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**Symposium Sponsors**
Dear Speakers and Delegates,

Welcome to the Fourth Annual Peptide Therapeutics Symposium. We are excited about the program we have assembled and are delighted that you were able to join us today.

This year’s program primarily focuses on state-of-the-art academic and commercial advances in the application of peptides at intracellular targets. While peptides, much like other macromolecules, have proven to be highly effective medicines, they have been predominantly used as injectable formulations directed to extracellular targets. The emergence of new chemistry and the application of peptide transport signals are expanding the breath of molecular targets where peptides and other macromolecules can be developed. A deeper molecular understanding of the basis for successful transport offers the potential for emergence of non-injectable forms of delivery and application to neurological diseases.

The program will begin with a comprehensive analysis of peptide therapeutics and a historical perspective regarding elements that ultimately influenced R&D success and failure. We are delighted to host this meeting and look forward to a stimulating set of lectures and associated discussions.

With kind regards,

Richard DiMarchi, Ph.D.
Symposium Chairman & Director
Peptide Therapeutics Foundation
Professor of Chemistry &
Gill Chair in Biomolecular Sciences
Indiana University

Pierre Rivière, Ph.D.
President
Peptide Therapeutics Foundation
Managing Director &
Vice President, Research
Ferring Research Institute Inc.

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Amylin Pharmaceuticals

Amylin Pharmaceuticals is a biopharmaceutical company committed to improving lives through the discovery, development and commercialization of innovative medicines. Amylin has developed and gained approval for two first-in-class medicines for diabetes, SYMLIN® (pramlintide acetate) injection and BYETTA® (exenatide) injection. Amylin’s research and development activities leverage the Company’s expertise in metabolism to develop potential therapies to treat diabetes and obesity. Amylin is headquartered in San Diego, California. Further information about Amylin Pharmaceuticals is available at www.amylin.com.

Ferring Research Institute Inc.

Ferring Pharmaceuticals is a specialty, research-driven biopharmaceutical company focused on biologics, peptides and proteins therapeutics, in three main core therapeutic areas: infertility, urology and gastroenterology. The company, headquartered in Saint-Prex (Switzerland), employs over 3,200 people worldwide and operates subsidiaries in over 45 countries. The company R&D centers are located in Saint-Prex (Switzerland), Copenhagen (Denmark), Mumbai (India), Beer Tuvia (Israel) and Parsippany and San Diego (United States).

The Ferring Research Institute, Inc. (FRI) was established in San Diego in 1996 as the company’s center of excellence for peptide research. FRI focuses its research on naturally-occurring peptides/hormones and receptors involved in the control of the body’s major biological functions and physio-pathological processes. Its peptide chemistry technology and expertise enabling in-depth modification of virtually all peptide/hormone families, allowing the custom design of peptide therapeutics with improved pharmacodynamics, pharmacokinetics and pharmaceutical properties. This has lead in recent years to the discovery of a number of innovative peptidic new chemical entities (NCEs) now at various stages of development, either in house or externally. The most advanced NCE, degarelix, was recently approved by the FDA for the treatment of patients with advanced prostate cancer.

IPSEN

Ipsen is an innovation-driven international specialty pharmaceutical group with over 20 products on the market and a total worldwide staff of nearly 4,000. Its development strategy is based on a combination of specialty products, which are growth drivers, in targeted therapeutic areas (oncology, endocrinology and neuromuscular disorders), and primary care products which contribute significantly to its research financing. The location of its four Research & Development centres (Paris, Boston, Barcelona, London) and its peptide and protein engineering platform give the Group a competitive edge in gaining access to leading university research teams and highly qualified personnel. More than 600 people in R&D are dedicated to the discovery and development of innovative drugs for patient care. This strategy is also supported by an active policy of partnerships. In 2005, Research and Development expenditure was about €205 million, in excess of 20% of consolidated sales, which amounted to €120.5 million while total revenues amounted to €163.8 million. Ipsen’s shares are traded on Segment A of Euronext Paris (stock code: IPN, ISIN code: FR0010259150). Ipsen’s shares are eligible to the Service de Règlement Différé (SRD) and the Group is part of the SBF 120 index. For more information on Ipsen, visit our website at www.ipsen.com.
The PolyPeptide Group

The PolyPeptide Group is a privately-held group of manufacturing companies that focuses on proprietary and generic GMP-grade peptides for the pharmaceutical, cosmetic and biotechnological market. With more than 50 years of experience, the Group is committed to the highest quality of peptide manufacturing, irrespective of whether this is for approved drug substances, GMP peptides in clinical trials, or small-scale non-GMP custom syntheses.

The PolyPeptide Group has six GMP facilities located across 3 continents: Denmark (Hillerød), France (Strasbourg), India (Mumbai), Sweden (Malmö), USA (San Diego and Torrance). As a multi-national company with about 450 employees worldwide, its diversity brings breadth and depth of knowledge and experience to the Group.

With over 20 approvals worldwide, the PolyPeptide Group works with companies of all sizes and can support peptide projects from early research through pre-GMP development, large-scale GMP manufacturing, and commercialization. With its increased capacity for GMP manufacturing and expanded capabilities for small-scale non-GMP custom peptides, the PolyPeptide Group has become stronger and better equipped to serve the needs of its customers at all stages of pharmaceutical peptide development. With its multinational organization, exclusive focus on peptides and solid financial base, the Group offers an almost unique security of supply to its customers.
Schedule of Events

Tuesday, November 17, 2009  Estancia La Jolla Hotel & Spa, La Jolla

4:30 p.m.  Registration Opens

5:00 – 5:15 p.m.  Opening Remarks
Jane Salik, Director, Peptide Therapeutics Foundation, CEO, PolyPeptide Laboratories Inc.

5:15 – 6:00 p.m.  Update on Development Trends for Peptide Therapeutics
Janice M. Reichert, Ph.D., Director, Peptide Therapeutics Foundation, Senior Research Fellow, Tufts Center for the Study of Drug Development

6:00 – 8:00 p.m.  Opening Reception

Wednesday, November 18, 2009  Salk Institute for Biological Studies, La Jolla

7:30 a.m.  Registration Opens

7:30 – 8:30 a.m.  Continental Breakfast

8:30 – 8:40 a.m.  Welcome Remarks
Richard DiMarchi, Ph.D., Symposium Chairman & Director, Peptide Therapeutics Foundation, Linda and Jack Gill Chair in Biomolecular Sciences & Professor of Chemistry, Indiana University

Session I Moderators  ● ● ●  Richard DiMarchi, Ph.D., Symposium Chairman & Director, Peptide Therapeutics Foundation, Linda and Jack Gill Chair in Biomolecular Sciences & Professor of Chemistry, Indiana University
Michael Hanley, Ph.D., Vice President, Discovery Research and CSO, Amylin Pharmaceuticals

8:40 – 9:30 a.m.  New Targets and Targeting Mechanisms for Contrast Agents and Therapeutics
Roger Tsien, Ph.D., HHMI Investigator & 2008 Nobel Laureate Chemistry, Professor, Pharmacology, Chemistry, and Biochemistry, UCSD

9:30 – 10:10 a.m.  NMR Structural Studies of Drug Receptors in Membranes
Stanley Opella, Ph.D., Professor, Chemistry and Biochemistry & Director, Nuclear Magnetic Resonance Resource Lab, UCSD

10:10 – 10:40 a.m.  Coffee Break

Session II Moderators  ● ● ●  Steve Kaldor, Ph.D., President & CEO, Ambrx
Janice M. Reichert, Ph.D., Director, Peptide Therapeutics Foundation, Senior Research Fellow, Tufts Center for the Study of Drug Development
Schedule of Events

Wednesday, November 18, 2009  Salk Institute for Biological Studies, La Jolla (continued)

10:40 – 11:20 a.m. Peptide Transduction Domain Delivery of Macromolecules: Tackling the siRNA Delivery Problem
Steven F. Dowdy, Ph.D., HHMI Investigator & Professor Cellular and Molecular Medicine, School of Medicine, UCSD

11:20 a.m. – 12:00 p.m. Peptide Inhibitors of Protein-Protein Interactions: from Rational Design to the Clinic
Daria Mochly-Rosen, Ph.D., Professor, Chemical and Systems Biology & Senior Associate Dean for Research, School of Medicine, Stanford University

12:00 p.m. – 1:30 p.m. Lunch

Session III Moderators ● ● ●
Jesse Dong, Ph.D., Director, Peptide Therapeutics Foundation, Vice President, Medicinal Chemistry, IPSEN
Curt Bradshaw, Ph.D., Vice President Chemistry, Pfizer-CovX

1:30 – 2:10 p.m. Stapled Helical Peptides: A New Drug Modality
Tomi Sawyer, Ph.D., CSO, Aileron Therapeutics

2:10 – 2:50 p.m. Clinically Validated Inhibitors of Intracellular Protein Interactions
Stephen D. Harrison, Ph.D., Senior Vice President, Research, Kai Pharmaceuticals

2:50 – 3:30 p.m. Pepducins – Novel Mediators of GPCR Signaling
Stephen Hunt, Ph.D., Senior Vice President, Discovery Research, Ascent Therapeutics

3:30 – 4:00 p.m. Coffee Break

Session IV Moderators ● ● ●
Jean Rivier, Ph.D., The Dr. Frederik Paulsen Chair in Neurosciences, Professor, Salk Institute for Biological Studies
Richard Dimarchi, Ph.D., Symposium Chairman & Director, Peptide Therapeutics Foundation, Linda and Jack Gill Chair in Biomolecular Sciences & Professor of Chemistry, Indiana University

4:00 – 4:40 p.m. Linaclotide: A Potential Therapy for IBS-C
Mark G. Currie, Ph.D., Senior Vice President, R&D & CSO, Ironwood Pharmaceuticals Inc.

4:40 – 5:20 p.m. Non-Invasive Systemic Delivery of Peptides with High Bioavailability
Edward T. Maggio, Ph.D., CEO & Director, Aegis Therapeutics

5:20 – 5:30 p.m. Closing Remarks
Pierre Riviere, Ph.D., President, Peptide Therapeutics Foundation, Managing Director & Vice President Research, Ferring Research Institute, Inc.

5:30 p.m. Closing Reception
Mark G. Currie, Ph.D. | Senior Vice President, R&D and Chief Scientific Officer, Ironwood Pharmaceuticals Inc.

**Linaclotide: A Potential Therapy for IBS-C**

Dr. Currie serves as Ironwood’s Senior Vice President of Research and Development and Chief Scientific Officer, and has led the Company’s R&D efforts since joining Ironwood in 2002. Prior to joining Ironwood, he directed cardiovascular and central nervous system disease research as Vice President of Discovery Research at Sepracor. Previously, Dr. Currie initiated, built, and led Discovery Pharmacology and also served as Director of Arthritis and Inflammation at Monsanto/Searle. Dr. Currie earned a B.S. in Biology from the University of South Alabama and holds a Ph.D. in Cell Biology from the Bowman-Gray School of Medicine of Wake Forest University.

Richard DiMarchi, Ph.D. | Linda & Jack Gill Chair in Biomolecular Sciences and Professor of Chemistry, Indiana University

**Welcome Remarks**

Dr. DiMarchi possesses over 30 years experience in peptide - protein drug discovery and development in academia, the pharmaceutical industry and biotech companies. He is the Linda & Jack Gill Chair in Biomolecular Sciences and Professor of Chemistry at Indiana University. His current research and commercial endeavors are focused on developing proteins with enhanced therapeutic properties through biochemical optimization with non-natural amino acids, an approach termed chemical-biotechnology.

Dr. DiMarchi is a retired Group Vice President at Eli Lilly & Company where for more than two decades he provided leadership in biotechnology, endocrine research and product development. He is readily recognized for discovery and development of rDNA-derived Humalog® (LisPro-human insulin). This designer insulin represents the first demonstration that structurally altered rDNA-derived biosynthetic proteins can improve pharmacological performance without increasing the risk of an abnormal immunological response. As scientist and manager, Dr. DiMarchi also contributed to the commercial development of Humulin®, Humatrope®, Xigris®, rGlucagon®, Evista® and Forteo®. He is a co-founder of Ambrx, Inc. and Marcadia Biotech. He serves as a board member to Ambrx, Marcadia, Isis and Pharmaceuticals. He is a scientific advisor to Ferring, Kai, Semafore Biotechnologies, Cadila Health Care and three venture funds; 5AM, TMP, and Twilight.

Dr. DiMarchi is the recipient of numerous awards including the 2005 AAPS Career Research Achievement Award in Biotechnology, the 2006 ACS Earle B. Barnes Award for Leadership in Chemical Research Management, the 2006 ACS Gustavus Esselen Award for Chemistry in the Service of Public Interest, and the 2007 Wallace Carothers Award for Excellence in Polymer Sciences.

Steven F. Dowdy, Ph.D. | HHMI Investigator and Professor, Cellular and Molecular Medicine, School of Medicine, UCSD

**Peptide Transduction Domain Mediated Delivery of siRNAs**

Dr. Dowdy is an Investigator of the Howard Hughes Medical Institute, and Professor of Cellular Medicine at the University of California, San Diego School of Medicine. He received his Ph.D. degree from the University of California, Irvine, where he studied with Professor Eric Stanbridge, and did his postdoctoral work with Professor Robert Weinberg at the Whitehead Institute, Massachusetts Institute of Technology. Prior to UCSD, Dr. Dowdy was an Assistant Investigator, HHMI, and an Assistant Professor at Washington University School of Medicine, St. Louis. His laboratory is focused on understanding G1 cell cycle progression during oncogenesis, and delivery of anti-cancer biologically active, macromolecular peptides, proteins and siRNAs by protein transduction domains (PTDs).
Stephen D. Harrison, Ph.D. | Senior Vice President of Research, KAI Pharmaceuticals

Clinically Validated Inhibitors of Intracellular Protein Interactions

Dr. Harrison joined KAI Pharmaceuticals in March 2005. As Senior Vice President of Research, he heads the Discovery and Preclinical efforts at KAI. This group supports the phase 2 clinical development of KAI-9803, a delta PKC inhibitor and has contributed the mechanistic, pharmacology, pharmacokinetic, CMC and ADME data in support of the successful regulatory filings for KAI-1455 and KAI-1678. He leads the pain program, and the development compound (KAI-1000) is currently in Phase 2a clinical trials in post-operative and neuropathic pain. The KAI research effort has recently identified KAI-4169, a compound designed to lower parathyroid hormone levels in renal dialysis patients. This compound is due to enter the clinic next year.

Prior to KAI, Steve held senior research leadership positions at Chiron Corporation and Thios Pharmaceuticals. He led multiple research programs at all stages from target identification to IND filing. Between 1994 and 2004, Steve held escalating positions at Chiron Corporation, most recently serving as Program Head of the kinase inhibitor program and member of the Research Management Team. At Chiron Steve was responsible for the evaluation of all new biological targets. As Chiron’s Director of Lead Optimization Biology, he led all hit-to-lead products and advanced many of these into full-scale lead optimization. Subsequently, as head of Chiron’s kinase inhibitor program he oversaw kinase inhibitor research and led the identification of Advanced Pre-clinical Candidates (APCs) for cancer indications as well as heading the outlicensing of a pre-clinical program in diabetes and neurodegeneration. In 2004, Steve joined Thios Pharmaceuticals as Vice President of Research, where he led efforts targeting biological sulfation, primarily for the treatment of inflammation. Steve directed the identification of the first lead series for inhibition of a sulfotransferase and his research group advanced two therapeutic antibodies to humanization. In these roles Steve also managed the identification and evaluation of pharmacogenomic technologies. Dr. Harrison holds a Ph.D. in Molecular Biology and an M.A. and B.A. in Biochemistry, all from the University of Cambridge, England. He is the author of numerous scientific publications and an inventor on sixteen issued U.S. patents.

Stephen Hunt, Ph.D. | Senior Vice President, Discovery Research, Ascent Therapeutics

Pepducins — Novel Mediators of GPCR Signaling

Dr. Hunt comes to Ascent with over 15 years of drug discovery and development experience at Pfizer. Most recently, he was Executive Director and Head of RNAi Development at Pfizer’s Research Technology Center. In this position, he led the development of, and helped implement the company’s RNAi strategy to turn the promise of RNAi technology into practical pharmaceutical products. Before assuming this role, Dr. Hunt held a series of positions of increasing responsibility within the Parke-Davis and later, Pfizer R&D organizations. Prior to joining industry, Dr. Hunt was a member of the faculty at the University of North Carolina Chapel Hill. Dr. Hunt received his Ph.D. from the University of Pittsburgh and was a NIH Postdoctoral Fellow in the laboratory of Dr. Leroy Hood at the California Institute of Technology.

Edward T. Maggio, Ph.D. | Chief Executive Officer & Director, Aegis Therapeutics

Non-Invasive Systemic Delivery of Peptides with High Bioavailability

Prior to co-founding Aegis, Dr. Maggio was founder and Chief Executive Officer of Structural Bioinformatics Inc., subsequently renamed Cengent Therapeutics. He was founder and Chief Executive Officer of ImmunoPharmaceutics, Inc. (IPI), which developed a number of endothelin antagonists, including Pfizer’s (formerly Encysive Pharmaceuticals’) Thelin® (Sitaxsentan), approved in 2003 for sale in Europe for cardiovascular disease and currently in US clinical trials.

Dr. Maggio has been a founder and board member of seven public and private life science companies in the San Diego area and one in Copenhagen, Denmark. He received his Ph.D. from the University of Michigan and was an NIH postdoctoral fellow at the University of California, San Francisco (UCSF) Department of Pharmaceutical Chemistry.
He is a member of the Advisory Board of the Polytechnic Institute of New York University, Department of Chemical and Biological Sciences, and serves on the University of California, San Diego Dean’s Board of Advisors for Biological Sciences, the California State University, San Marcos, Biotechnology Programs Advisory Board and the Industry Council of the San Diego Consortium for Regenerative Medicine. Dr. Maggio has edited and coauthored a number of books and scientific articles in the biotechnology area and is an author of more than three-dozen issued and pending U.S. and foreign patents.

Daria Mochly-Rosen, Ph.D. | Professor, Chemical and Systems Biology & Senior Associate Dean for Research, School of Medicine, Stanford University

Peptide Inhibitors of Protein-Protein Interactions: From Rational Design to the Clinic

Dr. Mochly-Rosen is the George D. Smith Professor of Translational Medicine, the Senior Associate Dean for Research and a Professor in the Department of Chemical and Systems Biology at Stanford University School of Medicine. She received her B.S. in life sciences from Tel Aviv University, her Ph.D. from the Weizmann Institute of Science, and was a postdoctoral fellow in the department of biochemistry at UC Berkeley.

Her research focuses on the rational design of peptides that interfere with protein-protein interactions to study signal transduction in normal and disease states. Also, her research encompasses the role of individual protein kinase C isozymes in eukaryotic cell signaling, with current emphasis on the intracellular trafficking of PKC isozymes. Her group has designed peptides that act as specific agonists and antagonists for individual PKC isozymes. These are applied to determine the role of PKC in cardiac hypertrophy and in protection from ischemia-induced cardiac infarction in stroke, cancer and Parkinson’s Disease. This research led to Dr. Mochly-Rosen’s foundation of KAI Pharmaceuticals Inc. The goal of the company is to test her peptides in the treatment of patients who suffer, for example, a heart attack. Although the data in animal models are promising, the challenge is to determine whether patients with heart attacks will equally benefit from this treatment.

Stanley Opella, Ph.D. | Professor, Chemistry and Biochemistry & Director, Nuclear Magnetic Resonance Resource Lab, UCSD

NMR Structural Studies of Drug Receptors in Membranes

Dr. Opella is Professor of Chemistry and Biochemistry at the University of California, San Diego, where is also the Director of the National Biotechnology Resource for Molecular Imaging of Proteins. Among other professional activities he is the Editor of the Journal of Magnetic Resonance. His research is focused on the development and application of NMR spectroscopy for the study of proteins in biological supramolecular assemblies, such as virus particles and membranes. Since most drug receptors including those that bind peptide hormones and effectors are membrane proteins, this research has the potential to impact the discovery of new drugs.

Stanley Opella was born in Summit, New Jersey, and received his B.S. degree in Chemistry from the University of Kentucky. He received his Ph.D. in Chemistry from Stanford University, and performed postdoctoral research at MIT before starting his academic career at the University of Pennsylvania. He moved to UCSD in 2000.
Janice M. Reichert, Ph.D. | Senior Research Fellow, Tufts Center for the Study of Drug Development
Update on Development Trends in Peptide Therapeutics

Dr. Reichert studies innovation in the pharmaceutical and biotechnology industries at the Tufts Center for the Study of Drug Development (CSDD). As Editor-in-Chief of mAbs, Dr. Reichert recruited a 72-member editorial board and launched the journal in January 2009.

At Tufts CSDD, Dr. Reichert's work focuses on strategic analyses of candidate and approved products, including clinical development and approval times, phase transition probabilities and approval success rates for new therapeutics and vaccines. Her recent work includes studies of peptide and human monoclonal antibody therapeutics. Dr. Reichert has presented her research results as an invited speaker at conferences in the United States, Canada, Europe and China.

Dr. Reichert is a member of the board of directors of the Peptide Therapeutics Foundation. In addition, she regularly provides input to various government, non-profit and industry organizations such as the National Institutes of Health, the Organization for Economic Cooperation and Development, the International AIDS Vaccine Initiative and the Biotechnology Industry Organization.

Dr. Reichert received her Ph.D. in organic chemistry from the University of Pennsylvania and her postdoctoral training as a National Institutes of Health Research Fellow at Harvard Medical School. Before joining Tufts CSDD, Dr. Reichert performed drug discovery research and preclinical development at several companies in the Boston area.

Pierre Rivière, Ph.D. | Managing Director & Vice President, Research, Ferring Research Institute
Closing Remarks

Dr. Pierre Rivière has served as Managing Director - Vice President, Research, of Ferring Research Institute Inc., in San Diego since January 2006. He joined Ferring in 1996 as Head of Biology and became Director of Research in 2002. In recent years, he contributed to the discovery of a number of peptidic new chemical entities now at various stages of development. Previously, Dr. Rivière occupied various research and managerial positions in drug discovery at the Institut de Recherche Jouvenal in Fresnes, France. He holds a Ph.D. in biology and physiology from the Institut National Polytechnique of Toulouse, followed by post-doctoral training in the Department of Pharmacology of the University of Arizona in Tucson. Dr. Rivière is scientific co-founder and advisor of Cara Therapeutics.

Tomi Sawyer, Ph.D. | Chief Scientific Officer, Aileron Therapeutics
Stapled Helical Peptides: A New Drug Modality

Dr. Sawyer is Chief Scientific Officer and Senior Vice-President of Drug Discovery - Innovative Technologies at Aileron Therapeutics, an emerging biotechnology company that is developing novel peptide drugs using proprietary stapling drug design and chemistry methods which exploit alpha-helical molecular recognition and structure-property relationships. Prior to joining Aileron Therapeutics, he rose through the scientific and management ranks at the Upjohn Company, Parke-Davis-Warner-Lambert and Pfizer Global Research - Development (most recent position was Senior Director, Pfizer Research Technology Center) as well as Ariad Pharmaceuticals (last position was Senior Vice-President, Drug Discovery). Tomi has successfully led several drug discovery campaigns into clinical trials, including peptide, peptidomimetic, natural product and small-molecule compounds for G protein-coupled receptor, aspartyl protease, protein kinase and protein-protein interaction therapeutic targets. He is credited with >300 publications and patents. Tomi is a past President of the American Peptide Society, a past co-chair of the 18th American Peptide Symposium, a DuVigneaud Award recipient, and is Editor-in-Chief of Chemical Biology - Drug Design. He holds Adjunct Professorship appointments at the University of Massachusetts and the University of Massachusetts Medical School, and Northeastern University Center for Drug Discovery.
Roger Tsien, Ph.D. | HHMI Investigator & 2008 Nobel Laureate Chemistry, Professor, Pharmacology, Chemistry, and Biochemistry, UCSD

New Targets and Targeting Mechanisms for Contrast Agents and Therapeutics

Dr. Tsien is the 2008 Nobel Laureate in chemistry, Investigator of the Howard Hughes Medical Institute and Professor in the Departments of Pharmacology and of Chemistry - Biochemistry at the University of California, San Diego. He received his A.B. in Chemistry and Physics summa cum laude from Harvard College in 1972. A Marshall Scholarship then took him to the Physiological Laboratory at the University of Cambridge, where he received his Ph.D. in 1977 and remained as a Research Fellow until 1981. He then became an Assistant, Associate, then full Professor in the Department of Physiology-Anatomy at the University of California, Berkeley.

Dr. Tsien’s honors include 1st prize in the Westinghouse Science Talent Search (1970), Searle Scholar Award (1983), Passano Foundation Young Scientist Award (1981), W. Alden Spencer Award in Neurobiology from Columbia University (1981), Artois-Baillet-Latour Health Prize (1985), Gairdner Foundation International Award (1985), American Heart Association Basic Research Prize (1985), Faculty Research Lectureship at UCSD (1985), the Herbert Sober Lectureship of the American Society of Biochemistry and Molecular Biology (2000), the Pearse Prize of the Royal Microscopical Society (2000), and Award for Creative Invention from the American Chemical Society (2002). He was elected to the Institute of Medicine in 1995, the National Academy of Sciences in 1998 and the Royal Academy in 2006. In 2008 he was awarded the Nobel Prize in Chemistry, along with Osamu Shimomura and Martin Chalfie, for his work with developing Green Fluorescent Protein into a tool that is now used by researchers around the world, in many disciplines, to view the inner workings of cells.

Dr. Tsien’s research has been at the interfaces between organic chemistry, cell biology, and neurobiology, starting long before such interdisciplinary efforts became fashionable. He is best known for designing and building molecules that either report or perturb signal transduction inside living cells. These molecules have enabled many laboratories including his own to gain new insights into signaling via calcium, sodium, pH, cyclic nucleotides, nitric oxide, inositol polyphosphates, membrane potential changes, protein phosphorylation, active export of proteins from the nucleus, and gene transcription. The optical reporter molecules are also valuable in miniaturized high-throughput screening of candidate drugs in the pharmaceutical industry.

In addition to Dr. Tsien’s traditional emphasis on trying to understand how the spatial and temporal dynamics of signal transduction orchestrate complex cellular responses such as synaptic plasticity, he is working on the design of molecules which specifically target cancer cells to deliver either imaging agents or therapeutics, greatly improving upon the specificity of current therapies.

Roger Tsien’s visionary merging of chemistry and biology has produced a dazzling array of designed molecules and fluorescent reporters to image and manipulate dynamic events in living cells. These nanoscale devices, of which the best known are the fluorescent proteins and genetically encoded indicators, have revolutionized biological research. Recently he has turned his attention to the problem of cancer, and is designing peptides to target tumors, both for imaging and therapeutics.
Linaclotide: A Potential Therapy for IBS-C

Mark G. Currie, Ph.D. | Senior Vice President, R&D and Chief Scientific Officer, Ironwood Pharmaceuticals Inc.
320 Bent Street, Cambridge, MA 02141 | (617) 621-7722

Linaclotide: First in Class for Lower GI Pain and Constipation

- >40 million potential U.S. patients
  - IBS-C, chronic constipation, opioid-induced constipation
- Targets defining attributes of IBS-C: abdominal pain and constipation
- Localized to site of action in gut
  - Systemic exposure below level of detection
- Clinical response within 1 – 3 days; sustained throughout treatment period
- Once-daily oral capsule
- Confirmatory Phase 3 program ongoing
  - 4 trials, >2500 patients, >200 centers
- Composition of matter patent > 2025

Pharmacology

- Stable peptide analog of guanylin and uroguanylin
- Activates GC-C receptor, increases intracellular and extracellular cGMP
- Inhibits afferent nerve firing
- Activates anion channels

Preclinical

- Alleviated visceral pain
- Stimulated anion and fluid secretion
- Accelerated intestinal transit
- ≤0.2% systemic exposure

Clinical (Phase 1 & 2 results)

- Dose-related reduction in pain (IBS-C)
- Dose-related improvement in bowel habits, abdominal symptoms, and global assessments
- Response within 1–3 days, sustained throughout treatment period
- Diarrhea only dose-dependent AE
- Systemic exposure below level of detection
Linaclotide Phase 2b Program: Summary

- Primaries and all secondary endpoints met (≥3 of 4 doses for each endpoint)
- Significant reduction in abdominal pain (IBS-C) and constipation symptoms in all dose groups
- Response occurred within 1–3 days; sustained through treatment period
- No evidence of rebound symptoms during the 2-week post-treatment period
- Diarrhea only dose-dependent AE

Linaclotide Summary

- First in class for functional GI disorders
  - Targets defining attributes of IBS-C: pain and constipation
  - Systemic exposure below level of detection
- Large market opportunities
- Phase 3 ongoing
Peptide Transduction Domain Delivery of Macromolecules: Tackling the siRNA Delivery Problem

Steven F. Dowdy, Ph.D. | Howard Hughes Medical Institute, Department of Cellular & Molecular Medicine, UCSD School of Medicine
9500 Gilman Dr. MC 0651, La Jolla, CA 92093-0651 | (858) 822-6258

Short interfering RNA (siRNA) induced RNA interference (RNAi) responses allow for discovery research and performing large scale screening; however, due to their size and anionic charge, siRNAs have no bioavailability to enter cells. However, current approaches fail to deliver siRNAs into a high percentage of primary cells in a non-cytotoxic fashion. We developed an efficient siRNA delivery approach that utilizes a Peptide Transduction Domain-dsRNA Binding Domain (PTD-DRBD) fusion protein. DRBDs bind to siRNAs with high avidity, masking the siRNA negative charge and allow for PTD-mediated cellular uptake via induction of macropinocytosis, a specialized form of fluid phase endocytosis. PTD-DRBD delivered siRNAs induced rapid RNAi responses in a non-cytotoxic manner in the entire cell population of all primary and transformed cell types assayed, including: T cells, HUVECs and hESCs. Whole genome microarray analysis showed minimal transcriptional changes by PTD-DRBD and we did not detect any innate immune responses in PBMCs. PTD-DRBD mediated siRNA delivery allows efficient RNAi manipulation of difficult cell types.

Glioblastoma multiforme (GBM) remains one of the most deadly and intractable human malignancies. Activation of multiple oncogenic pathways in glioblastoma leads to increased cellular growth, proliferation and tumor cell survival. In theory, RNAi responses can selectively target intersecting oncogenic pathways to induce a tumor cell specific RNAi synthetic lethal response. However, the concept of inducing in vivo RNAi synthetic lethal responses has not yet been addressed. We tested the in vivo ability of RNAi to induce a synthetic lethal response to kill tumor cells in an intracerebral glioblastoma mouse model. PTD-DRBD mediated delivery of combination therapeutic siRNAs targeting the EGF-Receptor and Akt2 synergized to induce tumor specific apoptosis in vitro. In vivo PTD-DRBD delivery of EGFR and Akt2 resulted in tumor specific apoptosis that resulted in a significant increased survival in intracerebral glioblastoma mouse models (P < 0.0005), whereas delivery of irrelevant control siRNAs did not alter longevity. These observations demonstrate the exquisite in vivo potential of PTD-DRBD to delivery siRNAs into tumors and the ability to simultaneously select and degrade multiple specific oncogenic mRNA targets to induce synthetic lethal RNAi responses.

Cationic PTD peptides are taken up into cells by macropinocytosis, a specialized form of fluid phase endocytosis that all cells appear to perform. Cationic PTD peptide binding to unknown cell surface proteins stimulates Rac1 resulting in an actin-dependent cell surface ruffle that pinches off into a vesicle that becomes coated in Rab5 and Rab34. Macropinosome vesicles have a decreased pH, but do not traffic to the lysosome. The PTD also mediates escape from macropinosome vesicles into the cytoplasm and is likely the current rate limiting step. We are currently identifying the proteins involved in this process.

PTD-dsRNA Binding Domain (DRBD) Fusion Protein
PTD-DRBD siRNA Knockdown of GFP

H1299 dGFP reporter cells treated with PTD-DRBD:siRNAs assayed by FACS

In Vivo Intracranial EGFR Knockdown by PTD-DRBD

<table>
<thead>
<tr>
<th>Tumor Innocation</th>
<th>PTD-DRBD siRNA</th>
<th>Sacrifice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day: 1</td>
<td>7</td>
<td>9</td>
</tr>
</tbody>
</table>

48 hr

PTD-DRBD

PBS EGFR siRNA
control siRNA EGFR siRNA

72 hr

H&E

Coronal LT

900 pmol siRNA
EGFR + Akt2 siRNAs Induces Synthetic Lethal

PTD-DRBD EGFR + Akt2 siRNA intracerebral glioblastoma model

% Survival

PTD-DRBD EGFR + Akt2 siRNA intracerebral glioblastoma model

600 pmol siRNA
day 3, 8, 13

Working Model of PTD-Mediated Transduction

1. Binding to Cell Surface

2. Stimulation of Macropinocytosis

3. Macropinosome Trafficking

4. Vesicle Escape

Rac1

Rab5
Rab34

Prion Protein
Vaccinia Virus

Cytoplasm

Lysosome
Clinically Validated Inhibitors of Intracellular Protein Interactions

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KAI Pharmaceuticals has developed a technology that uses small peptides (6-10 amino acids in length) to block intracellular interactions of therapeutically important proteins. This approach is demonstrated by two examples of peptidic PKC inhibitors that are being tested in phase 2 clinical trials. These inhibitors block the localization (translocation) of individual PKC isozymes to their intracellular sites of action by binding tightly to PKC receptors (so-called RACKs) at these sites. Previous work by KAI Pharmaceuticals' founding scientist, Dr. Daria Mochly-Rosen has demonstrated that peptides can be rationally designed to block translocation of an individual isozyme (e.g. εPKC or δPKC) without affecting other isozymes.

Historically peptides have been viewed as poor drugs because of their instability and their inability to penetrate cells. KAI Pharmaceuticals have improved cell penetration, and ultimately biodistribution, by conjugating their peptides to the TAT47-57 11-mer peptide via a variety of linkers. The resulting molecules have molecular weights of about 2.5 kD and demonstrate efficient cell penetration. The problems of protease susceptibility and chemical instability have been solved by simple chemical modifications of the peptides that have proven valuable in the stabilization of multiple peptides generated by this technology. The advantages of this general approach include minimal toxicity, stability, formulations developed for a variety of routes of administration, resin-based chemical synthesis ensuring reproducibility and predictability and low cost of goods.

The most advanced CPP-PKC inhibitor program is the development of the δPKC inhibitor (KAI-9803). KAI-9803 is a fusion of the 10-mer δPKC inhibitor peptide with TAT47-57 CPP. Conjugation is provided by a disulfide bond between N-terminal cysteines on both peptides. KAI-9803 is being developed as a treatment for acute myocardial infarction (AMI). Following myocardial ischemia, substantial further damage to the heart occurs following revascularization and restoration of blood flow to the organ. The resulting free-radical generation induces δPKC translocation to the mitochondria and ultimately leads to apoptosis and necrosis. Inhibition of this translocation by KAI-9803 reduces both apoptosis and necrosis. The ability to protect ischemic hearts from reperfusion-induced infarct has been demonstrated in the pig model of coronary artery occlusion. 15 μg of KAI-9803 administered to the coronary artery just before reperfusion reduced infarct size by 80% at three days post reperfusion.

These encouraging data prompted clinical testing in a Phase 2 clinical trial in 154 AMI patients. Four doses of KAI-9803 from 50 μg to 5 mg were tested by direct intracoronary administration at the time of balloon angioplasty. Substantial reductions in infarct were measured at all doses, quantitated by reduction in the levels of myocardial enzymes in circulation. Despite the small size of the trial, statistically significant reductions in electrocardiographic defects were observed and overall the composite measure of mortality and congestive heart failure post treatment was reduced, compared to placebo. Following demonstration of intravenous efficacy of KAI-9803 in pre-clinical models and confirmation of the safety of this route of administration of this peptide in a phase 1 clinical trial, KAI Pharmaceuticals has embarked on a 1,100 patient Phase 2b trial in AMI patients. This trial is being financed by Bristol Myers Squibb, who have licensed this compound.

The generality of the KAI approach is illustrated with KAI-1678 a linear peptide fusion of the ε-mer εPKC inhibitor and TAT47-57 CPP. δPKC is expressed in the nociceptor neurons that sense pain and forms an important link between pain receptors on these nociceptors and the ion channels that are responsible for transducing the pain signal to the spinal cord. KAI-1678 has been administered subcutaneously in multiple rat models of inflammatory and neuropathic pain and has shown rapid and complete reversal of pain behaviors at doses in the low μg range. Phase 1 clinical trials were conducted with subcutaneous infusions of KAI-1678 and it was shown to be safe and well tolerated at doses that generate plasma levels in excess of those associated with efficacy in animal models. KAI-1678 is currently being tested in phase 2 trials in post-operative pain and two neuropathic indications (post-herpetic neuralgia and spinal cord injury). Ultimately KAI-1678 is being developed as a subcutaneous injection for post-operative pain and as a patch formulation for chronic neuropathies. In support of the later product profile, it has been demonstrated that KAI-1678 can be effectively administered systemically by transdermal delivery from a topical patch.

Thus it appears that KAI peptides allow efficient modulation of PKCs in vivo and that conjugation to cell penetrating peptides provides adequate bioavailability to achieve high potency with large therapeutic indices. Initial clinical work appears to validate this approach.
KAI Technology Overview

Modular design of KAI peptides
- Highly potent
- Efficient cell penetration
- Design does not require a structure of the protein target
- Modular design makes SAR predictable
- Scale-up proven for three clinical programs
- Strong target biology

KAI’s Inhibitors Block PKC Intracellular Localization

Also: KAI activators can enhance the PKC/RACK Interaction
Intracellular Delivery Strategy

- Use of Cell Penetrating Peptides (e.g. TAT) to deliver PKC-inhibitors into cells.
- Clinical experience with arginine-rich carrier peptides (i.e., poly-R, TAT)
- Carrier, linker and peptide SAR

KAI-9803 Reduces Heart Damage in Acute Myocardial Infarction

KAI-9803 showed meaningful reductions in infarct size as assessed by cardiac enzymes
KAI-1678 Reverses Neuropathic Pain

Subcutaneous Infusion of KAI-1678 in CCI Model of neuropathic pain

- KAI-1678, 7.5 pg/kg/hr (n=5)
- KAI-1678, 0.75 pg/kg/hr (n=6)
- KAI-1678, 0.075 pg/kg/hr (n=7)
- Vehicle, (n=6)

Full reversal of allodynia and rapid onset of efficacy which can be maintained for > 5 days
Pepducins – Novel Mediators of GPCR Signaling

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GPCRs are the most commonly exploited targets among approved drugs (e.g. beta blockers, serotonin inhibitors, etc.). There are roughly 1000 GPCRs identified in the human genome. Of these, about half are putative drug targets (the other half are mainly taste and smell receptors). Of the 500 or so potential drug targets, only 40–50, or about 10%, of this large family of receptors has been successfully addressed with current drug discovery technologies.

Current GPCR-targeted products account for over $40 billion in annual sales. Thus, there is an enormous market opportunity among the many other GPCR targets. The unique sequence-based intracellular mechanism of pepducins overcomes many of the obstacles to current drug discovery approaches and thus provides an opportunity to pharmacologically address the entire family of GPCRs (Figure 1).

Pepducins are lipopeptides composed of a short sequence of amino acids (usually 10–15 residues) and a hydrophobic lipid moiety like palmitate or myristate (Figure 2). The peptide portion of the pepducin is derived from the amino acid sequence of a portion of one of the four intracellular loops of the target GPCR. These molecules are easily synthesized on solid support, adding the lipid in the final cycle before purification.

Pepducins work by a completely unique mechanism of cell entry and allosteric modulation of GPCR activity (Figure 3). Data from our laboratories and external academic researchers indicate that pepducins enter the cell in seconds to minutes via a mechanism that we call ‘insertion and inversion’. These data indicate that the lipid tail serves to insert and anchor the pepducin in the external leaflet of the lipid bilayer.
Charges in the peptidic portion of the molecule are neutralized, thus allowing the pepducin to traverse the membrane and anchor in the intracellular leaflet of the lipid bilayer. This process is similar to the "flip-flop" mechanism of fatty acid transport and thus would be expected to achieve equilibrium between the inner and outer leaflets of the membrane and thus allow for rapid movement of pepducins into, and out of, cells.

Once inside the cell, the pepducin is in a position to encounter its target GPCR. Anchoring of the pepducin in the membrane by the lipid moiety serves two functions: first, to increase the local concentration of pepducin in the area of the GPCR, and second, to present the peptidic portion in an appropriate manner to the target GPCR. Data from mechanistic studies including ligand binding, signal transduction, receptor internalization, β-arrestin recruitment, indicate that pepducins modulate signal transduction in a specific, receptor-mediated, manner. We believe that the peptide portion of the molecule interacts with its target GPCR to stabilize an active or inactive conformation of the receptor, alter the receptor's ability interact with the intracellular signaling machinery, or affect receptor dimerization in a positive or negative way resulting in agoallosteric or positive or negative modulator effects.

Our pepducin screening strategy is illustrated in Figure 4. The regions of the intracellular loops of any GPCR can be discerned by comparing the sequence of the target GPCR to the sequence coordinates derived from the crystal structure of rhodopsin. Small libraries (10–15 compounds) of pepducins are created from each of the loops using N- and C-terminal deletions and using palmitate as the lipid. The pepducins are screened in one of a number of primary functional screening assays that may include chemotaxis, calcium flux, cAMP modulation, or β-arrestin recruitment, followed by additional secondary functional and selectivity assays. Often, we have identified primary screening hits that possess sufficient potency, selectivity, and pharmaceutical properties to permit assessment of their activity in in vivo mechanistic studies. In the best case scenarios, we have been able to progress from paper target to in vivo activity in as little as 3–4 months. Ascent Therapeutics’ lead project, the chemokine receptor CXCR4, is just such a case.
CXCR4 is an attractive target for a variety of cancer indications involving stem cell mobilization prior to bone marrow transplantation, chemosensitization, and growth and survival, as well as for HIV. CXCR4 is the receptor for SDF1α, which is found in high concentrations in the bone marrow. Hematopoietic stem cells, along with many mature leukocytes, express CXCR4. The interaction of this receptor with its ligand leads to the retention of these cells in the marrow. Interrupting this interaction can facilitate release of the cells into the systemic circulation. Some types of malignant cells, for example certain leukemias, have high expression levels and it is thought that CXCR4-SDF-1α interaction leads to sequestration of the leukemia cells in the marrow. In December 2008, Genzyme’s Mozobil (plerixafor) was approved for mobilizing stem cells to improve yield during stem cell harvests prior to autologous stem cell transplants for certain tumors.

We have carried out a screening strategy for CXCR4 as described above. We identified hits from all four loops. Preliminary results suggest that the hits include agoallosteric modulators as well as positive and negative allosteric modulators. We have tested a subset of these compounds in in vivo mechanistic and efficacy models. As shown in Figure 5, ATI-2346, an i1 loop compound, caused release of bone marrow neutrophils in a dose-dependent manner with activity comparable to, if not better than, AMD3100 (Mozobil). Control peptide or negative pepducins showed no effect. Furthermore, this same compound mobilized bone marrow erythrocyte and granulocyte/monocytes progenitors to levels comparable to AMD3100 (Figure 6).

Summary

Ascent Therapeutics’ pepducin technology is a powerful new biologics platform aimed at the most important family of drug targets—GPCRs. Ascent has already built a solid drug discovery infrastructure based on the pepducin platform. Pepducins have achieved excellent preclinical results, with selective potency and drug-like pharmaceutical properties. Through a unique mechanism of allosteric modulation via the intracellular domains of a target GPCR, pepducins promise to tremendously expand the number of these well-validated targets that can be addressed pharmaceutically.
Non-Invasive Systemic Delivery of Peptides with High Bioavailability

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Aegis Therapeutics, located in San Diego, California, out licenses transmucosal peptide and protein drug delivery technology and protein stabilization technology originating at the University of Alabama Medical School in Birmingham. The technologies are covered by number of issued and pending US and foreign patents. At present, Aegis has issued product specific licenses covering 19 different APIs to 17 different pharmaceutical and biopharmaceutical companies in North America, Europe, and Asia. Aegis technologies are out-licensed under the separate trademarks Intravail® and ProTek® to allow for the differentiation of licensing terms associated with absorption enhancement vs. peptide or protein stabilization.

Increased transmucosal absorption and protein stabilization is accomplished through the use of selected alkylsaccharide excipients. While the University of Alabama patent coverage is quite broad covering essentially all alkylsaccharides within the activity class irrespective of the chemical bond joining the alkyl groups to the saccharide, alkylglycosides and alkyl esters exhibit particularly favorable characteristics for pharmaceutical applications. For example, they do not contain hetero atoms such as sulfur or nitrogen and metabolize cleanly to CO₂ and H₂O. Similar molecules, typically provided as mixtures having varying alkyl chain lengths from 6 to 18 carbons, are used extensively in the food and cosmetics industry. For food purposes, they are used as texturizers and emulsifiers. They are safe, odorless, tasteless, non-toxic, nonmutagenic and nonirritating. The NOEL for some of these molecules exceed 20,000 mg per kilogram (20 g per kilogram).

The Intravail alkylsaccharide excipients function by both paracellular and transcellular means, opening tight junctions and inducing transcytosis as seen in the figures below.
The alkyl saccharides exert their effect directly on the mucosal membrane rather than by interaction with the peptide or protein drugs. This can be seen in the figure below in which the polypeptides calcitonin and somatotropin are administered nasally to rodents. In this experiment, the administration of alkyl saccharides to the mucosal membrane can be separated in time from the administration of the peptide drugs. This figure demonstrates that for small proteins, such as calcitonin, the peptide may be administered up to two hours after exposure of the mucosal membrane to the Intravail excipient, representing eight half-lives of mucociliary clearance, and still demonstrate significant systemic absorption. For the larger protein, human growth hormone or somatotropin, the window is essentially closed within one hour following administration of the alkyl saccharide to mucosal membranes.

This experiment demonstrates the rapid reversibility of the effect of these alkyl saccharides on mucosal membranes, as well as the independence of the alkyl saccharides and the drug in the absorption enhancement phenomenon. (Buccal and oral peptide absorption data will also be presented).

The figure below shows intranasal bioavailability of various peptides or proteins having molecular weights ranging from less than 1000 daltons to 30,000 daltons as a function of alkyl saccharide excipient concentration. The data has been aggregated from a number of publications by the original University of Alabama inventors with the exception of the single data point for the >1000 Dalton anti-obesity peptide, which was tested at a single alkyl saccharide concentration of 0.125%. Note that the bioavailability for this small peptide actually exceeds that achieved by subcutaneous injection, presumably due to a depot effect at the injection site.
The figure below shows the results of a 10 patient three-way crossover study in which a 3-4kD peptide was administered intranasally in the presence of absence of Intravail alkylsaccharide and compared to subcutaneous administration. In this particular instance, there is an approximately 5 fold increase in the AUC upon addition of the Intravail alkylsaccharide.

3-Way Human Crossover Study — Intravail® Increases Calcitonin Bioavailability >5-fold

This last figure shows comparative bioavailability in primates for nasal administration of cyclic PTH 1-31 at low and high dose (i.e., 5 and 50 μg/kg) in the presence and absence of Intravail alkylsaccharide. The relative bioavailability with Intravail is 35-40%, compared to subcutaneous administration, essentially the same as that observed in humans for the similarly sized calcitonin molecule.

ZT-031 PK Profile in Monkeys Following IN Administration with 0.18% Intravail® A3

As new methods of highly efficient transmucosal (i.e., non-invasive) delivery of peptides and proteins emerge, whether by intranasal, sublingual, buccal, oral administration, we can expect to see substantial growth in the broader use of peptides as commercially and clinically viable human therapeutics, offering patients new, more convenient, and more effective, therapeutic options across the broad spectrum of human diseases.
Peptide Inhibitors of Protein-Protein Interactions: From Rational Design to the Clinic

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The importance of protein-protein interactions in cell signaling is now better understood and has been the basis of intense research aiming to generate new pharmacological tools to regulate cellular responses. Our interest in generating protein-protein interaction inhibitors, however, stemmed from a basic research question: why does each cell contain multiple members of the protein kinase C (PKC) family? Members of this family of signaling enzymes are highly homologous, activated by the same hormones and at least in vitro they phosphorylate the same substrates. If the enzymes do not serve redundant functions, how is their specificity determined? When we began this study, the research in the PKC field in academia as well as in the pharmaceutical industry largely employed non-specific pharmacological agents for the biological detection of the individual PKC isozymes, resulting in much misleading data. We therefore began our study, searching for new tools to determine the role of individual isozymes.

The first hint to what makes each isozyme unique came from an immunodluorescence microscopy study in 1990, when we found that in heart cells stimulated with norepinephrine, each PKC isozyme moves (or translocates) from the cytosol to unique subcellular sites. We hypothesized that this unique subcellular localization of each activated isozyme determines their specific function. We then proposed and subsequently demonstrated the presence of isozyme-specific anchoring proteins (which we termed RACKs); each RACK binds a different PKC isozyme. This hypothesis predicted that the location of each active PKC isozyme determines the protein substrate it can modify (e.g., an ion channel in the cell membrane vs. a promoter of gene expression in the nucleus.) Based on this hypothesis, we then developed a rational approach to generate novel isozyme-specific inhibitors.

These highly selective inhibitors of PKC isozymes were identified using sequence-mining techniques, which map regions in PKCs or their RACKs that are essential for protein-protein interactions. The inhibitors are short (6-10 amino acids) peptides and all were found to have the expected biological activities; the peptides act as competitive inhibitors of the protein-protein interaction between each PKC and its RACK and work in a simple mass action fashion. (The presentation will provide several rational approaches that were successfully used to identify these inhibitors.) The first design and in vivo application of a peptide inhibitor of protein-protein interaction for PKC was reported in 1991 (JBC); we generated an inhibitor of Xenopus oocyte maturation, a PKC-mediated function. We subsequently generated over twenty peptide inhibitors for the 4 conventional and 4 novel PKC isozymes (some of this work is reviewed in Nature Biotechnology, 1998). The peptides were converted into simple pharmacological tools by cross-linking them to short arginine-rich peptide carriers that can cross biological membranes.

These inhibitory peptides are highly selective. For example, a six amino acid peptide selectively inhibits the function of PKCβII, but not the function of PKCβI. (Note that these two PKC isozymes are identical throughout their 600 of the 750 amino acids.) The peptide inhibitors were used to study the role of PKC isozymes in a variety of normal signaling events. They enabled the identification of new molecular pathways down-stream of individual isozymes, and provided particularly useful tools to understand the molecular basis of a variety of diseases. In the presentation, I will provide a number of examples on the the use of these peptides as tools to identify the role of PKC isozymes in cell signaling.

We have also developed of a simple prediction method to generate allosteric agonists (activators) for each of the PKC isozymes. Again, using sequence mining, we found short sequences of homology between several PKC isozymes and their corresponding RACKs. We reasoned that, similar to pseudosubstrate sequences found in many protein kinases, which mimic a phosphorylatable sequence in their substrates and serves as an intra-molecular interaction site, the so-called pseudo-RACK sequence in a particular PKC isozyme binds to the RACK-binding site on the corresponding PKC to maintain that enzyme in its inactive state (PNAS, 1995). We predicted that activation of PKC by second messengers exposes the RACK-binding site, enabling intermolecular association of the enzyme with its anchoring RACK. We then predicted that the difference in charge between the pseudo-RACK sequence and the PKC-binding site on RACK (the RACK sequence) is critical for the displacement of the intramolecular interaction within PKC with the intermolecular interaction between PKC and its RACK. All these predictions were confirmed using site directed mutagenesis as well as the use of peptide analogs (e.g., JBC, 2004).
These peptide allosteric agonists together with the peptide antagonists provided a set of useful tools to identify the function of each isozyme. For example, the selective inhibitor of PKCδ reduced ischemia-induced cell death of heart cells whereas the selective activator of that same isozyme increased cell death. Importantly, adding the PKCδ peptide inhibitor and peptide activator together had the same effect as treatment with vehicle, confirming that it is PKCδ that mediates damage to the heart after myocardial infarction (PNAS, 2001).

Further, using the PKC-selective inhibitors and activators, we found that different PKC isozymes have unique and sometimes opposing roles under the same conditions. For example, both PKCδ and PKCε are activated in hearts subjected to ischemia (in a model of myocardial infarction). However, whereas PKCδ activation mediates cardiac injury (Circulation, 2003), PKCε activation protects from this damage (e.g., PNAS, 1999; Circulation, 2005).

These peptide regulators of PKC isozymes were used by us and by other investigators in a variety of animal models of human diseases. One peptide inhibitor reduced pain sensation (J Pain, 2005), another reduced stroke-induced damage when given up to eight hours after stroke onset. A third peptide inhibitor was synergistic with a standard of care (angiotensin receptor blocker) for the treatment of heart failure in rats. Other peptides supported survival of cardiac transplant rejection (Circulation, 2004) and inhibited hypertension-induced blood brain breakdown (J. Clinic. Invest., 2003). These basic research tools provided the basis for KAI Pharmaceuticals, a company that has developed the technology and uses small peptides PKC inhibitors in several phase 2 clinical trials. (Further discussion of the work at KAI Pharmaceuticals will be provided by Dr. Steve Harrison.)

The project

- The problem – protein kinase C (PKC) isozyme selectivity
- The solution – competitive antagonist and allosteric agonist
- The applications – animal models of human diseases
Activated PKC isozymes are localized to distinct subcellular sites by anchoring to selective RACKs (Receptors for Activated C-Kinase)

Review of the theory, Science, 1995

Testing the hypothesis

Hypothesis:
Location of each PKC determines its function.

Inhibitors of location should inhibit the function.
Activators of location should induce the function.
Generated isozyme-selective agonists & antagonists using a **rational design**.

6-10 amino acid peptides with biological activity - nM range.

Review of some rationales; Nature Biotechnology, 1998

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**PKC inhibitory peptides, for many human diseases**

- Heart failure
- Myocardial infarction
- Stroke
- Angiogenesis (DR, AMD)
- Metastasis
- etc, etc, etc.

NMR Structural Studies of Drug Receptors in Membranes

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NMR spectroscopy is a powerful tool for investigating proteins. However, most of the proteins that interact with therapeutic drugs reside in membranes, and because these proteins are immobilized by their interactions within phospholipid bilayers, they present extremely difficult targets for structural studies. In order to obtain high resolution NMR spectra of membrane proteins is necessary to apply the highest levels of four technologies, including sample preparation, instrumentation, experimental methods, and finally structure calculations (1).

The sample preparation is crucial for the success of the experiments, and it involves a number of steps. In order to obtain milligram amounts of isotopically labeled proteins, they are expressed in E. coli using optimized fusion partners so that they form inclusion bodies. In the purification scheme they are refolded into their active conformations. The proteins are reconstituted into phospholipid bilayers with mixtures of lipids that ensure sample stability and alignment by the magnetic field of the NMR spectrometer. The photograph of a typical sample in Figure 1 (left panel) shows that the protein-containing bilayers form a solution; nonetheless, the protein is immobile on the timescales of the NMR experiment. Without rapid reorientation of the protein, it is essential to use high power irradiation and experimental methods of solid-state NMR spectroscopy. Because the samples have very high dielectric properties due to the presence of salts and the phospholipids in aqueous solution, specialized coils are essential (middle panel). The tube containing the sample is inserted inside the coil, and then radiofrequency irradiations are applied as outlined in the right panel.

Membrane proteins in phospholipid bilayers require unique samples, instrumentation, and experimental methods.

Figure 1

Two-dimensional NMR spectra of four different membrane proteins are shown in Figure 2. All four polypeptides are uniformly 15N labeled by expression in bacteria grown on chemically defined media where the sole source of nitrogen is a labeled ammonium salt. The technologies shown in Figure 1 are successful in providing individual, resolved resonance for each backbone amide site in the proteins. Importantly, even though the spectrum of the 350-residue GPCR is very crowded, the linewidths of the signals are the same as in the smaller proteins. In the spectra of the proteins with one, two, or three transmembrane helices, a distinctive wheel-like pattern can be observed. The signals from residues in the transmembrane helices form regular patterns that follow exactly those of helical wheels. The other signals are from the terminal and inter-helical loops of the proteins. The presence of the wheel-like patterns and the segregation of the resonances into distinct spectral regions are consequences of the mapping of the protein structure onto the spectra. With the assignment of the resonances to individual residues and the appropriate calculations, it is possible to determine the three-dimensional structures of these membrane proteins in phospholipid bilayers from these data (2). Moreover, these signals can be used as spectroscopic monitors of drug binding, showing which residues are responsible for the interactions.
CXCR1 is a chemokine receptor involved in inflammatory disease. Like many other G-protein coupled receptors (GPCRs) it binds to peptide ligands, in this case the protein IL-8. As seen with the spectral comparisons in Figure 2, this membrane protein with seven trans-membrane helices presents significant challenges for obtaining the spectral resolution needed for detecting drug binding and structure determination. The results shown in Figure 3 demonstrate that we have been able resolve individual resonance associated with both inter-helical loop and trans-membrane helix residues with two different approaches. The spectral crowding results from the presence of about 350 overlapping signals. By labeling only one type of residue at a time, a tractable number of resonances are observed in the two-dimensional spectra. In the middle panel in Figure 3, the example is isoleucine (3). By using more sophisticated and higher dimensional spectroscopic experiments, it is possible obtain the necessary resolution from uniformly labeled samples. The orientationally dependent frequencies that are the sources of the spectral resolution also provide the input for drug-induced spectral changes and the constraints for structure calculations.

The presentation will describe the development and application of this approach to screening and structure-based drug discovery for GPCRs and other membrane-bound receptors.

References
Update on Development Trends for Peptide Therapeutics

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Introduction

Peptides are attracting increasing attention as therapeutics. To date, four of these products have reached global sales over US$1 billion: glatiramer acetate (Copaxone; $3.18 billion), leuprolide acetate (Lupron; $2.12 billion), goserelin acetate (Zoladex; $1.14 billion), and octreotide acetate (Sandostatin; $1.12 billion). In addition, exenatide (Byetta) and the recombinant peptide teriparatide (Forteo) are nearing the $1 billion benchmark, with global sales of $751 and $780 million, respectively, in 2008. The increasing interest by the pharmaceutical industry in developing peptides as drugs is at least partially a consequence of the now widespread acceptance of protein therapeutics by physicians and patients, and development of solutions to problems such as short half life and delivery of the molecules.

To track trends in the clinical development and marketing approval of peptides, Tufts Center for the Study of Drug Development and Ferring Research Institute compiled publically-available data for a total of 435 peptides that entered clinical study sponsored by commercial firms. We focused our analysis primarily on therapeutics, which comprised 77% of the data set, although peptide vaccines and diagnostics were also included. Our results provide a historical overview of peptide therapeutics development, and may inform strategic planning in this area.

Overview of therapeutic peptide development

A total of 334 therapeutic peptides were included in the data set. Of these, approximately equal numbers were in development (131 candidates) or had been terminated (149 candidates), while a total of 54 had been approved for marketing. The candidates in development included 41 at Phase 1, 72 at Phase 2, 16 at Phase 3 and two in regulatory review. Although peptides have been studied as drugs for decades, the rate of entry into clinical study was low prior to the 1980s. The average number of new candidates entering study per year has steadily increased; this number was 1.2 per year in the 1970s, 4.6 per year in the 1980s, 9.7 per year in the 1990s, and 16.8 per year so far in the 2000s (Figure 1).

Since the 1960s, peptides have been studied as treatments for a wide variety of indications. During 2000-2008, peptides entering study were most frequently treatments for cancer and metabolic disorders (18% and 17%, respectively). The percentage of candidates for metabolic disorders represents a notable increase from the 1980s and 1990s, when 2% and 11% of peptide therapeutics, respectively, were studied in this category. Indications such as diabetes, obesity, and osteoporosis are included in the metabolic category. Decreases were observed in the study of peptides as treatments for allergy and immunological disorders, as well as for cardiovascular disease.
The majority of peptide candidates targeted extracellular molecules, with less than 10% known to bind intracellular targets. The most common extracellular targets during the 1980s, 1990s, as well as 2000-2008 were G-protein coupled receptors (GPCRs). This family of receptors includes nearly 1000 transmembrane proteins that activate cellular responses. GPCRs are the target of numerous marketed drugs, as well as clinical candidates that are intended as treatments for a variety of indications. Of novel peptide candidates that entered clinical study during the 1980s, 1990s and 2000-2008, GPCRs were targeted by \( \text{1000} \), \( \text{950} \) and \( \text{800} \), respectively, and most of these had agonist activity.

**Approved products**

A total of 54 therapeutic peptides in the data set were approved by at least one regulatory agency, although four were subsequently withdrawn from their markets. Of the products now approved, 26 are currently marketed in the US and other countries and 28 are marketed only outside the US. Many (81%) of the products were approved during the 1990s and 2000s. Products were approved in an array of therapeutic categories, with the largest number of approvals in the oncology (17%), and obstetrics/gynecology (9%) and allergy and immunological categories (9%). Of the US-marketed products, 10 were approved after 2001 (Table 1).

### FDA approvals: 2001-08

<table>
<thead>
<tr>
<th>Company name</th>
<th>Generic name</th>
<th>Trade name</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Johnson &amp; Johnson</td>
<td>Nesiritide</td>
<td>Natrecor, Noratak</td>
<td>2001</td>
</tr>
<tr>
<td>Lilly</td>
<td>Teriparatide</td>
<td>Forteo</td>
<td>2002</td>
</tr>
<tr>
<td>Trimeris</td>
<td>Enfuvirtide</td>
<td>Fuzeon</td>
<td>2003</td>
</tr>
<tr>
<td>Praelis</td>
<td>Aprelixis</td>
<td>Plenaxis</td>
<td>2003*</td>
</tr>
<tr>
<td>Elan</td>
<td>Ziconotide</td>
<td>Prialt</td>
<td>2004</td>
</tr>
<tr>
<td>Amylin</td>
<td>Pramlintide</td>
<td>Symlin</td>
<td>2005</td>
</tr>
<tr>
<td>Amylin</td>
<td>Exenatide</td>
<td>Byetta</td>
<td>2006</td>
</tr>
<tr>
<td>Ipsen</td>
<td>Lanreotide</td>
<td>Somatuline, Angiopatin</td>
<td>2007</td>
</tr>
<tr>
<td>Amgen</td>
<td>Romiplostim</td>
<td>Nplate</td>
<td>2008</td>
</tr>
<tr>
<td>Ferring</td>
<td>Degarelix</td>
<td>Firmagon</td>
<td>2008</td>
</tr>
</tbody>
</table>

*Withdrawn from US market in May 2005 due to poor sales

Probabilities of approval success are important for strategic planning of product pipelines. Based on the data currently available for candidates with known fates (approval or termination), the approval success rates for peptide candidates that entered clinical study during 1980-2000 were in the range of 23-26%. Many of the candidates that entered clinical study during the 2000s remain under investigation.

Clinical and approval phase lengths are also important benchmark measures. To determine phase lengths that would be useful for planning purposes, we examined data for 15 peptide new molecular entities that were approved by FDA after enactment of the Prescription Drug User Fee Act (PDUFA) of 1992. This legislation, and subsequent reauthorization acts, defined timeline goals for FDA review of candidates based on a two-tier ranking system. Under the current guidelines, candidates are given either a priority or standard review, with performance goals of six and ten months, respectively, for the time to FDA’s first action on an application.

For all 15 products, the average clinical and approval phases were 103.0 and 24.8 months, respectively. The total time from the initiation of clinical studies to FDA approval was thus 127.8 months (Figure 2). The average clinical phase for the six priority reviewed products was 104.2 months, which was 28% shorter than the average for all 15 NME peptides. Standard-reviewed products had an average clinical phase of 122.3 months, which was 19% longer than the average for all products. Priority reviews are given to candidates that are treatments for serious or life-threatening diseases, or that might represent a significant improvement in the treatment of a disease. Candidates that fit this description are eligible for FDA programs such as Fast Track that may shorten the clinical phase. Indeed, three of the five products with the shortest clinical phases had either fast track designation or received an accelerated approval.
Future directions

Peptide therapeutics research and development is dynamic, with increasing numbers of candidates entering clinical study in a wide variety of therapeutic categories. We anticipate that the pharmaceutical and biotechnology industries will continue to focus on these versatile molecules because of the increased acceptance of injected drugs on the market, the availability of new formulation and delivery technologies, and the relatively high approval success rates. In particular, peptide candidates for metabolic disorders such as diabetes, obesity, osteoporosis that are prevalent in a sedentary, aging population are likely to enter study in increasing numbers. More than 15 candidates are in Phase 3 clinical studies or regulatory review, which suggests that peptide therapeutic products will continue to be approved at a steady pace in the future. Our research results represent a baseline against which future growth will be measured.
Stapled Helical Peptides: A New Drug Modality

Tomi Sawyer, Ph.D. | Chief Scientific Officer, Aileron Therapeutics
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The unique properties of stapled helical peptides are being advanced by Aileron Therapeutics and its collaborators to advance a new drug modality to address challenging intracellular therapeutic targets for varying diseases.

Stapled helical peptides exemplify synthetic biologics to bridge the gap in drug development between small molecules and more traditional biologics. Stapled helical peptides have high specificity, proteolytic stability, efficient cell penetration, excellent pharmacokinetics, and gain-of-function (dependent on the intracellular mechanism).
**Slide 3: α-Helical Protein-Protein Interaction Target Space**

A structural database has been created by Paramjit Arora to provide a framework for the systematic analysis of α-helical protein-protein interaction target space. The plethora of therapeutic targets may be functionally correlated to all major areas of human diseases.

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**Slide 4: Stapled Helical Peptides: A Wide Diversity of Promising Receptor, Cytosolic and Nuclear Therapeutic Targets**

Aileron and its collaborators are aggressively investigating stapled helical peptides relative to varying receptor, cytosolic and nuclear therapeutic targets. Noteworthy success in drug discovery have been achieved for Mcl-1 and Notch.
**Slide 5: Stapled Helical Peptides: Cell Penetration**

A working model of active transport (endocytosis) is emerging with respect to studies focused on understanding the cell penetration properties of stapled helical peptides. Currently, there is evidence showing energy- and temperature-dependence and receptor-independence.

**Stapled Helical Peptides: Cell Penetration**

A working model of active transport (endocytosis)

- Energy Dependent
- Temperature Dependent
- Receptor-Independent

**Slide 6: Cancer: Some Known ‘Hot’ Therapeutic Targets**

The structural and functional properties of several therapeutic targets having critical roles in cancer are being investigated by Aileron and its collaborators. In particular, Mcl-1 has been identified as a highly over-expressed protein in human cancers.

**Cancer: Some Known ‘Hot’ Therapeutic Targets**

- BIM:MCL-1
- MYC:MAX
- HIF-1α:p300
- NOTCH:CSL

*Leveraging α-helical protein–protein interactions*
The Bcl-2 family of anti- and pro-apoptotic proteins is well-understood to play critical roles in the pathology of human cancers. This family exists as a number of proteins having varying structural homologies relative to the prototype Bcl-2 protein. Stapled helical peptides corresponding to the BH3 domain of the BH3-only subfamily possess pro-apoptotic modulatory functional properties.

Aileron has developed a series of BIM BH3 stapled helical peptides which leverage Bcl-2 family modulatory properties with respect to multi-target selectivity, overcoming known resistance (as known for small molecule drugs such as related to ABT-737), and show promise for a wide range of human cancers.