Budding and transmission of negative-strand RNA viruses...such as Ebola, Marburg, Lassa Fever, Junin, rabies and vesicular stomatitis

Dr. Ronald N. Harty is an associate professor of microbiology (tenure-track) and head of the Laboratory of Infectious Diseases and Immunology in the Department of Pathobiology. Dr. Harty received a B.S. in biology from the University of Lowell, MA in 1985. After receiving his Ph.D. in microbiology/virology from Louisiana State University Medical Center in 1991, he worked as a post-doctoral fellow at Mount Sinai School of Medicine in New York before joining the Department of Pathobiology in 1998.

CHMI Symposium 2014

The Center for Host-Microbial Interactions (CHMI) will hold a two-day symposium on "Microbial Communities in Health and Disease" on October 15-16 in the Shirley and Vernon Hill Pavilion at the School of Veterinary Medicine. The symposium will be a joint presentation of CHMI with Penn’s Perelman School of Medicine and the School of Arts and Sciences. Carl Zimmer, acclaimed author and New York Times columnist will give a lecture on October 15 entitled "A Planet of Viruses: How Humans Can Live Safely on a Viral World". Online registration at http://bit.ly/CarlZimmer

SPEAKERS FOR THE UPCOMING CHMI SYMPOSIUM

The full program of featured speakers for the October 16th program are: Yasmine Belkaid, NIH/NIAID; Claire Fraser, University of Maryland; Jack Gilbert, University of Chicago; Sergio Lira, Mount Sinai (NY); John Rawls, Duke University; Martin F Polz, Massachusetts Institute of Technology; Ramnik Xavier, Harvard; and Janelle Ayres, Salk Institute. For more information: donza@vet.upenn.edu Register for the lectures at: http://bit.ly/RegisterCHMI
Dr. Ronald Harty...continued from page 1

Dr. Harty’s research interests are focused on understanding the molecular events that regulate budding and transmission of negative-strand RNA viruses such as Ebola, Marburg, Lassa Fever, Junín, rabies, and vesicular stomatitis virus (VSV). Dr. Harty’s main focus is to identify and characterize virus-host interactions that contribute to virus budding, with the long-term goal of developing broad-spectrum, host-oriented antiviral therapeutics to block the budding process.

**Viruses are ESCRT’d from the cell**—The ongoing Ebola epidemic in West Africa highlights the urgent need for safe and effective therapeutics against biodefense and NIAID Category A pathogens, including filoviruses (Ebola and Marburg) and arenaviruses (e.g. Lassa and Junín), all of which cause severe hemorrhagic fever syndromes with high mortality rates. The Harty laboratory along with others have shown that budding of filoviruses, arenaviruses, and rhabdoviruses is critically dependent on the subversion of host proteins (1, 2), such as Tsg101 and Nedd4, whose normal function in the cell is to regulate protein sorting and trafficking as part of the Endosomal Sorting Complex Required for Transport (ESCRT) pathway. Viral matrix proteins, like Ebola VP40, play a central role in virus budding, and interestingly, can bud independently from mammalian cells in the form of virus-like particles (VLPs) (1, 2). The viral matrix proteins contain one or more highly conserved motifs known as Late (L) domains that...continued on page 3
have the amino acid sequence of PTAP, PPxY (x is any amino acid), or YPxL and which function to hijack or recruit host ESCRT or ESCRT-associated proteins to facilitate the budding process (1–3). The Harty lab is attempting to target and block these critical virus-host interactions using small molecule probes (4–6). Dr. Harty postulates that these inhibitors will disrupt or slow-down virus budding, thereby providing the infected individual’s immune system more time to develop a robust and protective response to the virus and consequently reducing disease progression and transmission. According to Dr. Harty, it would be analogous to laying down spike strips (inhibitors) to slow down a criminal in a speeding car (virus trying to bud), thereby allowing the police (immune system) to catch up to and neutralize the perpetrator.

**No Exit: Toward the identification of virus budding inhibitors**—Recently, Dr. Harty and his multi-disciplinary team of expert collaborators have made substantive progress in identifying lead candidate inhibitors for two different series of compounds: one targeting the viral PTAP-host Tsg101 interaction (5) and the other targeting the viral PPxY-host Nedd4 interaction (6). The initial screen to identify virus budding inhibitors was performed by collaborators at USAMRIID, who used an *in silico* approach to screen approximately 5 million drug-like compounds from the ZINC database based on the known structural information for the PTAP-Tsg101 and PPxY-Nedd4 interactions. After multiple rounds of testing the top hits, Harty’s team identified the current lead PTAP inhibitor, compound 0013, which was shown to be most potent at blocking egress of both Ebola and Junín VLPs at nanomolar concentrations (5). They went on to demonstrate that inhibition of budding was PTAP-specific, and that compound 0013 blocked the PTAP-dependent interaction with host Tsg101 in bimolecular complementation and co-immunoprecipitation assays (5). Most importantly, compound 0013 inhibited budding of live Junín virus; a virus that buds solely via a single PTAP L-domain (5).

In addition to compound 0013, Dr. Harty’s team is pursuing two PPxY lead candidate budding inhibitors: compounds 4 and 5 (6). Compounds 4 and 5 were identified from the initial *in silico* screen followed by several rounds of iterative selection, and were found to be potent at blocking budding of VLPs derived from Ebola, Marburg, or Lassa fever virus (6). As mentioned above, a bimolecular complementation approach was used to demonstrate that compounds 4 and 5 specifically blocked the PPxY-Nedd4 interaction (6). Most exciting were the findings that compounds 4 and 5 blocked PPxY-dependent budding of live viruses including VSV, rabies (in collaboration with Dr. Matthias Schnell at Thomas Jefferson University), as well as Ebola (Kikwit) and Marburg (Ci67) (in collaboration with Dr. John Dye at the BSL-4 laboratory at USAMRIID (6; unpublished data)).

**Moving forward**—Dr. Harty has several areas of focus as he and his team move this research forward.
A wide range of students found their way to a Penn Vet research laboratory during the summer of 2014. They may have been participants in the NIH/Merial Veterinary Scholars Program; Summer Undergraduate Internship Program (SUIP); Penn's Life Sciences & Management Summer Program; Vagelos Scholars Program or simply due to individual exploration on the part of a motivated student. These programs provide an intense research experience to students interested in the biomedical and biological sciences. Some, not all, of the summer students are picture on these pages.  

See also Page 8.

**STUDENTS TO WATCH…they are on the move**

**Roger Rengert** V’17 worked in Dr. Makoto Senoo’s laboratory to examine the role of p63-interacting proteins in the control of the proliferative potential of keratinocytes. *(NIH Merial)*

**Megan Clark** V’18 just graduated from University of Delaware and starts Penn Vet this year. She worked this summer in Dr. Phillip Scott’s laboratory studying the effects of JAK3 inhibition on CD8 cytotoxicity in L. braziliensis infection.

**Derek Standlee**, Penn undergraduate, worked on identification and characterization of viral features and the host molecular mechanisms that determine the onset and quality of the antiviral immune response. *(López laboratory)*. *UPenn LSM summer internship program.*

**Beatriz Blanco** V’17 worked with Dr. Nicola Mason on re-directed T-cell therapy in dogs with B-cell lymphoma. *(NIH Merial)*

**Edward B. Irvine**, an undergraduate from Stanford University, worked in Dr. Carolina López’ laboratory generating mutant Sendai virus defective viral genome strains, and measured their relative antiviral cytokine production in vitro. *(SUIP Program)*

**Roger Rengert** V’17 worked in Dr. Makoto Senoo’s laboratory to examine the role of p63-interacting proteins in the control of the proliferative potential of keratinocytes. *(NIH Merial)*

**Amanda Watkins** V’16, worked with Dr. Julie Engiles, New Bolton Center, on a multimodal approach to the characterization of induced and natural laminitis cases. *(NIH Merial Program)*
Summer research at Penn Vet

Penn Vet faculty and faculty campus wide opened their basic research laboratories and/or their clinical research studies to engage students with summer projects. Those faculty with summer students were: Drs. Jaimo Ahn, Paul Axelsen, Kendra Bence, Seema Bhatnagar, Gudrun Debes, Julie Engiles, Hannah Galantino-Homer, Robert Greenberg, Ron Harty, Kurt Hankenson, Erika Krick, Sparky Lok, Carolina López, Nicola Mason, Cynthia Otto, Thomas Parsons, Ellen Puré, Shelley Rankin, Phillip Scott, Makoto Senoo, M Celeste Simon, Louis Soslowsky, Charles Vite and Susan W Volk. The mentors were truly welcoming and willing to teach students about their passion for research. Mentors are selected from the entire Penn biomedical community, in the city as well as from the large animal campus at New Bolton Center. It is through this collaboration between mentors and students that a trainee may be inspired to choose a career in research--basic, clinical or translational.

Raisa Glabman V’17 worked in Dr. Gudrun Debes’ laboratory investigating the role of CCR7 resident memory T cells in the skin. (NIH Merial)

Working in Dr. Kendra Bence’s laboratory, Alexandra Crooks V’16 examined differential gene expression in the amygdala of WT vs. PTP1B-deficient mice and found a possible molecular link between decreased anxiety in PTP1B-/- mice and elevated BDNF gene expression. (NIH Merial)

Arundhati Johri, a Millburn, New Jersey high school student worked with Dr. Nicola Mason on the isolation and expansion of CD40 activated B-cells to prime tumor specific T-cells.

Emily Shea V’17 is worked with Dr. Charles Vite on research comparing the neuropathology of Niemann-Pick type C disease and aging in cats. (NIH Merial)

continued on page 8

Wenzhi Song, an undergraduate student from Bryn Mawr College worked with Dr. Carolina López to investigate defective viral genome (DVG) polarity in correlation to the type I interferon response.

Michael Levenson V’15 is the 2014 recipient of the Veterinary Student Scholars Program at Morris Animal Foundation for his studies on canine osteosarcoma (OSA) with Dr. Nicola Mason. He is exploring the hypothesis that the incidence of canine osteosarcoma is much greater than previous estimates, and that delaying standard of care treatment does not influence overall survival - together his results aim to encourage earlier diagnosis of osteosarcoma and the use of neo-adjuvant therapy to prevent metastatic disease.

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**RECENT AWARDS at PENNVET**  
(Direct costs)

<table>
<thead>
<tr>
<th>Name</th>
<th>Funding Agency</th>
<th>Project Title</th>
<th>Grant ID/Details</th>
<th>Amount (USD)</th>
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<td>P. Jeremy Wang</td>
<td>NIH/NIGMS R01-GM089893</td>
<td>Regulation of meiotic recombination in mice</td>
<td>R01-GM089893</td>
<td>$780,000</td>
<td>8/1/2014-4/30/2018</td>
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<td>Narayan Avadhani</td>
<td>NIH/ROI AR067066-01</td>
<td>Ahr and osteoporosis</td>
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<td>$1,000,000</td>
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<td>Qi Fu (Wang Lab)</td>
<td>NIH R01-GM111384</td>
<td>The function of MOV101 in piRNA biogenesis</td>
<td>R01-GM111384 (Supplement)</td>
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<td>7/1/2014-6/30/2017</td>
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<td>Michaela Kristula</td>
<td>Merck Animal Health</td>
<td>Pilot assessment of four hydrogel teat sealant formulations in dairy cattle</td>
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<td>$20,381</td>
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<td>Thomas Parsons</td>
<td>ASPCA</td>
<td>Research fellowship and welfare training at Penn Vet Swine</td>
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<td>Thomas Parsons</td>
<td>PA Soybean Promotion Board</td>
<td>Anticipating the next welfare challenge: Optimizing controlled disease in loose housed sows</td>
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<td>Nicola Mason</td>
<td>At Penn Vet Swine</td>
<td>Evaluation of AT-014 in the treatment of canine osteosarcoma</td>
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<td>Host-Oriented Therapeutics Targeting Filovirus Budding</td>
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<td>Carolina Lopez</td>
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<td>A Novel Virus-Derived Adjuvant</td>
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<td>$1,000,000</td>
<td>7/1/14-6/30/18</td>
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<td>Phil Scott</td>
<td>NIH R01 AI066842</td>
<td>Protective and Pathologic Roles for CD8+ T cells in Leishmaniasis</td>
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<td>$1,000,000</td>
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<td>Brett Kaufman</td>
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<td>Molecular mechanisms of mitochondrial DNA deletion formation</td>
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<td>$1,740,000</td>
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<td>Michael Atchison</td>
<td>NIH/General Med</td>
<td>The role of YY1 in constitutive and inducible DNA loop formation</td>
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<td>9/1/14-8/31/18</td>
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</table>

**CNN Heroes**—Nicola Mason, BVetMed, PhD, assistant professor in the Departments of Clinical Studies PHL and Pathobiology, has been nominated as a CNN Hero. CNN looks for people who are changing the world—people who make a difference. If selected, voting for her will come next. Stay tuned; her story has been told.

**Changes on the horizon....**

Implementation of the NIH Genomic Data Sharing Policy for NIH Grant Applications and Awards (NOT-OD-14-111) Office of the Director, NIH

NIH Genomic Data Sharing Policy (NOT-OD-14-124) Office of the Director, NIH

Public Comments on Proposed Guidance Regarding Significant Changes to Ongoing Animal Activities (NOT-OD-14-125) Office of the Director, NIH

Guidance on Significant Changes to Animal Activities (NOT-OD-14-126) Office of the Director, NIH

**Publications**

- Intraoperative near-infrared imaging can distinguish cancer from normal tissue but not inflammation.  

- The diabetes susceptibility gene Clec16a regulates mitophagy.  
  S. Soleimanzour SP, Gupta A, Bakay M, Ferrari AM, Groff DN, Fadista J, Spruce LA, Kushner


- CXCR4 is dispensable for T cell egress from chronically inflamed skin via the afferent lymph. Geherin SA, Wilson RP, Jennrich S, Debes


- Ovine skin-recirculating gT cells express IFNγ and IL-17 and exit tissue independently of CCR7. Geherin SA, Lee MH, Wilson RP, Debes

First, Dr. Harty, in collaboration with medicinal chemists Drs. Jay Wrobel and Allen Reitz at the Fox Chase Chemical Diversity Center, will pursue a detailed structure-activity relationship (SAR) plan to further optimize and develop the current PTAP and PPxY lead compounds into more potent and less toxic antivirals. For example, this strategy will include evaluation of early ADME/PK properties and potency assessment of future compound analogs. Second, testing of future analogs against live BSL-4 viruses in collaboration with Dr. Dye at USAMRIID will be critical for the advancement of these compounds, and for eventually testing the protective efficacy of these compounds using small animal models of infection. Third, since these compounds are targeting, in part, a host protein(s), Dr. Harty will evaluate the effects of these compounds on the normal functions of their respective endogenous host target proteins, in addition to assessing the effects on cell viability and permeability. Lastly, with the expertise of Drs. Bruce Freedman and Gordon Ruthel, the Director and Manager, respectively, of the Veterinary School Core Imaging facility, Dr. Harty will use Fluorescence Lifetime Imaging Microscopy (FLIM) to monitor the dynamics of virus-host interactions in live mammalian cells, which will enable classification of lead inhibitors based upon their mode of action and provide a basis for developing multidrug therapies.

Dr. Harty’s research is funded by the NIH/NIAID (R21/R33 AI102104 and R21 AI103785). His laboratory and office are located in Room 412 of the Rosenthal building.

References


More summer student research experiences......

**Bryn Mawr’s STEM Posse--a Summer Student at Penn Vet--**

**Fransheska Clara**, a student from Bryn Mawr’s all-women’s STEM Posse program--students in science, technology, engineering and math--found a summer project in the laboratory of Dr. Robert Greenberg. She worked on identification of ion channel drug targets in schistosomes, parasitic worms that cause schistosomiasis, a disease affecting over 200 million people.

**Ashley Klein** V’16 was appointed as a pathology intern at Johnson & Johnson (J&J) this summer as a result of networking at the Merial Symposium last year! Ashley was their first intern and they hope to continue the program as an opportunity for more Penn Vet students. During her internship at J&J she learned to trim and embed tissues; prepare histology slides; began writing a manuscript for submission to a journal; and networked with people throughout the company. She had the opportunity to read histology slides to obtain final results in an important study in progress. Ashley will present her work at the November American College of Veterinary Pathologists (ACVP) meeting and at our **March 2015 Student Research Day**.

**Clint Kuban** - V’16 - and **EmmaRose Joffe** V’17 worked with Dr. Cindy Otto at Penn Vet’s Working Dog Center. Clint’s project was entitled: Quantification of Salivary Chemical Differences Associated With Type 1 Diabetic Low Blood Sugar Identified By Diabetic Alert Dogs. EmmaRose’s project was on canine olfactory detection of ovarian carcinoma in human plasma.

The Penn Vet Research Newsletter is distributed quarterly

Suggestions, requests, comments and story ideas may be directed to:
resnews@vet.upenn.edu

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Lobby for Science.....

In conjunction with Penn Science Policy Group, the **Biomedical Postdoctoral Council** is creating a network of Pennsylvania-based biomedical experts who may act as a liaison to our PA Congress members (Senators Casey, Toomey, & Representative Fattah). The Penn Science Policy Group would like to recruit members of the biomedical postdoctoral community to be available as scientific experts for our PA Congress members and their staff to contact in the future regarding scientific questions and concerns. Questions? Contact: Shaun O’Brien at 510-295-7619 or send an email: obriens@mail.med.upenn.edu