# Post genomic possibilities

## Diagnostic and therapeutic opportunities arising from the sequencing of animal genomes

Kevin Slater and Shahid Mian

The sequencing of the human genome, and that of a variety of animals, is now beginning to dramatically change the landscape for diagnostic testing and for new treatment regimens. Pharmaceutical companies are investing heavily in linking diagnostic tests to specific therapies in order to provide a new generation of personalised medicines. As always, the major investments are being made in the arena of human medicine, but there is a growing body of work starting in the veterinary field. With the recent announcement in the USA of the National Cancer Institute's Comparative Oncology Program, co-ordinated clinical trials in dogs of newly developed anticancer treatments are beginning. This work involves pulling together a range of post genomic technologies and is expected to make significant strides towards improving cancer therapy for animals and humans.

The term "post genomic" refers to the raft of technologies that have developed as a result of sequencing the human and other genomes. The genome represents the total genetic composition of an organism. It is the expression of these genes, both in terms of time (from conception onwards) and location (throughout the body), that results in the development of the cells, tissues and organs that comprise each individual. The term "individual", is key here, going beyond species and breed characteristics. For many years now, veterinary and human clinicians have recognised that the same treatment might be successful for one individual, but would fail for another. This problem is not only prevalent in clinical practice, it also plagues clinical trials, resulting in a huge attrition rate for new drug compounds with associated vast expenditure and time delays before a successful drug can be launched. The pharmaceutical industry and bioscience in general have therefore been forced to reassess this process in order to supply the treatments for major diseases that we now expect and demand. The developments that are occurring in this post genomic era are beginning to make this possible.

### A molecular view of the individual

Traditionally, the term phenotype was applied to the physical expression of an organism which resulted from its genotype (genome) and its immediate environment. However, we now view the phenotype more in molecular terms. The products of gene expression (RNA, protein, carbohydrate, lipids etc) are also viewed as components of the phenotype. It was originally thought that an individual's response to a drug would be governed by the genotype, and a considerable amount of work went into studying single nucleotide polymorphisms (SNPs) as a potential means of elucidating pharmacological differences between individual's. However, rapid progress in the field means that the range of technologies applied to this work is already diversifying.

| Markers | Cancer | Sensitivity | Specificity |
|---|---|---|---|
| PSA | Prostate | 65% | 35% |
| SELDI Multi Marker Profile | Prostate | 83% | 97% |
| CA15.3 | Breast | 23% | 69% |
| SELDI Multi Marker Profile | Breast | 93% | 91% |
| CA125 | Ovarian | 35% | 98% |
| SELDI Multi Marker Profile | Ovarian | 82% | 92% |

***Table 1:*** *Comparison between the true positive (sensitivity) and true negative (specificity) rates for three different human cancers as determined by traditional, single markers and by multiple marker analysis (SELDI). Data courtesy of Ciphergen Biosystems Inc.*

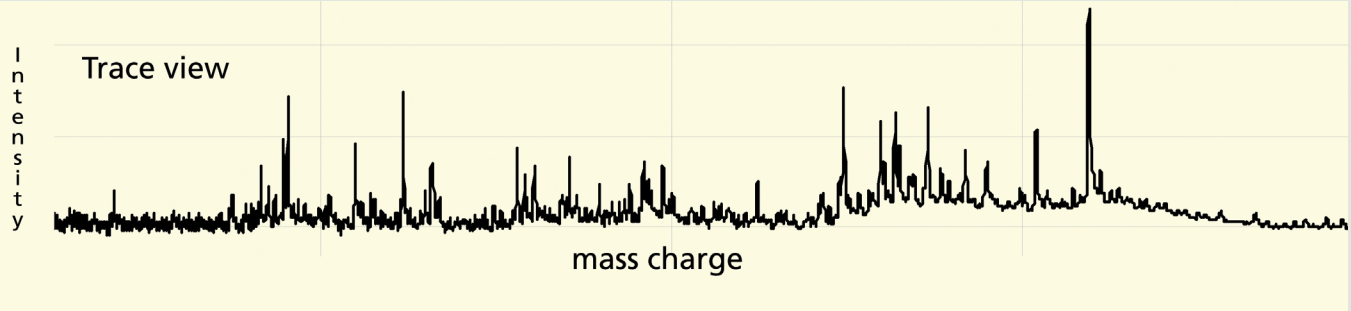


***Figure 1:*** *Proteomic profiling derived via the mass spectrometric analysis of proteins from a biological sample. The y-axis represents relative intensity value while the x-axis denotes the mass:charge value for each peak.*

Scientists are now turning their attention to the biological "workers", i.e. the structural and functional molecules that are directed by the "management" i.e. the genome. The power of the "omics" zoo is beginning to emerge, with disciplines looking at transcriptomics (total RNA composition), proteomics (all the different proteins in a biological specimen) and metabolomics (analysis of metabolite expression).

With the development of new, high throughput analytical techniques to study the various "omics", it is becoming both feasible and also convenient to monitor an organism's molecular phenotype and to look for changes associated with specific diseases.[1] For example, using a modern Mass Spectrometer, it is now possible to produce a profile of different proteins in a serum sample separated by the mass of each molecule. The resultant protein fingerprint can then be used as a complex biomarker for a given physiological state. Biomarkers are receiving considerable attention by human clinicians due to their translational application as diagnostic or prognostic indicators.[2] They have been used as phenotypic indicators for early disease detection and progression in a wide range of pathologies over several medical disciplines including oncology, renal, cardiac, immunology and neurodegeneration.

## A multi-marker approach to diagnostics

The key difference between this approach to diagnostics and traditional techniques is that the new technologies focus on multiple markers. In the example of proteomics, multiple peaks are analysed in order to make a diagnosis, whereas traditionally, the concentration of single molecule would be measured. This multiple marker approach takes into consideration the natural redundancy built into biological systems - a cell or organism will always have a number of mechanisms (for example biochemical pathways) for completing the same task. The use of multiple marker assays has been shown to make significant improvement in both true positive cases (sensitivity) and true negative cases (specificity)[3] as illustrated in Table 1.

It has also been suggested that identification of multiple markers (also known as biomarker patterns) from specific patients with defined pathologies could offer the potential for a personalised approach to healthcare.[4] The advantage of adopting such a strategy in clinical practice could facilitate the selection of treatment regimens to which a patient might possess a high probability of response, whilst avoiding therapies that are likely to be ineffectual or toxic to the patient. It is therefore not surprising that the pharmaceutical industry is already implementing this approach to help rationalise drug trials. This also signals the introduction of this technology for predictive medicine in the clinical practice.

## New screening technologies

The use of proteomics in health screening is already much nearer. In the USA, an ovarian cancer screening test will shortly be available for humans. At PetScreen, we have been adapting this technology in order to produce cancer screening for dogs. The objective is to provide a blood test for high incidence malignancies, which can be used either as a minimally invasive diagnostic test in situations where cancer might be suspected, or as a routine annual screen to help improve vigilance, particularly for those breeds with a high natural propensity for cancer.

The core technology is based around SELDI (Surface Enhanced Laser Desorption/Ionisation) protein chips and time-of-flight mass spectrometry (ToF MS), which is capable of accurately and rapidly measuring the mass of the different proteins in a biological sample. These instruments have already been extremely useful in the search for disease related biomarkers.[5-8] The sample, in this case serum, is bound to a specially designed SELDI chip which binds the constituent proteins. This is then placed into the mass spectrometer where the proteins are ionised by a high intensity laser. The ionised proteins are then accelerated through a flight chamber where they hit a detector. The speed at which the molecules "fly" is inversely proportional to their mass, resulting in the smaller molecules hitting the detector sooner than the larger ones. The output is then a spectrum comprising, in some cases, hundreds of peaks which represent the range of proteins present in the original sample (Figure 1).

Specific diseases result in the over expression of certain proteins and the under expression of others. These changes can be conveniently detected in the blood, making analysis of serum an ideal, minimally invasive source of diagnostic material. A simple comparison of two mass spectra, one derived from a dog without cancer, and the other from a dog with cancer, illustrates the difference showing both up regulation and down regulation of specific protein peaks (Figure 2). Although our focus has been on serum proteomics, the technology can be applied to other biological materials such as plasma, solid tissue extracts, urine and cerebrospinal fluid. Testing using time-of-flight mass spectrometers is very rapid, providing convenient, automated, high throughput analysis. The instrumentation is, however, very costly and requires dedicated and

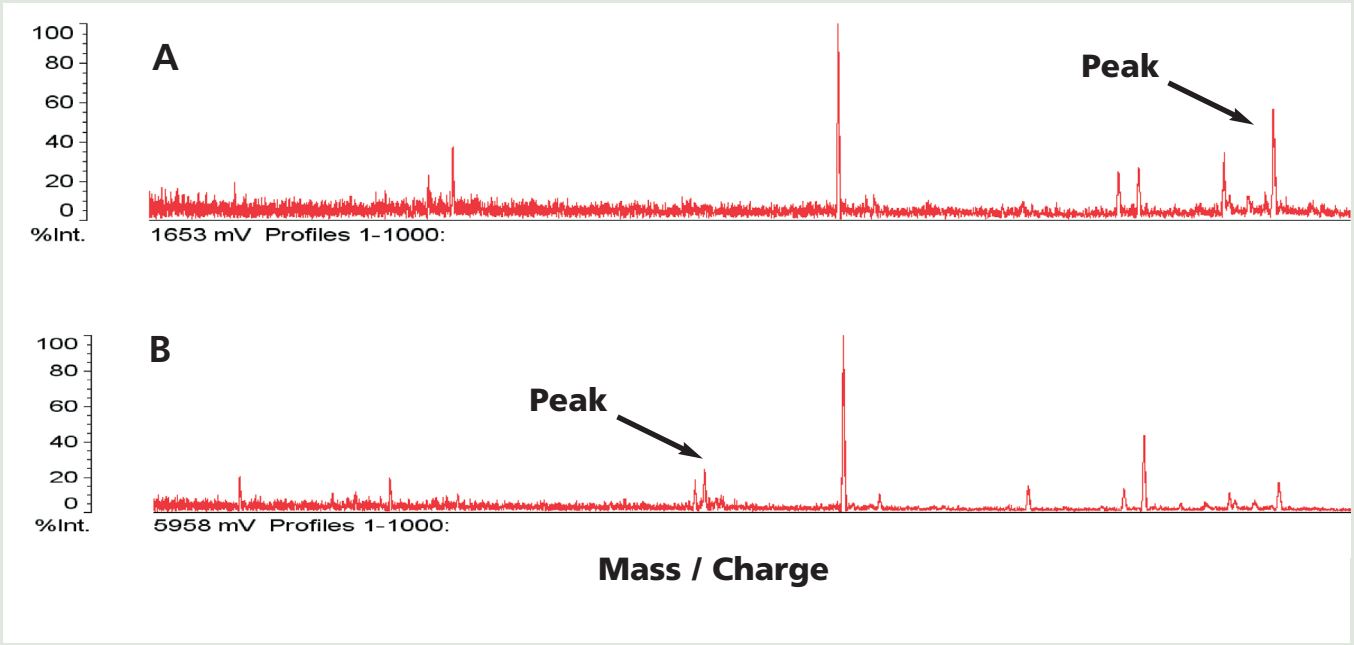


**Figure 2:** *A serum biomarker profile taken from a dog with no pathology (A) and a dog with confirmed cancer (B). In Figure 2A peak X is noted to have a higher relative intensity value than the animal with cancer. Figure 2B shows the*

precisely controlled laboratory conditions. This is not the technology for in-practice testing. Both in the human and veterinary fields, these types of tests will, for many years to come, be supplied by specialist service laboratories.

The data output from proteomic testing is vast. Analysis of these data requires advanced software run on very powerful PCs to produce meaningful interpretation. To further complicate the issue, identification of potential biomarker patterns requires the use of hundreds of specimens, from both healthy and diseased individuals, in order to develop statistically confident parameters that can form the basis of novel diagnostic assays. The power of computational analysis for clinical biomarkers lies in the fact that they can analyse literally millions of data points in relatively short time frames, providing a cohort of proteins that comprise the biomarker patterns. We have been collecting serum samples in the UK for two years now, but progress has been slow. Like many organisations, we have now turned our attentions to the United States, where we have, in a very short space of time, amassed sufficient samples for detailed and rigorous statistical analyses.

The data obtained from these analyses have demonstrated that serum biomarkers, capable of distinguishing dogs that either have or do not have cancer, can be rapidly identified. The availability of novel biomarker assays for companion animal diseases are likely to afford the same clinical benefits to the veterinary field as are emerging in human medicine. This is evidenced by the work now starting by the National Cancer Institute's Comparative Oncology Program in the United States, which is already bringing the benefits of post-genomic research to veterinary therapeutics.

## References


1. Bast RC Jr, Lilja H, Urban N, Rimm DL, Fritsche H, Gray J, Veltri R, Klee G, Allen A, Kim N, Gutman S, Rubin MA, Hruszkewycz A. Translational crossroads for *biomarkers. Clin Cancer Res* 2005 Sep 1; **11(17):** 6103-8.

2. Senzer N, Shen Y, Hill C, Nemunaitis J. "Individualised cancer therapeutics: dream or reality?" *Expert Opin Ther Targets* 2005 Dec; **9(6):** 1189-201.

3. Zhang Z, Bast RC Jr, Yu Y, Li J, Sokoll LJ, Rai AJ, Rosenzweig JM, Cameron B, Wang YY, Meng XY, Berchuck A, Van Haaften-Day C, Hacker NF, de Bruijn HW, van der Zee AG, Jacobs IJ, Fung ET, Chan DW. "Three biomarkers identified from serum proteomic analysis for the detection of early stage ovarian cancer." *Cancer Res* 2004 Aug 15; **64(16):** 5882-90.

4. Kirkwood SC, Hockett RD Jr. "Pharmacogenomic biomarkers." *Dis Markers* 2002; **18(2):** 63-71.

5. Le Naour F, Charrin S, Labas V, Le Caer JP, Boucheix C, Rubinstein E. "Tetraspanins connect several types of Ig proteins: IgM is a novel component of the tetraspanin web on B-lymphoid cells." *Cancer Immunol Immunother* 2004 Mar; **53(3):** 148-52.

6. Wulfkuhle JD, Paweletz CP, Steeg PS, Petricoin EF 3rd, Liotta L. "Proteomic approaches to the diagnosis, treatment, and monitoring of cancer." *Adv Exp Med Biol* 2003; **532:** 59-68.

7. Merchant M, Weinberger SR. "Recent advancements in surface-enhanced laser desorption/ionization-time of flight-mass spectrometry." *Electrophoresis* 2000 Apr; **21(6):** 1164-77.

8. Stanley BA, Gundry RL, Cotter RJ, Van Eyk JE. "Heart disease, clinical proteomics and mass spectrometry." *Dis Markers* 2004; **20(3):** 167-78.



**Kevin Slater BSc PhD** is Chief Executive, **Shahid Mian BSc PhD** is Technical Director, PetScreen Ltd.
www.pet-screen.com.