

Summary of Outcomes of ACC&D Scientific Think Tanks: Immunocontraception and Gene Silencing – *Rhodes*

In 2009, ACC&D organized two “think tanks” with support from the Found Animals Foundation and the Animal Assistance Foundation. The purpose of these think tanks was to bring together experts in research, development, cat and dog reproductive physiology, and drug and vaccine development to brainstorm about how emerging technologies might be brought to bear on the problem of developing a safe, effective and permanent (or long-term) contraceptive/sterilant for cats and dogs.

The first think tank, on the potential of the use of gene silencing, was held in October in Denver, Colorado, and focused on the use of interfering RNA or other gene silencing approaches. The second think tank, on the use of immunocontraception approaches, was held in Roanoke, Virginia.

Why these two topics? The concept of using interfering RNA or other gene silencing technologies is on the cutting edge of human medicine, and shows great promise in certain therapeutic areas, but researchers interested in the human medical applications and technology had not considered targeting genes involved in reproductive control, and particularly not in animals. Given the promise of this innovative approach, it made sense to begin to stimulate key researchers in this area to take an interest in the application of gene silencing to sterilizing cats and dogs.

For 40 years or more, research on immunocontraception has been extensive. One immunocontraceptive, a vaccine using porcine zona pellucida, has been used for years to control wild horse populations (Jay Kirkpatrick, Zoo Montana). A second immunocontraceptive, Gonacon®, a GnRH vaccine developed by the National Wildlife Research Center, has recently achieved regulatory approval from the Environmental Protection Agency (EPA) for use in deer. So why do a think tank on immunocontraception now? All current approaches either require multiple treatments, or have limited effectiveness long-term when given as a single injection. There are significant innovations that are being developed to improve the immune response, and to extend its duration. It was time to explore alternative approaches to immunocontraception for cats and dogs — seeking approaches for a single administration, and stimulating lasting immunity for use in feral cats and stray dogs, where multiple treatments are impractical.

Gene silencing think-tank

A distinguished panel of experts (see below) discussed pathways in which inhibition of a gene might interfere with reproduction and fertility, including master reproductive hormone, germ cells, pheromone signals, fertilization, embryo implantation, and trophoblast/embryo support. Because the goal is a permanent sterilant, participants discussed avoiding targets where cell regeneration would make a permanent treatment challenging.

Many potential targets were discussed, as well as the issues involved with delivery systems. Delivery of a gene silencing siRNA to cells and tissues is a significant challenge. Also, because of the novelty of the approach, regulatory approval will be more challenging.

The panel came to the following conclusions and research agenda (details are in the full think-tank summary report, posted on the ACC&D website). The physiology of cat and dog reproduction needs to be better characterized to help in validating targets for gene silencing. Particular areas of interest include timing of oocyte release, annotation of the cat and dog genome to help gene identification, including genetic diversity in populations, better understanding of GnRH receptor location and function, and characterization of toll-like receptor distribution and function, since these can impact the processing of RNA therapies.

The panel also recommended the creation of a resource of basic cat and dog molecular information and/or samples for interested researchers, and better characterization of molecular aspects of the cat and dog, male and female germ cells.

They suggested proof-of-principle experiments using mice or other model systems to demonstrate that RNAi or similar therapies can be targeted and shut down the systems of interest, and the characterization of long-term effects of lack of GnRH by using mouse models.

The group pointed out that there may be additional opportunities for a specially focused symposium to attract experts in various aspects of this research (e.g., gene therapy, stem cell biology), and to present to them what is known about cat and dog biology to stimulate interest in any feasible approach that is likely to be safe and could lead to permanent sterility.

Immunocontraception think-tank

The immunocontraceptive vaccines that have been developed were reviewed, including porcine zona pellucida (PZP) vaccines and gonadotropin-releasing hormone (GnRH) vaccines. Potential molecular targets were discussed, such as luteinizing hormone and its receptors, follicle stimulating hormone and its receptors, and germ-line or reproductive organ-specific proteins.

Safety is a significant concern when raising an immune response to a particular protein, and the experts emphasized the need to confirm that the expression of the target protein was restricted to the tissue of interest, to avoid adverse side effects.

Innovations in vaccine technology were discussed, including DNA vaccines, new delivery systems, vectors and adjuvants. The regulatory path for vaccine approval in the U.S. is complicated and any new technology will have to consider its potential regulatory path. The Environmental Protection Agency (EPA) is regulating immunocontraceptives for wildlife (and possibly for feral cats), the FDA has indicated they intend to regulate immunocontraceptives for pets (dogs and cats), and the USDA has in the past few years approved a GnRH vaccine (Pfizer) for treatment of benign prostatic hypertrophy in dogs.

The panel expressed enthusiasm for prospects of success with immunocontraception and came to the following conclusions and research agenda (details are in the full think-tank summary report, posted on the ACC&D website).

Prior to getting to a one-injection permanent sterilant, incremental research goals will be necessary, and creative approaches may include experts from engineering, reproductive biology, immunology, and virology. The group supported further investigation into the application of GnRH vaccines to dogs and cats, applying modern vaccine design strategies.

Short-term cell-mediated responses to destroy reproductive tissue could induce sterility, and this area should be further explored. Like the gene silencing group, the experts agreed that additional research on dog and cat reproductive biology was sorely needed, and added that research on dog and cat immunology would be very helpful in understanding how to stimulate and extend immunocontraceptive effectiveness. To avoid having to re-treat an animal, the group suggested research into technologies such as encapsulation/biomaterials or other methods of introducing a delayed release bolus for a second vaccine dose (booster).

If surrogate markers for long-term sterility could be found, they could shorten clinical trials. The experts emphasized that, unlike RNAi approaches, which are at a much earlier stage of research, vaccine research needs to be done in dogs and cats, since mice and rats are not good models for cat and dog immune responses.

Consideration of the safety of any approach is important for the treated animals, the humans administering the treatment, and the environment. The group suggested that ACC&D might consider other applications and partners — such as partnering with public health groups and organizations focused on controlling rodent pest populations.

Summary

Clearly, the results of these think tanks give innovative ideas into research directions for the future in these two promising areas, and will serve as guideposts as new researchers with new ideas using new technologies bring their expertise to bear to help develop new dog and cat contraceptive/sterilant approaches. ACC&D would like to thank the funders who made this work possible, and the experts who participated.

Gene silencing think-tank attendees

Foundation representatives:

Joyce Briggs, MS	President, Alliance for Contraception in Cats and Dogs, Portland, Oregon
Shirley Johnston, DVM, PhD	Director of Scientific Research, Found Animals Foundation, Los Angeles, California
Kevin Morris, PhD	Director of Research, Animal Assistance Foundation, Denver, Colorado

Scientific panel:

Greg Dissen, PhD	Staff Scientist, Oregon National Primate Research Center, Beaverton, Oregon
Norman Hecht, PhD	William Shippen Jr. Professor of Human Reproduction, Center for Research on

	Reproduction and Women's Health, Department of Obstetrics and Gynecology, University of Pennsylvania Medical Center, Philadelphia, Pennsylvania
John Herr, PhD	Professor of Cell Biology and Director of the Center for Research in Contraceptive and Reproductive Health, Department of Cell Biology, University of Virginia, Charlottesville, Virginia, CEO of ContraVac, Inc. (www.ContraVac.com)
Michelle Kutzler, DVM, PhD	Associate Professor of Theriogenology, Oregon State University College of Veterinary Medicine, Corvallis, Oregon
Dennis Lawler, DVM	Retired Veterinary Clinical and Research Scientist, St. Louis, Missouri
R. Michael Roberts, PhD	Curators' Professor of Animal Science and Biochemistry, University of Missouri, Columbia, Missouri
John Rossi, PhD	Chair and Professor, Molecular Biology, Dean, City of Hope Graduate School of Biological Sciences, Duarte, California
Michael Skinner, PhD	Professor, School of Molecular Biosciences, Director, Center for Reproductive Biology, School of Molecular Biosciences, Washington State University, Pullman, Washington

Dr. Carl Johnson, Executive Director for Science at the Hereditary Disease Foundation, moderated the think tank.

Immunocontraception think-tank attendees

Foundation representatives:

Joyce Briggs, MS	President, Alliance for Contraception in Cats and Dogs, Portland, Oregon
Shirley Johnston, DVM, PhD	Director of Scientific Research, Found Animals Foundation, Los Angeles, California

Scientific panel:

Harini Bagavant, MBBS, PhD	Assistant Professor, Department of Medicine, Division of Nephrology and Center for Immunity, Inflammation, and Regenerative Medicine, Virginia School of Medicine, Charlottesville, Virginia
Stephen Boyle, PhD	Professor of Microbiology, Director, Center for Molecular Medicine and Infectious Diseases, Department of Biomedical Sciences and Pathobiology, VA-MD Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, Virginia
David A. Brake, PhD	Founder and Principal, BioQuest Associates, LLC
Scott Coonrod, PhD	Associate Professor of Reproductive Biology, Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, New York
Roy Curtiss III, PhD	Director, Center for Infectious Diseases and Vaccinology, Biodesign Institute, Professor, School of Life Sciences, College of Liberal Arts and Sciences, Arizona State University, Tempe, Arizona
Gregg Dean, DVM, PhD	Professor and Director, Center for Comparative Medicine and Translational Research, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina
Julie Levy, DVM, PhD, DACVIM	Director, Maddie's Shelter Medicine Program, College of Veterinary Medicine, University of Florida, Gainesville, Florida
Colin R. Parrish, PhD	John M. Olin Professor of Virology, Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, New York
Beverly Purswell, DVM, PhD	Professor of Theriogenology, Department of Large Animal Clinic Sciences, VA-MD Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, Virginia
Paul Christopher Roberts, PhD	Associate Professor of Virology, Department of Biomedical Sciences and Pathobiology, Center for Molecular Medicine and Infectious Diseases, VA-MD Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, Virginia
John T. Schiller, PhD	Senior Investigator, National Cancer Institute, NIH, Bethesda, Maryland

Dr. Stephen Boyle and Dr. Scott Coonrod co-chaired the think tank.