

MEETING REPORT

# Biomarkers in veterinary medicine: establishing a new international forum for veterinary biomarker research

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BioCity Nottingham hosted the first international conference dedicated to biomarkers in veterinary medicine in July 2009. The conference attracted over 90 delegates from academia and the private sector. This 2-day meeting was intended to promote the field of veterinary biomarkers and their applications in veterinary medicine and science. The principal sponsors were Novartis Animal Health, Pfizer Animal Health and the WALTHAM Centre for Pet Nutrition. In addition, funding was provided by Nottingham City Council, Invest in Nottingham, Nottingham Science City, Healthcare and Bioscience iNet (East Midlands Innovation), Achiever Medical, the East Midlands Bioscience Knowledge Exchange (bioKneX) and BioCity itself. Petscreen and Histologix also supported the conference from within BioCity.

Focusing on the theme 'Biomarkers in Veterinary Medicine', this multidisciplinary 2-day conference brought together some of the world's leading experts in the field of biomarkers in companion animals, making it highly relevant to both clinicians and basic scientists interested in leading edge technologies and scientific approaches that are currently used in this area of research. The main objective of the meeting was to highlight current trends in this new and emerging area of research, with specific reference to veterinary diagnostic applications. Another objective was to initiate debate about the important but frequently neglected issue of standardisation in this rapidly expanding field.

The conference was opened on Wednesday 8 July by Dr Kevin Slater, CEO of Petscreen based in BioCity Nottingham. The first session was chaired by Dr Ali Mobasheri from the School of Veterinary Medicine

and Science at the University of Nottingham. The first presentation was given by Dr Peter O'Brien, Veterinary Clinical Pathologist and Conway Fellow from the School of Agriculture, Food Science and Veterinary Medicine at University College Dublin. Dr O'Brien discussed the applications of cardiac biomarkers in veterinary medicine. His research in the areas of cardiac and hepatic cell biology and toxicology has allowed him to assess novel bioassays for troponin as a marker of cardiac disease in animals. Although widely used in human medicine for the diagnosis and prognosis of myocardial infarction, to date, cardiac troponins have found limited application as biomarkers of myocardial injury in animals. Dr O'Brien pointed out that troponins are highly conserved between species, but demonstrated that commercially available immunoassays may produce different results in different species. Of particular interest was the application of troponins as biomarkers for monitoring myocardial damage induced by chemotherapeutic agents.

After the coffee break, Professor David Argyle from the Royal Dick School of Veterinary Medicine at the University of Edinburgh gave a presentation entitled 'Stem cells and cancer stem cells as biomarkers of disease'. Professor Argyle also gave a fascinating overview of his other main research area: telomere biology and how changes in canine telomere biology may be used as an alternative model system for studying human telomere biology and for developing telomerase-targeted anti-cancer therapies for both species. He emphasised the translational research theme that ran through the whole conference. He then moved on to discuss his work on

cancer stem cells, expounding the concept that the vast majority of tumour growth can be attributed to a small population of mutated stem cells. The majority of their progeny become regular tumour cells, while a small population of the cancer stem cells are maintained and continue to drive the growth of the tumour. Existing radio- or chemotherapy treatments may kill off the bulk of the tumour, but fail to destroy the small population responsible for the growth of the tumour. He emphasised the need to search for therapeutic target biomarkers within the cancer stem cell population in order to effect longer remission.

The next presentation was given by Professor Jolle Kirpensteijn from Utrecht University Vet School. Professor Kirpensteijn continued the theme of translational oncology by discussing his research on biomarkers in canine osteosarcomas and insulinomas and highlighted key similarities between these cancers in humans and dogs. His presentation was entitled: 'Biomarkers in two malignant canine tumours: osteosarcoma and insulinoma'. He pointed out that a standardised tissue repository could help in the search for important genomic and proteomic biomarkers through the provision of tissue arrays to the research community. Looking for differences in tissue arrays obtained from the two separate populations could provide vital information about the biology of diseases such as osteosarcoma. Professor Kirpensteijn illustrated this by showing differences in gene array patterns between different canine osteosarcoma patients.

The second session after lunch was chaired by Professor Jolle Kirpensteijn. Ray Dillon, Professor of Small Animal Surgery and Medicine at Auburn University gave a fascinating presentation entitled: 'Detection of heartworm infections in dogs and cats'. Professor Dillon introduced his talk with a commercial thought – the heartworm market is one of the biggest in the world, at over \$1.2 billion. Despite this, there is no reliable diagnostic test to diagnose immature (<3 months old) heartworm infection in a dog or cat. Current tests such as physical presence in the stool/blood, heart radiography and ECGs do not detect the immature worm; a CT scan or a lung radioisotope scan may, but these are far too expensive to be used as a screening procedure. The various antigen tests only detect mature worms, and some only mature females, hence a negative result is not a guaranteed rule out result. After Hurricane Katrina, the 20 million dogs that were relocated were screened after a few months for heartworm. The results were overwhelmingly negative, and these dogs were not treated. One year later there was an explosion of heartworm. An antibody test is not stage specific, and gives no indication of a current infection, nor is a DNA test, with the added complication that DNA does not last long enough in the blood to be

an effective screening marker. Professor Dillon's presentation highlighted the need for proteomic research to identify secreted proteins and glycoproteins in blood samples from infected animals.

Professor Carolyn J Henry from the College of Veterinary Medicine, University of Missouri gave a presentation entitled: 'Clinical application of veterinary cancer biomarkers'. Professor Henry provided an overview of a number of biomarkers that have been discovered and applied in veterinary oncology. Specifically, she focused on how evolving blood tests can be used to help detect cancer in dogs and how they can be used to monitor treatment. Her presentation highlighted the paucity of tests available to veterinarians in comparison with human clinicians. Focusing on tests for canine transitional cell carcinoma and canine lymphoma, Professor Henry provided examples of how new tests should be used to ensure clinical relevance of the data, specifically on how test performance (in terms of sensitivity and specificity), should dictate whether a test is best at either 'ruling in' or 'ruling out' the likelihood of disease during the diagnostic process. She then went on to discuss three separate proteomic studies, which have revealed new biomarkers for canine lymphoma and illustrated her work using the PetScreen Lymphoma blood test as a potential tool for monitoring remission in dogs undergoing treatment for lymphoma.

Dr Ali Mobasheri from the School of Veterinary Medicine and Science, University of Nottingham discussed proteomic strategies for identifying and validating biomarkers in osteoarthritis and focused specifically on biomarkers identified in an explant model of early osteoarthritis. Dr Mobasheri used examples from his recent research at the University of Nottingham's recently established vet school to highlight how proteomic strategies can be employed for the identification and validation of biomarkers in osteoarthritis. His talk included a brief summary of chondrocyte cell and matrix biology and an overview of articular cartilage degradation in osteoarthritis. He then outlined the objectives of the NIAMS Osteoarthritis Biomarkers Network, which aims to develop and validate standardised and sensitive assays for disease biomarkers in blood and urine in the preradiological stages of osteoarthritis. Dr Mobasheri summarised the similarities between arthritis in humans and companion animals and highlighted the fact that most of the biomarkers associated with arthritis are in fact markers of the late and irreversible stages of the disease when treatment can only be expected to decrease pain and slow progression. His talk reviewed the advantages and disadvantages of the osteoarthritis biomarkers established to date. He then reviewed the strengths and weaknesses of currently available *in vitro* models for arthritis research and provided an up-to-

date summary of the applications of systems biology and proteomic technologies to osteoarthritis research. He concluded his talk by summarising new data from a targeted proteomics study of an explant model of equine cartilage inflammation, a serum free model he has established in his laboratory in collaboration with industrial partners for identification of biomarkers in joint diseases.

Professor David Eckersall from the Faculty of Veterinary Medicine, University of Glasgow provided a comprehensive update of acute-phase proteins as biomarkers of inflammation and infection in veterinary medicine. His main focus of research has been on acute-phase proteins in domestic animals. He has pioneered the use of specific protein assays in veterinary clinical biochemistry. He has developed immunoassays for proteins such as canine C-reactive protein and feline alpha-1 acid glycoprotein. He has also been involved in work on haptoglobin and serum amyloid as biomarkers of mammary gland mastitis. Professor Eckersall is also a founder and director of a University of Glasgow spin-out company called Reactivlab Ltd, which focuses on the commercialisation of acute-phase protein tests enabling their application in veterinary diagnostics.

The first session of the second day was chaired by Professor David Eckersall. Nigel Otter, Head of Igenity for Europe (a division of Merial Limited, a joint venture between Merck & Co., Inc. and Sanofi-Aventis) gave a presentation entitled 'Achievement of enhanced breeding goals in *Bos taurus* using both genomic selection and the candidate gene approach'. Dr. Otter discussed his company's achievements in the area of enhanced breeding for cattle using genomic selection strategies and candidate gene approaches. He provided an update of the bovine genome, which has recently been sequenced, following on from the successes of the human genome project earlier in this decade. He argued that genomic profiling and selection may be used to produce more uniform pens of cattle for the packer by managing animals based on genetic potential rather than other criteria. This approach will allow companies to price the biggest variable input cost based on precise genetic potential, moving pricing from averages to pricing on specific genetic merit. He pointed out that this approach could reduce total days on feed and could reduce waste. In dairy herds this approach can be used to increase milk yield and quality and reduce somatic cell counts. It may even be used to determine susceptibility to mastitis. Dr Otter concluded that genetic profiling of animals early in life adds value within coordinated production chains by combining functional genomics with the health status of the animals. Genomics can provide valuable biomarkers for procurement, production or marketing at various stages in the production chain and DNA

collected at or shortly before weaning can provide vital information, which may be used later on in life.

Dr Melissa C. Paoloni, Deputy Director of the Comparative Oncology Trials Consortium, National Institutes of Health, National Cancer Institute Center for Cancer Research, Comparative Oncology Program discussed an integrative and comparative modelling approach to developing drugs for cancer. Dr Paoloni introduced the audience to the pioneering and valuable work being done by the Comparative Oncology Program, which emphasises the importance of using naturally occurring cancer models for the evaluation of novel drugs, devices and imaging for the benefit of human and veterinary cancer research. By bringing together a consortium of clinical research institutions, the programme has access to a wide range of methodologies to promote standardisation and help develop an 'essential reagent kit' for the study of comparative models in translational research. Dr Paoloni's presentation not only focused on the biomarker approach to diagnosis, but also on the significance of biomarker discovery to find new molecular targets for novel drugs. Pointing to the future, the presentation examined how biomarkers of toxicity could improve pharmacodynamic studies and how genomic biomarkers could ultimately lead to the potential for predicting a patient's resistance or sensitivity to a specific therapeutic agent.

Dr Alex German (Royal Canine Senior Lecturer in Small Animal Medicine, University of Liverpool) arrived just in time after coffee to remind us that obesity is now recognised as a major epidemic and medical disease. Dr German summarised the potential of adipokines as biomarkers and pointed out that adipose tissue is an endocrine organ capable of producing and secreting a diverse array of inflammatory mediators. He highlighted the hidden dangers of adipose tissue and shattered the myth that white adipose tissue is an inert tissue solely responsible for energy storage. He focused his talk on the obese state in humans, companion animals and animal models drawing on recent evidence in the literature for obesity as a medical disease. He summarised new information on the potential of using adipokines as biomarkers. His research has developed an *in vitro* model of canine adipocytes, which is ideal for understanding the role of adipose derived cytokines as inflammatory mediators.

The final presentation entitled: 'The pharmaceutical perspective on the potential of biomarkers in comparative translational medicine' was delivered by Dr Theo Kanellos, Associate Director of Global Alliances at Pfizer Animal Health. Dr Kanellos discussed the potential of biomarkers in comparative translational medicine and provided a crash course on the drug discovery process, highlighting the issues of attrition and regulatory affairs. Dr Kanellos

provided an overview of the pharmaceutical industry related to animal health comparing the human and animal pathways of drug development. He reminded the attendees of the long, arduous and expensive pathway that leads to the development of new pharmaceuticals, pointing to the high attrition rates that now dominate the R&D process in biopharma. Dr Kanellos defined translational medicine as 'activities and processes required to link *in vitro* or *in vivo* animal studies of a drug to human pharmacological activity'. He also provided a clear definition of how it relates to veterinary oncology patients by proposing that translational medicine is largely, but not exclusively, concerned with the development of pharmacodynamic measures (biomarkers) that can be used in the clinic as surrogates for clinical efficacy so that project progression decisions can be made earlier. His talk highlighted the need for biomarkers and current opportunities in biomarker research in the pharmaceutical industry. He concluded his talk by discussing the regulations governing investigational drug use in companion animals.

So why 'Biomarkers in veterinary medicine'? Traditionally biomarker discovery has been the domain of human clinical medicine where proteomics and mass spectrometry dominate the arena. However, this conference revealed a wide array of biomarker sources and many different platforms for the discovery and application of biomarkers in veterinary medicine. It also demonstrated that biomarker discovery, assay validation and evaluation of applications in veterinary diagnostics is a complex process requiring a multidisciplinary approach but that real progress is being made in veterinary biomarker development.

We were privileged to attract a range of internationally recognised speakers to illustrate the diversity of the field. With subjects ranging from oncology, cardiac disease, heartworm, osteoarthritis, infection and obesity, combined with a diverse audience, the meeting presented a significant challenge to the speakers. Apart from providing a broad overview of the current state of the art, a clear outcome of this conference was the realisation that research into veterinary biomarkers is perhaps more active than many might have originally thought. Proteins represent the principal biomarker candidates, but the methods for detecting them are diverse, involving physical, functional and immunological assays. Even images obtained using a variety of different imaging modalities can be useful biomarkers of disease (MRI, CT and ultrasound to name but a few). It is clear that these approaches are going to play an increasingly important role in the future of veterinary medicine. This is fertile territory for collaboration and interaction between researchers working in veterinary biomarker and human biomarker areas.

In summary, two recurring themes emerged during the meeting: (1) the importance of translational research for both veterinary and human medicine and (2) the need for gold standard measurements when comparing results obtained from established methodologies with the new tests that are emerging from biomarker research.

It is interesting and amusing to point out that almost all of the speakers had entered the term 'biomarker' into Google and Wikipedia while preparing their presentations. However, each speaker provided a modified definition of the term 'biomarker' as it was perceived by experts in their own areas of research. Despite the demands placed on the speakers by addressing a diverse audience on wide-ranging clinical subjects, the meeting provided a great opportunity for cross fertilisation of ideas and resulted in a number of new collaborations, which is always a sign of a successful meeting.

### Establishment of the Veterinary Biomarker society

At the close of the meeting, interested delegates were invited to attend a steering group meeting with the objective of forming a new Veterinary Biomarker Society. This meeting resulted in the development of draft plans for such a society to help support, promote and standardise the application of biomarker technology in veterinary research and clinical application. We intend to provide further information about this new group in the near future and are keen to hear from potential new members.

Professor David Eckersall also participated in the steering group meeting and encouraged the establishment of a Veterinary Biomarker Society as a cross-disciplinary group to include veterinary clinicians, clinical pathologists, biochemists, basic scientists and industry experts to facilitate cross-fertilisation between these disciplines. David is also a member of a group that meets once every 2 years to push forward research on acute-phase proteins as biomarkers for inflammation and infection in animals. The next meeting of this group will be held in Helsinki in 2010.

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