# *Plant-Based Therapeutics Symposium*

July 15 & 16, 2009
Louisville & Owensboro
Kentucky, USA

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# Welcome Message

***Welcome!***

The Sullivan University College of Pharmacy was established on October 10, 2006. Since then, we have admitted the Inaugural Class of 2011 and the second Class of 2012; both are studying furiously in our classrooms and on campus.

Since its opening on October 1, 2008, the Sullivan University College of Pharmacy was very pleased to host the First Annual Nanotechnology Symposium held on October 3 and 4, 2008. This year, we have had the pleasure to collaborate with Kentucky BioProcessing, LLC, to host the first Plant-Based Therapeutics Conference. We do hope that this conference will be a successful starting point to become a future event to be held yearly in the United States and to be a complement to the one held in Europe.

Dear Colleagues and Friends, thank you very much for coming to our beautiful city of Louisville and for taking the time to share your knowledge and accomplishments with us and other colleagues around the country. Thank you for choosing the Sullivan University College of Pharmacy to be the site for this historical event.

With Warm Regards,

Hieu T. Tran, Pharm.D.
Founding Dean and Professor
College of Pharmacy
Sullivan University
2100 Gardiner Lane
Louisville, KY 40205
502-413-8640
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# About
# Plant-Based Therapeutics

Many of today's new pharmaceutical products are actually proteins produced through a living organism. Some of these organisms include mammalian cells, yeast, and bacteria. The production technologies around each of these systems require development of expensive and complex bioreactors as a product transitions from phase II to phase III studies. Plant-made pharmaceuticals, or PMPs, are proteins that are expressed and purified from plants where the plant itself serves as the bioreactor. Utilizing a plant-based transient or transgenic gene expression system significantly reduces the time, risk, and huge capital expense of a typical bioreactor. In addition to this potential cost savings, PMPs offer a rapidly scalable platform that eliminates many of the concerns surrounding utilization of animal-based components.

# Many Thanks to our SPONSORS

# Symposium Chair

## Dr. Jeanne Novak

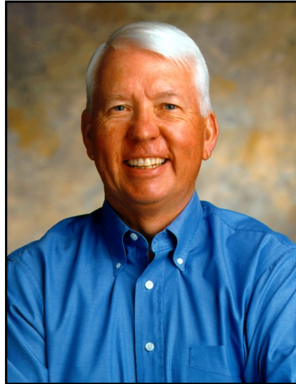
**President and CEO,**
**CBR International**

Dr. Jeanne Novak is a recognized authority in the biotechnology and pharmaceutical arenas with nearly 20 years of experience in regulatory affairs, clinical program design and strategy, quality program development and CMC process development. In addition to these areas of expertise, Dr. Novak has extensive experience providing US and international regulatory support for a variety of vaccine and pharmaceutical development programs, including plant-based systems, and participated in the development of the FDA's guidance document for drugs, biologics, and medical devices derived from bioengineered plants.

Dr. Novak received her Ph.D. in Experimental Pathology (Cell Biology) in the Department of Pathology from the University of Utah. She served as a Staff Research Scientist at USAMRIID in Frederick, Maryland developing novel vaccines for infectious diseases. In 1993, Dr. Novak accepted a regulatory scientist position at CDRH then CBER at FDA. As a FDA Senior Reviewer in DVRPA, CBER she served as the primary reviewer on numerous vaccine and therapeutic product INDs and multiple license applications. Dr. Novak was the primary reviewer on the first IND received by FDA for a plant-based vaccine. Also while at CBER, Dr. Novak was a credentialed PAI/GMP FDA Investigator and served as an assistant to the Center Director regarding biologics policy.

Dr. Novak and the twenty-plus members of the CBR International Corp.® team provide global, full-service product, clinical, and regulatory compliance support. The team is responsible for designing and overseeing successful product development programs and pivotal clinical trials that have resulted in numerous approved vaccine, biotech and pharmaceutical products in indications such as endocrine, oncology, infectious disease and others.

# Keynote Speaker



## Dr. Charles Arntzen

### Regents' Professor and Florence Ely Nelson Presidential Chair School of Life Sciences, and The Biodesign Institute Arizona State University

Dr. Arntzen was appointed to the Florence Ely Nelson Presidential Endowed Chair at Arizona State University (ASU) in Tempe in 2000. He served as the Founding Director of the Biodesign Institute (previously identified as the Arizona Biomedical Institute) from 2001-2003, and served as the Co-Director of the Center for Infectious Diseases and Vaccinology with Professor Roy Curtiss until 2007. Prior to joining ASU, Dr. Arntzen served as President and CEO of Boyce Thompson Institute -- a not-for-profit corporation affiliated with Cornell University. Earlier administrative experience included service as Director of Research at the Dupont Company. He was also Deputy Chancellor for Agriculture; Dean, College of Agriculture and Life Sciences; and Director of the Texas Agricultural Experiment Station in the Texas A&M University System.

Dr. Arntzen was elected to the U.S. National Academy of Sciences in 1983 and to the National Academy of Sciences in India the following year. He is a fellow of The American Association for the Advancement of Science and received the Award for Superior Service from the U.S. Department of Agriculture for international project leadership in India. He was a member of the Executive Committee of the Board of Governors of the University of Chicago for Argonne National Laboratory and served as chairperson of their Science and Technology Advisory Committee. He served as chairman of the National Biotechnology Policy Board of the National Institutes of Health, as chairman of the National Research Council's Committee on Biobased Industrial Products, and on the National Research Council's Committee on Space Biology and Medicine. He served for eight years on the Editorial Board of SCIENCE. He currently serves as a Distinguished Advisor on the Council for Biotechnology, and on the Board of Directors of the National Center for Genome Resources. In 2001 he was appointed as a member of President George W. Bush's Council of Advisors on Science & Technology, and in 2004 received a Presidential appointment to serve on the National Nanotechnology Oversight Board; both of these appointments continued until 2009.

Dr. Arntzen's private sector service includes past membership on the Board of Directors of DeKalb Genetics (prior to sale to Monsanto), and Board of Directors and Board of Scientific Advisors for Axis Genetics in Cambridge, UK. He currently serves on the Board of Directors of VAXX, Inc, Advanced BioNutrition, Inc. and Performance Plants, Inc. He serves on the Advisory Board of the Burrill and Company's Agbio Capital Funds and The Nutraceuticals Fund.

Dr. Arntzen has received honorary doctorate degrees from Purdue University, the University of Minnesota, and the Hebrew University in Jerusalem.

# Keynote Speaker

## Dr. Yuri Gleba

### Managing Director, Icon Genetics

Dr. Gleba has graduated from Kiev University in 1971 (M. Sc., Biology, Genetics); he received his Ph. D. from the Institute of Botany, Academy of Sciences of Ukraine; and he received a D.Sc. from Leningrad University in 1980).

Dr. Gleba's pioneering research in plant cell biology, genetics, physiology and biotechnology were published in more than 200 research papers, 3 books and over 30 patent families, and has earned the respect of the international scientific community as is evidenced by his election to the World Academy of Arts and Science (Rome), the European Academy (*Academia Europaea*, London), the National German Academy *Leopoldina* (Halle), the National Academy of Sciences of Ukraine (Kiev), the Lithuanian Academy of Science (Vilnius) and the Bavarian Academy of Sciences (Munich). In recognition of his outstanding scientific contributions, he also received numerous international and national awards and prizes, including Koerber Prize (Hamburg), A. von Humboldt Prize (Bonn), USSR State Prize (Moscow), State Prize of Ukraine (Kiev), etc.

He founded the Institute of Cell Biology and Genetic Engineering, National Academy of Sciences of Ukraine, Kiev, Ukraine, in 1989, and is still serving as its Director. Dr. Gleba joined American Cyanamid Company, Princeton, NJ in 1992, where he developed research efforts in plant biotechnology, genomics and crop engineering, first as a group leader, and later as a Director of the Crop Engineering Department. In 1999, Dr. Gleba has founded Icon Genetics, Princeton/Munich/Halle, a plant biotechnology company; he has been serving since its inception as Icon's CEO. After Icon Genetics has been acquired by Bayer AG in 2006, Dr. Gleba continued to manage the company. In 2008, Dr. Gleba founded Nomad Bioscience GmbH, Munich, and is currently managing both companies.

# Schedule of Events

Wednesday, July 15

| Registration and Continental Breakfast | 9:00 a.m. — 9:30 a.m. |
|---|---|

| Welcome | 9:30 a.m. — 9:45 a.m. |
|---|---|

- **Yashwant Pathak, Ph.D.**
  Symposium Coordinator
  Assistant Dean of Academic Affairs and Chair,
  Department of Pharmaceutical Sciences
  Sullivan University College of Pharmacy
  Louisville, KY
- **Hieu T. Tran, Pharm.D.**
  Founding Dean and Professor
  Sullivan University College of Pharmacy
  Louisville, KY
- **Glenn Sullivan**
  President
  Sullivan University System
  Louisville, KY

| Symposium Inauguration | 9:45 a.m. — 10:45 a.m. |
|---|---|

- **Hugh Haydon**
  Chairman
  Kentucky BioProcessing, LLC
  Owensboro, KY

"Regulatory State of Affairs for Plant-Based Pharmaceuticals"

- **Jeanne Novak, Ph.D.**
  President and CEO
  CBR International
  Boulder, CO

"Plant-Based Therapeutics: Technical Advancements and Business Opportunities"

- **Charles Arntzen, Ph.D.**
  Regents' Professor and Florence Ely Nelson Presidential Chair
  School of Life Sciences, and The Biodesign Institute
  Arizona State University
  Mesa, AZ

| Break | 10:45 a.m. — 10:55 a.m. |
|---|---|

** Note that presenters for each abstract are underlined

# Schedule of Events

## Wednesday, July 15

**Plant-Based Antivirals — 10:55 a.m. — 1:00 p.m.**

- *Scalable Manufacture of HIV-1 Entry Inhibitor Griffithsin and Validation of its Safety and Efficacy as a Topical Microbicide Component*

  Kenneth E. Palmer[1,2,3], Barry R. O'Keefe[4], Fakhrieh Vojdani[3], Viviana Buffa[5], Robin J. Shattock[5], David C. Montefiori[6], Steven D. Hume[7], and Barry Bratcher[7]

  1. Department of Pharmacology and Toxicology and James Graham Brown Cancer Center, University of Louisville School of Medicine, Louisville, KY
  2. Owensboro Cancer Research Program, Owensboro, KY
  3. Intrucept Biomedicine LLC, Owensboro, KY
  4. Molecular Targets Development Program, National Cancer Institute, Frederick, MD
  5. St. George's Hospital Medical School, University of London, London, United Kingdom
  6. Department of Surgery, Duke University Medical School, Durham, NC
  7. Kentucky BioProcessing LLC, Owensboro, KY

- *Plant Expression of Chimeric Gag/gp41 Virus-Like Particles as a Mucosally-Targeted Subunit Vaccine Against HIV-1*

  Sarah Kessans, John Frater, Nobuyuki Matoba, and Tsafrir Mor
  School of Life Sciences and Biodesign Institute, Arizona State University, Tempe, AZ

- *Chloroplast-Derived Oral and Injectable Vaccines*

  Henry Daniell
  University of Central Florida College of Medicine, Orlando, FL

- *Development of a Robust and Rapid Plant Expression System for Actinohivin, A Novel Anti-HIV-1 Protein Targeting the Envelope High-Mannose Cluster*

  Brian Barnett[1], Hillary Conway[1], Adam Husk[1], Michelle Pickel[2], Charles Arntzen[2], Pengfei Zhang[3], Gerald Quinnan[3], Jason Mooney[4], Carl Hanson[4], Atsushi Takahashi[5], Haruo Tanaka[5, 6], and Nobuyuki Matoba[1]

  1. Owensboro Cancer Research Program, James Graham Brown Cancer Center, University of Louisville School of Medicine, Owensboro and Louisville, KY
  2. Center for Infectious Diseases and Vaccinology, Biodesign Institute at Arizona State University, Tempe, AZ
  3. Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, Bethesda, MD
  4. Viral and Rickettsial Diseases Laboratory, California State Department of Public Health, Berkeley, CA
  5. Iwaki Meisei University, Japan
  6. KIIM Pharmaceutical Laboratory, Japan

- *Plant-Produced L2 Vaccines Induce Protective Immune Responses Against Mucosotropic Papillomavirus in the Dog Model and HPV Cross-Neutralizing Antibodies in Immunized Animals*

  Amanda B. Lasnik[1,2,3], Shonna K. Riedell[1,3], Mark L. Smith[4], Nathaniel W. Waldron[1,3], Kyle V. Conway[1,3], Janice M. Walker[1,3], Janice Ditslear[2], Tanya E. Franklin[2], Sherry Willer[1], Shin-je Ghim[1], A. Bennett Jenson[1], and Kenneth E. Palmer[1,2,3]

  1. James Graham Brown Cancer Center, University of Louisville School of Medicine, Louisville, KY
  2. University of Louisville School of Medicine, Louisville, KY
  3. Owensboro Cancer Research Program, Owensboro, KY
  4. Genentech Inc., Vacaville, CA

- *Development of Plant-Based Vaccine Candidate Against Avian Influenza Virus*

  Angela Natilla and Lev G. Nemchinov
  USDA/ARS/PSI, Molecular Plant Pathology Laboratory, Beltsville, MD

# Schedule of Events

## Wednesday, July 15

| Lunch, served in 1st floor Student Lounge | 1:00 p.m.—2:00 p.m. |
|---|---|

| Plant-Based Therapeutics: General Applications | 2:00 p.m.—3:45 p.m. |
|---|---|

- *Genetic Tools for Expression of Single and Multiple Foreign Genes in Transgenic Plants*
  Indu B. Maiti, Sumita Raha, Narayan C. Das, Sitakanta Pattanaik, Somnath Bhattacharyya, and Nrisingha Dey
  Kentucky Tobacco Research & Development Center, Lexington, KY
- *Economics and Applications of Tobacco Biomass for the Production of Plant-Based Pharmaceuticals and Industrial Materials*
  Orlando Chambers, Richard Mundell, James O'Daniel, and Maelor Davies
  Kentucky Tobacco Research & Development Center, Lexington, KY
- *An Immunoadhesin Therapy for Inhalation Anthrax*
  A. Belle[1], L. Schaefer[1], D. Deppe[1], S. Neuss[1], A. Trilling[1], J. Maclean[1], K. Bush[2], J. Pawlik[2], L. Sower[2], J. W. Peterson[2], K. L Wycoff[1], and L.Yu[1]
  1. Planet Biotechnology, Hayward, CA
  2. University of Texas Medical Branch, Galveston, TX
- *Proteins and Biologics Purified Under cGMP from Animals and Transgenic Plants*
  Ronald Bassuner
  SAFC-Pharma-SIGMA ALDRICH, St. Louis, MO
- *Progress in the Development of Plant-Based Vaccine for Mucosal Delivery*
  S. Spitsin, H. Koprowski, and M. Golovkin
  Biotechnology Foundation Laboratories at Thomas Jefferson University, Philadelphia, PA

| Break and Poster Presentations | 3:45 p.m.— 4:15 p.m. |
|---|---|

- *Challenges in Plant-Based Therapeutics*
  Yashwant V. Pathak, and Hieu T. Tran
  Sullivan University College of Pharmacy, Louisville, KY
- *Plant Produced Antibodies as Therapeutics*
  Carolyn F. Hughes, Hieu T. Tran and Yashwant V. Pathak
  Sullivan University College of Pharmacy, Louisville, KY
- *Anacardic Acid Induces Apoptosis and Inhibits DNA Synthesis and Invasion in Breast Cancer Cell Lines Independent of Estrogen Receptor Status*
  Nalinie S Wickramasinghe[1], Leslie Schier[2], Jeremy S. Harbour[2], Joan E. Magnusen[3], Apsara K. Wickramasinghe[2], Abirami Krishnasamy[2], Susan M. Dougherty[1], Piyumika S. Suriyampola[2], David J. Schultz[2,4], and Carolyn M. Klinge[1,4]
  1. University of Louisville School of Medicine, Louisville, KY
  2. Department of Biology, University of Louisville, Louisville, KY
  3. Keuka College, Keuka Park, NY
  4. Center for Genetics and Molecular Medicine, University of Louisville, Louisville, KY

# Schedule of Events

## Wednesday, July 15

### Plant-Based Antibodies and Anti-Cancer Agents 4:15 p.m.—5:30 p.m.

- *Isolation and Analysis of the Cancer-Preventive Peptide Lunasin*
Brian W. Barnett[2], Lauren E. Seber[2], and Keith R. Davis[1,2]
1. James Graham Brown Cancer Center and University of Louisville, Louisville, KY
2. Owensboro Cancer Research Program, Owensboro, KY

- *Preventing Sexual Transmission: Mapp66, An Antibody-Based Vaginal Microbicide*
Natasha Bohorova, Larry Zeitlin, Andrew Hiatt, Michael Pauly, Do Kim, Andy Ho, Jesus Velasco, and Kevin Whaley
Mapp Biopharmaceutical Inc., San Diego, CA

- *Anti-Ebola Virus Human Monoclonals Produced in Nicotiana Benthamiana: Development as an Immunoprotectant for Human Use*
Larry Zeitlin[1], Natasha Bohorova[1], Andrew Hiatt[1], Andy Ho[1], Kati Hnath[2], Gene Olinger[2], Do Kim[1], Michael Pauly[1], Jesus Velasco[1], Herta Steinkellner[3], and Kevin Whaley[1]
1. Mapp Biopharmaceutical Inc., San Diego, CA
2. USAMRIID, Frederick, MD
3. Center of Applied Genetics, BOKU Vienna, Austria

### Dinner On Your Own

Please see suggested restaurants on pages 40-41

# Schedule of Events

## Thursday, July 16

| | |
|---|---|
| Meet at Sullivan University COP for Breakfast | 7:00 a.m.– 7:30 a.m. EST |
| Load Buses and Depart for Owensboro | 7:30 a.m.– 7:45 a.m. EST |

***Owensboro is located in Central Time Zone. Please take note of the time zones for each event. ***

| | |
|---|---|
| Welcome to Owensboro & Keynote Presentations | 9:15 a.m.—10:45 a.m. CDT |

- **Danny Ebelhar**
  Owensboro Biotechnology Alliance
  Owensboro, KY
- **Nick Brake**
  Greater Owensboro Economic Development Corporation
  Owensboro, KY
- **Alex Day, Moderator**
  Director of Business Development
  Kentucky BioProcessing, LLC
  Owensboro, KY

"Transgenic Systems for Expression of Foreign Proteins in Plants"

- **Ernie Hiatt, Ph.D.**
  Principal Scientist
  Kentucky BioProcessing, LLC
  Owensboro, KY

"Plant-Based Therapeutics: Contributing to Human Health"

- **Yuri Gleba, Ph.D.**
  Managing Director, Icon Genetics
  Founder Nomad Biosciences
  Berlin, Germany

| | |
|---|---|
| Coffee Break | 10:45 a.m.—11:00 a.m. CDT |
| Tours of Kentucky BioProcessing, LLC | 11:00 a.m.—11:45 a.m. CDT |
| Lunch (Question & Answer Session) | 11:45 a.m.—12:45p.m. CDT |
| Workshop | 12:45 p.m.—2:15 p.m. CDT |

"Quality Systems Development for Plant Made Pharmaceuticals"

- **Jeanne Novak, Ph.D. and Kathy Hanley, Ph.D.**
  CBR International
  Boulder, CO

| | |
|---|---|
| Travel to Louisville, KY | 2:15 p.m.—5:00 p.m. CDT |
| Arrive in Louisville | 6:00 p.m. EST |

# Collection of Abstracts

# Regulatory State of Affairs for Plant-Based Pharmaceuticals

**CBR International Corp®, Boulder, CO**
**Presented by Jeanne M. Novak Ph.D.**

Successful development and manufacture of recombinant drug products is highly dependent on the use of appropriate gene expression systems. An appropriate production system is one that can produce a drug as expected and at as low a cost as possible. Plant-based expression systems have been developed as alternatives to more traditional animal and microbial cell-based systems. Although still considered novel by many accounts, the use of the plant's molecular scaffolding as a production vehicle needs to be positioned as a viable option for drug manufacture.

This presentation will provide an overview of the past and present state of affairs for plant-made pharmaceuticals (PMP). This will include a discussion on the pioneers and innovators that laid the ground work for development and use of plant-based production systems.

This overview will also provide an insider's perspective on the regulatory process for the approval of PMP. In spite of the groundbreaking work, commercial and academic institutions have faced a number of unique regulatory challenges associated with plant-based production of pharmaceuticals. As a result, there have been no PMP approved by the FDA or internationally for human use. During this discussion, the main regulatory obstacles for plant-based pharmaceuticals will be presented along with what to expect from the regulators and the regulatory environment moving forward.

# Plant-Based Antivirals

# Scaleable Manufacture of HIV-1 Entry Inhibitor Griffithsin and Validation of its Safety and Efficacy as a Topical Microbicide Component

**Kenneth E. Palmer[1,2,3], Barry R. O'Keefe[4], Fakhrieh Vojdani[3], Viviana Buffa[5], Robin J. Shattock[5], David C. Montefiori[6], Steven D. Hume[7], and Barry Bratcher[7]**

1) Department of Pharmacology and Toxicology and James Graham Brown Cancer Center, University of Louisville School of Medicine, Louisville, KY
2) Owensboro Cancer Research Program, Owensboro, KY
3) Intrucept Biomedicine LLC, Owensboro, KY
4) Molecular Targets Development Program, National Cancer Institute, Frederick, MD
5) St. George's Hospital Medical School, University of London, London, United Kingdom
6) Department of Surgery, Duke University Medical School, Durham, NC
7) Kentucky Bioprocessing LLC, Owensboro, KY

To prevent sexually transmitted HIV, the most desirable active ingredients of microbicides are antiretrovirals (ARVs) that directly target viral entry and avert infection at mucosal surfaces. However, most promising ARV entry inhibitors are biologicals, which are costly to manufacture and deliver to resource-poor areas where effective microbicides are urgently needed. Here, we report a manufacturing breakthrough for Griffithsin (GRFT), one of the most potent HIV entry inhibitors. This red algal protein was produced in multi-gram quantities after extraction from *Nicotiana benthamiana* plants transduced with a tobacco mosaic virus vector expressing GRFT. Plant-produced GRFT (GRFT-P) was shown as active against HIV at picomolar concentrations; directly virucidal via binding to HIV envelope glycoproteins and capable of blocking cell-to-cell HIV transmission. GRFT-P has broad-spectrum activity against HIV Clades A, B, and C with utility as a microbicide component for HIV prevention in established epidemics in subSaharan Africa, South Asia, China, and the industrialized west. Cognizant of the imperative that microbicides not induce epithelial damage or inflammatory responses, we also show that GRFT-P is non-irritating and non-inflammatory in human cervical explants and *in vivo* in the rabbit vaginal irritation model. Moreover, GRFT-P is potently active in preventing infection of cervical explants by HIV-1 and has no mitogenic activity on cultured human lymphocytes.

# Plant Expression of Chimeric Gag/gp41 Virus-Like Particles as a Mucosally-Targeted Subunit Vaccine Against HIV-1

**Sarah Kessans, John Frater, Nobuyuki Matoba, and Tsafrir Mor**
School of Life Sciences and Biodesign Institute, Arizona State University, Tempe, AZ

The transmembrane HIV-1 envelope protein gp41 has been shown to play critical roles in the viral mucosal transmission and infection of CD4+ cells. Gag is a structural protein configuring the enveloped virus particles, and has been suggested to constitute a target of the cellular immunity potentially controlling the viral load. The goal of this project is to express HIV enveloped virus-like particles (eVLPs) consisting of Gag and the deconstructed, critical membrane proximal domain of gp41 (dgp41) in plants towards an inexpensive subunit vaccine inducing a broad anti-HIV immune response. Using a PCR-based de novo gene synthesis method, a plant optimized HIV-1 *gag* gene was constructed based on a primary subtype C R5 HIV-1 isolate. Gag protein was expressed in *Nicotiana benthamiana* using a modified tobacco mosaic virus-based transient expression system. Western blotting using anti-p24 Abs demonstrated the expression of the 55 kDa Gag protein in the leaf tissue. Sucrose gradient sedimentation showed that the full-length Gag protein migrated at a density corresponding to that reported for Gag VLPs. Furthermore, examination of leaf material and the extract in transmission electron microscopy (TEM) showed potential budding as well as the formation of VLPs with a diameter of approximately 100 nm. These results suggest that plant cells can support the formation of HIV-1 Gag VLPs. Stable lines harboring the gag gene were created using a binary vector system, and also expressed the Gag protein. The dgp41 gene was then transiently expressed in these stable lines, and expression was confirmed with Western blotting using anti-2F5 Abs. Preliminary evidence based on sucrose gradient sedimentation suggests that the two proteins may assemble into eVLPs.

# Chloroplast-Derived Oral and Injectable Vaccines

**Henry Daniell**
Department of Molecular Biology and Microbiology, College of Medicine,
University of Central Florida, Orlando, FL

Plant derived injectable vaccines eliminate expenses associated with fermenters. However, oral vaccines eliminate prohibitively expensive purification, cold storage/transportation and sterile delivery. Green vaccines expressed in chloroplasts have advantages of hyper-expression of therapeutic proteins (10,000 copies of transgene per cell), efficient oral delivery and transgene containment via maternal inheritance. To date, 23 vaccine antigens against 16 different bacterial, viral or protozoan pathogens have been expressed in chloroplasts. Mice subcutaneously immunized with the chloroplast derived injectable anthrax protective antigen conferred 100% protection against lethal doses of the anthrax toxin. Oral immunization of F1-V plague antigens without adjuvant conferred greater protection (88%) against 50-fold lethal dose of aerosolized plague (*Yersinia pestis*) than subcutaneous (SQV) immunization (33%). Oral immunization of malarial vaccine antigens fused to the cholera antigen (CTB-AMA1/CTB-Msp1) conferred prolonged immunity (50% life span), 100% protection against cholera toxin challenge and inhibited proliferation of the malarial parasite. Protection was correlated with antigen-specific titers of intestinal, serum IgA & IgG1 in ORV and only IgG1 in SQV mice, but no other immunoglobulin. Dehydrated lettuce leaves showed minimal degradation of vaccine antigens when stored at room temperature for several months. High level expression in lettuce chloroplasts ideal for oral delivery and long-term immunity observed should facilitate development of low cost green vaccines for large populations, at times of out break of infectious diseases.

# Development of a Robust and Rapid Plant Expression System for Actinohivin, A Novel Anti-HIV-1 Protein Targeting the Envelope High-Mannose Cluster

**Brian Barnett[1], Hillary Conway[1], Adam Husk[1], Michelle Pickel[2], Charles Arntzen[2], Pengfei Zhang[3], Gerald Quinnan[3], Jason Mooney[4], Carl Hanson[4], Atsushi Takahashi[5], Haruo Tanaka[5,6], and Nobuyuki Matoba[1]**

1) Owensboro Cancer Research Program, James Graham Brown Cancer Center & Department of Pharmacology and Toxicology, University of Louisville School of Medicine, Owensboro and Louisville, KY
2) Center for Infectious Diseases and Vaccinology, Biodesign Institute at Arizona State University, Tempe, AZ
3) Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, Bethesda, MD
4) Viral and Rickettsial Diseases Laboratory, California State Department of Public Health, Berkeley, CA
5) Faculty of Pharmacy, Iwaki Meisei University, Japan
6) KIIM Pharmaceutical Laboratory, Japan

Actinohivin (AH) is a novel actinomycete-derived lectin that exhibits anti-human immunodeficiency virus type-1 (HIV-1) activity. This small 12.5 kDa protein has high affinity for a cluster of high-mannose glycans on HIV-1 envelope (Env) gp120, but not to other sugar units present on endogenous host glycoproteins. To discern the characteristics of AH's anti-HIV-1 effect, we performed a human cell line-based Env pseudotyped single-round infectivity assay. AH neutralized all four tested R5-type B clade viruses with half maximal inhibitory concentrations ($IC_{50}$s) in the nanomolar range. The antiviral activity appeared to correlate with the number of N-linked glycans in the Env region between V1 and pre-V2. Moreover, AH was shown to potently and dose-dependently prevent the infection of human peripheral blood mononuclear cells by a primary R5-type HIV-1 isolate from B and C clades. These results suggest that AH has a broad antiviral activity against different R5-type HIV-1 subtypes. Given these promising properties of AH as a prototype HIV-1 microbicide, we attempted to establish a robust and rapid recombinant (r)AH expression system that can facilitate AH's molecular characterization, activity enhancement, and large-scale production. We utilized the magnICON tobacco mosaic virus-based vector system to express rAH and a translational rAH-rAH fusion ("rAH dimer") in *Nicotiana benthamiana* plants. Our preliminary analysis revealed that plants can express functional, gp120-binding rAH and the rAH dimer with an expression level of up to 150 mg/kg of leaf materials. Purification and detailed quality analysis of the plant-made rAHs as well as optimization of expression conditions are currently underway.

# Plant-Produced L2 Vaccines Induce Protective Immune Responses Against Mucosotropic Papillomavirus in the Dog Model and HPV Cross-Neutralizing Antibodies in Immunized Animals

**Amanda B. Lasnik[1,2,3], Shonna K. Riedell[1,3], Mark L. Smith[4], Nathaniel W. Waldron[1,3], Kyle V. Conway[1,3], Janice M. Walker[1,3], Janice Ditslear[5], Tanya E. Franklin[6], Sherry Willer[1], Shin-je Ghim[1], A. Bennett Jenson[1], and Kenneth E. Palmer[1,2,3]**

1) James Graham Brown Cancer Center, University of Louisville School of Medicine, Louisville, KY
2) Department of Pharmacology and Toxicology, University of Louisville School of Medicine, Louisville, KY
3) Owensboro Cancer Research Program, Owensboro, KY
4) Genentech Inc., Vacaville, CA
5) Research Resource Facilities, University of Louisville School of Medicine, Louisville, KY
6) Department of Obstetrics, Gynecology and Women's Health, University of Louisville School of Medicine, Louisville, KY

The minor capsid protein (L2) of human papillomavirus (HPV) contains epitopes that can induce antibodies with cross-neutralizing activity. The recently-licensed HPV vaccines are expensive, and hence might not be available for use in developing countries where the burden of cervical cancer is highest. Additionally, the L1-based virus-like particle vaccines do not induce strong cross-protective immune responses. An inexpensive L2-based monovalent vaccine could reduce the global incidence of HPV-linked cancers of mucosal epithelia. The present study first aimed to determine whether a prototype Canine oral papillomavirus (COPV) L2 vaccines could induce protective immune responses in the canine oral papillomavirus (COPV) model. Two overlapping domains, COPV $L2_{61\text{-}170}$ and COPV $L2_{5\text{-}260}$, were fused to the streptavidin (SA) protein, which acted as a carrier and affinity tag for the COPV vaccine. The vaccines were manufactured in *Nicotiana benthamiana* using a tobacco mosaic virus (TMV)-based gene expression system, purified and used as immunogens in beagle dogs. Animals were vaccinated three times, at two week intervals. Study endpoints included serology; analysis of COPV neutralizing titers in a pseudovirus-based neutralization assay; and development of oral papillomas after challenge with a high titer stock of infectious COPV. All vaccinated animals produced antibodies to L2 and the SA carrier protein. The COPV $L2_{5\text{-}260}$ vaccine induced good levels of neutralizing antibodies and protected all 5 vaccinated animals against challenge with COPV, while the COPV $L2_{61\text{-}170}$ vaccine induced protective immunity in 4 out of 7 vaccinated animals, with the remaining 3 partially protected. The degree of protection against challenge in this cohort was correlated with L2-reactive antibody titers. Two L1 VLP-vaccinated animals were protected from challenge, and two mock-vaccinated animals developed large oral warts. These data provide strong proof of concept that a plant-produced L2 vaccine can protect against mucosal papillomavirus challenge in a relevant animal model. The second goal of the study was

(Continuation)

# Plant-Produced L2 Vaccines Induce Protective Immune Responses Against Mucosotropic Papillomavirus in the Dog Model and HPV Cross-Neutralizing Antibodies in Immunized Animals

**Amanda B. Lasnik[1,2,3], Shonna K. Riedell[1,3], Mark L. Smith[4], Nathaniel W. Waldron[1,3], Kyle V. Conway[1,3]; Janice M. Walker[1,3]; Janice Ditslear[5], Tanya E. Franklin[6], Sherry Willer[1], Shin-je Ghim[1], A. Bennett Jenson[1], and Kenneth E. Palmer[1,2,3]**

to determine whether a prototype HPV 16 L2 vaccine could induce antibodies that cross-neutralize different HPV types in vaccinated animals. A peptide encompassing amino acids 11 to 130 of HPV-16 L2 was fused to the streptavidin carrier, and manufactured in *N. benthamiana*. Guinea pigs were vaccinated with the HPV-16L2-streptavidin fusion protein, and HPV-16, -18, -31,-45 and -6 neutralizing titers were measured. The plant-produced HPV-16 L2 vaccine induced high titers of HPV-16 neutralizing antibodies that had broad cross-neutralizing activity against all of the HPV types assayed, unlike the licensed L1-based vaccine, which induced type-specific neutralizing antibodies (HPV-16, HPV-18, HPV-6 and HPV-11) in guinea pigs. We conclude that the plant-produced HPV L2 vaccine is superior to the licensed HPV L1 vaccine in its capacity to induce broadly cross-neutralizing antibody responses, and is a candidate for safety and immunogenicity testing in humans.

# Development of Plant-Based Vaccine Candidate Against Avian Influenza Virus

**Angela Natilla and Lev G. Nemchinov**
USDA/ARS/PSI, Molecular Plant Pathology Laboratory, Beltsville, MD

Expression of heterologous epitopes using plant viruses as carriers have been shown to be one of the most advantageous and useful technologies for the production of therapeutics and vaccines in plants. We have previously reported a plant-derived vaccine platform based on the capsid protein gene (CP) of *Cucumber mosaic virus,* Ixora strain (CMV-Ix), placed under transcriptional control of a *Potato virus X* (PVX)-based vector. PVX-expressed CMV CP formed virus-like particles, which served as carriers for heterologous antigens of *Newcastle disease virus* (Natilla, et al, 2006) and *Avian influenza virus* (AIV) (Nemchinov and Natilla, 2007). As a continuation of our effort to develop a plant-derived vaccine candidate against AIV, we have expressed a 23 amino acid M2e epitope of AIV as an N-terminal fusion to the coat protein of PVX. The fused M2e epitope critically affected formation of virus particles and movement of the virus throughout *Nicotiana benthamiana* plants, causing a significant delay of PVX-like symptoms. Sequence analysis of RT-PCR products confirmed that the epitope coding sequences are replicated with high fidelity during PVX infection for a period of up to two months. Both crude plant extracts and purified chimeric virus particles (CVPs) were immunoreactive with PVX antibodies and antibodies specific to M2e peptide. Modified PVX particles were visualized by electron microscopy. Chicken immunized with CVPs developed an M2e-specific response.

# Plant-Based Therapeutics: General Applications

# Genetic Tools for Expression of Single and Multiple Foreign Genes in Transgenic Plants

**Indu B. Maiti, Sumita Raha, Narayan C. Das, Sitakanta Pattanaik, Somnath Bhattacharyya, and Nrisingha Dey**
Molecular Plant Virology and Plant Genetic Engineering Laboratory
Kentucky Tobacco Research & Development Center, University of Kentucky, Lexington, KY

Prospects of agricultural biotechnology, plant-based manufacturing of recombinant proteins including high-value biopharmaceuticals, antibodies and vaccines have become very clear. There is a rapidly growing interest worldwide in improved new technology and production systems. We have designed and analyzed plant engineering tools and genetic promoters for expression of single and multiple genes in plants. For metabolic engineering, expression of multiple genes in a single cell will be necessary. The use of different promoters with non-homologous sequences may be useful in order to avoid genetic instability due to recombination between identical promoter sequences. Genetic promoters were isolated from six different members of the genus *Caulimovirus* belonging to the *Caulimoviridae* family- a group of plant double stranded DNA virus. These are *Cauliflower mosaic virus* (CaMV), *Figwort mosaic virus* (FMV), *Peanut chlorotic streak virus* (PClSV), *Mirabilis mosaic virus* (MMV), *Cassava vein mosaic virus* (CVMV) and *Strawberry vein banding virus* (SVBV). Strength of MMV FLt promoter was highest in tobacco whereas CaMV 35S promoter showed maximal activity in maize protoplasts compared to other promoters analyzed. The MMV Sgt promoter fragment is a strong constitutive promoter, with strength comparable to that of the MMV FLt promoter. The MMV Sgt promoter and FMV Sgt promoter also demonstrated much greater activity compared to the CaMV 19S promoter and CaMV 35S promoter both in tobacco protoplasts and in transgenic tobacco plants. These promoters are very useful for expressing foreign genes in transgenic plants. There is very limited sequence homology among the caulimovirus-promoters although they are functionally analogous. In addition, using PCLSV genetic elements we have developed chimeric constructs for intron-mediated enhanced expression (IME) of genes, and expression of multiple genes from a single promoter in transgenic plants. Designed constructs have been tested both in transient protoplast expression experiments and in stably transformed transgenic plants. The efficient translation of polycistronic mRNAs has potential value in plant metabolic engineering.

# Economics and Applications of Tobacco Biomass for the Production of Plant-Based Pharmaceuticals and Industrial Materials

**Orlando Chambers, Richard Mundell, James O'Daniel, and Maelor Davies**
Kentucky Tobacco Research & Development Center, University of Kentucky, Lexington, KY

Tobacco has long been considered a potential crop for producing novel materials using biotechnology. High biomass production and ease of genetic transformation suggest that tobacco-based production of plant-made pharmaceutical and industrial products (PMPs) may be economically viable.

Research at the Kentucky Tobacco Research and Development Center focuses on developing technologies to improve the economic viability of PMPs. The program emphasizes applications-oriented research designed to facilitate the development of new crop=based businesses and technologies. Research areas include technology development such as new expression systems and plant genetic engineering technologies, novel purification methods, etc. This presentation will focus on agricultural and economic containment, optimized production methods such as mechanized, multiple harvesting and the development of regulatory systems to meet USDA-mandated regulations. KTRDC has conducted numerous transgenic field tests including 8 USDA approved field trial of transgenic tobacco plants expressing pharmaceutical and industrial products.

An analysis of the cost of producing tobacco biomass in the field and an evaluation of the commercialization of this technology highlights where improvement have been made as PMPs move toward commercial production. Increased density of planting, high fertilizer rates, significant labor for mandatory USDA monitoring and the cost of mechanized harvesting are the areas where production costs are high relative to conventional tobacco production. The development of *Nicotiana* hybrids with improved traits such as better disease resistance, identity preservation, male and female sterility , etc., play an important role in our efforts to assist with commercialization. In addition, the development of containment methods and production procedures that meet USDA regulatory requirements will be discussed. KTRDC's efforts also include the development of methods for the greenhouse production of tobacco biomass where containment costs are significantly lower. Commercial testing of PMPs in an open field setting has declined in the United States in the past few years as USDA regulations have tightened, but improvements in the technology and commercialization of both field and greenhouse production systems are continuing to advance. This presentation will highlight our research efforts and provide examples of the commercial testing of tobacco grown for the production of plant-based pharmaceuticals and industrial materials.

# An Immunoadhesin Therapy for Inhalation Anthrax

**A. Belle[1], L. Schaefer[1], D. Deppe[1], S. Neuss[1], A. Trilling[1], J. Maclean[1], K. Bush[2], J. Pawlik[2], L. Sower[2], J. W. Peterson[2], and K. L Wycoff [1]**

1) Planet Biotechnology Inc., Hayward, CA
2) Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX

Planet Biotechnology is developing PBI-220 as a safe, effective and inexpensive therapeutic countermeasure to *Bacillus anthracis*, a category A threat agent and the causal agent of anthrax. PBI-220, a recombinant, chimeric protein, is an immunoadhesin comprised of a fusion of CMG2, a human receptor for anthrax toxins, and the Fc of human IgG1, for long circulating half-life and immune effector cell interaction. PBI-220 is expressed in stably transformed tobacco and processed to a high level of purity for use as a therapy for anthrax. Preliminary studies with the plant-made PBI-220, using *in vitro* cell based macrophage assays and in *in vivo* rabbit models for inhalation anthrax, indicate that PBI-220 is at least as effective as any anti-PA monoclonal antibody under development, and furthermore, may be the best-in-class biological against anthrax as it neutralizes engineered forms of the anthrax toxin that are not neutralized by a monoclonal antibody.

# Proteins and Biologics Purified Under cGMP from Animals and Transgenic Plants

**Ronald Bassuner**
SAFC Pharma-SIGMA-ALDRICH, St. Louis, MO

SAFC Pharma, a business division of Sigma-Aldrich, St. Louis, Missouri, specializes in custom cGMP bulk manufacturing of biomolecules from natural and transgenic plant source material for diverse pharmaceutical applications. Key considerations to successfully develop a cGMP product for a customer according to regulatory guidelines are discussed in this presentation, that focus on technology in-transfer from the client, purification process development with analytical support, and QC assay development for drug product release and stability testing. Responsibilities of QA and a body of internal quality systems are discussed in view of support and oversight of the manufacturing process, for regulatory document submissions on behalf of the client, and in context of a customer-oriented project management. Examples of process scale-up and projections of process economics will be presented on pharmaceutically relevant proteins extracted and purified from natural and transgenic plant sources.

# Progress in the Development of Plant-Based Vaccine for Mucosal Delivery

**S. Spitsin, H. Koprowski, and M. Golovkin**
Biotechnology Foundation Laboratories at Thomas Jefferson University, Philadelphia, PA

Our interests lay in the integrative area of biomedical research that utilizes plants for production of recombinant vaccine components. The main advantages of plants as an alternative bioreactors are low-cost and a greater potential for scalability comparing to microbial or animal systems. An additional advantage, from the public health point of view, is a higher safety, because plants do not contain mammalian pathogens and can be used as directly in needle-free immunization routes.

Vaccination against smallpox was resumed a few years ago in the US among high risk groups including military personnel, health care workers etc. This was mainly done in response to the threat of bioterrorism. Though very efficacious - the current live vaccine can have serious and potentially lethal complications; not only in the recently immunized individual, but also among people in close contact (e.g. service people, family members etc.).

The development of plant-based smallpox vaccine was initiated first by utilizing single Vaccinia virus antigen B5. Production of plant-derived B5 (pB5) was very efficient (especially when using Magnicon). Injections with pB5 fully protected mice against the lethal challenge with live virus [Golovkin et al., PNAS. 2007]. Later, we found pB5 preparations can be also administered intranasally (needle-free) [Portocarrero et al., Vaccine 2008].

Recently, we have added another Vaccinia virus protein, L1, to the preparation. It is known to also induce strong immune response and protect mice and primates against pox virus infections. Immunogenic properties of plant-derived L1 (pL1) are confirmed to these standards. Various levels of antigen-specific antibodies for pB5 and pL1 were observed after intranasal immunization with several potential mucosal adjuvants. No antigen-specific antibodies were detected when adjuvant was omitted. Application to mucosal surfaces induced both IgG response in sera and IgA in lungs while immunization by injections did not evoke any IgA.

In sum: our results substantiate feasibility of needle-free mucosal administration of recombinant subunit smallpox vaccine in regards to the purity, dosage and adjuvant formulation.

# Poster Presentations

# Challenges in Plant-Based Therapeutics

**Yashwant Pathak and Hieu Tran**
Sullivan University College of Pharmacy, Louisville, KY

The creation of the first monoclonal antibody (mAb) by Kohler and Milstein in 1975 paved the way for the development of a new class of pharmaceutical drugs. Due to the nature of the immune repertoire, it is theoretically possible to design a mAb that can selectively bind to virtually any target. This attribute has made mAbs quite useful in research and diagnostics and highlights their enormous potential in pharmacotherapy. In view of a rapidly expanding market, upscaled production will be needed to meet the demand for therapeutic mAbs. Although several transgenic systems are used to produce mAbs, transgenic plants show the greatest potential for large-scale production. Plant produced antibodies (plant produced mAbs and their derived antibody fragments) have been investigated for the treatment of infectious disease, inflammation, autoimmune disease and cancer and some have progressed to clinical trials. Additionally, advances in plant biotechnology will bring plant produced antibodies as therapeutics even closer to reality. Plants also have shown extensive potential to develop plant based antiviral therapeutic products.

The challenges which the plant based therapeutics are facing and will have to address before it goes for wide spread application and full scale manufacturing can be grouped into:

- Regulatory challenges
- Technical challenges
- Economic challenges
- Public perception of the technology
- Environmental concerns about eh genetically modified plants
- Disposal of the final biomass when manufactured on large scale

The Plant based therapeutics research has been around for more than two decades and very few products are in the market being approved any FDA authorities around the world.

The present paper will discuss various challenges and will try to overcome the challenges.

# Plant Produced Antibodies as Therapeutics

**Carolyn F. Hughes, Hieu T. Tran, and Yashwant V. Pathak**
Sullivan University College of Pharmacy, Louisville, KY

Pharmaceutical recombinant proteins can be produced in a variety of expression systems including bacteria, yeast, insect cells, mammalian cells, the milk of transgenic animals, egg albumin from transgenic chickens and transgenic plants. Monoclonal antibodies are complex glycoproteins that require correct protein post-translational modifications best achieved in eukaryotic cells; most therapeutic monoclonal antibodies are produced in mammalian cells [10]. Due to demand for mAbs and the high cost of mammalian cell systems, production of mAbs in plants is being developed as an alternative.

In the traditional sense, a plant antibody would be an antibody produced by a plant for the protection of that plant. Although plants do have defenses against pathogens, these are not antibody-mediated. Therefore, any antibody produced in a plant is the result of genetic engineering. Transgenic plants can be genetically engineered to produce mammalian antibody to protect that plant from herbicides or pathogens; or to produce mammalian antibody for medical research, diagnostics or therapy, including veterinary applications.

Fab and scFv antibody fragments have been explored as alternatives to mAbs because they can bind antigen but do not require as much post-translational modification as mAbs. It has been noted that small scFvs, in comparison with IgGs, have a higher target binding capacity per equivalent weight thus requiring less acreage of transgenic tobacco and lower costs for growing and purification . In this abstract "plant produced antibodies" we refer inclusively to mAbs, scFvs or other antibody fragments.

This paper will discuss various aspects of plant based antibody therapeutics, examples of plant based therapeutics being researched, unique advantages of plant based production platforms, and future possibilities.

# Anacardic Acid Induces Apoptosis and Inhibits DNA Synthesis and Invasion in Breast Cancer Cell Lines Independent of Estrogen Receptor Status

**Nalinie S. Wickramasinghe[1], Leslie Schier[2], Jeremy S. Harbour[2], Joan E. Magnusen[3], Apsara K. Wickramasinghe[2], Abirami Krishnasamy[2], Susan M. Dougherty[1], Piyumika S. Suriyampola[2], David J. Schultz[2,4], and Carolyn M. Klinge[1,4]**

1) Department of Biochemistry and Molecular Biology, University of Louisville School of Medicine, Louisville, KY
2) Department of Biology, University of Louisville, Louisville, KY
3) Keuka College, Keuka Park, NY
4) Center for Genetics and Molecular Medicine, University of Louisville, Louisville, KY

Epidemiological evidence indicates that the consumption of diets rich in plant materials is associated with reduced cancer risk. Anacardic acid (2-hydroxy -6-alkylbenzoic acid) is formed by a combination of fatty acid and polyketide synthesis pathways in plants and is structurally related to salicylic acid and aspirin that have proven cancer chemoprevention activities. Human dietary exposure to anacardic acid includes the cashew apple and ginkgo (leaves and fruits). Plants within the *Anacardiaceae* family have been used in traditional medicine and show anti-cancer activity in cell culture and animal studies. However, the molecular mechanism(s) by which anacardic acid inhibits tumor cell proliferation have not been elucidated. To investigate the anti-cancer activity of anacardic acid, cell proliferation was assessed by analyzing both metabolic activity (MTT assay) and DNA synthesis (BrdU incorporation). Effects of anacardic acid on cell invasion, cyclooxygenase (COX) activity, and PGE2 synthesis were also determined. We found that anacardic acid 24:1$^{w5}$ (AnAc 24:1$^{w5}$), purified from geranium (*Pelargonium ´hortorum*) trichomes, inhibited MCF-7 (ER positive, endocrine-sensitive), LCC9 (ER positive, endocrine-resistant), and MDA-MB-231 (ER negative, endocrine-resistant) breast cancer cell proliferation in a concentration-dependent manner with $IC_{50}$ value estimates in the mM range. AnAc 24:1$^{w5}$ induced apoptosis in MCF-7 cells and co-treatment with $E_2$ was not sufficient to abolish anacardic acid-mediated apoptosis. AnAc 24:1$^{w5}$ inhibited COX-1 and COX-2 activities *in vitro* with $IC_{50}$ values 34 and 7 µM respectively. AnAc 24:1$^{w5}$ also inhibited TPA-induced-PGE2 synthesis and invasion in MDA-MB-231 breast cancer cells. In conclusion, our studies indicate that AnAc 24:1$^{w5}$ inhibits cell growth and invasion and induces apoptosis in breast cancer cell lines in part by inhibiting COX-2 activity and expression.

*Supported by a University of Louisville Research Initiation Grant to D.J.S., a University of Louisville Research on Women grant to C.M.K., and by NIH R01 DK 53220 to C.M.K*

# Plant-Based Antibodies and Anti-Cancer Agents

# Isolation and Analysis of the Cancer-Preventative Peptide Lunasin

**Brian W. Barnett[2], Lauren E. Seber[2], and Keith R. Davis[1,2]**
1) James Graham Brown Cancer Center and University of Louisville, Louisville, KY
2) Owensboro Cancer Research Program, Owensboro, KY

Lunasin is a small peptide consisting of 43 amino acids with a C-terminal end of nine consecutive aspartic acid residues. Lunasin was originally isolated from soybean but has been found in a variety of plant species at relatively low levels. Initial studies demonstrated that lunasin can prevent the transformation of mammalian cells by chemical carcinogens or viral oncogenes, however, lunasin has little effect on normal or established cancer cell lines. This chemopreventive effect on cells undergoing a transformation event is thought to be mediated by the disruption of mitosis and induction of apoptosis. More recent studies suggest that lunasin binds to deacetylated core histones and exerts its effects via an epigenetic mechanism that disrupts the normal dynamics of histone acetylation-deacetylation. Although the potential cancer-chemopreventive activity has been known for almost a decade, little progress has been made to demonstrate clinical relevance. Attempts to express lunasin in *E. coli*, yeast, and animal cells have been unsuccessful, thus limiting studies to quantities of lunasin that can be synthesized or purified from natural sources. To obtain sufficient lunasin to conduct large-scale animal studies, and ultimately human trials, we are pursuing two complimentary approaches for generating gram-kilogram quantities of lunasin. First, we are utilizing a transient, tobacco-based expression system based on the Tobacco Mosaic Virus vector, Geneware®, for large-scale production of lunasin. We have successfully expressed lunasin in this system as a GFP fusion at levels approaching 100 mg/kg fresh weight tissue. The second approach is to develop a cost effective purification procedure using soybean seed materials that are the byproduct of soybean oil production. We anticipate that one of these approaches will allow production of sufficient quantities of purified lunasin to determine the precise mechanism(s) of lunasin's chemopreventive activity and large-scale animal studies on efficacy. Results will be presented on the expression levels of lunasin in tobacco and the current status of developing a purification procedure to obtain purified lunasin from soy-based materials.

# Preventing Sexual Transmission: Mapp66, An Antibody-Based Vaginal Microbicide

**Natasha Bohorova, Larry Zeitlin, Andrew Hiatt, Michael Pauly, Do Kim, Andy Ho, Jesus Velasco, and Kevin Whaley**
Mapp Biopharmaceutical Inc., San Diego, CA

**Background:** Unsafe sex is one of the highest risk factors for disability and death worldwide. Every year, 340 million people acquire one of the four primary curable sexually transmitted infections, another 4.3 million become infected with HIV, and unknown numbers acquire chronic viral and bacterial infections. In addition, recent research estimates that over 200 million women in developing countries have an unmet need for effective contraceptives. There is an urgent need to develop safe, effective, and accessible *multi-purpose* technologies that will prevent pregnancy, sexually transmitted infections, and other common reproductive tract infections. Because of their versatility and specificity, monoclonal antibodies (Mabs) are appealing as candidate microbicides. However, traditional manufacturing systems cannot meet the demands of a large, cost-sensitive market. Further, the formulated product must be stable (years at ambient temperature) and convenient.

**Methods:** The magnifection transient expression system (Icon Genetics/Bayer) is a fast, scalable and economical approach to high-level production of Mabs in *Nicotiana benthamiana*. Mabs for sexual and reproductive health (SRH) expressed in *N. benthamiana* with two and five vectors systems are: (a) HSVgD Mab-N (binds glycoprotein D of HSV); (b) CCR5 Mab-N (an inhibitor that blocks HIV's use of CCR5 as a co-receptor for viral entry)**;** (c) b12 Mab-N (binds gp120 on HIV); (d) CD52g Mab-N (agglutinates sperm). Antibodies are spray dried (Buchi, B-290) for formulation as vaginal tablets and film.

**Results:** Depending on two and five vectors systems, expression levels of Mabs vary (100ug/g – 800 ug/g fresh leaf weight) under scaleable laboratory conditions. HSVgD Mab-N is equivalent to CHO cell derived HSVgD Mab by gD binding ELISA, HSV neutralization assay ($IC_{90}$ = 9.2 /ml) and in vivo protection of mice against vaginal HSV transmission. CCR5 Mab-N demonstrates HIV neutralizing activity against a panel of isolates (0.017 ug/ml to 30 ug/ml), similar to mammalian Mab. The b12 Mab-N has been expressed also in *N. benthamiana*. The CD52g Mab-N co-agglutinates 100% of sperm and other seminal cells in less than thirty seconds at 100 ug/ml. The SRH Mabs were produced aglycosylated, with conventional plant glycans, and in a transgenic line with xylosyltransferase and fucosyltransferase knocked out, resulting in mammalian-like glycosylation patterns.

**Conclusions:** Human SRH Mabs to pathogens and sperm have been produced in *Nicotiana* using the magnifection system. Now that a large scale, cost effective manufacturing system is in place, IND-enabling rabbit vaginal irritation studies were conducted in mid 2009. In addition, the safety (symptoms, colposcopy, pro-inflammatory cytokines) of candidate Mabs can be evaluated in a Phase 1a safety trial in late 2009; vaginal residence time and ex vivo neutralization are secondary objectives of this trial.

# Anti-Ebola Virus Human Monoclonals Produced in Nicotiana Benthamiana: Development as an Immunoprotectant for Human Use

**Larry Zeitlin[1], Natasha Bohorova[1], Andrew Hiatt[1], Andy Ho[1], Kati Hnath[2], Gene Olinger[2], Do Kim, Michael Pauly[1], Jesus Velasco[2], Herta Steinkellner[3], and Kevin Whaley[1]**

1) Mapp Biopharmaceutical Inc., San Diego, CA
2) USAMRIID, Frederick, MD
3) Center of Applied Genetics, BOKU Vienna, Austria

**Introduction:** Ebola, a filovirus, causes severe morbidity and mortality during episodic outbreaks in regions where it is endemic. In addition, there are concerns that Ebola virus could be used in a bioterror attack. No prophylaxis or therapy currently exists. Three mammalian produced anti-Ebola monoclonals (mAbs) for use as an immunoprotectants have been developed; these mAbs are highly potent in a mouse challenge model. A transient expression system was used to produce the Ebola mAbs in wildtype *N. benthamiana* as well as in a transgenic line that yields humanized N-glycoforms.

**Methods:** The magnifection transient expression system (Icon Genetics/Bayer) is a fast, scalable and economical approach to high-level production of mAbs in *Nicotiana benthamiana*. The three Ebola mAbs were expressed in *N. benthamiana* and the transgenic with two and five vector systems as glycosylated and aglycosylated molecules. C1q binding ELISAs were performed to test the ability of the mAbs to initiate the complement cascade. Antibodies were also tested in vivo using a mouse challenge model.

**Results:** Differences in glycosylation between the wildtype and transgenic *N. benthamiana* had a significant impact on the ability of mAbs to bind to human C1q, with superior binding displayed by mAb from the transgenic line. Antibodies from both plant lines were capable of protecting mice from lethal challenge, whereas aglycosylated mAb provided only partial protection. Dose response experiments are ongoing to determine the relative in vivo potency of the different glycan forms of the mAbs.

**Conclusions:** In vitro and in vivo comparisons are allowing us to understand the mechanisms of protection by these Ebola mAbs, especially with respect to glycan-related effector functions. In addition to these data, progress in advancing these mAbs towards clinical evaluation will be presented.

# Workshop:
# Plant-Made Pharmaceuticals and Quality Oversight

**Presented by Jeanne M. Novak Ph.D. and Kathy M. Hanley, Ph.D.**

Successful development and manufacture of recombinant drug products using a plant-based expression system must include development of an appropriate Quality System. The level of Quality oversight required is generally dependent on the stage of clinical development and operational concerns. It also should be based on forward-thinking and what will be required for future plant-made pharmaceutical manufacture at your facility.

This workshop will provide an introduction to the regulations and guidance provided by regulatory agencies for the development and support of a Quality System. Special emphasis will be placed on FDA regulatory and compliance requirements for novel expression systems. Specifically, the workshop will include a discussion of concepts and policies that should be considered in order to provide adequate Quality oversight for both plant-made pharmaceuticals and biotech in general.

# About...

## Sullivan University College of Pharmacy

Sullivan University has been preparing students for careers for more than 45 years. The College of Pharmacy is the most recent addition to the University, where the first classes began in July 7, 2008. The College of Pharmacy offers an accelerated program, allowing students to earn their Doctor of Pharmacy degree in three years (after 2 years of pre-requisite courses) rather than the typical four professional years. The College currently consists of two departments, Clinical & Administrative Sciences and Pharmaceutical Sciences. The College of Pharmacy program is under the leadership of the Founding Dean Hieu T. Tran, Pharm.D., who joined the Sullivan University in October 10, 2006 to launch the new College of Pharmacy in Louisville, KY. All faculty members and administrators were hand-picked from across the country to ensure that the College of Pharmacy provides to its students a quality and meaningful learning experiences.

## Kentucky BioProcessing, LLC

KBP (www.kbpllc.com) is located in Owensboro, Kentucky and maintains a highly experienced staff and facilities focused on expression, extraction, purification and commercial scale production of proteins and other products from plants. KBP offers clients and collaborators access to controlled plant growth facilities along with bench, pilot and commercial scale production under cGMP conditions. KBP's proprietary gene expression technology, GENEWARE®, offers a robust and highly scalable platform for plant based protein expression.

# Restaurant List

Note: All directions are based on taking a right out of the main parking lot. The light (Bakery on your Right, Heine Brothers on your left) is Bardstown Road.

**Winston's Restaurant**
Address: 3101 Bardstown Road
Phone: (502) 456-0980
Directions: Go straight through light to main Sullivan Campus

**Buckhead Bar & Grill**
Address: 3008 Bardstown Road
Phone: (502) 456-6680
Directions: Before arriving at light, take Left into Parking Lot by Heine Brothers

**Chili's Grill & Bar**
Address: 3623 Bardstown Road
Phone: (502) 301-8888
Directions: Right on Bardstown Rd, 1 mile up on Left (Near Arby's)

**Tumbleweed**
Address: 3602 Bardstown Road
Phone: (502) 454-2727
Directions: Right on Bardstown Rd, 1 mile on up on right

**John E's Restaurant and Lounge**
Address: 3708 Bardstown Road
Phone: (502) 456-1111
Directions: Turn Right on Gardiner Lane, Right on Bardstown Rd, 1.2 miles on left

**Steak 'n Shake**
Address: 3232 Bardstown Road
Phone: (502) 456-2670
Directions: Turn Right on Gardiner Lane, Right on Bardstown Rd, .3 miles on right

**Mr. Gatti's Pizza**
Address: 3319 Bardstown Road
Phone: (502) 451-0540
Directions: Turn Right on Gardiner Lane, Right on Bardstown Rd, .3 miles on left

**Bristol Bar & Grille**
Address: 1321 Bardstown Road
Phone: (502) 456-1702
Directions: Turn Right on Gardiner Lane, Left on Bardstown Rd, 2.3 miles on right

# Restaurant List Continued

**Mark's Feed Store**
Address: 1514 Bardstown Road
Phone: (502) 458-1570
Directions: Left on Bardstown Road, 2.5 miles on left

**Kashmir Indian Restaurant**
Address: 1285 Bardstown Road
Phone: (502) 473-8765
Directions: Left on Bardstown Road, 3 miles on right

**Just Fresh Bakery**
Address: 1255 Bardstown Road
Phone: (502) 451-2324
Directions: Left on Bardstown Road, 3.1 miles on right

**De La Torre's Restaurant**
Address: 1606 Bardstown Road
Phone: (502) 456-4955
Directions: Left on Bardstown Road, 2.3 miles on right

**Sapporo Japanese Steakhouse**
Address: 1706 Bardstown Road
Phone: (502) 479-5550
Directions: Left on Bardstown Road, 2.5 miles on right

**Seviche: A Latin Restaurant**
Address: 1538 Bardstown Road
Phone: (502) 473-8560
Directions: Left on Bardstown Road, 2.5 miles on right

# Quick Service Restaurants

Directions: Turn Right on Gardiner Lane, Right on Bardstown Road

**Sonic Drive-In**
**Taco Bell**
**Pizza Hut**
**Penn Station**
**Arby's**
**Wendy's**
**Kentucky Fried Chicken**
**Popeye's Chicken & Biscuits**
**White Castle**
**Subway (Inside WalMart)**
**Dairy Queen (turn left at Thornton's)**
**Captain D's Seafood**

# Map of Kentucky

**Owensboro, KY**
**Home of: Kentucky BioProcessing, LLC**

**Louisville, KY**
**Home of: Sullivan University COP**