

# Discovery

The Newsletter of  
the Institute of  
Human Virology

FROM LABORATORY TO CLINIC

**ROBERT  
C. GALLO, M.D.**  
*Director of  
the Institute*



## Message From The Director

The Institute of Human Virology extends a warm welcome to Kathleen Kennedy-Townsend as chair of its Board of Advisors. She will succeed Stewart Greenebaum, who vacates the post after years of dedicated service and who will be recognized later this fall as a recipient of the IHV's Lifetime Achievement Award. Historically, it will be the first IHV Lifetime Achievement Award presented to a non-scientist. The award will be in recognition of Stewart's significant contributions to the Institute's AIDS research, treatment and prevention efforts.

This transition marks a time of major milestones and turning points at the IHV. Founded in 1996, the Institute was the first center to combine the disciplines of research, treatment and prevention under a single roof with scientists, clinicians and epidemiologists working side-by-side in a concerted effort to cut delays and speed the pace of progress.

Eight years later, the Institute has covered historic ground with impressive results. A handful of employees has grown to more than 250. Our annual budget has grown from \$9 million to \$40 million. Our reliance on state funding has decreased from more than half to just over 10 percent of total budget. In less than a decade, the IHV has been awarded more than 21 patents for innovative ideas and inventions, our patient base has grown from fewer than 300 to more than 3,000 and on the international front, we have work underway at 109 sites in 36 countries.

Despite all the growth and success, much work lies ahead. The AIDS epi-  
*(continued on page 2)*



**DRS. NIEL CONSTANTINE AND JANET BARLETTA, HIV researchers**

## New diagnostic method detects HIV earlier and more effectively

Researchers at the Institute of Human Virology have developed an ultra-sensitive testing technique that has shown the ability to detect HIV infection earlier than all current methods. This new diagnostic approach combines two existing models and is 25 times more sensitive than the best technology currently available.

"This new ultra-sensitive testing method, known as Real-Time Immuno-PCR, will allow us to detect HIV earlier and at much lower levels," according to Dr. Niel Constantine, a researcher at the IHV who has pioneered diagnostic research for more than two decades. He currently heads the Laboratory of Viral Diagnostics at the IHV, is Director of the Clinical Immunology Laboratory at the University of Maryland Medical Center and is a professor in the Department of Pathology at the University of Maryland School of Medicine.

Findings were published in the July issue of the American Journal of Clinical Pathology in an article co-authored by Janet M. Barletta, Ph.D., and Daniel C. Edelman, MS. The IHV is a center of the University of Maryland Biotechnology Institute and is

affiliated with the University of Maryland School of Medicine and University of Maryland Medical Center.

### Utilizing Existing Technology in New Ways

Real-Time Immuno-PCR, a novel method that detects ultra-low levels of proteins, has been applied for the detection of the AIDS virus. Specifically, it detects an inner protein of the virus known as p24, rather than detecting antibodies or viral nucleic acids. "Each virus particle contains about 3,000 molecules of p24 as compared with only two copies of nucleic acid. So there's a greater amount of target to detect," says Constantine of the new testing method, which combines the traditional Enzyme-Linked Immunosorbent Assay (ELISA)

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**KATHLEEN KENNEDY-TOWNSEND,**  
*Chair, IHV Board of Advisors*

less toxic therapeutic agents with fewer side effects for the patients who live with HIV, and a national pilot program that incorporates HIV education with directly observed therapy to combat a growing problem of drug resistance is showing potential to be adapted globally to ensure better long-term patient success. The IHV also has developed an ultra-sensitive testing technology that detects HIV much earlier than was previously possible; five concepts utilizing IHV science have progressed from bench to bedside, and we are poised to move forward with commercialization efforts later this year.

I would like to take this opportunity to thank Stewart for his steadfast dedication and support to the IHV's mission. We were fortunate and are grateful to

democ is getting worse, not better. Since the turn of the year we have become the single most funded academic research institute under the President's Emergency Plan for AIDS Relief, recognized for our strengths and success working with and in those developing countries hardest hit by the epidemic. Continued requests for assistance are mounting and, to meet these challenges and deliver the assistance needed, the IHV will have to continue to expand. Major additions to the staff will be implemented, international training efforts will broaden and scientific and community partnerships will continue to be the foundation from which we build.

Within the Institute, scientists have developed one of the most promising HIV vaccine candidates that exist, one that shows potential to be effective worldwide. Clinicians are pushing for



**STEWART GREENEBAUM,**  
*Former Chair, IHV Board of Advisors*

have had the leadership and vision of one who has walked with us every step of the way and never faltered. Kathleen, also a long-time member of our board, exemplifies Stewart's own philosophy of life: that we are here to better the human condition. Stewart will remain as a consultant and Kathleen will step up to the challenges that lie ahead. It is a defining moment in many ways. For us as an institute; for the world at large as it continues to confront the deadliest of epidemics; and, most especially, for those we treat and protect from HIV infection.

## New diagnostic method, *continued from page 1*

approach with another method known as PCR (polymerase chain reaction). "It's an advance over current methods in that we can detect down to the equivalent of two copies of RNA as compared with current methods which have been validated to only 50 copies," says Dr. Barletta.

The new approach has important implications for both HIV identification and treatment. Traditional testing methods detect HIV about 12 days after infection. Earlier detection could result not only in earlier diagnosis, but in improved protection of the blood supply and more effective treatment options for patients. "Monitoring HIV infection during antiretroviral treatment is the standard of care, and better monitoring of virus in the blood can more effectively address the growing trend of drug resistance," says Dr. Constantine.

In the U.S., HIV patients are living longer and better lives since the introduction of effective antiretroviral therapy. It is estimated, however, that as many as half of all HIV-infected patients in care experience treatment complications due to antiretroviral drug resistance, drastically reducing their odds of long-term success.

### Simpler is sometimes essential

Constantine's laboratory also is making strides in developing and maximizing HIV monitoring efforts that can be effectively utilized around the world, even in the most resource-constrained developing nations.

In a \$200,000 project funded by the Doris Duke Foundation, Constantine's team is utilizing a portable, battery-operated HIV monitoring system that weighs less than five pounds and can be powered by an automotive battery to address the critical issues of unstable electricity and high temperatures that often occur in most of the world's laboratories.

Some 90 percent of all HIV infections occur in the developing world. However, few can afford therapeutics needed for the masses and, in those areas where even emergency aid is available, therapeutics often come in the absence of effective treatment monitoring.

"Monitoring the response of HIV-infected persons to drugs is essential for managing care and increasing life expectancy," says Constantine. "Drug regimens must often be changed in response to resurgence of virus in blood, and thus, viral levels need to be monitored at regular intervals. Currently, the tests used to monitor active replication of the virus (viral load tests) are expensive and rigorous to perform; thus, these tests cannot be easily used in resource-limited countries and in laboratories that lack sufficient technical capability or infrastructure."

The portable battery-operated tests are simple to perform with fast results. "The proposed method incorporates a magnetic bead support and an antigen-antibody reaction that will be visualized by color production after the initial signal has been multiplied many times by using a special reagent. This "boosted" method allows a simple test to compete with more sophisticated technologies for detecting low levels of virus.

"The test will be much less expensive than current viral load tests," says Constantine, "much simpler to perform, portable, and will address a current and important void in the ability of developing country laboratories to assist health care professionals in determining if drug therapy for HIV infection is effective."

**Discovery** is a quarterly  
newsletter of the Institute of Human  
Virology. Copies are available upon request.  
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# Alpha-Interferon may provide missing piece in HIV/AIDS immunosuppression puzzle

**H**IV-1, like other debilitating viruses, has evolved into a highly opportunistic foe. It commandeers cellular machinery and processes to outmaneuver the body's immune system, step by step, with one simple goal—survival. Amazingly, HIV induces massive immunosuppression while infecting under one percent of target immune cells (CD4+ T cells). How can HIV produce large T cell declines and ultimately cripple the entire immune system, when so many cells remain uninfected?

Fabio Romerio, Assistant Professor in the Basic Science Division of the IHV, in collaboration with Dr. Robert Gallo, IHV Director, and with Dr. Daniel Zagury of Paris, is working to address that question. Although previous research has provided partial answers, it doesn't account for the magnitude or mechanism of HIV's de-

structive effects. "We want to determine even one mechanism of how HIV suppresses protective immune responses," says Romerio, who believes more research should be focused on uninfected, "bystander" T cells, which may be indirectly controlled by HIV-induced processes or "chemical messages."

From previous work with alpha-interferon—a natural, immune-modulating molecule—Romero knew HIV/AIDS patients show excessively and persistently high blood levels of alpha-interferon, predictors of a poor disease

outcome. "Normally, alpha-interferon is part of an innate, localized response for combating microbes," says Romerio, "for example, in acute tissue injury, such as a skin cut." He is working to understand how high alpha-interferon levels associated with AIDS contribute to immunosuppression.

"During a protective immune response, alpha-interferon promotes maturation of immune T cells into regulatory T cells (Treg), within lymph nodes," Romerio states. "These mature T cells suppress immune responses, via secretion of various immune modulating

molecules called cytokines." It seemed logical to Romerio, then, to wonder whether HIV-associated overproduction of alpha-interferon was having a similar effect in patients, generating a type(s) of suppressive cell from uninfected cells.

After modifying a cell culture system developed by Zagury, Romerio tested whether culturing mixed HIV-infected immune cells (peripheral blood mononuclear cells, PBMCs) with uninfected PBMCs could suppress proliferation of the

uninfected immune cells, and what role alpha-interferon played.

He discovered proliferation was vastly suppressed by exposure to infected cells, as well as exposure to the liquid medium the infected cells had grown in, and that alpha-interferon was mandatory. "HIV infection of PBMC induces alpha-interferon production and generates suppressive cells which potentially inhibit proliferation of uninfected PBMC, via release of unknown soluble factors," Romerio states. "This could be one way HIV suppresses immune function of uninfected cells." Romerio, Gallo, Zagury and co-workers are now close to identifying the suppressor cells and characterizing the soluble factors.

"Our ultimate goal," says Romerio "is the patient. We are trying to derive strategies for restoring their immune system and designing new therapeutic approaches, while still offering more classical vaccines."

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- FABIO ROMERIO



**FABIO ROMERIO**

*Assistant Professor, IHV Basic Science Division*

## Can removing a sugar be crucial for immune function and HIV-1 infection?

**NICHOLAS STAMATOS**

*Assistant Professor, IHV Clinical Division*

Sialic acid, a sugar found throughout nature, dots the exterior of microorganisms such as viruses and bacteria as well as mammalian cells, such as those in humans. Added last to an external sugar chain, sialic acid may serve as a “mask”—hiding a molecule and its function until needed. Sialic acid is removed naturally from mammalian cells by proteins called sialidases. In certain immune cells, removal increases immune recognition and response, even as it enhances the ability of HIV-1 to infect them.

Nicholas Stamatos, Assistant Professor in the Clinical Division at the IHV, is exploring the “yin-yang” role of sialidases in HIV/AIDS. Working with human monocytes, Stamatos has measured the activities of four sialidases as the monocytes are activated and mature into one of two final immune cells types, macrophages or dendritic cells (mature and immature). “During this differentiation of monocytes, the activities and gene expression of two sialidases, one found on the cell’s surface (Neu3) and one found within the cell (Neu1), are greatly increased,” he says. Interestingly, HIV cannot productively infect monocytes, but does infect and grow within macrophages and immature dendritic cells. HIV infection after differentiation is probably advantageous for the virus, since removal of sialic acids from HIV has proven to substantially increase

its ability to infect. Both HIV and host cell receptors for HIV binding are heavily coated with sialic acids. “Viruses are smart,” says Stamatos. “They continually maximize their ability to usurp cellular machinery.

As HIV approaches the cell, the cell surface sialidase may cleave off HIV sialic acid residues, inducing changes that increase binding to and facilitate fusion with the cell membrane.” Stamatos then wondered whether using inhibitors to block the activ-

ity of cellular sialidase might alter HIV’s ability to infect macrophages. “I discovered that continuous inhibition of sialidase activity (before, during, and after HIV was added

to cultured cells) with either of two sialidase inhibitors greatly decreased growth of HIV in macrophages,” he states.

He has since begun to examine similar endpoints in dendritic cells, “siblings” of the macrophage. “I intend to focus on these cells, determining what regulates sialidase activities across monocyte differentiation and what role sialidases play in the process itself,” Stamatos states. Loss of sialic acids on the cell surface also appears to turn on certain intracellular processes needed for viral integration and growth. Greater understanding of sialidase function and regulation will hopefully result in better HIV therapeutics.

“It’s possible to develop a new class of anti-viral drugs to block a host protein (sialidase), thereby affecting a previously untargeted viral growth stage,” he says. Such drugs, by theoretically reducing HIV entry into host cells, could be both preventative (for high risk populations) as well as therapeutic (for HIV-positive patients).



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-NICHOLAS STAMATOS



Third from left, Assistant Prof. Alash’le Abimiku, at the dedication of the Gallo House in Jos, Nigeria. The dedication was attended by dignitaries including Plateau State Governor Chief Joshua Dariye, the Honorable Commissioner of Health and Her Excellency Mrs. Obasanjo.

## Finding a “Cure” for HIV/AIDS: Shifting the Focus to HIV-1 Reservoirs

Reservoirs are typically envisioned as vast storage basins for fresh water, tapped during periods of need. Similarly, the immune system has reservoirs—“compartments” within the body that harbor reserve immune cells, lying quiet until activated for use. It is in these compartments, as well as others, that HIV takes up residence, within days of infection and detection in the bloodstream, and becomes “shielded” from even today’s most aggressive anti-viral therapeutics.

Roger Pomerantz, Professor at Thomas Jefferson University and Director of the University’s Division of Infectious Diseases and Environmental Medicine as well as the Center for Human Virology and Biodefense, has been working on HIV/AIDS his entire career. He now focuses on identifying and understanding the role played by various HIV reservoirs in thwarting eradication measures.

“There are two major types of HIV reservoirs,” says Pomerantz. “One is resting immune (CD4+) T cells, monocyte cells, and macrophage cells found throughout the body.” Resting immune cells harbor HIV that has integrated into cellular DNA and also maintains a low level of viral replica-

tion—so low that anti-viral drugs aimed at blocking replication have no effect.

“The second is a “sanctuary” site, such as the brain, retina, or testes,” he says. These so-called sanctuary sites represent body compartments that severely and selectively limit entry of cells, drugs, or vaccines, thereby sheltering HIV.

Several other cell types can also be infected by HIV, although at very low levels. “Cells found in the brain, blood vessels, kidneys, and muscle can harbor HIV,” notes Pomerantz, “although these are not likely to be part of the disease state.”

“Current HAART (Highly Active Anti-Retroviral Therapy) can reduce viral replication by 99.9 percent,” Pomerantz says. “Our problem is the residual virus in reservoirs that HAART cannot reach. It only takes one competent virus to emerge out of 1000 defective viruses within host DNA to re-infect a patient.” Pomerantz believes that even after HAART “these reservoir cells reignite a viral brush fire.”

“With the drugs configured at this time, HIV cannot be cured,” Pomerantz states bluntly. “But before we get depressed about the lack of a cure, we have to appreciate that we have succeeded in turning a fatal illness into a chronic (long-term), treatable one.”

Still, he readily acknowledges that HIV/AIDS is still a “bear” of a disease. “You’re on drugs for life, at high cost, with numerous side effects, and unknown long term consequences.”

Pomerantz knows that future treatment strategies must shift their focus toward “getting every last virus.” In addition to more intensive HAART-like drugs designed to block all viral replication, novel approaches that target and kill infected monocytes/macrophages, and stimulate latent, integrated HIV to replicate (and become vulnerable to HAART) will be needed.

“Some treatments may even need to be sequential, but they shouldn’t entail life-long therapy. If we’re able to cure it, a person will simply be cured,” he states succinctly. “We should know if this is possible within the next decade.”



**ROGER POMERANTZ**  
Professor,  
Thomas Jefferson  
University



### Wang Recipient of New Investigator Award

Lai-Xi Wang, an assistant professor of chemistry and researcher, will be honored as the second recipient of the New Investigator Award in Carbohydrate Chemistry by the American Chemical Society’s Division of Carbohydrate Chemistry. The award recognizes outstanding

contributions to research in carbohydrate chemistry by scientists at their first independent faculty position. Wang is being recognized for his work on bioorganic synthesis of glycoproteins and the design of carbohydrate-based HIV vaccines.

Wang received a Ph.D. in organic chemistry from Shanghai Institute of Organic Chemistry at the Chinese Academy of Sciences.

During his graduate study, he also spent three years at Japan’s Institute of Physical & Chemical Research studying oligosaccharide synthesis. After completing postdocs at Johns Hopkins University and Massachusetts Institute of Technology, Wang joined the Institute of Human Virology in 2000 to help establish a bioorganic chemistry program.

The award carries a prize of \$750 and will be presented at the Fall National ACS Meeting, to be held in Philadelphia, Aug. 22-26.



Dr. Robert Gallo was recently awarded honorary degrees from the Hebrew University of Jerusalem, Israel; the University of Hamburg, Germany; and Monterey University of Mexico.

**THE INSTITUTE OF HUMAN VIROLOGY (IHV)** at the University of Maryland was established to create and develop a world-class center of excellence focusing on chronic diseases and virally linked cancers. The IHV is dedicated to discovery, research, treatment, and prevention of these diseases and cancers. Its unique structure seeks to connect cohesive, multidisciplinary research and clinical programs so that new treatments are streamlined from discovery to patient. The IHV serves patients locally and the scientific community globally.

## **Planning for the day after access: Putting HIV vaccine access on the global agenda**

Working from the premise that assuring HIV vaccine access will require the same kind of commitment and timeline as vaccine science, a group of experts at the International AIDS Conference gathered for a satellite symposium, *The Day after Efficacy: Planning for Success in Assuring Access to AIDS Vaccines in the Developing World*.

### **Thai Perspectives**

Speakers from Thailand offered the perspective of a country that is host to the world's largest AIDS vaccine trial. Dr. Vallop Thaineau, Permanent Secretary of the Thai Ministry of Public Health (MOPH), laid out critical issues that need to be addressed: regulatory issues, demand forecasting, defining vaccination strategies and building infrastructure, procurement and production of vaccines, and political will and commitment.

Demand forecasting for HIV vaccines is crucial to ensuring rapid availability of a vaccine. Thailand has led the way in researching demand. Dr. Viroj Tangcharoensathien of the MOPH presented a Thai case study on demand forecasting which looked at different scenarios for effectiveness and cost of a vaccine.

Dr. Supamit Chunsuttiwat, of Thailand's MOPH and Co-Principal Investigator of the Prime-Boost HIV Vaccine Phase III trial currently being conducted in Thailand, outlined the challenges inherent in a study of this type. These include establishing communication strategies to secure community

awareness and engagement. He noted that public information at national and community levels and education and advocacy forums with local authorities, community leaders, NGOs and local AIDS support groups are all essential tools

### **Global Perspectives**

Giving a global overview of the vaccine field, NIAID's Dr. Peggy Johnston outlined the spectrum of strategies currently being studied. She pointed out that with an expanded HIV vaccine pipeline (more than 30 vaccines in trials) comes new challenges, for example, working to identify the most promising prime boost combinations.

Regulatory issues are key to eventual licensure of a vaccine. Dr. Helen Rees, of the University of Witwatersrand in Johannesburg, South Africa addressed the need for formal mechanisms that allow for national regulatory authorities (NRAs) to exchange information and the challenges posed by lack of NRA harmonization.

Dr. Jim Tartaglia, head of Aventis Pasteur's global HIV vaccine program, offered industry perspectives on AIDS vaccine access. He discussed how HIV vaccines will differ from traditional vaccine access models. He pointed out that no company, government or NGO alone will be able to carry the burden of access and that governments, NGOs, donors and industry must work together to design access programs that will allow a vaccine to get to those who need it the most as

quickly as possible.

The International AIDS Vaccine Initiatives' Dr. Seth Berkley addressed the challenges of getting the world to care about an HIV vaccine. He noted that scientific progress, mobilizing financing and generating policy change all depend on political will and for this, leadership is critical. He stressed that building a global political constituency for an HIV vaccine requires reaching new and emerging leaders in the North and the South, tapping into the rich network of AIDS NGOs and building a coalition of powerful developing country voices.

Symposium co-sponsors represented the different sectors that need to come together to ensure access. They included Aventis Pasteur, the U.S. Army Medical Research and Materiel Command, the Thai Ministry of Public Health, the U.S. National Institute of Allergy and Infectious Diseases, the Bill and Melinda Gates Foundation and the International AIDS Vaccine Initiative.

Copies of the presentations and more information about HIV vaccine access will be available at the website [www.day-after-efficacy.com](http://www.day-after-efficacy.com).

-- contributed by AP



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**DISCOVERY would like to thank its corporate sponsor, Aventis Pasteur, for continued support of the IHV and its mission.**