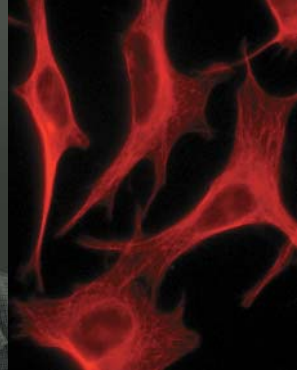
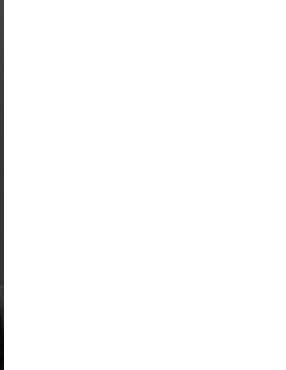
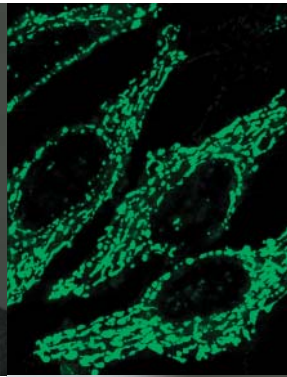
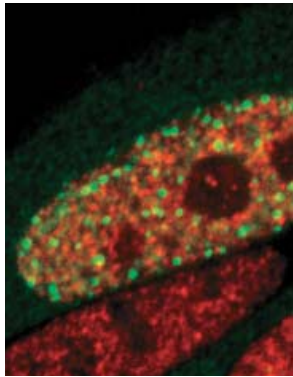


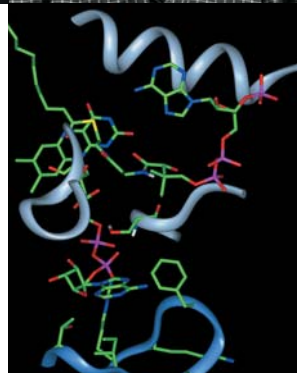
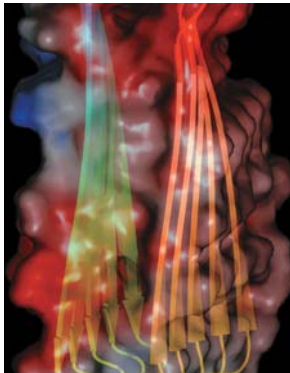
# Gladstone Institute of Virology and Immunology

Director's Report

Warner C. Greene, MD, PhD



*Left to right*  
Marielle Cavrois  
Ya-Lin Chiu  
Robert M. Grant  
Warner C. Greene  
Melanie Ott  
Eric Verdin



**H**IV/AIDS will certainly go down in history as one of the worst infectious disease epidemics to afflict humankind. Even today, when spread of the virus is perceptibly slowing, the epidemic continues to expand with 7400 new infections a day coupled with 5500 AIDS-related deaths. Seventy percent of all new HIV infections are occurring in one region of the world—sub-Saharan Africa. The good news is that HIV infections are now declining in select sub-Saharan countries, including Zimbabwe, Botswana, Malawi, and Zambia. However, the overall percentage of the population that is infected remains very high. The tide of HIV disease is receding due to programs like the President's Emergency Plan for AIDS Relief (PEPFAR)—the largest international health initiative in history dedicated to a single disease. PEPFAR has provided lifesaving antivirals to 2.4 million infected individuals and HIV testing and counseling to nearly 29 million people in 30 developing countries.

This progress is heartening, but much work remains to be done. Currently, we are still only reaching one third of Africans who are in need of antiviral drug treatment and only one third of pregnant infected mothers who need antiviral drug prophylaxis to protect their babies from acquiring HIV. Currently, for each individual placed on antiviral therapy in Africa, there are three to four new infections. Thus, it is clear that we cannot easily treat our way out of this pandemic.

How to move forward? One approach would be to place a **greater emphasis on preventing new HIV infections**. The search for an HIV vaccine, the “holy grail” of HIV research, has yielded one disappointment after another. However, a glimmer of hope emerged this past

year in a prime-boost (canary pox/HIV gp120) vaccine trial performed in Thailand (RV144). Individuals receiving the experimental vaccine experienced a significant 31.2% decline in HIV infections compared to placebo when analyzed using a modified intent-to-treat approach. This U.S. Army-sponsored trial was supervised by Colonel Jerome Kim based in the Military Office of HIV Research. Dr. Kim had previously trained as a postdoctoral fellow in my

**“I AM ENCOURAGED THAT A NEW WAVE OF BREAKTHROUGHS WILL OCCUR ON THE FRONTIERS OF HIV RESEARCH .... I FURTHER PREDICT THAT GIVI SCIENTISTS WILL PLAY IMPORTANT ROLES IN THESE ADVANCES.”**

—WARNER C. GREENE

laboratory. While the level of protection with this vaccine was quite low, making it unusable for the general public, **the results, if confirmed, certainly raise the possibility that a safe and effective HIV vaccine may be an achievable goal.**

Another preventive approach involves the preemptive administration of antivirals to uninfected individuals at high risk for HIV (**pre-exposure prophylaxis or**

**PreP**) in an effort to prevent these individuals from acquiring the virus. Several international PreP trials are now under way, including one fully enrolled trial directed by Dr. Robert Grant, a senior investigator in the Gladstone Institute of Virology and Immunology (GIVI). Hopefully, we will learn whether PreP is a viable prevention strategy within the next 12 months. As a part of Dr. Grant's PreP trial, he and his colleagues produced a video documentary called *Voices of Hope*, which describes the epidemic in men who have sex with men around the world, the new hope that the PreP prevention trial is engendering, and the heroic efforts of individuals who have elected to personally fight HIV by joining to create and participate in an HIV prevention clinical trial. The North American premiere of this video, which required 140,000 miles of travel across four continents, occurred on Tuesday, December 15 at the Gladstone Institutes.

Other studies are exploring various **female-controlled microbicides** that might be able to block HIV infection. Thus far, no effective microbicides have been identified. Indeed, many of the microbicides tested actually increased HIV infection rates in women using them. Recently, Dr. Nadia Roan in the GIVI reported the identification of surfen as a small molecule that blocks the action of SEVI (semen enhancer of viral infection). SEVI is a fibrillar peptide found in human semen that can boost HIV infection thousands of fold under certain conditions (Roan et al. *J. Biol. Chem.* 2009). It will be interesting to consider in the future the construction of combination microbicides, somewhat like combination antiviral drugs, that attack both the virus and the host factors that promote its transmission. Whether surfen or a

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surfen-related compound can be safely used in the female reproductive tract is now under study.

The most stunning advance in HIV prevention relates to **circumcision**. Circumcised males display an approximately 70% decreased risk of acquiring HIV from an HIV-infected woman compared with uncircumcised males. However, the introduction of adult circumcision is neither an easy nor straightforward task. Cultural and societal issues currently are stymieing the introduction of this surprisingly effective biomedical prevention approach.

A potential new approach for combating the global HIV epidemic under active discussion involves a **“test and treat” strategy**. The strategy entails implementation of an annual program of volunteer HIV testing for people older than 15 years of age. If an individual tests positive, they are immediately started on antiretroviral therapy regardless of their CD4 T cell count or viral load. The goal is to prevent HIV transmission. Mathematical modeling of such an intervention predicts that the HIV pandemic could be reduced within 10 years from 20 cases per 1000 (approximately the frequency of HIV infection in South Africa) to just 1 incident case per 1000 people. Theoretically, the pandemic could be brought to a complete end within 50 years with this approach. However, the feasibility of mounting and sustaining such an aggressive testing and treating program remains entirely unknown.

Another topic occupying great attention in current HIV medicine in the United States and throughout the developed world is the potential link between **HIV infection and accelerated aging**. Most HIV-infected patients in the United

States are now dying not of AIDS-related diseases but rather of non-AIDS diseases. These diseases, including cardiovascular disease, chronic kidney disease, bone disease, dementia, and liver disease, appear to occur approximately 10 years earlier in patients with HIV infection than in uninfected controls. A number of cohort studies also indicate a higher incidence of non-AIDS malignancies in HIV-infected patients, compared to age-matched uninfected individuals. These conditions appear to be on the rise even in the setting of successful antiretroviral therapy with suppressed viremia and stable CD4 T-cell counts.

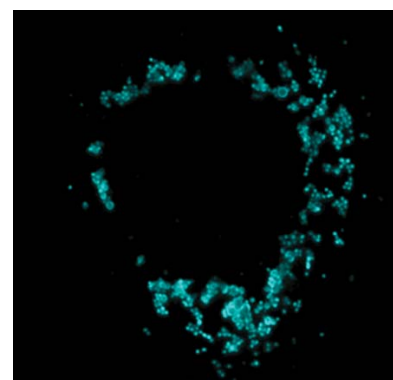
Gladstone investigators Drs. Eric Verdin and Melanie Ott will be joining Nobel laureate and GIVI SAB member Dr. Elizabeth Blackburn and Dr. Steven Deeks of the UCSF Positive Health Program to study the underlying cause(s) of this accelerated aging phenomenon in HIV-infected subjects.

This issue of the annual report provides a snapshot view of work under way in each of the GIVI laboratories. However, given the limitations of space, the described work represents only a small part of the compendium of research spanning virology, immunology, stem cell biology, and metabolomics under way in the GIVI. I invite you to read about the work of our investigators and, if possible, to visit the institute to learn more.

In addition to its basic science, educational outreach is a high priority within the GIVI. This year on the occasion of World AIDS Day, 18 members of the Institute visited San Francisco high schools to raise awareness about HIV/AIDS emphasizing aspects of the biology of this virus, how it is transmitted, and what steps each student should be

taking to avoid becoming infected. Also in recognition of World AIDS Day, Dr. Beatrice Hahn delivered the Merle Sande Memorial Lecture entitled “The Prehistory of HIV: Understanding the Primate Roots of Human AIDS.” This captivating presentation traced the remarkable story of how the precursor virus to HIV was identified in chimpanzees.

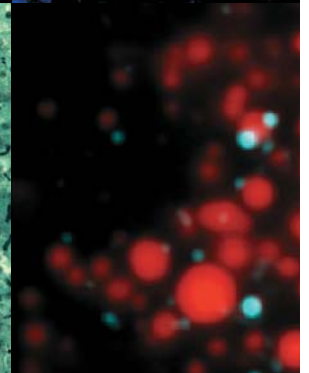
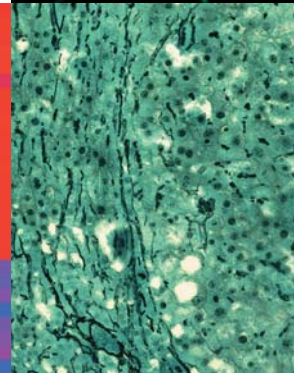
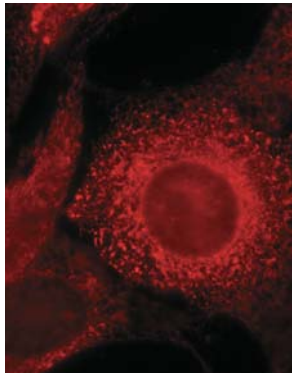
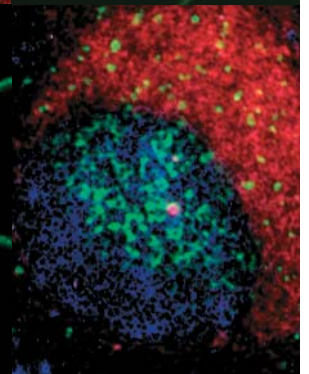
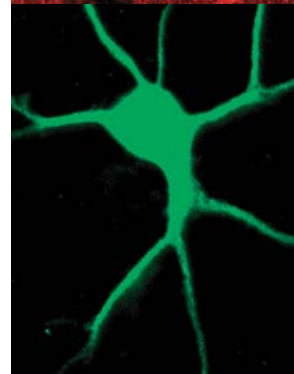
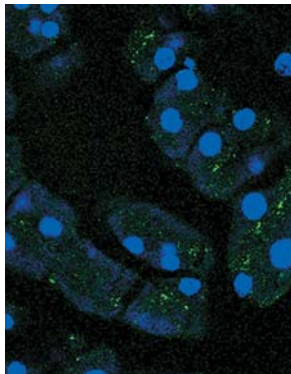
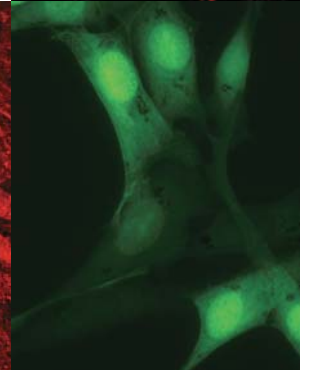
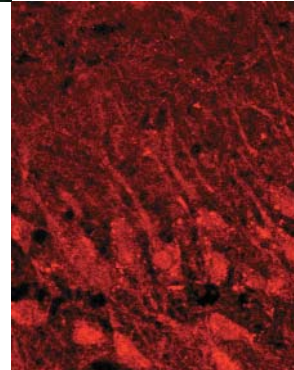
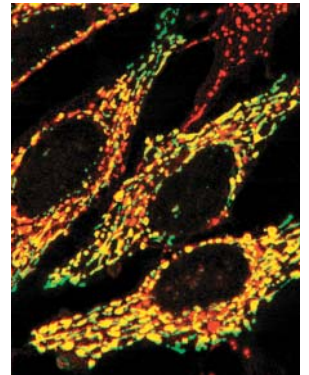
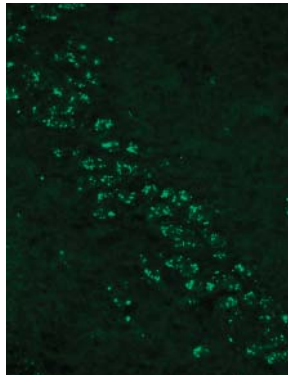
The GIVI continues its close interactions with the UCSF-GIVI Center for AIDS Research codirected by Dr. Paul Volberding and myself. This CFAR, one of the oldest in the country, provides important scientific cores that are used by a broad spectrum of HIV investigators at UCSF, an active mentoring program that helps ensure the success of young investigators interested in HIV biology, and a small grants program for fellows and junior faculty that distributes approximately \$750,000/year. In close-



ing, I also wanted to note with pride that Dr. Eric Verdin was elected as a 2009 Fellow of the American Association for the Advancement of Science.

This represents my 18th GIVI Director's overview. As I reflect back over the past 18 years of progress in the fight against HIV, I marvel at how far we have come. HIV is no longer a death sentence. The creation of multiple classes of anti-HIV drugs will certainly go down

as a milestone in the history of modern medicine. However, disparities in the distribution of these drugs remains, and we lack a truly effective strategy for HIV prevention. Whether at long last an efficacious vaccine will emerge or whether “test and treat” will be implemented, or whether PreP will become a standard in HIV prevention is hard to predict. However, I am encouraged that a new wave of breakthroughs will occur on the frontiers of HIV research involving prevention, pathogenesis, transmission, and drug development. I further predict that GIVI scientists will play important roles in these advances.



# Marielle Cavrois, PHD

## Investigating the Viral Determinants of HIV Transmission



**MUCOSAL TRANSMISSION OF HIV** is the major force driving the expansion of the global AIDS epidemic. Understanding the initial events of infection in a new host is key to developing an effective vaccine and microbicides.

HIV-1 diversifies throughout infection, leading to the emergence of multiple viral genotypes in chronically infected subjects. Nevertheless, a newly infected person harbors relatively homogeneous strains of the virus. The selection of quasi-species appears to be at least directed against the viral envelope and leads to the transmission of viruses whose envelope genes encode proteins with compact variable regions and fewer glycosylation sites. These viruses are uniquely sensitive to neutralization by antibodies from the transmitting partner. Thus, losing resistance to antibodies seems to confer greater transmissibility or better fitness for HIV in establishing infection in a new host, possibly by increasing the efficiency of fusion to target cells, the primary function of the HIV envelope protein.

We compared fusion mediated by envelope proteins from HIV isolated in newly infected individuals to fusion mediated by envelopes of the transmitting partner. Our collaborator, E. Hunter from the Emory Vaccine Center, established a cohort of couples discordant for HIV infection. These couples were followed every 3 months. Despite counseling, HIV was transmitted in several couples. The HIV envelope gene was sequenced from virus in blood samples harvested near the time of the transmission event. The phylogenetic tree highlighted the cluster of envelopes from a newly infected woman (purple) and the diversity of envelope sequences

from her transmitting partner (green). These envelope genes were subcloned into a laboratory-adapted strain of HIV to measure their ability to mediate fusion to primary CD4 T cells and dendritic cells. In our pilot study, the fusion phenotype was analyzed for four transmission pairs. In some pairs, the transmitted envelopes fused with higher efficiencies; in others, the phenotypes were more similar. More envelopes are being characterized to establish statistical significance.

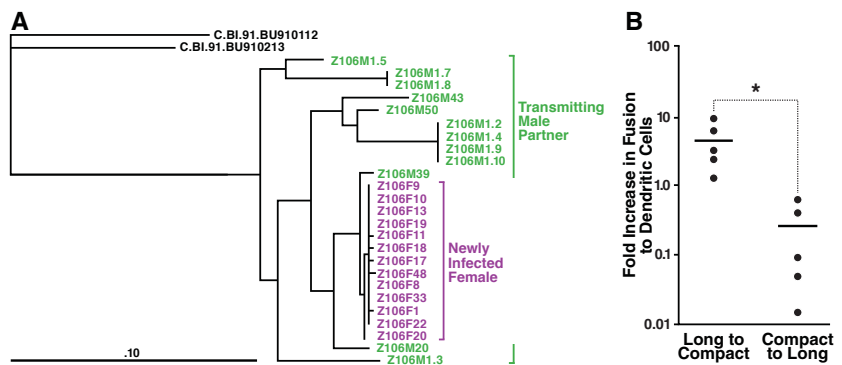
We noticed that envelopes encoding compact V1 domains consistently mediated higher fusion than envelopes with longer V1 domains. To formally investigate whether the V1 domain length directly influences fusion, we engineered mutant envelopes containing V1 loops of

varying length. Shortening the V1 loop consistently increased fusion, whereas lengthening the V1 loop decreased fusion. Since envelopes isolated from newly infected individuals encode proteins with shorter variable loops, this finding suggests that transmission to a new host may involve HIV virions containing highly fusiogenic envelopes.

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Cavrois M, Neidleman J, Greene WC (2008) The Achilles heel of the Trojan horse model of HIV-1 trans-infection. *PLoS Pathog.* 4:e1000051.



(A) Maximum likelihood tree (V1–V4 envelope sequences). (B) Fusion mediated by mutant envelopes. The variable loop V1 of HIV envelope proteins was either shortened or lengthened using as a matrix long or compact envelopes found in the same transmission pair. Fold increase in fusion compared to wildtype is reported. \* $p < 0.01$

### Cavrois Laboratory Members

Marielle Cavrois  
Jason Neidleman

# Ya-Lin Chiu, PHD

## Key Anti-HIV Enzyme Also Controls Gene-jumping Activities in Humans



**THE APOBEC3G (A3G) ENZYME** protects human cells in two ways. It provides immunity to exogenous HIV-1 infection, and it restricts the excessive movement of endogenous retroelements. The antiviral action of A3G involves its effective incorporation into budding viral particles and subsequent hypermutation of nascent viral DNA formed during the next round of infection. It is unclear how A3G controls mobile retroelements.

A3G is stored in cells in two forms: a low-molecular-mass form and a high-molecular-mass (HMM) RNA-protein complex. We found that HMM A3G complexes correspond to RNA granules with retroelement RNAs, specifically Alu and hY RNAs. These retroelements jump from place to place in the human genome, if they are assisted by the nuclear LINE-1 enzymatic machinery. When the jumping process is successful, lethal mutations and genetic instability may occur. We showed that expression of A3G restricts the jumping activity of Alu retroelements in cell-based assays.

To further understand how A3G controls Alu retroelements, we developed a method for visualizing Alu RNA in living cells. This method uses MS2-GFP fusion proteins containing a nuclear localization signal (NLS) and an Alu RNA reporter containing tandemly repeated MS2-binding sites. When the MS2-GFP-NLS fusion protein was expressed alone in HeLa cells, the NLS confined the protein to the nucleus, showing homogeneous nuclear GFP signals. When the fusion protein was co-expressed with the Alu RNA reporter, the GFP appeared as particle-like signals predominantly in the nucleus, but also in the cytoplasm. In their life cycles, Alu RNAs normally spend time in both the

nucleus and cytoplasm. Strikingly, when HA-A3G is co-expressed, MS2-GFP-NLS bound to the Alu RNA accumulated only in cytoplasmic granule-like structures. This finding suggests that A3G restricts Alu retroelements by locking the RNAs in these structures, thereby preventing them from accessing the nuclear LINE-1 enzymatic machinery required for their gene-jumping activity.

### RECENT PUBLICATIONS

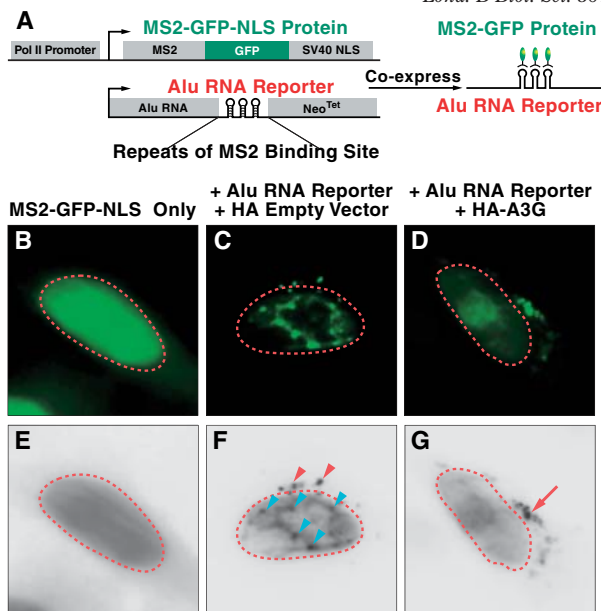
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### Chiu Laboratory Members

Shih-Shin Chang  
Ya-Lin Chiu  
Kotaro Shirakawa  
Shawn Sun



Visualizing A3G-restricting activity on Alu retroelements. **(A)** Constructs expressing the MS2-GFP-NLS fusion protein and the Alu RNA reporter. **(B–G)** Visualizing the Alu RNA reporter with the MS2-GFP-NLS fusion protein in HeLa cells. Red dashed lines, boundary of the nucleus; green, GFP signal; E–G, black and white images of B–D. **(B, E)** The MS2-GFP-NLS fusion alone shows only a homogeneous nuclear signal. **(C, F)** A cell expressing the MS2-GFP-NLS fusion and the Alu RNA reporter, showing particle-like GFP signals predominantly in the nucleus (blue arrowheads), but also in the cytoplasm (red arrowheads). **(D, G)** A cell expressing the MS2-GFP-NLS fusion and the Alu RNA reporter with A3G. A3G restricts movements of the Alu RNA reporter, and MS2-GFP-NLS bound to the reporter accumulates in cytoplasmic granule-like structures (red arrow).

## Robert M. Grant, MD, MPH

### Does a Pill a Day Prevent HIV Infection?



**OUR LABORATORY FOCUSES ON** the possibility that antiretroviral agents prevent acquisition of HIV infection. This concept, called “chemoprophylaxis,” is well established for the prevention of infectious diseases for which there is no effective vaccine, including malaria, tuberculosis, pneumonia, and perioperative infections. Antiretroviral agents prevent mother-to-child transmission, and the HIV epidemic among children has virtually vanished in settings where this concept is widely used.

Whether antiretroviral agents prevent sexual transmission of HIV is unknown. We are leading a global clinical trial to find out. We focus on pre-exposure prophylaxis, or PreP, because this approach was more effective in preclinical research in animals and is more forgiving when exposure to HIV is not recognized due to human limitations: denial, misinformation, and misperception. Our study is called the International PreP Initiative, or iPrEx, and is sponsored by the United States National Institutes of Health, with co-funding from the Bill and Melinda Gates Foundation, and drug donated by Gilead Sciences. Enrollment began in Lima, Peru, in 2007 and was completed in December 2009 at all sites in Brazil, Ecuador, Peru, South Africa, Thailand, and the United States. The study involves 425 workers, 2500 participants, and thousands of friends and families. PreP research has become the focal point of prevention research, intersecting with vaccine development, microbicide research, pharmacology, and social and behavioral science.

The iPrEx study has a unique role in the international portfolio of PreP research. The study is the only effi-

cacy study of PreP in gay men, transgendered women, and other men who have sex with men. These communities carry a major burden of the HIV-1 epidemic throughout the Americas and parts of Asia. The burden carried in Africa is increasingly appreciated in part because of surveillance studies supported by iPrEx. Cellular specimens collected for iPrEx will allow us to seek surrogate markers of the protective effect of PreP. Such surrogate markers, if any are found, will greatly accelerate further developments in the field.

Immunological, virological, metabolic, and qualitative substudies are under way to understand how PreP may impact antiviral immune responses, bone and fat, and social behavior.

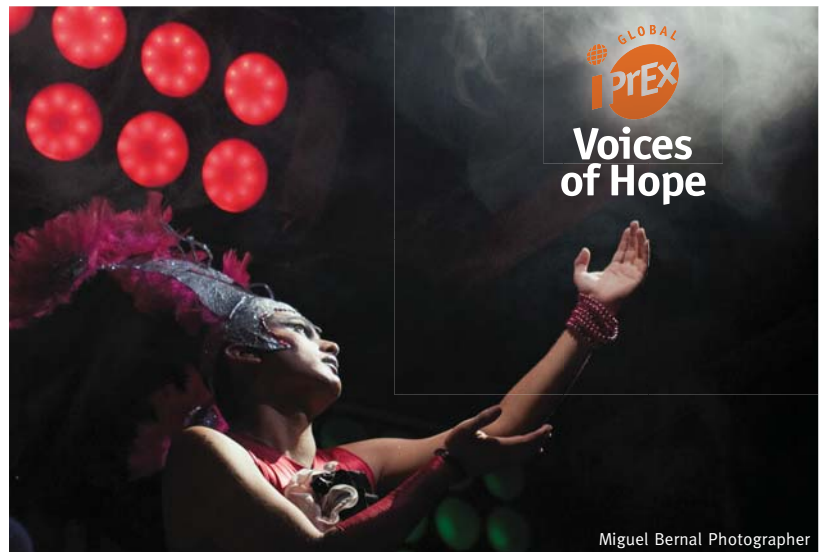
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#### Grant Laboratory Members

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Pedro Augusto  
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Jeanny Lee  
Jeff McConnell  
Vanessa McMahan  
Megha Mehrotra  
Mohamed Abdel  
Mohsen  
Aliza Monroe-Wise



Miguel Bernal Photographer

A documentary film was made to explain our collaborative approach to clinical research with communities who are on the front lines of the epidemic. More than 130 interviews were conducted on video in English, Spanish, Portuguese, and Thai. Scientists, community leaders, and participants speak of their vision for research that aims to arm people's struggle to stop the spread of HIV. The film premiered in the United States at The J. David Gladstone Institutes on December 15, 2009.

# Warner C. Greene, MD, PHD

## Murder on the HIV Express



**DESPITE MORE THAN 20 YEARS** of study, the mechanism by which HIV so effectively depletes CD4 T cells in untreated, infected subjects remains a mystery. It is precisely this progressive elimination of CD4 T cells that leads to AIDS. Understanding the underlying killing pathway might provide new approaches for preserving CD4 T cells in HIV-infected individuals.

Dr. Gilad Doitsh in my laboratory launched an exciting series of studies designed to explore how HIV depletes CD4 T cells in a biologically relevant tissue, human tonsil. These studies revealed that tonsil CD4 T cells are readily killed in the presence of HIV, but the dying cells are not productively infected with HIV. Rather, the dying CD4 T cells are abortively infected with the virus, reflecting arrest of the viral life cycle during the reverse transcription step. The accumulation of incomplete reverse transcripts is “sensed” within the cell, triggering caspase-3 activation and apoptosis. This killing requires the budding of HIV virions from the productively infected cell plus close cell-cell contact, implying that a host factor on the surface of cells may be required as well. Thus, a few productively infected cells in lymphoid tissue appear to be capable of mounting a murderous form of abortive viral infection involving many surrounding CD4 T cells that leads to their demise and the progressive loss of CD4 T cells.

Key future directions are to define the nature of the cellular sensor that trig-

gers apoptosis and the host cell factor that appears to be uniquely produced in lymphoid tissue.

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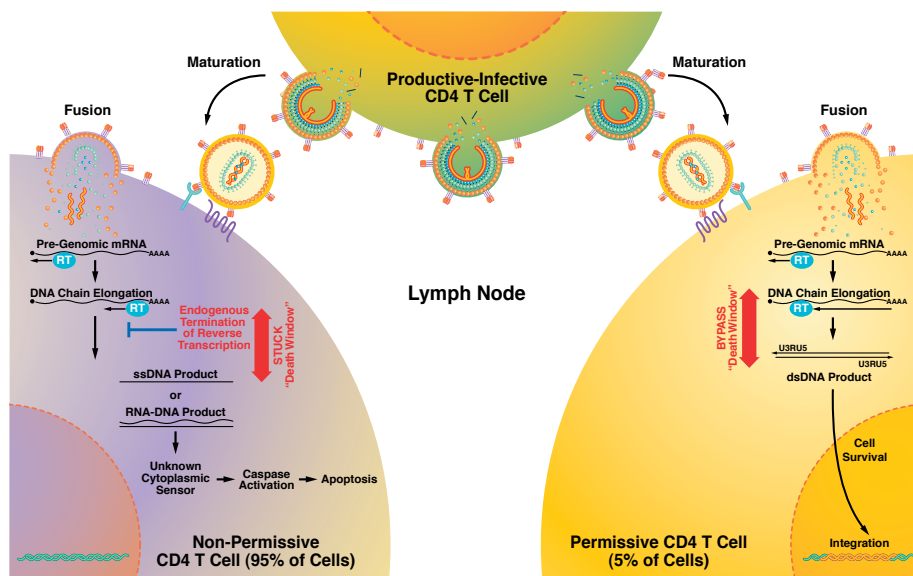
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### Greene Laboratory Members

- Robert Benitez
- Brianna Burden
- Nicole Byers
- Jonathan Chan
- Duy Dao
- Gilad Doitsh
- Analise Farmer
- Warner Greene
- Andrew Hebbeler
- Kara Lassen
- David Lim
- Michael Lobritz
- Aaron Middlebrook
- Mauricio Montano
- Isa Munoz-Arias
- Jason Neidleman
- Nadia Roan
- Debbie Ruelas
- Mario Santiago
- Nathan Schoeneck
- Stefanie Sowinski
- Matthew Strelau
- Brian Webster
- Silke Wissing
- Zhiyuan Yang
- Orlando Zepeda



Overview of HIV-induced CD4 T-cell killing showing how the fusion and entry of virions to quiescent nonpermissive CD4 T cells results in an arrest of reverse transcription, sensing of these cytoplasmic nucleic acids, and triggering of caspase-3-dependent apoptosis. These cytopathic events occurring as a result of abortive HIV infection appear to play an important role in CD4 T-cell depletion.

# Melanie Ott, MD, PHD

## Small Modifications Make a Big Difference: HIV Tat Protein Is Methylated by Set7/9



**AS WITH MOST INFECTIONS,** HIV involves a complex interaction of multiple factors. Understanding the choreography of these interactions is essential for finding new therapies for AIDS. We are interested in the complex interactions of HIV-1 with the cellular transcription machinery. HIV's Tat protein is required for efficient transcription of the virus. Previously, we showed that Tat is regulated by reversible acetylation. Recently, we found that Tat is a substrate of Set7/9, an enzyme that transfers a single methyl group to lysine residues. We also showed that methylation by Set7/9 stimulates Tat-mediated activation of HIV gene expression and is a critical early step in the Tat transactivation cycle.

Set7/9, recently renamed KMT7, was originally discovered as an enzyme that methylates lysine 4 within histone H3, an important marker for transcriptionally active genes. It was later found that Set7/9 also methylates other proteins, including the tumor suppressor p53, estrogen receptor  $\alpha$ , and the transcription factor NF- $\kappa$ B. The emerging importance of Set7/9-KMT7 as a central regulator of gene expression in diseases such as cancer and diabetes prompted us to investigate whether the enzyme also plays a role in HIV infection.

We found that Set7/9-KMT7 associates with the HIV promoter in HIV-infected T cells and methylates lysine 51 in Tat. Lysine 51 is a highly conserved residue located in the RNA-binding region of Tat. Methylation of lysine 51 enhances the affinity of Tat to its target RNA, a stem-loop structure called TAR that recruits the Tat protein to nascent HIV transcripts. Experimental knock-down of Set7/9-KMT7 suppresses Tat-

mediated activation of HIV gene expression, but does not affect the transcriptional activity of methylation-deficient Tat (K51A).

Surprisingly, our findings revealed that Set7/9-KMT7 itself binds to TAR RNA and engages in a complex formed by Tat, TAR RNA, and the cellular transcription elongation factor P-TEFb. We speculate that during the formation of this complex, lysine 51 in Tat becomes methylated, a process that stably tethers the complex to TAR RNA. Since formation of this complex is a key step in the HIV life cycle, we are exploring ways to block methylation of Tat by Set7/9-KMT7 as a potential new therapeutic for HIV infection.

### RECENT PUBLICATIONS

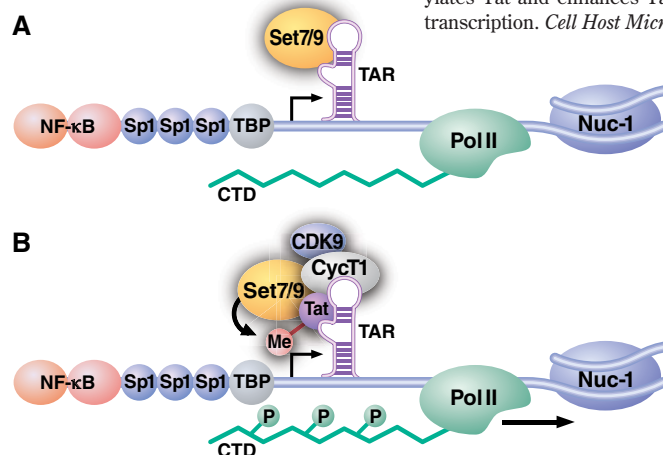
Cho S, Schroeder S, Kaehlcke K, Pedal A, Kwon H-S, Herker E, Schnoelzer M, Ott M (2009) Acetylation of the cyclin T1 regulates the equilibrium between active and inactive P-TEFb in cells. *EMBO J.* 28:1407–1417.

Cho S, Schroeder S, Ott M. Posttranslational modifications of P-TEFb regulate transcription elongation. *Cell Cycle.* In press.

Kwon HS, Brent MM, Getachew R, Jayakumar P, Chen LF, Schnoelzer M, McBurney MW, Marmorstein R, Greene WC, Ott M (2008) Human immunodeficiency virus type 1 Tat protein inhibits the SIRT1 deacetylase and induces T-cell hyperactivation. *Cell Host Microbe* 3:158–167.

Kwon HS, Ott M (2008) The ups and downs of SIRT1. *Trends Biochem. Sci.* 33:517–525.

Pagans S, Kauder SE, Kaehlcke K, Sakane N, Schroeder S, Dormeyer W, Trievel RC, Verdin E, Schnoelzer M, Ott M. Set7/9 (KMT7) binds HIV-1 TAR RNA, monomethylates Tat and enhances Tat-dependent HIV transcription. *Cell Host Microbe.* In press.



Model of Set7/9-KMT7 recruitment to the HIV LTR. (A) Set7/9-KMT7 binds TAR RNA within newly synthesized HIV transcripts. (B) When Tat is produced, Set7/9-KMT7 engages in complex formation with Tat and P-TEFb and methylates K51 in Tat.

### Ott Laboratory Members

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## Eric Verdin, MD

### Aging and Age-Associated Diseases: SIRT3 in Mitochondria



#### UNIQUE GENES HAVE BEEN IDENTIFIED

that regulate life span. Sirtuins are protein deacetylases that regulate life span in yeast, worms, flies, and mice. Another way to increase life span is by calorie restriction. Mice consistently fed 30% fewer calories than normal live about 30% longer. Importantly, calorie restriction fails to increase life span when sirtuin genes are lacking. Short-term studies in humans indicate that calorie restriction is likely to afford the same advantages, including increased life span and decreased diabetes, cancer, atherosclerosis, and neurodegeneration with aging. Resveratrol, a polyphenolic compound in red wine, is a direct activator of a sirtuin called SIRT1. Treating mice with resveratrol increases life span and protects mice from the deleterious effects of a high-fat diet.

Our laboratory focuses on SIRT3, one of seven human sirtuin proteins. SIRT3 is located in mitochondria in mammalian cells. Mitochondria are critical in aging: they are the metabolic center of the cell and the site of production of radical oxygen species, byproducts of the respiration system that contribute to aging. We hypothesized that SIRT3 regulates acetylation levels of key mitochondrial proteins. We recently observed that SIRT3 expression is upregulated in liver and brown adipose tissues during fasting. Livers from mice lacking SIRT3 show higher levels of intermediate products of fatty acid oxidation and triglycerides during fasting associated with decreased levels of fatty acid oxidation than wildtype mice. Mass spectrometry analysis of mitochondrial proteins shows a critical enzyme in fatty acid oxidation, long-chain acyl CoA dehydrogenase (LCAD), is hyperacetylated at lysine 42 in the absence of SIRT3.

LCAD is deacetylated by SIRT3 in wild-type mice under fasting conditions. Decreases in acetylation levels of LCAD by overexpression of SIRT3, incubation of recombinant LCAD with SIRT3 in vitro, or mutation of LCAD lysine 42 into arginine enhance its enzymatic activity. Mice lacking SIRT3 exhibit other hallmarks of fatty acid oxidation disorders: reduced ATP levels during fasting and intolerance to cold exposure upon fasting. These findings identify acetylation as a novel regulatory mechanism for mitochondrial fatty acid oxidation and show SIRT3 modulates mitochondrial intermediary metabolism and fatty acid utilization during fasting.

We continue our characterization of mice lacking SIRT3 by measuring their life span and sensitivity to metabolic disturbances, such as obesity and diabetes. Our working model is that SIRT3, like SIRT1, is

an important link between calorie restriction and its beneficial effects in mammals.

#### RECENT PUBLICATIONS

Blazkova J, Trejbalova K, Gondois-Rey F, Halfon P, Philibert P, Guiguen A, Verdin E, Olive D, Van Lint C, Hejnar J, Hirsch I (2009) CpG methylation controls reactivation of HIV from latency. *PLoS Pathog.* 5:e1000554.

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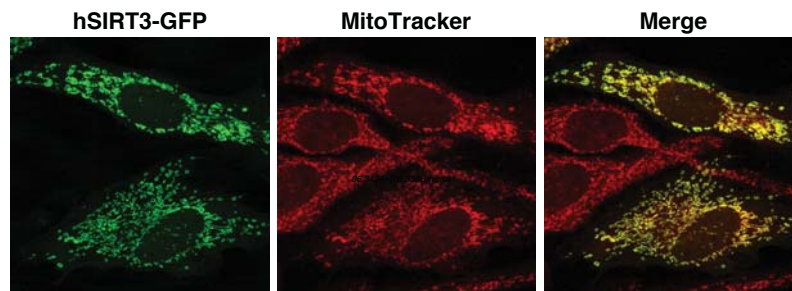
Hirschey MD, Shimazu T, Huang J-Y, Verdin E (2009) Acetylation of mitochondrial proteins. *Methods Enzymol.* 457:137-147.

Hirschey MD, Shimazu T, Goetzman E, Jing E, Schwer B, Lombard DB, Grueter CA, Harris C, Biddinger S, Ilkayeva OR, Stevens RD, Li Y, Saha AK, Ruderman NB, Bain JR, Newgard CB, Farese RV Jr, Alt FW, Kahn CR, Verdin E. SIRT3 regulates fatty acid oxidation via reversible enzyme deacetylation. *Nature*. In press.

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#### Verdin Laboratory Members

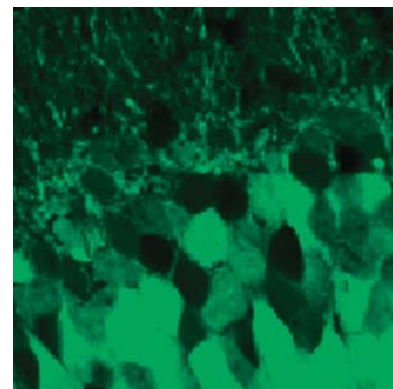
Leonard Chavez  
Amy Collins  
Liang Guo  
Shweta Hakre  
Matt Hirschey  
Linh Ho  
Jing-Yi Huang  
Glen Adams Huynh  
Herbert Kasper  
Steven Kauder  
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Hyungwook Lim  
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A fluorescent version of SIRT3 was introduced into cells (green-hSIRT3-GFP, left) and shows a unique distribution around the nucleus of the cells and throughout the cytoplasm of two cells. Staining of the same cells to highlight mitochondria (red-MitoTracker, middle) reveals the same staining distribution, indicating that SIRT3 is located in mitochondria. This is further confirmed by the perfect overlap (yellow) of the two merged pictures (right).

# Gladstone Institute of Virology and Immunology Publications

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