humanized SCID mouse
Irradiate SCID mouse, transplanted with human thymus and foetal liver fragments
e Humanized BLT mouse
Irradiate NSG mice, transplanted with foetal human thymus and liver tissue, and CD34+ stem cells
f Macaque
Infect animals with:
SIV
RT-SHIV
SHIV

Figure 38: Current model systems to study HIV-1 latency. A-C. Cell models. Cell line models (part a) are derived from immortalized T cell clones (for example, from Jurkat-derived cell lines) or pro-monocyte clones (for example, U1), and they have uniformly integrated copies of proviral HIV-1 DNA. By contrast, primary cell models (part b) are derived from HIV-1-negative donor CD4+ T cells, and latency is established following infection using different protocols. Studies in cells obtained from aviremic, antiretroviral therapy (ART)-treated patients (part c) can be studied ex vivo for their response to putative latency-reversing agents and other stimuli. **D-E.** Humanized mouse models. Several humanized mouse models have been developed by engraftment of mice with various human tissues. Humanized severe combined immunodeficiency (SCID) mice (part d) are generated by transplanting irradiated SCID mice with human thymus and fetal liver tissue that develops into a human thymic organoid and supports HIV-1 infection, but only within this organoid. As HIV-1 replication is limited to the thymus, latency is only established in naive T cells. Engraftment of the human immune system was vastly improved with the development of the humanized NSG (NOD SCID gamma) mouse (not shown), which is generated by transplanting irradiated NOD/SCID/IL-2Rγ chain knockout mice with human $CD34^+$ stem cells. Humanized BLT (bone marrow–liver–thymus) mice are generated by implanting human fetal thymus and liver cells into NOD SCID or NSG mice and transplantation of human $CD34^+$ stem cells (part e). The reconstitution of the human immune system and the systemic modelling of HIV infection and latency is most robust in this mouse model. **F.** Non-human primate models. The course of SIV infection in rhesus and pig-tailed macaques reflects the progression of HIV-1 infection in humans. SIV-infected animals also respond to ART. However, SIV is not susceptible to non-nucleoside reverse transcriptase inhibitors (NNRTIs) and its envelope sequence is functionally divergent from that of HIV-1. The recombinant SIV viruses RT-SHIV and SHIV are aimed at overcoming these limitations using HIV-1 reverse transcriptase and envelope, respectively. RT-SHIV enables the use of clinically relevant ART combinations, and SHIV models have wider immunotherapeutic potential and can use both CC-chemokine receptor 5 (CCR5) and CXC-chemokine receptor 4 (CXCR4) co-receptors. PBMCs, peripheral blood mononuclear cells. Reprinted by permission from Macmillan Publishers Ltd: Nat. Rev. Microbiol., Archin, N.M., Sung, J.M., Garrido, C., Soriano-Sarabia, N., and Margolis, D.M. (2014). Eradicating HIV-1 infection: seeking to clear a persistent pathogen. *12*, 750–764. (Figure 2). Copyright 2009

6.5. The use of OV as a "kill" strategy in combination with latency reversal agents

Due to the complex mechanisms underlying the establishment and maintenance of the latent HIV-1 reservoir, a multi-pronged approach will likely be necessary in designing therapies aimed at HIV-1 eradication. The "shock and kill" strategy is founded on the idea that reactivation of HIV replication, in combination with augmentation of HIV-specific immune responses, can facilitate the elimination of latently HIV-1 infected cells.

In keeping with this, latency reversal was investigated as a potential strategy to further accentuate IFN-I impairments and enhance OV infection. SAHA (Vorinostat), a potent inhibitor of HDACs, was observed to significantly and selectively enhance VSVΔ51 and MG1 infection, as well as MG1-mediated cytopathic effects in U1 and OM10.1 cells (Chapter 4). In addition to inducing HIV-1 replication in these latently infected cell lines, SAHA may also directly augment OV infection and killing by dampening the cellular IFN-I response. These results are consistent with previous studies that showed that treatment of human prostate, breast, and ovarian cancer xenograft models with SAHA significantly improved VSV replication, dissemination, and oncolysis (Nguyên et al., 2008b).

Interestingly, SAHA may also play a crucial role in augmenting systemic tumor or HIV-1 specific CTL responses. For instance, SAHA in combination with an Adenovirus prime-VSV boost vaccine was shown to significantly improve the elimination of intracranial melanoma by enhancing tumor-specific immune responses *in vivo* (Bridle et al., 2013). Going forward, SAHA and other HDACi may be useful as chemical switches to not only reactivate HIV-1 replication within latently HIV-1 infected cells, but also to

potentiate the killing of these cells by promoting OV infection and indirect enhancement of HIV-1/OV-specific immune responses.

Modulation of immune checkpoint proteins may also boost OV-mediated targeting of the latent HIV-1 reservoir (Marchini et al., 2016). Recently, checkpoint proteins, including Cytotoxic T-Lymphocyte Associated Protein 4 (CTLA-4), PD-1, TIGIT, and LAG-3, were identified to be important in the establishment and/or maintenance of the latent HIV-1 reservoir (Barouch and Deeks, 2014; Fromentin et al., 2016). Consistent with this, immune checkpoint blockers (ICBs) such as the anti-CTLA4 antibody, ipilimumab, were reported to induce reactivation of HIV-1 in infected individuals on HAART (Wightman et al., 2015). Interestingly, the use of anti-CTLA4 antibody has also been shown to potentiate VSV-mediated elimination of mammary tumors through the recruitment of tumor-specific $CD4^+$ and $CD8^+$ T cell responses (Gao et al., 2009). Therefore, the use of ICBs in combination with OV may represent a viable approach to reversing HIV-1 latency and improving the killing of the latently HIV-1 infected cells.

Ultimately, a multifaceted approach using latency reversal or immune modulation agents in combination with augmentation of killing via direct OV-mediated oncolysis and recruitment of immune responses may represent the most practical approach to eliminating the latent HIV-1 reservoir.

6.6. Practical benefits and limitations to using OV as a strategy to eliminate the latent HIV-1 reservoir

6.6.1. Can OV effectively target latently HIV-1 infected cells that are systemically disseminated *in vivo*?

In HIV-1 infected individuals on HAART, latently HIV-infected cells are disseminated throughout the body and can be isolated in samples from the peripheral blood and lymphoid tissues including lymph nodes, spleen, and GALT (Chun et al., 1998) (Chun et al., 2008; Churchill et al., 2016; Haase et al., 1996). However, despite the capacity of these cells to circulate systemically, the majority of cells carrying proviral DNA are present within distinct anatomic reservoirs (Chun et al., 2008). Within these compartments, resting memory $CD4^+$ T cells and T follicular helper cells (T_{fh}) are capable of sustaining persistent HIV-1 infection (Deleage et al., 2016; Perreau et al., 2013). Enrichment of latently HIV-1 infected cells within these reservoirs may provide a microenvironment that, like the tumor milieu, can facilitate the establishment of a locus of OV infection. This is important because OV, as biological agents, have the unique capacity to self-propagate; allowing even low titers of virus to rapidly reach the therapeutic threshold needed to eliminate latently HIV-1 infected cells within the lymphoid tissues (Parato et al., 2005).

OV may also be capable of eliminating latently HIV-1 infected cells disseminated on a systemic level. *In vivo* models of leukemia and metastatic tumors provide an interesting parallel to latent HIV-1 infection (Cody and Hurst, 2015a; Patel et al., 2011a). Administration of measles virus has been reported to effectively eliminate tumor cells in murine models of disseminated acute lymphoblastic leukemia (ALL) and chronic

lymphocytic leukemia (CLL) (Castleton et al., 2014; Patel et al., 2011a). Similarly, VSV and MG1 have been shown to selectively reduce the tumor burden in metastatic breast cancer (Bourgeois-Daigneault et al., 2016; Cody and Hurst, 2015a). The capacity of OV to potentially replicate within tissue reservoirs enriched with latently HIV-1 cells, as well as target susceptible cells in circulation, therefore represents an important asset in purging the latent reservoir.

6.6.2. Determining the route and therapeutic dose needed to eliminate latently HIV-1 infected cells *in vivo*

Both intravenous (IV) and intratumoral (IT) injection of VSVΔ51 and MG1 have been demonstrated to be safe and efficacious in various xenograft models of melanoma, colon, ovarian, and lung cancers (Brun et al., 2010; Stojdl et al., 2003). For the treatment of disseminated tumors, however, IV injection represents the preferred route of administration (Cody and Hurst, 2015b; Patel et al., 2011b). Given the similarities between the models, the IV route likely represents the most viable approach in the context of HIV latency (Figure 39).

The challenge with IV administration of OV, however, is that greater doses of OV may be required to reach the HIV reservoirs disseminated *in vivo*. In both resting $CD4^+$ T cells and memory $CD4^+$ T cells isolated from HIV-infected individuals, a significant reduction in integrated HIV-1 DNA and/or inducible HIV-1 was observed following MG1 infection at MOI 1 and 10, as well as VSVΔ51 infection at MOI as low as 0.1 to 1. Within our models these doses of OV correspond to PFU ranging from ~10^4 to 10^7. In clinical trials

studying OV, IV doses of up to 10^{11} to 10^{12} PFU have been successfully administered with tolerable safety and efficacy profiles (Aghi and Martuza, 2005). Consistent with this, the phase I/II clinical trial assessing MG1 oncolytic virotherapy as a strategy to reduce tumor burden in advanced metastatic melanoma is evaluating the efficacy and tolerability of repeated MG1 dosing at 10^{10} PFU/dose (ClinicalTrials.gov Identifier: NCT02285816). Similar viral concentrations will likely be required to reach and establish OV infection within the various anatomic and physiologic compartments that encompass the latent HIV-1 reservoir.

Determining the appropriate dosing, however, will require further understanding of the mechanisms by which OV target and eliminate HIV-infected cells. In addition, the cellular microenvironment *in vivo*, capacity of OV to replicate in various tissues, the presence of endogenous cells, and the immune response to OV will need to be accounted for when translating doses from pre-clinical to *in vivo* studies (Aghi and Martuza, 2005). Nevertheless, the ability of OV to eliminate latently HIV-1 infected cells warrants further study using *in vivo* models of HIV-1 latency.

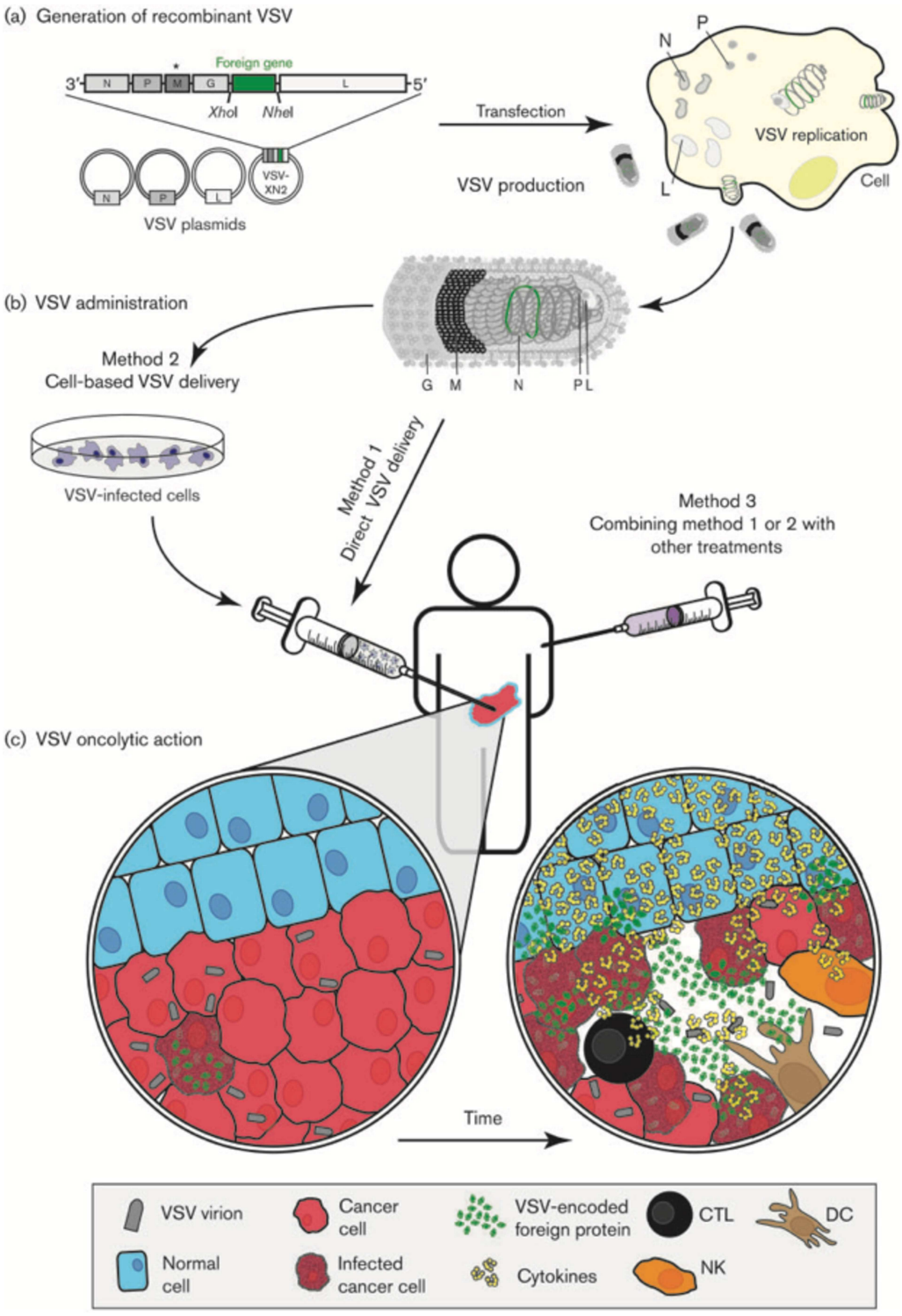

(a) Generation of recombinant VSV
Foreign gene
3′
N
P
M
G
L
5′
XhoI
NheI
VSV-XN2
VSV plasmids
Transfection
VSV production
N
P
L
VSV replication
Cell
(b) VSV administration
Method 2
Cell-based VSV delivery
G M N P L
VSV-infected cells
Method 1
Direct VSV delivery
Method 3
Combining method 1 or 2 with
other treatments
(c) VSV oncolytic action
Time
VSV virion
Cancer cell
VSV-encoded foreign protein
CTL
DC
Normal cell
Infected cancer cell
Cytokines
NK

Figure 39. Scheme of VSV-based OV therapy. A. Reverse genetics allows generation of a recombinant VSV encoding a foreign gene of interest between the VSV G and L genes. The asterisk above M indicates M protein mutation(s) resulting in VSV attenuation in normal cells. Plasmids encoding VSV replication machinery and the modified genome are co-transfected into a cell line, and complete virions are produced and amplified using good manufacturing practices. **B.** For evaluation of oncolytic efficacy, VSV can be administered directly, via cell-based delivery, or in combination with other treatments (chemotherapy, radiotherapy or other OVs). **C.** In infected cells, VSV recombinants may express a foreign gene that facilitates killing of the adjacent uninfected cancer cells (e.g. suicide-gene approach or immunostimulation). Innate antiviral responses and other mechanisms prevent cell death in normal cells. Ideally, stimulation of innate and adaptive immune cells by VSV and/or the foreign gene product should lead to tumour-specific immune responses, including memory responses that prevent cancer recurrence. The Journal of general virology by FEDERATION OF EUROPEAN MICROBIOLOGICAL SOCIETIES; SOCIETY FOR GENERAL MICROBIOLOGY Reproduced with permission of Microbiology Society in the format Republish in a thesis/dissertation via Copyright Clearance Center. Hastie, E., and Grdzelishvili, V.Z. (2012). Vesicular stomatitis virus as a flexible platform for oncolytic virotherapy against cancer. J Gen Virol *93*, 2529–2545.

6.6.3. Barriers to the systemic delivery of OV

Systemic administration and efficient delivery of OV *in vivo* poses potential immunologic and physiologic challenges. VSVΔ51 and MG1 represent biological agents that are foreign to the body. Consequently, immune responses mediated by circulating antibodies or complement proteins, as well as sequestration of virus in various organs such as the liver can impede effective OV delivery (Hastie and Grdzelishvili, 2012). Both VSVΔ51 and MG1 are non-human pathogens, and the presence of pre-existing antibodies against the viruses should be low in most populations (Roberts et al., 1999). Nevertheless, innate and humoral immunity represents an important challenge in the context of prolonged administration of OV or repeated dosing regimens.

Interestingly, several strategies have been developed in cancer virotherapy to overcome these specific barriers, including the use of cell-based OV delivery methods that prevent neutralization or sequestration (Nakashima et al., 2010) (Figure 39). For instance, Kottke and colleagues utilized tumor-specific $CD8^+$ T cells pre-loaded with VSV to demonstrate enhanced delivery of VSV to tumor sites and improved immune-mediated tumor clearance in mice containing pre-existing antibodies against VSV (Kottke et al., 2008). Importantly, this 'Trojan horse' delivery system not only evades the immune response to OV, but also significantly enhances specific delivery of OV to target sites *in vivo* and reduces the initial concentration of OV required (Nakashima et al., 2010). By utilizing cells native to the latent HIV-1 reservoir, including circulating monocytes, DCs, $CD4^+$ or $CD8^+$ T cells, a similar approach could be used to effectively deliver OV to anatomical compartments carrying latent HIV-1.

6.7. Recombinant engineering of OV to enhance the selectivity and efficacy of OV in HIV-1 infected cells

To effectively translate VSVΔ51 or MG1 to clinical models, exceptional efficacy and safety will need to be established and demonstrated. One of the major advantages of biological agents such as OV is that they can be genetically engineered to deliver immunomodulatory factors and cytokines to target cells, while improving specificity and potency (Lichty et al., 2004b; 2014).

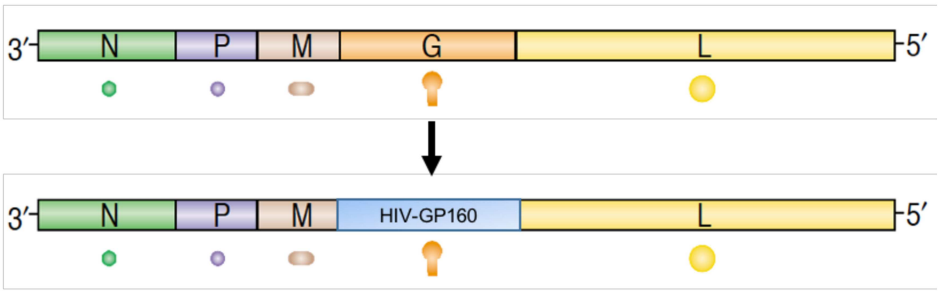
VSVΔ51 and MG1 are replication selective viruses that exploit cellular defects in IFN-I pathways. However, selectivity of these viruses can be further enhanced by altering the viral tropism. HIV-1 predominantly targets and infects cells that express CD4 and the co-receptors CXCR4 or CCR5. Accordingly, VSVΔ51 or MG1 can be designed to specifically infect cellular targets of HIV-1 by replacing the OV G protein with ligands for CD4, CXCR4, and CCR5. Bortiz and colleagues demonstrated the feasibility of this approach by developing a recombinant VSV strain that expressed the HIV-1 envelope (gp120/gp41) in place of the VSV G protein (Figure 40A). This modification was shown to significantly improve selective targeting of $CD4^+$ T cells by VSV (Boritz et al., 1999). Introduction of similar genetic modifications to VSVΔ51 or MG1 can restrict the tropism of the viruses to HIV-1 susceptible cells.

Another strategy to enhance OV selectivity would be to design conditionally replicating viruses that are dependent on the presence of HIV-1 proteins to initiate efficient OV replication (Connolly, 2003). For instance, a fusion peptide that is bridged by

the HIV-1 protease cleavage site can be introduced into the genome of VSVΔ51 or MG1 (Brik and Wong, 2003; Lebon and Ledecq, 2000) (Figure 40B). This would ensure that the replication and synthesis of OV mRNA and proteins would occur only in the presence of HIV-1 protease, thereby minimizing the non-specific effects of OV replication and killing. Importantly however, the application of such OV to target latently HIV-1 infected cells would necessitate the use of latency reversal agents that induce HIV-1 replication and production of viral proteins including HIV-1 protease.

Lastly, VSVΔ51 and MG1 can be redesigned as viral vectors to introduce therapeutic genes that can further potentiate elimination of latently HIV-1 infected cells. IFNα-2a represents an interesting candidate because administration of pegylated IFNα-2a to HIV-1 infected individuals on HAART has been shown to significantly decrease integrated HIV-1 DNA and enhance antiviral ISG expression in resting $CD4^+$ T cells (Azzoni et al., 2012; Hubbard et al., 2012). Consequently, design and delivery of IFN-expressing OV directly to the site of latently HIV-1 infected cells may represent a unique combinatorial approach to further target and eliminate the reservoir.

A.



B.

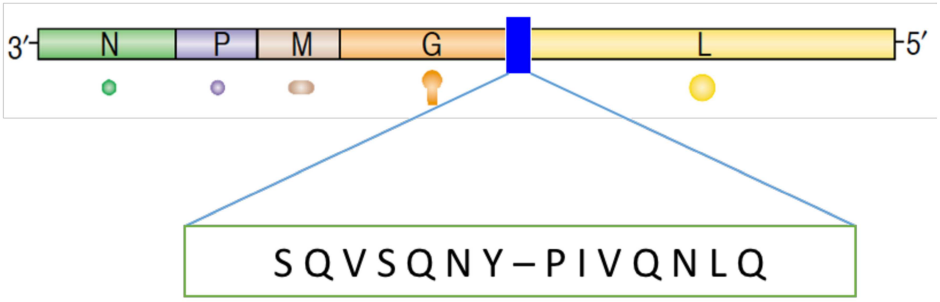


Figure 40. Recombinant strategies to enhance selective OV replication in HIV-infected cells. A. Strategy to develop a recombinant OV strain that expresses the HIV-1 envelope (gp160) in place of the VSV-G protein. **B.** An RNA sequence can be inserted between the G and L in the OV genome to encode a fusion peptide that can only be cleaved by HIV-1 protease, thereby creating an OV that selectively replicates in cells carrying HIV-1 protease. Reprinted with modifications from Trends in Molecular Medicine, 10, Lichty, B.D., Power, A.T., Stojdl, D.F., and Bell, J.C., Vesicular stomatitis virus: re-inventing the bullet, 210-216, Copyright 2004, with permission from Elsevier.

6.8. Conclusion

The objective of this dissertation was to further characterize features of HIV-1 latency, evaluate a novel therapeutic strategy designed to target and eliminate the latent HIV-1 reservoir, and to create a logical and systematic approach with which to investigate the therapeutic efficacy of novel HIV-1 eradication strategies.

In this study, we demonstrated that defects in IFN-I responses represent an important feature of latently HIV-infected cell lines. *In vivo*, a complex interplay exists between the IFN-I system and HIV-1, with significant viral countermeasures present to escape the antiviral effects of IFN-I. Consequently, the findings presented here warrant further validation in primary cell models of HIV infection as it represents a potentially significant contributor to the establishment and maintenance of the reservoir.

We next investigated the capacity of OV to target and eliminate latently HIV-1 infected cells using cell lines, *in vitro* latently infected resting $CD4^+$ T cells, and *ex vivo* memory $CD4^+$ T cells isolated from HIV-infected individuals. We demonstrated a significant reduction in integrated HIV-1 DNA and/or inducible HIV-1 following both MG1 and VSVΔ51 infection, albeit with differences in efficacy between the viruses. Reduction in HIV-1 DNA and inducible replication have been identified to closely correlate with a decrease in the size of the latent HIV-1 reservoir. The work presented here therefore provides a proof-of-principle that oncolytic virotherapy may represent a novel approach to target and eliminate the latent HIV-1 reservoir.

VSVΔ51 and MG1 have proven effective in selective elimination of IFN-defective tumors and are now being studied in patients with metastatic melanoma and non-small

cell lung cancers (ClinicalTrials.gov Identifier: NCT02285816 and NCT02879760). This has laid the groundwork for rapid translation of our findings to *in vivo* studies investigating efficacy of OV in eliminating the latent HIV-1 reservoir. We therefore have a unique opportunity to develop a curative strategy, which in combination with current therapies, can significantly alleviate the global clinical and socioeconomic burden of HIV-1 infection.

References

Adachi, A., Gendelman, H.E., Koenig, S., Folks, T., Willey, R., Rabson, A., and Martin, M.A. (1986). Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone. Journal of Virology *59*, 284–291.

Aghi, M., and Martuza, R.L. (2005). Oncolytic viral therapies - the clinical experience. Oncogene *24*, 7802–7816.

Agosto, L.M., Yu, J.J., Dai, J., Kaletsky, R., Monie, D., and O'Doherty, U. (2007). HIV-1 integrates into resting CD4+ T cells even at low inoculums as demonstrated with an improved assay for HIV-1 integration. Virology *368*, 60–72.

Alexopoulou, L., Holt, A.C., Medzhitov, R., and Flavell, R.A. (2001). Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. Nature *413*, 732–738.

Altfeld, M., and Gale, M., Jr (2015). Innate immunity against HIV-1 infection. Nat Immunol *16*, 554–562.

Ananworanich, J., Dubé, K., and Chomont, N. (2015). How does the timing of antiretroviral therapy initiation in acute infection affect HIV reservoirs? Current Opinion in HIV and AIDS *10*, 18–28.

Antiretroviral Therapy Cohort Collaboration (2008). Life expectancy of individuals on combination antiretroviral therapy in high-income countries: a collaborative analysis of 14 cohort studies. Lancet *372*, 293–299.

Antonio Chiocca, E. (2002). Oncolytic viruses. Nat Rev Cancer *2*, 938–950.

Archin, N.M., Liberty, A.L., Kashuba, A.D., Choudhary, S.K., Kuruc, J.D., Crooks, A.M., Parker, D.C., Anderson, E.M., Kearney, M.F., Strain, M.C., et al. (2012). Administration of vorinostat disrupts HIV-1 latency in patients on antiretroviral therapy. Nature *487*, 482–485.

Archin, N.M., Sung, J.M., Garrido, C., Soriano-Sarabia, N., and Margolis, D.M. (2014). Eradicating HIV-1 infection: seeking to clear a persistent pathogen. Nat. Rev. Microbiol. *12*, 750–764.

Arfi, V., Rivière, L., Jarrosson-Wuillème, L., Goujon, C., Rigal, D., Darlix, J.-L., and Cimarelli, A. (2008). Characterization of the early steps of infection of primary blood monocytes by human immunodeficiency virus type 1. Journal of Virology *82*, 6557–6565.

Azzoni, L., Foulkes, A.S., Papasavvas, E., Mexas, A.M., Lynn, K.M., Mounzer, K., Tebas, P.,

Jacobson, J.M., Frank, I., Busch, M.P., et al. (2012). Pegylated Interferon Alfa-2a Monotherapy Results in Suppression of HIV Type 1 Replication and Decreased Cell-Associated HIV DNA Integration. Journal of Infectious Diseases *207*, 213–222.

Baer, A., and Kehn-Hall, K. (2014). Viral Concentration Determination Through Plaque Assays: Using Traditional and Novel Overlay Systems. JoVE 1–19.

Balachandran, S., Roberts, P.C., Brown, L.E., Truong, H., Pattnaik, A.K., Archer, D.R., and Barber, G.N. (2000). Essential role for the dsRNA-dependent protein kinase PKR in innate immunity to viral infection. Immunity *13*, 129–141.

Baldauf, H.-M., Pan, X., Erikson, E., Schmidt, S., Daddacha, W., Burggraf, M., Schenkova, K., Ambiel, I., Wabnitz, G., Gramberg, T., et al. (2012). SAMHD1 restricts HIV-1 infection in resting CD4+ T cells. Nat Med *18*, 1682–1689.

Baltimore, D., and Feinberg, M.B. (1989). HIV revealed: toward a natural history of the infection. N. Engl. J. Med. *321*, 1673–1675.

Baltzis, D., Qu, L.-K., Papadopoulou, S., Blais, J.D., Bell, J.C., Sonenberg, N., and Koromilas, A.E. (2004). Resistance to vesicular stomatitis virus infection requires a functional cross talk between the eukaryotic translation initiation factor 2alpha kinases PERK and PKR. Journal of Virology *78*, 12747–12761.

Barber, G.N. (2004). Vesicular Stomatitis Virus as an Oncolytic Vector. Viral Immunol. *17*, 516–527.

Barouch, D.H., and Deeks, S.G. (2014). Immunologic strategies for HIV-1 remission and eradication. Science *345*, 169–174.

Barré-Sinoussi, F., Chermann, J.C., Rey, F., Nugeyre, M.T., Chamaret, S., Gruest, J., Dauguet, C., Axler-Blin, C., Vézinet-Brun, F., Rouzioux, C., et al. (1983). Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science *220*, 868–871.

Battcock, S.M., Collier, T.W., Zu, D., and Hirasawa, K. (2006). Negative regulation of the alpha interferon-induced antiviral response by the Ras/Raf/MEK pathway. Journal of Virology *80*, 4422–4430.

Belardelli, F. (1995). Role of interferons and other cytokines in the regulation of the immune response. Apmis *103*, 161–179.

Bidwell, B.N., Slaney, C.Y., Withana, N.P., Forster, S., Cao, Y., Loi, S., Andrews, D., Mikeska, T., Mangan, N.E., Samarajiwa, S.A., et al. (2012). Silencing of Irf7 pathways in breast cancer cells promotes bone metastasis through immune escape. Nat Med *18*, 1224–1231.

Black, B.L., and Lyles, D.S. (1992). Vesicular stomatitis virus matrix protein inhibits host cell-directed transcription of target genes in vivo. Journal of Virology *66*, 4058–4064.

Blagoveshchenskaya, A.D., Thomas, L., Feliciangeli, S.F., Hung, C.H., and Thomas, G. (2002). HIV-1 Nef downregulates MHC-I by a PACS-1- and PI3K-regulated ARF6 endocytic pathway. Cell *111*, 853–866.

Bluming, A.Z., and Ziegler, J.L. (1971). Regression of Burkitt's lymphoma in association with measles infection. Lancet *2*, 105–106.

Boritz, E., Gerlach, J., Johnson, J.E., and Rose, J.K. (1999). Replication-competent rhabdoviruses with human immunodeficiency virus type 1 coats and green fluorescent protein: entry by a pH-independent pathway. Journal of Virology *73*, 6937–6945.

Bos, J.L. (1989). ras oncogenes in human cancer: a review. Cancer Res. *49*, 4682–4689.

Bosinger, S.E., and Utay, N.S. (2015). Type I Interferon: Understanding Its Role in HIV Pathogenesis and Therapy. Curr HIV/AIDS Rep *12*, 41–53.

Bosque, A., and Planelles, V. (2009). Induction of HIV-1 latency and reactivation in primary memory CD4+ T cells. Blood *113*, 58–65.

Bourgeois-Daigneault, M.-C., St-Germain, L.E., Roy, D.G., Pelin, A., Aitken, A.S., Arulanandam, R., Falls, T., Garcia, V., Diallo, J.-S., and Bell, J.C. (2016). Combination of Paclitaxel and MG1 oncolytic virus as a successful strategy for breast cancer treatment. Breast Cancer Res. *18*, 83.

Brand, S.R., Kobayashi, R., and Mathews, M.B. (1997). The Tat protein of human immunodeficiency virus type 1 is a substrate and inhibitor of the interferon-induced, virally activated protein kinase, PKR. Journal of Biological Chemistry *272*, 8388–8395.

Breitbach, C.J., De Silva, N.S., Falls, T.J., Aladl, U., Evgin, L., Paterson, J., Sun, Y.Y., Roy, D.G., Rintoul, J.L., Daneshmand, M., et al. (2011). Targeting tumor vasculature with an oncolytic virus. Mol Ther *19*, 886–894.

Breitbach, C.J., Paterson, J.M., Lemay, C.G., Falls, T.J., McGuire, A., Parato, K.A., Stojdl, D.F., Daneshmand, M., Speth, K., Kirn, D., et al. (2007). Targeted inflammation during oncolytic virus therapy severely compromises tumor blood flow. Mol Ther *15*, 1686–1693.

Brenchley, J.M., Price, D.A., and Douek, D.C. (2006). HIV disease: fallout from a mucosal catastrophe? Nat Immunol *7*, 235–239.

Brenchley, J.M., Schacker, T.W., Ruff, L.E., Price, D.A., Taylor, J.H., Beilman, G.J., Nguyen, P.L., Khoruts, A., Larson, M., Haase, A.T., et al. (2004). CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. Journal of

Experimental Medicine *200*, 749–759.

Brennan, R.O., and Durack, D.T. (1981). Gay compromise syndrome. Lancet *2*, 1338–1339.

Bridle, B.W., Chen, L., Lemay, C.G., Diallo, J.-S., Pol, J., Nguyen, A., Capretta, A., He, R., Bramson, J.L., Bell, J.C., et al. (2013). HDAC inhibition suppresses primary immune responses, enhances secondary immune responses, and abrogates autoimmunity during tumor immunotherapy. Mol Ther *21*, 887–894.

Brik, A., and Wong, C.-H. (2003). HIV-1 Protease: Mechanism and Drug Discovery. ChemInform *34*.

Britto, A.M.A., Amoedo, N.D., Pezzuto, P., Afonso, A.O., Martínez, A.M.B., Silveira, J., Sion, F.S., Machado, E.S., Soares, M.A., and Giannini, A.L.M. (2013). Expression levels of the innate response gene RIG-I and its regulators RNF125 and TRIM25 in HIV-1-infected adult and pediatric individuals. Aids *27*, 1879–1885.

Brooks, D.G., and Zack, J.A. (2002). Effect of latent human immunodeficiency virus infection on cell surface phenotype. Journal of Virology *76*, 1673–1681.

Brun, J., McManus, D., Lefebvre, C., Hu, K., Falls, T., Atkins, H., Bell, J.C., McCart, J.A., Mahoney, D., and Stojdl, D.F. (2010). Identification of Genetically Modified Maraba Virus as an Oncolytic Rhabdovirus. Mol Ther *18*, 1440–1449.

Budhiraja, S., Famiglietti, M., Bosque, A., Planelles, V., and Rice, A.P. (2013). Cyclin T1 and CDK9 T-loop phosphorylation are downregulated during establishment of HIV-1 latency in primary resting memory CD4+ T cells. Journal of Virology *87*, 1211–1220.

Butera, S.T., Perez, V.L., Besansky, N.J., Chan, W.C., Wu, B.Y., Nabel, G.J., and Folks, T.M. (1991a). Extrachromosomal human immunodeficiency virus type-1 DNA can initiate a spreading infection of HL-60 cells. J. Cell. Biochem. *45*, 366–373.

Butera, S.T., Perez, V.L., Wu, B.Y., Nabel, G.J., and Folks, T.M. (1991b). Oscillation of the human immunodeficiency virus surface receptor is regulated by the state of viral activation in a CD4+ cell model of chronic infection. Journal of Virology *65*, 4645–4653.

Butera, S.T., Roberts, B.D., Lam, L., Hodge, T., and Folks, T.M. (1994). Human immunodeficiency virus type 1 RNA expression by four chronically infected cell lines indicates multiple mechanisms of latency. Journal of Virology *68*, 2726–2730.

Cai, R., Carpick, B., Chun, R.F., Jeang, K.T., and Williams, B.R. (2000). HIV-I TAT inhibits PKR activity by both RNA-dependent and RNA-independent mechanisms. Archives of Biochemistry and Biophysics *373*, 361–367.

Calvanese, V., Chavez, L., Laurent, T., Ding, S., and Verdin, E. (2013). Dual-color HIV

reporters trace a population of latently infected cells and enable their purification. Virology *446*, 283–292.

Cameron, P.U., Saleh, S., Sallmann, G., Solomon, A., Wightman, F., Evans, V.A., Boucher, G., Haddad, E.K., Sekaly, R.-P., Harman, A.N., et al. (2010). Establishment of HIV-1 latency in resting CD4+ T cells depends on chemokine-induced changes in the actin cytoskeleton. Proc. Natl. Acad. Sci. U.S.a. *107*, 16934–16939.

Carr, A., and Cooper, D.A. (2000). Adverse effects of antiretroviral therapy. Lancet *356*, 1423–1430.

Carter, C.C., Onafuwa-Nuga, A., McNamara, L.A., Riddell, J., Bixby, D., Savona, M.R., and Collins, K.L. (2010). HIV-1 infects multipotent progenitor cells causing cell death and establishing latent cellular reservoirs. Nat Med *16*, 446–451.

Cassol, E., Alfano, M., Biswas, P., and Poli, G. (2006). Monocyte-derived macrophages and myeloid cell lines as targets of HIV-1 replication and persistence. Journal of Leukocyte Biology *80*, 1018–1030.

Castleton, A., Dey, A., Beaton, B., Patel, B., Aucher, A., Davis, D.M., and Fielding, A.K. (2014). Human mesenchymal stromal cells deliver systemic oncolytic measles virus to treat acute lymphoblastic leukemia in the presence of humoral immunity. Blood *123*, 1327–1335.

Chan, D.C., and Kim, P.S. (1998). HIV entry and its inhibition. Cell *93*, 681–684.

Chan, J.K., Bhattacharyya, D., Lassen, K.G., Ruelas, D., and Greene, W.C. (2013). Calcium/calcineurin synergizes with prostratin to promote NF-κB dependent activation of latent HIV. PLoS ONE *8*, e77749.

Chang, H.-M., Paulson, M., Holko, M., Rice, C.M., Williams, B.R.G., Marié, I., and Levy, D.E. (2004). Induction of interferon-stimulated gene expression and antiviral responses require protein deacetylase activity. Pnas *101*, 9578–9583.

Chavez, L., Calvanese, V., and Verdin, E. (2015). HIV Latency Is Established Directly and Early in Both Resting and Activated Primary CD4 T Cells. PLoS Pathog. *11*, e1004955–21.

Chehimi, J., Campbell, D.E., Azzoni, L., Bacheller, D., Papasavvas, E., Jerandi, G., Mounzer, K., Kostman, J., Trinchieri, G., and Montaner, L.J. (2002). Persistent decreases in blood plasmacytoid dendritic cell number and function despite effective highly active antiretroviral therapy and increased blood myeloid dendritic cells in HIV-infected individuals. J. Immunol. *168*, 4796–4801.

Chéné, du, I., Basyuk, E., Lin, Y.-L., Triboulet, R., Knezevich, A., Chable-Bessia, C., Mettling, C., Baillat, V., Reynes, J., Corbeau, P., et al. (2007). Suv39H1 and HP1gamma are responsible for chromatin-mediated HIV-1 transcriptional silencing and post-

integration latency. Embo J. *26*, 424–435.

Chomont, N., El-Far, M., Ancuta, P., Trautmann, L., Procopio, F.A., Yassine-Diab, B., Boucher, G., Boulassel, M.-R., Ghattas, G., Brenchley, J.M., et al. (2009). HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. Nat Med *15*, 893–900.

Chun, T.W., Carruth, L., Finzi, D., Shen, X., DiGiuseppe, J.A., Taylor, H., Hermankova, M., Chadwick, K., Margolick, J., Quinn, T.C., et al. (1997a). Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. Nature *387*, 183–188.

Chun, T.W., Carruth, L., Finzi, D., Shen, X., DiGiuseppe, J.A., Taylor, H., Hermankova, M., Chadwick, K., Margolick, J., Quinn, T.C., et al. (1997b). Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. Nature *387*, 183–188.

Chun, T.W., Engel, D., Berrey, M.M., Shea, T., Corey, L., and Fauci, A.S. (1998). Early establishment of a pool of latently infected, resting CD4(+) T cells during primary HIV-1 infection. Pnas *95*, 8869–8873.

Chun, T.W., Engel, D., Mizell, S.B., Hallahan, C.W., Fischette, M., Park, S., Davey, R.T., Dybul, M., Kovacs, J.A., Metcalf, J.A., et al. (1999). Effect of interleukin-2 on the pool of latently infected, resting CD4+ T cells in HIV-1-infected patients receiving highly active anti-retroviral therapy. Nat Med *5*, 651–655.

Chun, T.W., Finzi, D., Margolick, J., Chadwick, K., Schwartz, D., and Siliciano, R.F. (1995). In vivo fate of HIV-1-infected T cells: quantitative analysis of the transition to stable latency. Nat Med *1*, 1284–1290.

Chun, T.W., Stuyver, L., Mizell, S.B., Ehler, L.A., Mican, J.A., Baseler, M., Lloyd, A.L., Nowak, M.A., and Fauci, A.S. (1997c). Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. Pnas *94*, 13193–13197.

Chun, T.-W., Justement, J.S., Lempicki, R.A., Yang, J., Dennis, G., Hallahan, C.W., Sanford, C., Pandya, P., Liu, S., McLaughlin, M., et al. (2003). Gene expression and viral prodution in latently infected, resting CD4+ T cells in viremic versus aviremic HIV-infected individuals. Pnas *100*, 1908–1913.

Chun, T.-W., Justement, J.S., Murray, D., Hallahan, C.W., Maenza, J., Collier, A.C., Sheth, P.M., Kaul, R., Ostrowski, M., Moir, S., et al. (2010). Rebound of plasma viremia following cessation of antiretroviral therapy despite profoundly low levels of HIV reservoir: implications for eradication. Aids *24*, 2803–2808.

Chun, T.-W., Nickle, D.C., Justement, J.S., Meyers, J.H., Roby, G., Hallahan, C.W., Kottilil, S., Moir, S., Mican, J.M., Mullins, J.I., et al. (2008). Persistence of HIV in gut-associated lymphoid tissue despite long-term antiretroviral therapy. Journal of Infectious Diseases *197*, 714–720.

Churchill, M.J., Deeks, S.G., Margolis, D.M., Siliciano, R.F., and Swanstrom, R. (2016). HIV reservoirs: what, where and how to target them. Nat. Rev. Microbiol. *14*, 55–60.

Clerzius, G., Gélinas, J.-F., and Gatignol, A. (2010). Multiple levels of PKR inhibition during HIV-1 replication. Rev. Med. Virol. *21*, 42–53.

Cocchi, F., DeVico, A.L., Garzino-Demo, A., Arya, S.K., Gallo, R.C., and Lusso, P. (1995). Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by CD8+ T cells. Science *270*, 1811–1815.

Cody, J.J., and Hurst, D.R. (2015a). Promising oncolytic agents for metastatic breast cancer treatment. Oncolytic Virother *4*, 63–73.

Cody, J.J., and Hurst, D.R. (2015b). Promising oncolytic agents for metastatic breast cancer treatment. Oncolytic Virother *4*, 63–73.

Coffin, J.M., Hughes, S.H., and Varmus, H.E. (1997). Retroviruses. N. Engl. J. Med. *339*, 1646–1646.

Cohen, G.B., Gandhi, R.T., Davis, D.M., Mandelboim, O., Chen, B.K., Strominger, J.L., and Baltimore, D. (1999). The selective downregulation of class I major histocompatibility complex proteins by HIV-1 protects HIV-infected cells from NK cells. Immunity *10*, 661–671.

Colamonici, O.R., Domanski, P., Platanias, L.C., and Diaz, M.O. (1992). Correlation between interferon (IFN) alpha resistance and deletion of the IFN alpha/beta genes in acute leukemia cell lines suggests selection against the IFN system. Blood *80*, 744–749.

Collins, S.J., Gallo, R.C., and Gallagher, R.E. (1977). Continuous growth and differentiation of human myeloid leukaemic cells in suspension culture. Nature *270*, 347–349.

Connolly, J.B. (2003). Conditionally replicating viruses in cancer therapy. Gene Ther. *10*, 712–715.

Cooper, D.A., Imrie, A.A., and Penny, R. (1987). Antibody response to human immunodeficiency virus after primary infection. Journal of Infectious Diseases *155*, 1113–1118.

Corbeau, P., and Reynes, J. (2011). Immune reconstitution under antiretroviral therapy: the new challenge in HIV-1 infection. Blood *117*, 5582–5590.

Côté, S.C., Plante, A., Tardif, M.R., and Tremblay, M.J. (2013). Dectin-1/TLR2 and NOD2 Agonists Render Dendritic Cells Susceptible to Infection by X4-Using HIV-1 and Promote cis-Infection of CD4+ T Cells. PLoS ONE *8*, e67735–12.

Critchley-Thorne, R.J., Simons, D.L., Yan, N., Miyahira, A.K., Dirbas, F.M., Johnson, D.L., Swetter, S.M., Carlson, R.W., Fisher, G.A., Koong, A., et al. (2009). Impaired interferon signaling is a common immune defect in human cancer. Pnas *106*, 9010–9015.

Dalgleish, A.G., Beverley, P.C., Clapham, P.R., Crawford, D.H., Greaves, M.F., and Weiss, R.A. (1984). The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. Nature *312*, 763–767.

Davey, R.T., Bhat, N., Yoder, C., Chun, T.W., Metcalf, J.A., Dewar, R., Natarajan, V., Lempicki, R.A., Adelsberger, J.W., Miller, K.D., et al. (1999). HIV-1 and T cell dynamics after interruption of highly active antiretroviral therapy (HAART) in patients with a history of sustained viral suppression. Pnas *96*, 15109–15114.

de Pee, S., Grede, N., Forsythe, M., and Bloem, M.W. (2012). Antiretroviral therapy requires life-long adherence. Lancet Infect Dis *12*, 661–2–authorreply662.

DeChristopher, B.A., Loy, B.A., Marsden, M.D., Schrier, A.J., Zack, J.A., and Wender, P.A. (2012). Designed, synthetically accessible bryostatin analogues potently induce activation of latent HIV reservoirs in vitro. Nat Chem *4*, 705–710.

Deeks, S.G., and Phillips, A.N. (2009). HIV infection, antiretroviral treatment, ageing, and non-AIDS related morbidity. Bmj *338*, a3172–a3172.

Deleage, C., Wietgrefe, S.W., Del Prete, G., Morcock, D.R., Hao, X.P., Piatak, M., Bess, J., Anderson, J.L., Perkey, K.E., Reilly, C., et al. (2016). Defining HIV and SIV Reservoirs in Lymphoid Tissues. Pathog Immun *1*, 68–106.

Deng, K., Pertea, M., Rongvaux, A., Wang, L., Durand, C.M., Ghiaur, G., Lai, J., McHugh, H.L., Hao, H., Zhang, H., et al. (2015). Broad CTL response is required to clear latent HIV-1 due to dominance of escape mutations. Nature *517*, 381–385.

Denton, P.W., Olesen, R., Choudhary, S.K., Archin, N.M., Wahl, A., Swanson, M.D., Chateau, M., Nochi, T., Krisko, J.F., Spagnuolo, R.A., et al. (2012). Generation of HIV latency in humanized BLT mice. Journal of Virology *86*, 630–634.

Der, S.D., Zhou, A., Williams, B.R., and Silverman, R.H. (1998). Identification of genes differentially regulated by interferon alpha, beta, or gamma using oligonucleotide arrays. Pnas *95*, 15623–15628.

Descours, B., Petitjean, G., López-Zaragoza, J.-L., Bruel, T., Raffel, R., Psomas, C., Reynes, J., Lacabaratz, C., Levy, Y., Schwartz, O., et al. (2017). CD32a is a marker of a CD4 T-cell HIV reservoir harbouring replication-competent proviruses. Nature *94*, 13193–13198.

Dinoso, J.B., Kim, S.Y., Wiegand, A.M., Palmer, S.E., Gange, S.J., Cranmer, L., O'Shea, A., Callender, M., Spivak, A., Brennan, T., et al. (2009). Treatment intensification does not reduce residual HIV-1 viremia in patients on highly active antiretroviral therapy. Proc.

Natl. Acad. Sci. U.S.a. *106*, 9403–9408.

Doehle, B.P., Chang, K., Rustagi, A., McNevin, J., McElrath, M.J., and Gale, M. (2012). Vpu Mediates Depletion of Interferon Regulatory Factor 3 during HIV Infection by a Lysosome-Dependent Mechanism. Journal of Virology *86*, 8367–8374.

Doehle, B.P., Hladik, F., McNevin, J.P., McElrath, M.J., and Gale, M. (2009). Human immunodeficiency virus type 1 mediates global disruption of innate antiviral signaling and immune defenses within infected cells. Journal of Virology *83*, 10395–10405.

Doitsh, G., Galloway, N.L.K., Geng, X., Yang, Z., Monroe, K.M., Zepeda, O., Hunt, P.W., Hatano, H., Sowinski, S., Muñoz-Arias, I., et al. (2013). Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection. Nature *505*, 509–514.

Donahue, D.A., and Wainberg, M.A. (2013). Cellular and molecular mechanisms involved in the establishment of HIV-1 latency. Retrovirology *10*, 11.

Doyle, T., Goujon, C., and Malim, M.H. (2015). HIV-1 and interferons: who's interfering with whom? Nat. Rev. Microbiol. *13*, 403–413.

Doyon, G., Zerbato, J., Mellors, J.W., and Sluis-Cremer, N. (2013). Disulfiram reactivates latent HIV-1 expression through depletion of the phosphatase and tensin homolog. Aids *27*, F7–F11.

Dragic, T., Litwin, V., Allaway, G.P., Martin, S.R., Huang, Y., Nagashima, K.A., Cayanan, C., Maddon, P.J., Koup, R.A., Moore, J.P., et al. (1996). HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5. Nature *381*, 667–673.

Dunn, G.P., Bruce, A.T., Sheehan, K.C.F., Shankaran, V., Uppaluri, R., Bui, J.D., Diamond, M.S., Koebel, C.M., Arthur, C., White, J.M., et al. (2005a). A critical function for type I interferons in cancer immunoediting. Nat Immunol *6*, 722–729.

Dunn, G.P., Koebel, C.M., and Schreiber, R.D. (2006). Interferons, immunity and cancer immunoediting. Nat Rev Immunol *6*, 836–848.

Dunn, G.P., Sheehan, K.C.F., Old, L.J., and Schreiber, R.D. (2005b). IFN unresponsiveness in LNCaP cells due to the lack of JAK1 gene expression. Cancer Res. *65*, 3447–3453.

Durack, D.T. (1981). Opportunistic infections and Kaposi's sarcoma in homosexual men. N. Engl. J. Med. *305*, 1465–1467.

Durand, C.M., Ghiaur, G., Siliciano, J.D., Rabi, S.A., Eisele, E.E., Salgado, M., Shan, L., Lai, J.F., Zhang, H., Margolick, J., et al. (2012). HIV-1 DNA is detected in bone marrow populations containing CD4+ T cells but is not found in purified CD34+ hematopoietic progenitor cells in most patients on antiretroviral therapy. J Infect Dis *205*, 1014–1018.

Duverger, A., Jones, J., May, J., Bibollet-Ruche, F., Wagner, F.A., Cron, R.Q., and Kutsch, O. (2009). Determinants of the establishment of human immunodeficiency virus type 1 latency. Journal of Virology *83*, 3078–3093.

Eisele, E., and Siliciano, R.F. (2012). Redefining the Viral Reservoirs that Prevent HIV-1 Eradication. Immunity *37*, 377–388.

Elliott, J.H., Wightman, F., Solomon, A., Ghneim, K., Ahlers, J., Cameron, M.J., Smith, M.Z., Spelman, T., McMahon, J., Velayudham, P., et al. (2014). Activation of HIV transcription with short-course vorinostat in HIV-infected patients on suppressive antiretroviral therapy. PLoS Pathog. *10*, e1004473.

Emiliani, S., Fischle, W., Ott, M., Van Lint, C., Amella, C.A., and Verdin, E. (1998). Mutations in the tat gene are responsible for human immunodeficiency virus type 1 postintegration latency in the U1 cell line. Journal of Virology *72*, 1666–1670.

Eriksson, S., Graf, E.H., Dahl, V., Strain, M.C., Yukl, S.A., Lysenko, E.S., Bosch, R.J., Lai, J., Chioma, S., Emad, F., et al. (2013). Comparative analysis of measures of viral reservoirs in HIV-1 eradication studies. PLoS Pathog. *9*, e1003174.

Feng, Q., Sekula, D., Guo, Y., Liu, X., Black, C.C., Galimberti, F., Shah, S.J., Sempere, L.F., Memoli, V., Andersen, J.B., et al. (2008). UBE1L causes lung cancer growth suppression by targeting cyclin D1. Mol. Cancer Ther. *7*, 3780–3788.

Feng, Y., Broder, C.C., Kennedy, P.E., and Berger, E.A. (1996). HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. Science *272*, 872–877.

Fernandez, M., Porosnicu, M., Markovic, D., and Barber, G.N. (2002). Genetically engineered vesicular stomatitis virus in gene therapy: application for treatment of malignant disease. Journal of Virology *76*, 895–904.

Finkel, T.H., Tudor-Williams, G., Banda, N.K., Cotton, M.F., Curiel, T., Monks, C., Baba, T.W., Ruprecht, R.M., and Kupfer, A. (1995). Apoptosis occurs predominantly in bystander cells and not in productively infected cells of HIV- and SIV-infected lymph nodes. Nat Med *1*, 129–134.

Finkelshtein, D., Werman, A., Novick, D., Barak, S., and Rubinstein, M. (2013). LDL receptor and its family members serve as the cellular receptors for vesicular stomatitis virus. Proc. Natl. Acad. Sci. U.S.a. *110*, 7306–7311.

Finzi, D., and Siliciano, R.F. (1998). Viral dynamics in HIV-1 infection. Cell *93*, 665–671.

Finzi, D., Blankson, J., Siliciano, J.D., Margolick, J.B., Chadwick, K., Pierson, T., Smith, K., Lisziewicz, J., Lori, F., Flexner, C., et al. (1999). Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination

therapy. Nat Med *5*, 512–517.

Finzi, D., Hermankova, M., Pierson, T., Carruth, L.M., Buck, C., Chaisson, R.E., Quinn, T.C., Chadwick, K., Margolick, J., Brookmeyer, R., et al. (1997). Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. Science *278*, 1295–1300.

Fitzgerald-Bocarsly, P., and Jacobs, E.S. (2010). Plasmacytoid dendritic cells in HIV infection: striking a delicate balance. Journal of Leukocyte Biology *87*, 609–620.

Folks, T.M., Justement, J., Kinter, A., Dinarello, C.A., and Fauci, A.S. (1987). Cytokine-induced expression of HIV-1 in a chronically infected promonocyte cell line. Science *238*, 800–802.

Folks, T.M., Justement, J., Kinter, A., Schnittman, S., Orenstein, J., Poli, G., and Fauci, A.S. (1988). Characterization of a promonocyte clone chronically infected with HIV and inducible by 13-phorbol-12-myristate acetate. J. Immunol. *140*, 1117–1122.

Forsman, A., and Weiss, R.A. (2008). Why is HIV a pathogen? Trends in Microbiology *16*, 555–560.

Foster, J.L., Denial, S.J., Temple, B.R.S., and Garcia, J.V. (2011). Mechanisms of HIV-1 Nef function and intracellular signaling. J Neuroimmune Pharmacol *6*, 230–246.

Frankel, A.D., and Young, J.A.T. (1998). HIV-1: Fifteen Proteins and an RNA. Annu. Rev. Biochem. *67*, 1–25.

Friedman, J., Cho, W.-K., Chu, C.K., Keedy, K.S., Archin, N.M., Margolis, D.M., and Karn, J. (2011). Epigenetic silencing of HIV-1 by the histone H3 lysine 27 methyltransferase enhancer of Zeste 2. Journal of Virology *85*, 9078–9089.

Friis-Møller, N., Sabin, C.A., Weber, R., d'Arminio Monforte, A., El-Sadr, W.M., Reiss, P., Thiébaut, R., Morfeldt, L., De Wit, S., Pradier, C., et al. (2003). Combination antiretroviral therapy and the risk of myocardial infarction. N. Engl. J. Med. *349*, 1993–2003.

Fromentin, R., Bakeman, W., Lawani, M.B., Khoury, G., Hartogensis, W., DaFonseca, S., Killian, M., Epling, L., Hoh, R., Sinclair, E., et al. (2016). CD4+ T Cells Expressing PD-1, TIGIT and LAG-3 Contribute to HIV Persistence during ART. PLoS Pathog. *12*, e1005761.

Furió, V., Garijo, R., Durán, M., Moya, A., Bell, J.C., and Sanjuán, R. (2012). Relationship between within-host fitness and virulence in the vesicular stomatitis virus: correlation with partial decoupling. Journal of Virology *86*, 12228–12236.

Gale, M., and Sen, G.C. (2009). Viral evasion of the interferon system. J. Interferon Cytokine Res. *29*, 475–476.

Gallo, R.C., Salahuddin, S.Z., Popovic, M., Shearer, G.M., Kaplan, M., Haynes, B.F., Palker,

T.J., Redfield, R., Oleske, J., and Safai, B. (1984). Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. Science *224*, 500–503.

Gallo, R.C., and Montagnier, L. (2003). The discovery of HIV as the cause of AIDS. N. Engl. J. Med. *349*, 2283–2285.

Gandhi, R.T., Bosch, R.J., Aga, E., Albrecht, M., Demeter, L.M., Dykes, C., Bastow, B., Para, M., Lai, J., Siliciano, R.F., et al. (2010). No evidence for decay of the latent reservoir in HIV-1-infected patients receiving intensive enfuvirtide-containing antiretroviral therapy. J Infect Dis *201*, 293–296.

Ganesh, L., Burstein, E., Guha-Niyogi, A., Louder, M.K., Mascola, J.R., Klomp, L.W.J., Wijmenga, C., Duckett, C.S., and Nabel, G.J. (2003). The gene product Murr1 restricts HIV-1 replication in resting CD4+ lymphocytes. Nature *426*, 853–857.

Gao, D., Wu, J., Wu, Y.-T., Du, F., Aroh, C., Yan, N., Sun, L., and Chen, Z.J. (2013). Cyclic GMP-AMP synthase is an innate immune sensor of HIV and other retroviruses. Science *341*, 903–906.

Gao, Y., Whitaker-Dowling, P., Griffin, J.A., Barmada, M.A., and Bergman, I. (2009). Recombinant vesicular stomatitis virus targeted to Her2/neu combined with anti-CTLA4 antibody eliminates implanted mammary tumors. Cancer Gene Ther. *16*, 44–52.

Garcia, J.V. (2016). In vivo platforms for analysis of HIV persistence and eradication. J. Clin. Invest. *126*, 424–431.

Glushakova, S., Baibakov, B., Margolis, L.B., and Zimmerberg, J. (1995). Infection of human tonsil histocultures: a model for HIV pathogenesis. Nat Med *1*, 1320–1322.

Glushakova, S., Baibakov, B., Zimmerberg, J., and Margolis, L.B. (1997). Experimental HIV infection of human lymphoid tissue: correlation of CD4+ T cell depletion and virus syncytium-inducing/non-syncytium-inducing phenotype in histocultures inoculated with laboratory strains and patient isolates of HIV type 1. AIDS Res. Hum. Retroviruses *13*, 461–471.

Gottlieb, M.S., Schroff, R., Schanker, H.M., Weisman, J.D., Fan, P.T., Wolf, R.A., and Saxon, A. (1981). Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. N. Engl. J. Med. *305*, 1425–1431.

Goujon, C., and Malim, M.H. (2010). Characterization of the alpha interferon-induced postentry block to HIV-1 infection in primary human macrophages and T cells. Journal of Virology *84*, 9254–9266.

Gresser, I., Belardelli, F., Maury, C., Maunoury, M.T., and Tovey, M.G. (1983). Injection

of mice with antibody to interferon enhances the growth of transplantable murine tumors. Journal of Experimental Medicine *158*, 2095–2107.

Haase, A.T., Henry, K., Zupancic, M., Sedgewick, G., Faust, R.A., Melroe, H., Cavert, W., Gebhard, K., Staskus, K., Zhang, Z.Q., et al. (1996). Quantitative image analysis of HIV-1 infection in lymphoid tissue. Science *274*, 985–989.

Haase, A.T. (2005). Perils at mucosal front lines for HIV and SIV and their hosts. Nat Rev Immunol *5*, 783–792.

Han, Y., Lassen, K., Monie, D., Sedaghat, A.R., Shimoji, S., Liu, X., Pierson, T.C., Margolick, J.B., Siliciano, R.F., and Siliciano, J.D. (2004). Resting CD4+ T cells from human immunodeficiency virus type 1 (HIV-1)-infected individuals carry integrated HIV-1 genomes within actively transcribed host genes. Journal of Virology *78*, 6122–6133.

Han, Y., Lin, Y.B., An, W., Xu, J., Yang, H.-C., O'Connell, K., Dordai, D., Boeke, J.D., Siliciano, J.D., and Siliciano, R.F. (2008). Orientation-dependent regulation of integrated HIV-1 expression by host gene transcriptional readthrough. Cell Host & Microbe *4*, 134–146.

Hanson, R.P., Rasmussen, A.F., Brandly, C.A., and Brown, J.W. (1950). Human infection with the virus of vesicular stomatitis. J. Lab. Clin. Med. *36*, 754–758.

Harman, A.N., Nasr, N., Feetham, A., Galoyan, A., Alshehri, A.A., Rambukwelle, D., Botting, R.A., Hiener, B.M., Diefenbach, E., Diefenbach, R.J., et al. (2015). HIV Blocks Interferon Induction in Human Dendritic Cells and Macrophages by Dysregulation of TBK1. Journal of Virology *89*, 6575–6584.

Harrison, S.C. (2008). Viral membrane fusion. Nat. Struct. Mol. Biol. *15*, 690–698.

Hasan, M., and Yan, N. (2014). Safeguard against DNA sensing: the role of TREX1 in HIV-1 infection and autoimmune diseases. Front Microbiol *5*, 193.

Hastie, E., and Grdzelishvili, V.Z. (2012). Vesicular stomatitis virus as a flexible platform for oncolytic virotherapy against cancer. J Gen Virol *93*, 2529–2545.

Haverkos, H.W., and Curran, J.W. (1982). The current outbreak of Kaposi's sarcoma and opportunistic infections. CA Cancer J Clin *32*, 330–339.

Heil, F., Hemmi, H., Hochrein, H., Ampenberger, F., Kirschning, C., Akira, S., Lipford, G., Wagner, H., and Bauer, S. (2004). Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. Science *303*, 1526–1529.

Hemmi, H., Takeuchi, O., Kawai, T., Kaisho, T., Sato, S., Sanjo, H., Matsumoto, M., Hoshino, K., Wagner, H., Takeda, K., et al. (2000). A Toll-like receptor recognizes bacterial DNA. Nature *408*, 740–745.

Hermankova, M., Siliciano, J.D., Zhou, Y., Monie, D., Chadwick, K., Margolick, J.B., Quinn, T.C., and Siliciano, R.F. (2003). Analysis of human immunodeficiency virus type 1 gene expression in latently infected resting CD4+ T lymphocytes in vivo. Journal of Virology *77*, 7383–7392.

Hershey, J.W. (1991). Translational control in mammalian cells. Annu. Rev. Biochem. *60*, 717–755.

Heyman, M., Grandér, D., Bröndum-Nielsen, K., Cederblad, B., Liu, Y., Xu, B., and Einhorn, S. (1994). Interferon system defects in malignant T-cells. Leukemia *8*, 425–434.

Hladik, F., and McElrath, M.J. (2008). Setting the stage: host invasion by HIV. Nature Publishing Group *8*, 447–457.

Ho, Y.-C., Shan, L., Hosmane, N.N., Wang, J., Laskey, S.B., Rosenbloom, D.I.S., Lai, J., Blankson, J.N., Siliciano, J.D., and Siliciano, R.F. (2013). Replication-Competent Noninduced Proviruses in the Latent Reservoir Increase Barrier to HIV-1 Cure. Cell *155*, 540–551.

Hocqueloux, L., Prazuck, T., Avettand-Fenoel, V., Lafeuillade, A., Cardon, B., Viard, J.-P., and Rouzioux, C. (2010). Long-term immunovirologic control following antiretroviral therapy interruption in patients treated at the time of primary HIV-1 infection. Aids *24*, 1598–1601.

Homann, S., Tibroni, N., Baumann, I., Sertel, S., Keppler, O.T., and Fackler, O.T. (2009). Determinants in HIV-1 Nef for enhancement of virus replication and depletion of CD4+ T lymphocytes in human lymphoid tissue ex vivo. Retrovirology *6*, 6.

Hornung, V., Ellegast, J., Kim, S., Brzózka, K., Jung, A., Kato, H., Poeck, H., Akira, S., Conzelmann, K.-K., Schlee, M., et al. (2006). 5'-Triphosphate RNA is the ligand for RIG-I. Science *314*, 994–997.

Hsu, T.-H., Chu, C.-C., Jiang, S.-Y., Hung, M.-W., Ni, W.-C., Lin, H.-E., and Chang, T.-C. (2012). Expression of the class II tumor suppressor gene RIG1 is directly regulated by p53 tumor suppressor in cancer cell lines. FEBS Lett. *586*, 1287–1293.

Huang, J., Wang, F., Argyris, E., Chen, K., Liang, Z., Tian, H., Huang, W., Squires, K., Verlinghieri, G., and Zhang, H. (2007). Cellular microRNAs contribute to HIV-1 latency in resting primary CD4+ T lymphocytes. Nat Med *13*, 1241–1247.

Hubbard, J.J., Greenwell-Wild, T., Barrett, L., Yang, J., Lempicki, R.A., Wahl, S.M., Asmuth, D.M., Murphy, R.L., Pollard, R.B., and Kottilil, S. (2012). Host gene expression changes correlating with anti-HIV-1 effects in human subjects after treatment with peginterferon Alfa-2a. J Infect Dis *205*, 1443–1447.

Igarashi, T., Brown, C.R., Endo, Y., Buckler-White, A., Plishka, R., Bischofberger, N.,

Hirsch, V., and Martin, M.A. (2001). Macrophage are the principal reservoir and sustain high virus loads in rhesus macaques after the depletion of CD4+ T cells by a highly pathogenic simian immunodeficiency virus/HIV type 1 chimera (SHIV): Implications for HIV-1 infections of humans. Pnas *98*, 658–663.

Ilkow, C.S., Marguerie, M., Batenchuk, C., Mayer, J., Ben Neriah, D., Cousineau, S., Falls, T., Jennings, V.A., Boileau, M., Bellamy, D., et al. (2015). Reciprocal cellular cross-talk within the tumor microenvironment promotes oncolytic virus activity. Nat Med *21*, 530–536.

Imbeault, M., Lodge, R., Ouellet, M., and Tremblay, M.J. (2009a). Efficient magnetic bead-based separation of HIV-1-infected cells using an improved reporter virus system reveals that p53 up-regulation occurs exclusively in the virus-expressing cell population. Virology *393*, 160–167.

Imbeault, M., Ouellet, M., and Tremblay, M.J. (2009b). Microarray study reveals that HIV-1 induces rapid type-I interferon-dependent p53 mRNA up-regulation in human primary CD4+ T cells. Retrovirology *6*, 5–14.

Isaacs, A., and Lindenmann, J. (1987). Virus interference. I. The interferon. By A. Isaacs and J. Lindenmann, 1957.

Ivashkiv, L.B., and Donlin, L.T. (2014). Regulation of type I interferon responses. Nature Publishing Group *14*, 36–49.

J Buzón, M., Massanella, M., Llibre, J.M., Esteve, A., Dahl, V., Puertas, M.C., Gatell, J.M., Domingo, P., Paredes, R., Sharkey, M., et al. (2010). HIV-1 replication and immune dynamics are affected by raltegravir intensification of HAART-suppressed subjects. Nat Med *16*, 460–465.

Jakobsen, M.R., Bak, R.O., Andersen, A., Berg, R.K., Jensen, S.B., Tengchuan, J., Jin, T., Laustsen, A., Hansen, K., Østergaard, L., et al. (2013). IFI16 senses DNA forms of the lentiviral replication cycle and controls HIV-1 replication. Proc. Natl. Acad. Sci. U.S.a. *110*, E4571–E4580.

Jakobsen, M.R., Olagnier, D., and Hiscott, J. (2015). Innate immune sensing of HIV-1 infection. Current Opinion in HIV and AIDS *10*, 96–102.

Joos, B., Fischer, M., Kuster, H., Pillai, S.K., Wong, J.K., Böni, J., Hirschel, B., Weber, R., Trkola, A., Günthard, H.F., et al. (2008). HIV rebounds from latently infected cells, rather than from continuing low-level replication. Proc. Natl. Acad. Sci. U.S.a. *105*, 16725–16730.

Kahn, J.O., and Walker, B.D. (1998). Acute human immunodeficiency virus type 1 infection. N. Engl. J. Med. *339*, 33–39.

Kamga, I., Kahi, S., Develioglu, L., Lichtner, M., Marañón, C., Deveau, C., Meyer, L., Goujard, C., Lebon, P., Sinet, M., et al. (2005). Type I interferon production is profoundly and transiently impaired in primary HIV-1 infection. J Infect Dis *192*, 303–310.

Katlama, C., Deeks, S.G., Autran, B., Martinez-Picado, J., van Lunzen, J., Rouzioux, C., Miller, M., Vella, S., Schmitz, J.E., Ahlers, J., et al. (2013). Barriers to a cure for HIV: new ways to target and eradicate HIV-1 reservoirs. The Lancet *381*, 2109–2117.

Kaushik, S., Teque, F., Patel, M., Fujimura, S.H., Schmidt, B., and Levy, J.A. (2013). Plasmacytoid dendritic cell number and responses to Toll-like receptor 7 and 9 agonists vary in HIV Type 1-infected individuals in relation to clinical state. AIDS Res. Hum. Retroviruses *29*, 501–510.

Kelly, E., and Russell, S.J. (2007). History of oncolytic viruses: genesis to genetic engineering. Mol Ther *15*, 651–659.

Kinoshita, S., Su, L., Amano, M., Timmerman, L.A., Kaneshima, H., and Nolan, G.P. (1997). The T cell activation factor NF-ATc positively regulates HIV-1 replication and gene expression in T cells. Immunity *6*, 235–244.

Kinter, A., Moorthy, A., Jackson, R., and Fauci, A.S. (2003). Productive HIV infection of resting CD4+ T cells: role of lymphoid tissue microenvironment and effect of immunomodulating agents. AIDS Res. Hum. Retroviruses *19*, 847–856.

Kiselinova, M., De Spiegelaere, W., Buzon, M.J., Malatinkova, E., Lichterfeld, M., and Vandekerckhove, L. (2016). Integrated and Total HIV-1 DNA Predict Ex Vivo Viral Outgrowth. PLoS Pathog. *12*, e1005472.

Klatzmann, D., Barré-Sinoussi, F., Nugeyre, M.T., Danquet, C., Vilmer, E., Griscelli, C., Brun-Veziret, F., Rouzioux, C., Gluckman, J.C., and Chermann, J.C. (1984a). Selective tropism of lymphadenopathy associated virus (LAV) for helper-inducer T lymphocytes. Science *225*, 59–63.

Klatzmann, D., Champagne, E., Chamaret, S., Gruest, J., Guetard, D., Hercend, T., Gluckman, J.C., and Montagnier, L. (1984b). T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. Nature *312*, 767–768.

Kloke, O., and Niederle, N. (1990). Development and mechanisms of interferon resistance. Cancer Treat. Rev. *17 Suppl A*, 81–88.

Kopecky, S.A., Willingham, M.C., and Lyles, D.S. (2001). Matrix protein and another viral component contribute to induction of apoptosis in cells infected with vesicular stomatitis virus. Journal of Virology *75*, 12169–12181.

Kottke, T., Diaz, R.M., Kaluza, K., Pulido, J., Galivo, F., Wongthida, P., Thompson, J., Willmon, C., Barber, G.N., Chester, J., et al. (2008). Use of biological therapy to enhance

both virotherapy and adoptive T-cell therapy for cancer. Mol Ther *16*, 1910–1918.

Koup, R.A., Safrit, J.T., Cao, Y., Andrews, C.A., McLeod, G., Borkowsky, W., Farthing, C., and Ho, D.D. (1994). Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. Journal of Virology *68*, 4650–4655.

Krishnan, V., and Zeichner, S.L. (2004). Host cell gene expression during human immunodeficiency virus type 1 latency and reactivation and effects of targeting genes that are differentially expressed in viral latency. Journal of Virology *78*, 9458–9473.

Kulkosky, J., Nunnari, G., Otero, M., Calarota, S., Dornadula, G., Zhang, H., Malin, A., Sullivan, J., Xu, Y., DeSimone, J., et al. (2002). Intensification and stimulation therapy for human immunodeficiency virus type 1 reservoirs in infected persons receiving virally suppressive highly active antiretroviral therapy. Journal of Infectious Diseases *186*, 1403–1411.

la Fuente, de, C., Santiago, F., Deng, L., Eadie, C., Zilberman, I., Kehn, K., Maddukuri, A., Baylor, S., Wu, K., Lee, C.G., et al. (2002). Gene expression profile of HIV-1 Tat expressing cells: a close interplay between proliferative and differentiation signals. BMC Biochem. *3*, 14.

Laguette, N., Sobhian, B., Casartelli, N., Ringeard, M., Chable-Bessia, C., Ségéral, E., Yatim, A., Emiliani, S., Schwartz, O., and Benkirane, M. (2011). SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. Nature *474*, 654–657.

Laird, G.M., Bullen, C.K., Rosenbloom, D.I.S., Martin, A.R., Hill, A.L., Durand, C.M., Siliciano, J.D., and Siliciano, R.F. (2015). Ex vivo analysis identifies effective HIV-1 latency-reversing drug combinations. J. Clin. Invest. *125*, 1901–1912.

Laird, G.M., Eisele, E.E., Rabi, S.A., Lai, J., Chioma, S., Blankson, J.N., Siliciano, J.D., and Siliciano, R.F. (2013). Rapid quantification of the latent reservoir for HIV-1 using a viral outgrowth assay. PLoS Pathog. *9*, e1003398.

Lassen, K.G., Bailey, J.R., and Siliciano, R.F. (2004). Analysis of human immunodeficiency virus type 1 transcriptional elongation in resting CD4+ T cells in vivo. Journal of Virology *78*, 9105–9114.

Lassen, K.G., Hebbeler, A.M., Bhattacharyya, D., Lobritz, M.A., and Greene, W.C. (2012). A flexible model of HIV-1 latency permitting evaluation of many primary CD4 T-cell reservoirs. PLoS ONE *7*, e30176.

Lassen, K.G., Ramyar, K.X., Bailey, J.R., Zhou, Y., and Siliciano, R.F. (2006). Nuclear retention of multiply spliced HIV-1 RNA in resting CD4+ T cells. PLoS Pathog. *2*, e68.

Lebon, F., and Ledecq, M. (2000). Approaches to the Design of Effective HIV-1 Protease Inhibitors. Cmc *7*, 455–477.

Lewin, S.R., and Rouzioux, C. (2011). HIV cure and eradication: how will we get from the laboratory to effective clinical trials? Aids *25*, 885–897.

Li, P., Kaiser, P., Lampiris, H.W., Kim, P., Yukl, S.A., Havlir, D.V., Greene, W.C., and Wong, J.K. (2016a). Stimulating the RIG-I pathway to kill cells in the latent HIV reservoir following viral reactivation. Nat Med *22*, 807–811.

Li, P., Kaiser, P., Lampiris, H.W., Kim, P., Yukl, S.A., Havlir, D.V., Greene, W.C., and Wong, J.K. (2016b). Stimulating the RIG-I pathway to kill cells in the latent HIV reservoir following viral reactivation. Nat Med *22*, 807–811.

Li, Q., Smith, A.J., Schacker, T.W., Carlis, J.V., Duan, L., Reilly, C.S., and Haase, A.T. (2009). Microarray analysis of lymphatic tissue reveals stage-specific, gene expression signatures in HIV-1 infection. J. Immunol. *183*, 1975–1982.

Lichty, B.D., Breitbach, C.J., Stojdl, D.F., and Bell, J.C. (2014). Going viral with cancer immunotherapy. Nat Rev Cancer *14*, 559–567.

Lichty, B.D., Power, A.T., Stojdl, D.F., and Bell, J.C. (2004a). Vesicular stomatitis virus: re-inventing the bullet. Trends in Molecular Medicine *10*, 210–216.

Lichty, B.D., Stojdl, D.F., Taylor, R.A., Miller, L., Frenkel, I., Atkins, H., and Bell, J.C. (2004b). Vesicular stomatitis virus: a potential therapeutic virus for the treatment of hematologic malignancy. Human Gene Therapy *15*, 821–831.

Lin, L., Su, Z., Lebedeva, I.V., Gupta, P., Boukerche, H., Rai, T., Barber, G.N., Dent, P., Sarkar, D., and Fisher, P.B. (2006). Activation of Ras/Raf protects cells from melanoma differentiation-associated gene-5-induced apoptosis. Cell Death Differ. *13*, 1982–1993.

Liu, Y.-J. (2005). IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. Annu. Rev. Immunol. *23*, 275–306.

Lubben, N.B., Sahlender, D.A., Motley, A.M., Lehner, P.J., Benaroch, P., and Robinson, M.S. (2007). HIV-1 Nef-induced down-regulation of MHC class I requires AP-1 and clathrin but not PACS-1 and is impeded by AP-2. Mol. Biol. Cell *18*, 3351–3365.

Malim, M.H., and Emerman, M. (2008). HIV-1 accessory proteins--ensuring viral survival in a hostile environment. Cell Host & Microbe *3*, 388–398.

Mangeat, B., Turelli, P., Caron, G., Friedli, M., Perrin, L., and Trono, D. (2003). Broad antiretroviral defence by human APOBEC3G through lethal editing of nascent reverse transcripts. Nature *424*, 99–103.

Marchini, A., Scott, E.M., and Rommelaere, J. (2016). Overcoming Barriers in Oncolytic Virotherapy with HDAC Inhibitors and Immune Checkpoint Blockade. Viruses *8*, 9.

Marincola, F.M., Jaffee, E.M., Hicklin, D.J., and Ferrone, S. (2000). Escape of human solid tumors from T-cell recognition: molecular mechanisms and functional significance. Adv. Immunol. *74*, 181–273.

Marozin, S., Altomonte, J., Stadler, F., Thasler, W.E., Schmid, R.M., and Ebert, O. (2008). Inhibition of the IFN-β Response in Hepatocellular Carcinoma by Alternative Spliced Isoform of IFN Regulatory Factor-3. Mol Ther *16*, 1789–1797.

Marsden, M.D., Kovochich, M., Suree, N., Shimizu, S., Mehta, R., Cortado, R., Bristol, G., An, D.S., and Zack, J.A. (2012). HIV latency in the humanized BLT mouse. Journal of Virology *86*, 339–347.

Martinelli, E., Cicala, C., Van Ryk, D., Goode, D.J., Macleod, K., Arthos, J., and Fauci, A.S. (2007). HIV-1 gp120 inhibits TLR9-mediated activation and IFN-α secretion in plasmacytoid dendritic cells. Pnas *104*, 3396–3401.

Martinson, J.A., Roman-Gonzalez, A., Tenorio, A.R., Montoya, C.J., Gichinga, C.N., Rugeles, M.T., Tomai, M., Krieg, A.M., Ghanekar, S., Baum, L.L., et al. (2007). Dendritic cells from HIV-1 infected individuals are less responsive to toll-like receptor (TLR) ligands. Cell. Immunol. *250*, 75–84.

Martuza, R.L., Malick, A., Markert, J.M., Ruffner, K.L., and Coen, D.M. (1991). Experimental therapy of human glioma by means of a genetically engineered virus mutant. Science *252*, 854–856.

Mattapallil, J.J., Douek, D.C., Hill, B., Nishimura, Y., Martin, M., and Roederer, M. (2005). Massive infection and loss of memory CD4+ T cells in multiple tissues during acute SIV infection. Nature *434*, 1093–1097.

McMillan, N.A., Chun, R.F., Siderovski, D.P., Galabru, J., Toone, W.M., Samuel, C.E., Mak, T.W., Hovanessian, A.G., Jeang, K.T., and Williams, B.R. (1995). HIV-1 Tat directly interacts with the interferon-induced, double-stranded RNA-dependent kinase, PKR. Virology *213*, 413–424.

McNab, F., Mayer-Barber, K., Sher, A., Wack, A., and O'Garra, A. (2015). Type I interferons in infectious disease. Nature Publishing Group *15*, 87–103.

Mellors, J.W., Muñoz, A., Giorgi, J.V., Margolick, J.B., Tassoni, C.J., Gupta, P., Kingsley, L.A., Todd, J.A., Saah, A.J., Detels, R., et al. (1997). Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. Ann. Intern. Med. *126*, 946–954.

Mellors, J.W., Rinaldo, C.R., Gupta, P., White, R.M., Todd, J.A., and Kingsley, L.A. (1996). Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science *272*,

1167–1170.

Moore, J.P., and Klasse, P.J. (2007). HIV-1 pathogenesis: the complexities of the CCR5-CCL3L1 complex. Cell Host & Microbe *2*, 281–283.

Moss, A.R., and Bacchetti, P. (1989). Natural history of HIV infection. Aids *3*, 55–61.

Mousseau, G., Clementz, M.A., Bakeman, W.N., Nagarsheth, N., Cameron, M., Shi, J., Baran, P., Fromentin, R., Chomont, N., and Valente, S.T. (2012). An analog of the natural steroidal alkaloid cortistatin A potently suppresses Tat-dependent HIV transcription. Cell Host & Microbe *12*, 97–108.

Mousseau, G., Kessing, C.F., Fromentin, R., Trautmann, L., Chomont, N., and Valente, S.T. (2015). The Tat Inhibitor Didehydro-Cortistatin A Prevents HIV-1 Reactivation from Latency. MBio *6*, e00465.

Müller, U., Steinhoff, U., Reis, L.F., Hemmi, S., Pavlovic, J., Zinkernagel, R.M., and Aguet, M. (1994). Functional role of type I and type II interferons in antiviral defense. Science *264*, 1918–1921.

Nakashima, H., Kaur, B., and Chiocca, E.A. (2010). Directing systemic oncolytic viral delivery to tumors via carrier cells. Cytokine & Growth Factor Reviews *21*, 119–126.

Neil, S.J.D., Zang, T., and Bieniasz, P.D. (2008). Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. Nature *451*, 425–430.

Nguyên, T.L.-A., Abdelbary, H., Arguello, M., Breitbach, C., Leveille, S., Diallo, J.-S., Yasmeen, A., Bismar, T.A., Kirn, D., Falls, T., et al. (2008a). Chemical targeting of the innate antiviral response by histone deacetylase inhibitors renders refractory cancers sensitive to viral oncolysis. Proc. Natl. Acad. Sci. U.S.a. *105*, 14981–14986.

Nguyên, T.L.-A., Abdelbary, H., Arguello, M., Breitbach, C., Leveille, S., Diallo, J.-S., Yasmeen, A., Bismar, T.A., Kirn, D., Falls, T., et al. (2008b). Chemical targeting of the innate antiviral response by histone deacetylase inhibitors renders refractory cancers sensitive to viral oncolysis. Proc. Natl. Acad. Sci. U.S.a. *105*, 14981–14986.

Noser, J.A., Mael, A.A., Sakuma, R., Ohmine, S., Marcato, P., WK Lee, P., and Ikeda, Y. (2007). The RAS/Raf1/MEK/ERK Signaling Pathway Facilitates VSV-mediated Oncolysis: Implication for the Defective Interferon Response in Cancer Cells. Mol Ther *15*, 1531–1536.

Obuchi, M., Fernandez, M., and Barber, G.N. (2003). Development of recombinant vesicular stomatitis viruses that exploit defects in host defense to augment specific oncolytic activity. Journal of Virology *77*, 8843–8856.

Okumura, A., Lu, G., Pitha-Rowe, I., and Pitha, P.M. (2006). Innate antiviral response

targets HIV-1 release by the induction of ubiquitin-like protein ISG15. Pnas *103*, 1440–1445.

Oliere, S., Arguello, M., Mesplede, T., Tumilasci, V., Nakhaei, P., Stojdl, D., Sonenberg, N., Bell, J., and Hiscott, J. (2008). Vesicular stomatitis virus oncolysis of T lymphocytes requires cell cycle entry and translation initiation. Journal of Virology *82*, 5735–5749.

Otsuki, A., Patel, A., Kasai, K., Suzuki, M., Kurozumi, K., Chiocca, E.A., and Saeki, Y. (2008). Histone deacetylase inhibitors augment antitumor efficacy of herpes-based oncolytic viruses. Mol Ther *16*, 1546–1555.

Pacanowski, J., Kahi, S., Baillet, M., Lebon, P., Deveau, C., Goujard, C., Meyer, L., Oksenhendler, E., Sinet, M., and Hosmalin, A. (2001). Reduced blood CD123+ (lymphoid) and CD11c+ (myeloid) dendritic cell numbers in primary HIV-1 infection. Blood *98*, 3016–3021.

Pace, M.J., Graf, E.H., Agosto, L.M., Mexas, A.M., Male, F., Brady, T., Bushman, F.D., and O'Doherty, U. (2012). Directly Infected Resting CD4+T Cells Can Produce HIV Gag without Spreading Infection in a Model of HIV Latency. PLoS Pathog. *8*, e1002818–15.

Palella, F.J., Delaney, K.M., Moorman, A.C., Loveless, M.O., Fuhrer, J., Satten, G.A., Aschman, D.J., and Holmberg, S.D. (1998). Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. N. Engl. J. Med. *338*, 853–860.

Palmer, S., Maldarelli, F., Wiegand, A., Bernstein, B., Hanna, G.J., Brun, S.C., Kempf, D.J., Mellors, J.W., Coffin, J.M., and King, M.S. (2008). Low-level viremia persists for at least 7 years in patients on suppressive antiretroviral therapy. Proc. Natl. Acad. Sci. U.S.a. *105*, 3879–3884.

Palmer, S., Wiegand, A.P., Maldarelli, F., Bazmi, H., Mican, J.M., Polis, M., Dewar, R.L., Planta, A., Liu, S., Metcalf, J.A., et al. (2003). New real-time reverse transcriptase-initiated PCR assay with single-copy sensitivity for human immunodeficiency virus type 1 RNA in plasma. J. Clin. Microbiol. *41*, 4531–4536.

Parato, K.A., Senger, D., Forsyth, P.A.J., and Bell, J.C. (2005). Recent progress in the battle between oncolytic viruses and tumours. Nat Rev Cancer *5*, 965–976.

Park, S.Y., Waheed, A.A., Zhang, Z.-R., Freed, E.O., and Bonifacino, J.S. (2014). HIV-1 Vpu accessory protein induces caspase-mediated cleavage of IRF3 transcription factor. J. Biol. Chem. *289*, 35102–35110.

Parker, B.S., Rautela, J., and Hertzog, P.J. (2016). Antitumour actions of interferons: implications for cancer therapy. Nat Rev Cancer *16*, 131–144.

Patel, B., Dey, A., Ghorani, E., Kumar, S., Malam, Y., Rai, L., Steele, A.J., Thomson, J.,

Wickremasinghe, R.G., Zhang, Y., et al. (2011a). Differential cytopathology and kinetics of measles oncolysis in two primary B-cell malignancies provides mechanistic insights. Mol Ther *19*, 1034–1040.

Patel, B., Dey, A., Ghorani, E., Kumar, S., Malam, Y., Rai, L., Steele, A.J., Thomson, J., Wickremasinghe, R.G., Zhang, Y., et al. (2011b). Differential cytopathology and kinetics of measles oncolysis in two primary B-cell malignancies provides mechanistic insights. Mol Ther *19*, 1034–1040.

Perelson, A.S., Essunger, P., Cao, Y., Vesanen, M., Hurley, A., Saksela, K., Markowitz, M., and Ho, D.D. (1997). Decay characteristics of HIV-1-infected compartments during combination therapy. Nature *387*, 188–191.

Perelson, A.S., Neumann, A.U., Markowitz, M., Leonard, J.M., and Ho, D.D. (1996). HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time. Science *271*, 1582–1586.

Perreau, M., Savoye, A.-L., De Crignis, E., Corpataux, J.-M., Cubas, R., Haddad, E.K., De Leval, L., Graziosi, C., and Pantaleo, G. (2013). Follicular helper T cells serve as the major CD4 T cell compartment for HIV-1 infection, replication, and production. J. Exp. Med. *210*, 143–156.

Pestka, S., Krause, C.D., and Walter, M.R. (2004). Interferons, interferon-like cytokines, and their receptors. Immunol Rev *202*, 8–32.

Petersen, J.M., Her, L.S., and Dahlberg, J.E. (2001). Multiple vesiculoviral matrix proteins inhibit both nuclear export and import. Pnas *98*, 8590–8595.

Pfeffer, L.M., and Donner, D.B. (1990). The down-regulation of alpha-interferon receptors in human lymphoblastoid cells: relation to cellular responsiveness to the antiproliferative action of alpha-interferon. Cancer Res. *50*, 2654–2657.

Pierson, T.C., Zhou, Y., Kieffer, T.L., Ruff, C.T., Buck, C., and Siliciano, R.F. (2002). Molecular characterization of preintegration latency in human immunodeficiency virus type 1 infection. Journal of Virology *76*, 8518–8531.

Piller, S.C., Caly, L., and Jans, D.A. (2003). Nuclear import of the pre-integration complex (PIC): the Achilles heel of HIV? Curr Drug Targets *4*, 409–429.

Pincetic, A., Kuang, Z., Seo, E.J., and Leis, J. (2010). The interferon-induced gene ISG15 blocks retrovirus release from cells late in the budding process. Journal of Virology *84*, 4725–4736.

Platanias, L.C. (2005). Mechanisms of type-I- and type-II-interferon-mediated signalling. Nat Rev Immunol *5*, 375–386.

Pol, J.G., Zhang, L., Bridle, B.W., Stephenson, K.B., Rességuier, J., Hanson, S., Chen, L., Kazdhan, N., Bramson, J.L., Stojdl, D.F., et al. (2014a). Maraba virus as a potent oncolytic vaccine vector. Mol Ther *22*, 420–429.

Pol, J.G., Zhang, L., Bridle, B.W., Stephenson, K.B., Rességuier, J., Hanson, S., Chen, L., Kazdhan, N., Bramson, J.L., Stojdl, D.F., et al. (2014b). Maraba virus as a potent oncolytic vaccine vector. Mol Ther *22*, 420–429.

Pomerantz, R.J., Trono, D., Feinberg, M.B., and Baltimore, D. (1990). Cells nonproductively infected with HIV-1 exhibit an aberrant pattern of viral RNA expression: a molecular model for latency. Cell *61*, 1271–1276.

Power, A.T., Wang, J., Falls, T.J., Paterson, J.M., Parato, K.A., Lichty, B.D., Stojdl, D.F., Forsyth, P.A.J., Atkins, H., and Bell, J.C. (2007). Carrier cell-based delivery of an oncolytic virus circumvents antiviral immunity. Mol Ther *15*, 123–130.

Prins, J.M., Jurriaans, S., van Praag, R.M., Blaak, H., van Rij, R., Schellekens, P.T., Berge, ten, I.J., Yong, S.L., Fox, C.H., Roos, M.T., et al. (1999). Immuno-activation with anti-CD3 and recombinant human IL-2 in HIV-1-infected patients on potent antiretroviral therapy. Aids *13*, 2405–2410.

Rasmussen, T.A., Søgaard, O.S., Brinkmann, C., Wightman, F., Lewin, S.R., Melchjorsen, J., Dinarello, C., Østergaard, L., and Tolstrup, M. (2014). Comparison of HDAC inhibitors in clinical development. Human Vaccines & Immunotherapeutics *9*, 993–1001.

Rea, I.M., McNerlan, S.E., and Alexander, H.D. (1999). CD69, CD25, and HLA-DR activation antigen expression on CD3+ lymphocytes and relationship to serum TNF-alpha, IFN-gamma, and sIL-2R levels in aging. Exp. Gerontol. *34*, 79–93.

Ritchie, K.J., Hahn, C.S., Kim, K.I., Yan, M., Rosario, D., Li, L., la Torre, de, J.C., and Zhang, D.-E. (2004). Role of ISG15 protease UBP43 (USP18) in innate immunity to viral infection. Nat Med *10*, 1374–1378.

Roberts, A., Buonocore, L., Price, R., Forman, J., and Rose, J.K. (1999). Attenuated vesicular stomatitis viruses as vaccine vectors. Journal of Virology *73*, 3723–3732.

Rosner, M., Schipany, K., and Hengstschläger, M. (2013). Merging high-quality biochemical fractionation with a refined flow cytometry approach to monitor nucleocytoplasmic protein expression throughout the unperturbed mammalian cell cycle. Nat Protoc *8*, 602–626.

Rotger, M., Dang, K.K., Fellay, J., Heinzen, E.L., Feng, S., Descombes, P., Shianna, K.V., Ge, D., Günthard, H.F., Goldstein, D.B., et al. (2010). Genome-wide mRNA expression correlates of viral control in CD4+ T-cells from HIV-1-infected individuals. PLoS Pathog. *6*, e1000781.

Ruelas, D.S., and Greene, W.C. (2013). An Integrated Overview of HIV-1 Latency. Cell *155*, 519–529.

Rustagi, A., and Gale, M. (2014). Innate antiviral immune signaling, viral evasion and modulation by HIV-1. J. Mol. Biol. *426*, 1161–1177.

Sadler, A.J., and Williams, B.R.G. (2008). Interferon-inducible antiviral effectors. Nat Rev Immunol *8*, 559–568.

Saleh, S., Solomon, A., Wightman, F., Xhilaga, M., Cameron, P.U., and Lewin, S.R. (2007). CCR7 ligands CCL19 and CCL21 increase permissiveness of resting memory CD4+ T cells to HIV-1 infection: a novel model of HIV-1 latency. Blood *110*, 4161–4164.

Saleh, S., Wightman, F., Ramanayake, S., Alexander, M., Kumar, N., Khoury, G., Pereira, C., Purcell, D., Cameron, P.U., and Lewin, S.R. (2011). Expression and reactivation of HIV in a chemokine induced model of HIV latency in primary resting CD4+ T cells. Retrovirology *8*, 80.

Sanchez, D.J., Miranda, D., Marsden, M.D., Dizon, T.M.A., Bontemps, J.R., Davila, S.J., Del Mundo, L.E., Ha, T., Senaati, A., Zack, J.A., et al. (2015). Disruption of Type I Interferon Induction by HIV Infection of T Cells. PLoS ONE *10*, e0137951.

Sandler, N.G., Bosinger, S.E., Estes, J.D., Zhu, R.T.R., Tharp, G.K., Boritz, E., Levin, D., Wijeyesinghe, S., Makamdop, K.N., del Prete, G.Q., et al. (2014). Type I interferon responses in rhesus macaques prevent SIV infection and slow disease progression. Nature *511*, 601–605.

Sato, M., Hata, N., Asagiri, M., Nakaya, T., Taniguchi, T., and Tanaka, N. (1998a). Positive feedback regulation of type I IFN genes by the IFN-inducible transcription factor IRF-7. FEBS Lett. *441*, 106–110.

Sato, M., Suemori, H., Hata, N., Asagiri, M., Ogasawara, K., Nakao, K., Nakaya, T., Katsuki, M., Noguchi, S., Tanaka, N., et al. (2000). Distinct and essential roles of transcription factors IRF-3 and IRF-7 in response to viruses for IFN-alpha/beta gene induction. Immunity *13*, 539–548.

Sato, M., Tanaka, N., Hata, N., Oda, E., and Taniguchi, T. (1998b). Involvement of the IRF family transcription factor IRF-3 in virus-induced activation of the IFN-beta gene. FEBS Lett. *425*, 112–116.

Schröder, A.R.W., Shinn, P., Chen, H., Berry, C., Ecker, J.R., and Bushman, F. (2002). HIV-1 integration in the human genome favors active genes and local hotspots. Cell *110*, 521–529.

Seliger, B., Maeurer, M.J., and Ferrone, S. (2000). Antigen-processing machinery breakdown and tumor growth. Immunol. Today *21*, 455–464.

Seu, L., Sabbaj, S., Duverger, A., Wagner, F., Anderson, J.C., Davies, E., Wolschendorf, F., Willey, C.D., Saag, M.S., Goepfert, P., et al. (2015). Stable Phenotypic Changes of the Host T Cells Are Essential to the Long-Term Stability of Latent HIV-1 Infection. Journal of Virology *89*, 6656–6672.

Shan, L., Rabi, S.A., Laird, G.M., Eisele, E.E., Zhang, H., Margolick, J.B., and Siliciano, R.F. (2013). A Novel PCR Assay for Quantification of HIV-1 RNA. Journal of Virology *87*, 6521–6525.

Shan, L., and Siliciano, R.F. (2013). From reactivation of latent HIV-1 to elimination of the latent reservoir: the presence of multiple barriers to viral eradication. Bioessays *35*, 544–552.

Shan, L., Deng, K., Shroff, N.S., Durand, C.M., Rabi, S.A., Yang, H.-C., Zhang, H., Margolick, J.B., Blankson, J.N., and Siliciano, R.F. (2012). Stimulation of HIV-1-Specific Cytolytic T Lymphocytes Facilitates Elimination of Latent Viral Reservoir after Virus Reactivation. Immunity *36*, 491–501.

Shan, L., Yang, H.-C., Rabi, S.A., Bravo, H.C., Shroff, N.S., Irizarry, R.A., Zhang, H., Margolick, J.B., Siliciano, J.D., and Siliciano, R.F. (2011). Influence of host gene transcription level and orientation on HIV-1 latency in a primary-cell model. Journal of Virology *85*, 5384–5393.

Sheehy, A.M., Gaddis, N.C., Choi, J.D., and Malim, M.H. (2002). Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. Nature *418*, 646–650.

Shulak, L., Beljanski, V., Chiang, C., Dutta, S.M., Van Grevenynghe, J., Belgnaoui, S.M., Nguyên, T.L.-A., Di Lenardo, T., Semmes, O.J., Lin, R., et al. (2014). Histone deacetylase inhibitors potentiate vesicular stomatitis virus oncolysis in prostate cancer cells by modulating NF-κB-dependent autophagy. Journal of Virology *88*, 2927–2940.

Shyu, R.-Y., Chang, S.-C., Yu, J.-C., Hsu, S.-J., Chou, J.-M., Lee, M.-S., and Jiang, S.-Y. (2005). Expression and regulation of retinoid-inducible gene 1 (RIG1) in breast cancer. Anticancer Res. *25*, 2453–2460.

Siegal, F.P., Fitzgerald-Bocarsly, P., Holland, B.K., and Shodell, M. (2001). Interferon-alpha generation and immune reconstitution during antiretroviral therapy for human immunodeficiency virus infection. Aids *15*, 1603–1612.

Siegal, F.P., Kadowaki, N., Shodell, M., Fitzgerald-Bocarsly, P.A., Shah, K., Ho, S., Antonenko, S., and Liu, Y.J. (1999). The nature of the principal type 1 interferon-producing cells in human blood. Science *284*, 1835–1837.

Siliciano, J.D., and Siliciano, R.F. (2005). Enhanced culture assay for detection and quantitation of latently infected, resting CD4+ T-cells carrying replication-competent

virus in HIV-1-infected individuals. Methods Mol. Biol. *304*, 3–15.

Siliciano, J.D., Kajdas, J., Finzi, D., Quinn, T.C., Chadwick, K., Margolick, J.B., Kovacs, C., Gange, S.J., and Siliciano, R.F. (2003). Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T cells. Nat Med *9*, 727–728.

Siliciano, R.F., and Greene, W.C. (2011). HIV Latency. Cold Spring Harbor Perspectives in Medicine *1*, a007096–a007096.

Simon, V., and Ho, D.D. (2003). HIV-1 dynamics in vivo: implications for therapy. Nat. Rev. Microbiol. *1*, 181–190.

Simon, V., Bloch, N., and Landau, N.R. (2015). Intrinsic host restrictions to HIV-1 and mechanisms of viral escape. Nat Immunol *16*, 546–553.

Simon, V., Ho, D.D., and Abdool Karim, Q. (2006). HIV/AIDS epidemiology, pathogenesis, prevention, and treatment. The Lancet *368*, 489–504.

Sloan, R.D., and Wainberg, M.A. (2011). The role of unintegrated DNA in HIV infection. Retrovirology *8*, 52.

Smithgall, T.E., and Thomas, G. (2013). Small molecule inhibitors of the HIV-1 virulence factor, Nef. Drug Discovery Today: Technologies *10*, e523–e529.

Smyth, M.J. (2005). Type I interferon and cancer immunoediting. Nat Immunol *6*, 646–648.

Solis, M., Nakhaei, P., Jalalirad, M., Lacoste, J., Douville, R., Arguello, M., Zhao, T., Laughrea, M., Wainberg, M.A., and Hiscott, J. (2011). RIG-I-mediated antiviral signaling is inhibited in HIV-1 infection by a protease-mediated sequestration of RIG-I. Journal of Virology *85*, 1224–1236.

Soumelis, V., Scott, I., Gheyas, F., Bouhour, D., Cozon, G., Cotte, L., Huang, L., Levy, J.A., and Liu, Y.J. (2001). Depletion of circulating natural type 1 interferon-producing cells in HIV-infected AIDS patients. Blood *98*, 906–912.

Spina, C.A., Anderson, J., Archin, N.M., Bosque, A., Chan, J., Famiglietti, M., Greene, W.C., Kashuba, A., Lewin, S.R., Margolis, D.M., et al. (2013). An In-Depth Comparison of Latent HIV-1 Reactivation in Multiple Cell Model Systems and Resting CD4+ T Cells from Aviremic Patients. PLoS Pathog. *9*, e1003834.

Spivak, A.M., Andrade, A., Eisele, E., Hoh, R., Bacchetti, P., Bumpus, N.N., Emad, F., Buckheit, R., McCance-Katz, E.F., Lai, J., et al. (2014). A pilot study assessing the safety and latency-reversing activity of disulfiram in HIV-1-infected adults on antiretroviral therapy. Clin. Infect. Dis. *58*, 883–890.

Stacey, A.R., Norris, P.J., Qin, L., Haygreen, E.A., Taylor, E., Heitman, J., Lebedeva, M., DeCamp, A., Li, D., Grove, D., et al. (2009). Induction of a striking systemic cytokine cascade prior to peak viremia in acute human immunodeficiency virus type 1 infection, in contrast to more modest and delayed responses in acute hepatitis B and C virus infections. Journal of Virology *83*, 3719–3733.

Stetson, D.B., and Medzhitov, R. (2006). Type I Interferons in Host Defense. Immunity *25*, 373–381.

Stojdl, D.F., Abraham, N., Knowles, S., Marius, R., Brasey, A., Lichty, B.D., Brown, E.G., Sonenberg, N., and Bell, J.C. (2000a). The murine double-stranded RNA-dependent protein kinase PKR is required for resistance to vesicular stomatitis virus. Journal of Virology *74*, 9580–9585.

Stojdl, D.F., Lichty, B., Knowles, S., Marius, R., Atkins, H., Sonenberg, N., and Bell, J.C. (2000b). Exploiting tumor-specific defects in the interferon pathway with a previously unknown oncolytic virus. Nat Med *6*, 821–825.

Stojdl, D.F., Lichty, B.D., tenOever, B.R., Paterson, J.M., Power, A.T., Knowles, S., Marius, R., Reynard, J., Poliquin, L., Atkins, H., et al. (2003). VSV strains with defects in their ability to shutdown innate immunity are potent systemic anti-cancer agents. Cancer Cell *4*, 263–275.

Strain, M.C., Little, S.J., Daar, E.S., Havlir, D.V., Günthard, H.F., Lam, R.Y., Daly, O.A., Nguyen, J., Ignacio, C.C., Spina, C.A., et al. (2005). Effect of treatment, during primary infection, on establishment and clearance of cellular reservoirs of HIV-1. (Oxford University Press), pp. 1410–1418.

Sun, W.H., Pabon, C., Alsayed, Y., Huang, P.P., Jandeska, S., Uddin, S., Platanias, L.C., and Rosen, S.T. (1998). Interferon-alpha resistance in a cutaneous T-cell lymphoma cell line is associated with lack of STAT1 expression. Blood *91*, 570–576.

Sun, X., Yau, V.K., Briggs, B.J., and Whittaker, G.R. (2005). Role of clathrin-mediated endocytosis during vesicular stomatitis virus entry into host cells. Virology *338*, 53–60.

Sundström, C., and Nilsson, K. (1976). Establishment and characterization of a human histiocytic lymphoma cell line (U-937). Int. J. Cancer *17*, 565–577.

Swiggard, W.J., Baytop, C., Yu, J.J., Dai, J., Li, C., Schretzenmair, R., Theodosopoulos, T., and O'Doherty, U. (2005). Human Immunodeficiency Virus Type 1 Can Establish Latent Infection in Resting CD4+ T Cells in the Absence of Activating Stimuli. Journal of Virology *79*, 14179–14188.

Søgaard, O.S., Graversen, M.E., Leth, S., Olesen, R., Brinkmann, C.R., Nissen, S.K., Kjaer, A.S., Schleimann, M.H., Denton, P.W., Hey-Cunningham, W.J., et al. (2015). The Depsipeptide Romidepsin Reverses HIV-1 Latency In Vivo. PLoS Pathog. *11*, e1005142.

Taura, M., Kudo, E., Kariya, R., Goto, H., Matsuda, K., Hattori, S., Vaeteewoottacharn, K., McDonald, F., Suico, M.A., Shuto, T., et al. (2015). COMMD1/Murr1 Reinforces HIV-1 Latent Infection through IκB-α Stabilization. Journal of Virology *89*, 2643–2658.

Thompson, A.J.V., and Locarnini, S.A. (2007). Toll-like receptors, RIG-I-like RNA helicases and the antiviral innate immune response. Immunol. Cell Biol. *85*, 435–445.

Tong, J.G., Valdes, Y.R., Barrett, J.W., Bell, J.C., Stojdl, D., McFadden, G., McCart, J.A., DiMattia, G.E., and Shepherd, T.G. (2015). Evidence for differential viral oncolytic efficacy in an in vitro model of epithelial ovarian cancer metastasis. Mol Ther Oncolytics *2*, 15013.

Tyagi, M., Pearson, R.J., and Karn, J. (2010). Establishment of HIV latency in primary CD4+ cells is due to epigenetic transcriptional silencing and P-TEFb restriction. Journal of Virology *84*, 6425–6437.

UNAIDS (2016). Fact sheet, People living with HIV, HIV, antiretroviral therapy, new HIV infections, AIDS, tuberculosis, facts. 1–8.

van Sighem, A.I., Gras, L.A.J., Reiss, P., Brinkman, K., de Wolf, F., ATHENA national observational cohort study (2010). Life expectancy of recently diagnosed asymptomatic HIV-infected patients approaches that of uninfected individuals. Aids *24*, 1527–1535.

Vandergeeten, C., Fromentin, R., DaFonseca, S., Lawani, M.B., Sereti, I., Lederman, M.M., Ramgopal, M., Routy, J.P., Sekaly, R.P., and Chomont, N. (2013). Interleukin-7 promotes HIV persistence during antiretroviral therapy. Blood *121*, 4321–4329.

Vandergeeten, C., Fromentin, R., Merlini, E., Lawani, M.B., DaFonseca, S., Bakeman, W., McNulty, A., Ramgopal, M., Michael, N., Kim, J.H., et al. (2014). Cross-clade ultrasensitive PCR-based assays to measure HIV persistence in large-cohort studies. Journal of Virology *88*, 12385–12396.

Vatakis, D.N., Nixon, C.C., Bristol, G., and Zack, J.A. (2009). Differentially Stimulated CD4+ T Cells Display Altered Human Immunodeficiency Virus Infection Kinetics: Implications for the Efficacy of Antiviral Agents. Journal of Virology *83*, 3374–3378.

Verdin, E., Paras, P., and Van Lint, C. (1993). Chromatin disruption in the promoter of human immunodeficiency virus type 1 during transcriptional activation. Embo J. *12*, 3249–3259.

Wang, F.-X., Xu, Y., Sullivan, J., Souder, E., Argyris, E.G., Acheampong, E.A., Fisher, J., Sierra, M., Thomson, M.M., Najera, R., et al. (2005). IL-7 is a potent and proviral strain–specific inducer of latent HIV-1 cellular reservoirs of infected individuals on virally suppressive HAART. J. Clin. Invest. *115*, 128–137.

Wei, D.G., Chiang, V., Fyne, E., Balakrishnan, M., Barnes, T., Graupe, M., Hesselgesser, J.,

Irrinki, A., Murry, J.P., Stepan, G., et al. (2014). Histone Deacetylase Inhibitor Romidepsin Induces HIV Expression in CD4 T Cells from Patients on Suppressive Antiretroviral Therapy at Concentrations Achieved by Clinical Dosing. PLoS Pathog. *10*, e1004071.

Wheelock, E.F., and Dingle, J.H. (1964). Observations on the repeated administration of viruses to a patient with acute leukemia. N. Engl. J. Med. *271*, 645–651.

Whitney, J.B., Hill, A.L., Sanisetty, S., Penaloza-MacMaster, P., Liu, J., Shetty, M., Parenteau, L., Cabral, C., Shields, J., Blackmore, S., et al. (2014). Rapid seeding of the viral reservoir prior to SIV viraemia in rhesus monkeys. Nature *512*, 74–77.

Wightman, F., Solomon, A., Khoury, G., Green, J.A., Gray, L., Gorry, P.R., Ho, Y.S., Saksena, N.K., Hoy, J., Crowe, S.M., et al. (2010). Both CD31 +and CD31 −Naive CD4 +T Cells Are Persistent HIV Type 1–Infected Reservoirs in Individuals Receiving Antiretroviral Therapy. Journal of Infectious Diseases *202*, 1738–1748.

Wightman, F., Solomon, A., Kumar, S.S., Urriola, N., Gallagher, K., Hiener, B., Palmer, S., Mcneil, C., Garsia, R., and Lewin, S.R. (2015). Effect of ipilimumab on the HIV reservoir in an HIV-infected individual with metastatic melanoma. Aids *29*, 504–506.

Williams, S.A., Chen, L.-F., Kwon, H., Ruiz-Jarabo, C.M., Verdin, E., and Greene, W.C. (2006). NF-kappaB p50 promotes HIV latency through HDAC recruitment and repression of transcriptional initiation. Embo J. *25*, 139–149.

Wong, J.K., Hezareh, M., Günthard, H.F., Havlir, D.V., Ignacio, C.C., Spina, C.A., and Richman, D.D. (1997a). Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. Science *278*, 1291–1295.

Wong, L.H., Krauer, K.G., Hatzinisiriou, I., Estcourt, M.J., Hersey, P., Tam, N.D., Edmondson, S., Devenish, R.J., and Ralph, S.J. (1997b). Interferon-resistant human melanoma cells are deficient in ISGF3 components, STAT1, STAT2, and p48-ISGF3gamma. Journal of Biological Chemistry *272*, 28779–28785.

Yan, N., Regalado-Magdos, A.D., Stiggelbout, B., Lee-Kirsch, M.A., and Lieberman, J. (2010). The cytosolic exonuclease TREX1 inhibits the innate immune response to human immunodeficiency virus type 1. Nat Immunol *11*, 1005–1013.

Yang, H.-C., Xing, S., Shan, L., O'Connell, K., Dinoso, J., Shen, A., Zhou, Y., Shrum, C.K., Han, Y., Liu, J.O., et al. (2009). Small-molecule screening using a human primary cell model of HIV latency identifies compounds that reverse latency without cellular activation. J. Clin. Invest. *119*, 3473–3486.

Yerly, S., and Hirschel, B. (2012). Diagnosing acute HIV infection. Expert Rev Anti Infect Ther *10*, 31–41.

Yoneyama, M., Kikuchi, M., Natsukawa, T., Shinobu, N., Imaizumi, T., Miyagishi, M., Taira, K., Akira, S., and Fujita, T. (2004). The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. Nat Immunol *5*, 730–737.

Zhang, J., Tai, L.-H., Ilkow, C.S., Alkayyal, A.A., Ananth, A.A., de Souza, C.T., Wang, J., Sahi, S., Ly, L., Lefebvre, C., et al. (2014). Maraba MG1 virus enhances natural killer cell function via conventional dendritic cells to reduce postoperative metastatic disease. Mol Ther *22*, 1320–1332.

Zhang, K.-X., Matsui, Y., Hadaschik, B.A., Lee, C., Jia, W., Bell, J.C., Fazli, L., So, A.I., and Rennie, P.S. (2010). Down-regulation of type I interferon receptor sensitizes bladder cancer cells to vesicular stomatitis virus-induced cell death. Int. J. Cancer *127*, 830–838.

Zhang, L., and Su, L. (2012). HIV-1 immunopathogenesis in humanized mouse models. Cell. Mol. Immunol. *9*, 237–244.

Zhao, C., Collins, M.N., Hsiang, T.-Y., and Krug, R.M. (2013). Interferon-induced ISG15 pathway: an ongoing virus-host battle. Trends in Microbiology *21*, 181–186.

Zygiert, Z. (1971). Hodgkin's disease: remissions after measles. Lancet *1*, 593.

Contributions of Collaborators

A portion of the results in this thesis was only made possible with the tireless work and collaboration of Dr. Sandra C. Côté and Teslin S. Sandstrom. I am ever grateful for their support in helping take the work presented in this thesis to completion. Listed below are the specific contributions of the collaborators.

1. In Figure 32A, the integrated HIV-1 DNA PCR following MG1 infection (n=8) of *in vitro* latently HIV-1 infected resting $CD4^+$ T cells was performed by the writer. An additional n=4 of integrated HIV-1 DNA PCR was performed by Dr. Sandra C. Côté (Post-doctoral Fellow) for a total n=12.

2. In Figure 34A, the n=6 of the integrated HIV-1 DNA PCR for MG1 infection with UV-inactivated MG1 was performed by Dr. Sandra C. Côté (Post-doctoral Fellow).

3. In Figure 34C, n=2 of p24 expression following viral outgrowth (VOA) of resting $CD4^+$ T cells infected with MG1 or UV-inactivated MG1 was performed by the writer. An additional n=2 of p24 expression following VOA in MG1-infected resting $CD4^+$ T cells was performed by Teslin S. Sandstrom (PhD Student).

Curriculum Vitae

EDUCATION

2011-Present	**Doctor of Medicine and Philosophy (MD/PhD), University of Ottawa, Ottawa, ON**
2007-2011	Bachelor of Health Sciences (BHSc Hon.), McMaster University, Hamilton, ON

RESEARCH

2012-Present	**Oncolytic viruses as a potential approach to eliminate cells that constitute the HIV reservoir** Dr. Jonathan Angel, Ottawa Hospital Research Institute, Ottawa, Ontario
2012	Assessment of the role of remyelination for spinal cord repair through implantation of hollow fibre channels Dr. Eve C. Tsai, Ottawa Hospital Research Institute, Ottawa, Ontario
2009-2011	Canadian Longitudinal Thrombosis in End-Stage Renal Disease Study: Evaluating the incidence and risk of atherotrhombotic and cardiovascular disease in patients on dialysis Dr. Catherine Clase, McMaster University, Hamilton, Ontario
2011	Prevalence, incidence, and risk factors for gastrointestinal bleeding in an incident cohort of patients on dialysis (CAG/CFCC Studentship) Dr. David Morgan and Dr. Catherine Clase, Canadian Association of Gastroenterology, Hamilton, Ontario
2010	Prevalence of Atrial Fibrillation and Anticoagulation for Stroke prophylaxis in patients with Atrial Fibrillation Dr. Catherine Clase, McMaster University, Hamilton, Ontario

RESEARCH AWARDS AND HONOURS

2014-2016	**The Vanier Canada Graduate Scholarship: Doctoral Award** ▪ Salary award to support research aimed at examining oncolytic viruses as a potential approach to eliminate cells that constitute the HIV reservoir ▪ *Status: Awarded and accepted*
2014-2016	Canadian Institute of Health Research: Doctoral Award ▪ Research aimed at examining oncolytic viruses as a potential approach to eliminate cells that constitute the HIV reservoir ▪ *Status: Awarded and declined*
2015	International AIDS Society 2015: Towards a HIV Cure Scholarship ▪ Award to support travel and presentation at the 8th IAS 2015: Towards a HIV Cure Symposium

2015 Canadian Association of HIV Research 2015 Academic Scholarship in Vaccine Research

- One of eight awards to recognize research excellence in HIV/AIDS Vaccine research, as well as knowledge dissemination and oral presentation at the 24th Canadian Association of HIV Research Conference on HIV/AIDS Research

2014 Canadian Institute of Health Research - HIV/AIDS ICS Travel Award

- Award to support travel and presentation at the 23rd Annual Canadian Association of HIV Research Conference on HIV/AIDS Research

2013-2014 Queen Elizabeth II graduate scholarships in science and technology (QEII - GSST)

- Research aimed at examining oncolytic viruses as a potential approach to eliminate cells that constitute the HIV reservoir

PUBLICATIONS

Ranganath N, Sandstrom TS, Burke-Schinkel S, Côté SC, Angel, JB. (December, 2017). The oncolytic virus, MG1, targets and eliminates latently HIV-1-infected cells: implications for an HIV cure. *In Press* Journal of Infectious Disease (MS #JID-63221R1).

Sandstrom TS, **Ranganath N**, Angel JB. (July 2017). Impairment of the type I interferon response by HIV-1: Potential targets for HIV eradication. Cytokine & Growth Factor Reviews. DOI: 10.1016/j.cytogfr.2017.04.004.

Ranganath N, Sandstrom TS, Fadel S, Côté S, Angel JB. (August, 2016) Type I Interferon response are impaired in latently HIV infected cells. Retrovirology, 13 (66). DOI 10.1186/s12977-016-0302-9

Rochwerg B, Cheung JH, Ribic CM, France JC, Ganta S, **Ranganath N**, Walele A, MacDonald E, Meade MO, Cook DJ, Wilkieson TT, Clase CM, Margetts PJ, Gangji AS. (August, 2016) Assessment of Postresuscitation Volume Status by Bioimpedance Analysis in Patients with Sepsis in the Intensive Care Unit: A Pilot Observational Study. Canadian respiratory journal: journal of the Canadian Thoracic Society, (11), pp 1-8.

Wilkieson TJ, Rahman MO, Voss MD, Ingram AJ, **Ranganath N**, Goldsmith CH, Kotsamanes CZ, Gangji AS, Crowther MA, Rabbat CG, Clase CM. (2015). Coronary artery calcification, cardiovascular events, and death: a prospective cohort study of incident patients on hemodialysis. Canadian Journal of Kidney Health and Disease. 2 (29). DOI: 10.1186/s40697-015-0065-6

Ranganath N, Noel A. (2015). Integrating Administration, the Clinic, and Research: Interview with Dr. Jacques Bradwejn. University of Ottawa Journal of Medicine, 5 (II), pp. 12-15.

Ranganath N, Noel A. (2014). Development of a mobile application, ImmunizeCA, for the management of patient immunization records: A discussion with Dr. Kumanan Wilson and Katherin Atkinson. University of Ottawa Journal of Medicine, 4 (II), pp. 9-12.

Ranganath N, Larocque N. (2013). The Faculty of Medicine Student Mentorship Program: A new initiative. University of Ottawa Journal of Medicine, 3 (I), pp. 14-16.

PRESENTATIONS (PRESENTING AUTHOR UNDERLINED)

Ranganath N, Côté S, Angel JB. (May 2016). "Latently HIV-1 infected cells have defects in type I interferon responses that can be exploited for killing by oncolytic viruses". 25th Annual Canadian Conference on HIV/AIDS Research – CAHR 2016. Winnipeg, Manitoba.

- *Awarded the Canadian Association of HIV Research 2016 Academic Scholarship in Vaccine Research*

Ranganath N, Côté S, Angel JB. (November 2015). "Latently HIV-1 infected cells have defects in type I IFN response that can be exploited by VSVΔ51 and MG1 viruses". Ottawa Hospital Research Institute 2015 Research Meeting. Ottawa, Ontario.

Ranganath N, Côté S, Angel JB. (May 2015). "MG1 and VSVΔ51 viruses target and kill latently HIV-infected myeloid cells". 24th Annual Canadian Conference on HIV/AIDS Research – CAHR 2015. Toronto, Ontario.

- *Awarded the Canadian Association of HIV Research 2015 Academic Scholarship in Vaccine Research*

Ranganath N, Côté S, Angel JB. (November 2014). "The oncolytic virus MG1 preferentially targets and kills latently HIV-infected U1 cells". 2014 Meeting of Canadian Society for Clinical Investigation. Toronto, Ontario.

- *Awarded the Banting Research Foundation Prize for Best Oral Presentation at the 2014 CSCI Meeting*

Ranganath N, Côté S, Angel JB. (November 2014) "Oncolytic viruses as a potential approach to eliminate cells that constitute the HIV reservoir". 4th Annual Identification of Marketable Products, Applications and Commercializable Technologies (IMPACT) Competition. Ottawa, Ontario

- *Awarded Third Place in the Identification of Marketable Products, Applications and Commercializable Technologies (IMPACT) 4th Annual Competition.*

Ranganath N, Cohen J, Larocque N. (November 2013). Mentoring: How it can be applied to medical education? 3rd Canadian Conference on Physician Health. Calgary, Canada.

ABSTRACTS & POSTERS (PRESENTING AUTHOR UNDERLINED)

Ranganath N, Sandstrom T, Côté S, Angel JB. (November 2015). "VSV-Δ51 selectively targets and kills latently HIV-infected myeloid cells". Canadian HIV Cure Enterprise (CanCure) 2nd Annual Meeting. Montreal, Quebec.

- *Awarded the Merck, Canada 2015 Award for Best PhD Poster Presentation at CanCure Meeting*

Ranganath N, Côté S, Angel JB. (July 2015). "MG1 and VSVΔ51 viruses target and kill latently HIV-infected myeloid cells". 8th International AIDS Society: Towards a HIV Cure Symposium 2015. Vancouver, British Columbia.

- *Awarded the International AIDS Society 2015: Towards a HIV Cure Scholarship*

Ranganath N, Côté S, Angel JB. (April 2015). "MG1 and VSVΔ51 viruses target and kill latently HIV-infected myeloid cells". Mechanisms of HIV Persistence: Implications for a Cure. Boston, Massachusetts, USA.

Ranganath N, Côté S, Angel JB. (November 2014). "VSV-Δ51 preferentially targets latently HIV-infected U1 and OM10.1 cells". Ontario HIV Trials Network Conference. Toronto, Ontario.

Ranganath N, Côté S, Angel JB. (November 2014). "VSV-Δ51 selectively targets and kills latently HIV-infected myeloid cells". Canadian HIV Cure Enterprise (CanCure) 1st Annual Meeting. Montreal, Quebec.

Ranganath N, Côté S, Angel JB. (November 2014). "The oncolytic virus MG1 preferentially targets and kills latently HIV-infected OM10.1 cells". Ottawa Hospital Research Institute 2014 Research Meeting. Ottawa, Ontario.

Ranganath N, Côté S, Angel JB. (May 2014). "The oncolytic virus MG1 preferentially targets and kills latently HIV-infected U1 cells". Can Journal of infectious Disease Medical Microbiology 25 (SupplA): (Abstract # P026). 23rd Annual Canadian Conference on HIV/AIDS Research – CAHR 2014. St. John's, Newfoundland

Guzman J, Gibson A, Sandhu A, Yu R, Ranganath N, Zimmerman D, Wilkieson TJ, Clase CM. (November, 2010). Prevalence of Atrial Fibrillation and Anticoagulation for Stroke prophylaxis in patients with Atrial Fibrillation: A cross-sectional study of patients on Hemodialysis. American Society of Nephrology. Denver, USA.

- *Awarded McMaster Postgraduate Medical Education Quality Assurance Award 2010*