protein BIOMARKER strategies

The potential for parallel protein-based analyses to define a new era in diagnostics and drug development is generating considerable excitement in the field of proteomics. However, the discovery of differentially expressed proteins that correlate with a disease state or a drug response is only one aspect of the process. In this article we examine the biomarker discovery and validation approaches and the technologies employed, setting them in the context of the diagnostics and drug development markets.

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In the world of proteomics research, potential protein biomarkers (see box for definition) are low-hanging fruit. Harvesting them is easy, but bringing them to market is the challenge. In fact, the number of new protein biomarker diagnostics launched each year has slowed down to a trickle (**Figure 1**). This is an unfortunate trend because of the positive impact biomarkers can have on the drug discovery and development process and the healthcare industry. There is no proven strategy for reversing this trend, but the key decision points are rapidly taking shape. The first strategic decision is whether to adopt an empirical or a hypothesis-driven approach. The second is whether to pursue single protein biomarkers or patterns. The third is whether to use currently available technologies for validation or to take a more aggressive approach with emerging methods. And the fourth is whether to develop products and tools for the diagnostics market and/or the drug development market. All of these decisions, however, are meaningless unless the biomarkers show clear clinical benefit. The optimal decisions will likely become clear over the next two or three years, but those companies which make them now will be well ahead of the competition.

Empirical versus hypothesis-driven research

The empirical approach is based on the premise that more efficient data generation and analysis yields more efficient protein biomarker discovery. This approach relies on high-output methods,

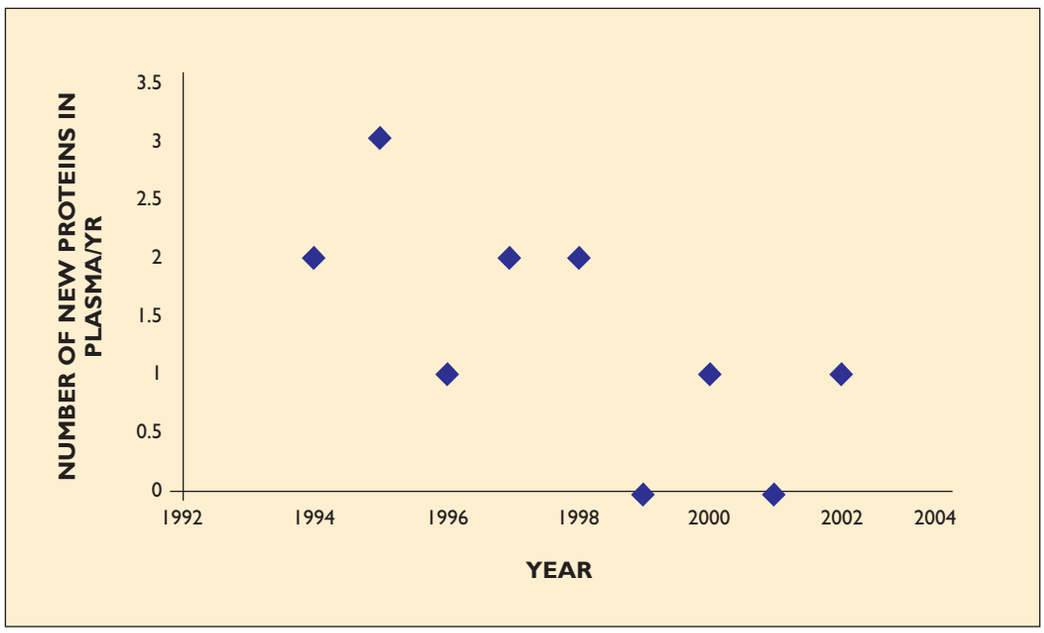

Figure 1
Rate of introduction of new FDA-approved diagnostic protein analytes[1]

such as mass spectrometry, chromatography and 2D gel electrophoresis, to generate the data and informatics systems to store and analyse the data. The technology dramatically increases output, but also dramatically reduces the role of human analysis and extrapolation. Human analysis is critical because the results of high-output proteomics studies are highly dependent on the capabilities – and biases – of the technologies (see below). Human extrapolation is critical because current proteomics technologies, although high-output, only query a small fraction of the total proteome of any individual cell type or biological fluid, let alone an entire organism. But despite these limitations, the success of proteomics technologies in identifying candidate protein biomarkers is beyond question.

Virtually every proteomics research programme is identifying potential protein biomarkers. In fact, a quantitative study reveals that for every 2,000 protein features studied, 50-300 might be identified that are uniquely or differentially expressed[2]. Current technologies routinely analyse ~2,000 proteins per experiment. Further evidence can be obtained through surveys of proteomics programmes. At a recent proteomics conference, for example, (IBC Life Sciences' Proteomics and the Proteome Conference in Basel, Switzerland, June 30-July 2, 2003), all of the researchers, from both industry and academia, who used 2D gel electrophoresis and/or mass spectrometry reported the discovery of potential biomarkers[3]. Hanno Langen, Head of Proteomics at F. Hoffman-LaRoche AG, Germany, reported the identification of more than 100 potential biomarkers from a single research programme. Similar levels of success were also documented in a recent survey of proteomics experts[4]. In fact, the rate of discovery is so high that validation is rapidly becoming a problem.

Any company with a proteomics programme is therefore in the protein biomarker discovery business, although the goals and markets may be different. Pharmaceutical companies, including Roche, have in-house programmes and are also outsourcing. The results of these programmes are not always publicised, but several biotechnology companies have placed their dedicated proteomics programs in the public eye. Ciphergen Biosystems, Inc (Fremont, CA, USA) has five Biomarker Discovery Centers® (Fremont, Baltimore, Philadelphia, Denmark, and Japan) that use the company's proprietary ProteinChip® technology, which is based on surface-enhanced laser desorption/ionisation (SELDI) time-of-flight mass spectrometry (TOF-MS). In addition, more than 45 studies using the ProteinChip platform in cancer research were published in the proceedings of the American Association for Cancer Research's 2003 meeting; candidate biomarkers with high sensitivity and specificity (see glossary for definition) were reported for lung, colorectal, breast, hepatocellular, prostate and ovarian cancers. Furthermore, Ciphergen and Biosite Inc (San Diego, CA, USA) entered into a collaboration to identify biomarkers for diagnostic assays or for therapeutic develop-

ment. Biosite will provide clinical samples and Ciphergen will search for biomarkers.

SELDI is a variation of matrix-assisted laser/desorption ionisation-mass spectrometry (MALDI-MS) that uses selective surfaces to enrich for broad classes of proteins (thereby examining a subset of the proteome) prior to mass analysis by MALDI-MS. The system is easy to operate with the attendant sample handling robotics, yielding a higher throughput than most mass spectrometry systems. Many companies, however, prefer the higher resolution and better protein identification capabilities of MALDI- or electrospray-based mass spectrometers (equipped with tandem MS/MS capabilities for identification of individual ions) combined with more defined protein enrichment strategies. SurroMed, Inc (Mountain View, CA, USA), for example, has an integrated suite of mass spectrometry-based technologies, specifically dedicated to biomarker discovery. The company has proprietary normalisation algorithms that increase the accuracy of differential LC-MS analyses and can track and quantify tens of thousands of molecular ions for each sample (**Figure 2**). The company has used this technical prowess to sign nine deals with top academic institutions and biotechnology and pharmaceutical companies. Caprion Pharmaceuticals Inc (Montreal, Quebec, Canada) recently announced biomarker discovery deals with AstraZeneca Plc (London, England) and Wyeth (Madison, NJ, USA). The company uses mass spectrometry (LC-MS and LC-MS/MS) to identify proteins from highly purified organelle preparations and serum fractions. The technology has been validated by numerous publications, including a comprehensive description of the proteins that make up the phagosome, which led to the discovery of a novel cell pathway that is used as a route of entry for pathogens into host cells[5].

All of these approaches use fractionation to reduce the complexity of the proteome, increasing both signal-to-noise and dynamic range. But the tradeoff is that biomarker discovery takes place in a subset of the proteome and, depending on the methodology employed, may be a very limited subset. This in turn increases the importance of reproducible fractionation between samples so that observed differences are not due to experimental variability. GeneProt (Geneva, Switzerland), by contrast, has attempted a comprehensive proteomic analysis of all generated fractions (not a subset of them). The company increases both signal-to-noise and dynamic range by pooling serum samples from cases and controls such that litres of material are reproducibly separated into thousands of fractions and then each of these is compared to its partner fraction. The results of this analysis will provide an assessment of the merits of comprehensive analysis and pooling, at least in the case of the serum proteome.

In addition, protein biomarker discovery need not be isolated from other discovery modalities and results. SurroMed, for example, integrates its protein data with profiles of metabolites (LC-MS) and cell-surface markers (flow cytometry) to broaden its search for biomarkers. Beyond Genomics, Inc (Waltham, MA, USA) integrates protein (peptide LC-MS), metabolite (NMR and LC-MS), and mRNA (microarrays) data. The company uses proprietary algorithms and a systems biology approach to uncover the pathways that are perturbed in specific disease states. Moreover, biomarker discovery need not be separate from drug target discovery. The her-2/neu growth factor, for example, which is present in 25% to 30% of breast tumours, is both a marker of aggressive disease and the target for Genentech's therapeutic antibody Herceptin[6].

The empirical approaches to identification of differentially expressed proteins are conceptually straightforward but technically challenging. Of the challenges, including the technical complexities associated with reproducible sample collection and fractionation, the most difficult is developing software for accurate analysis of LC-MS,

The FDA's definition of an ideal biomarker[16]

- **Specific**
  Association to a particular disease or disease state and able to differentiate among similar physiological conditions.
- **Sensitive**
  Measurable and standard baseline to act as reference point.
- **Predictive**
  Clear association between measurable state and potential conditions.
- **Robust**
  Rapid, simple, accurate and inexpensive detection of the relevant marker.
- **Bridges preclinical and clinical trials**
  Enables development of the relevant measurements and then application of those measurements in a clinical development context.
- **Non-invasive/accessible**
  Allows for use of standard biological sources such as serum and urine as the basis of measurement.

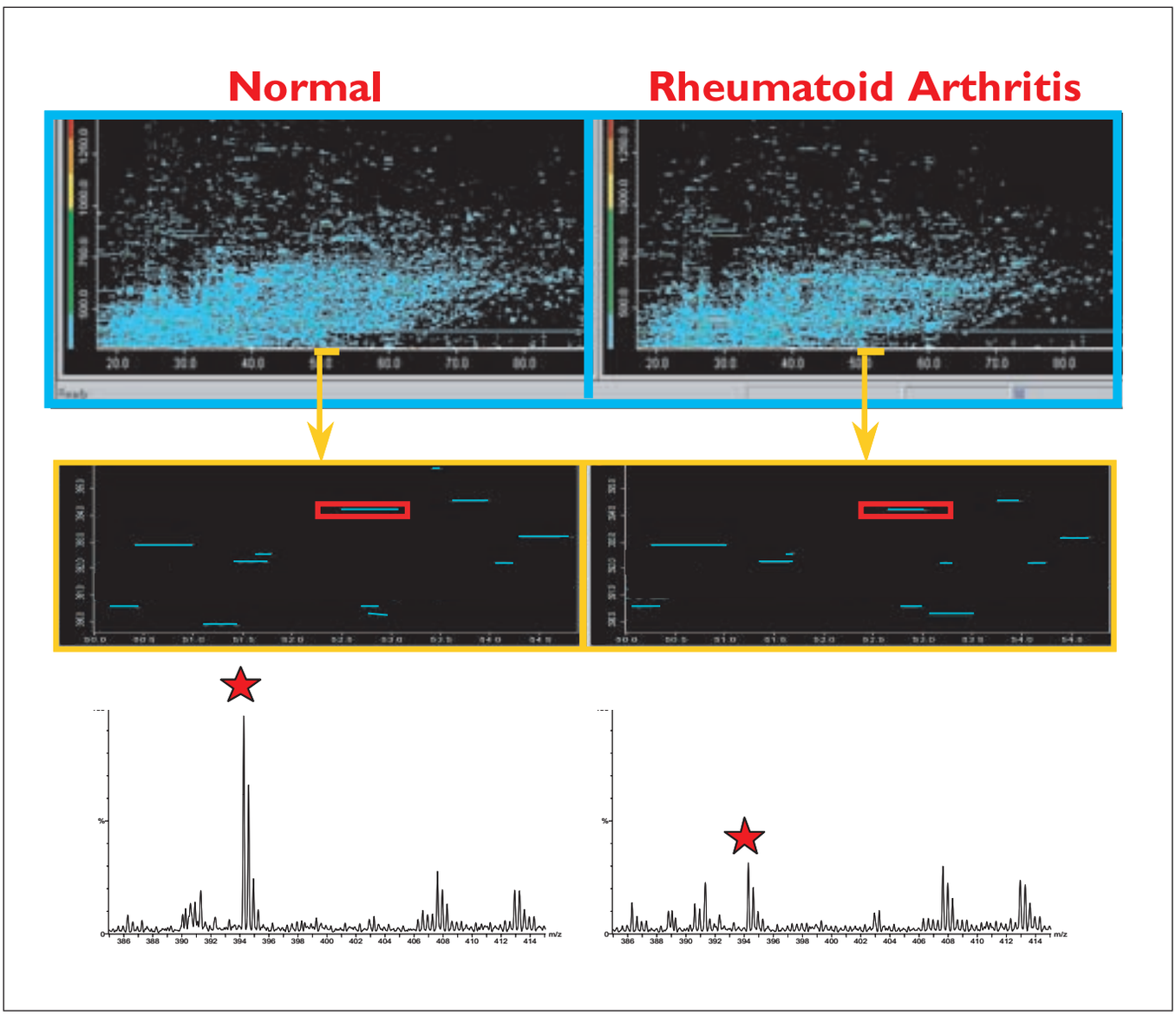

Figure 2

DeepLook™ MS (2D-LC-MS) from SurroMed: Differential expression profiling of low-abundance proteins in human serum. Six patients diagnosed with rheumatoid arthritis (RA) and not taking any medication were compared with six age- and sex-matched healthy individuals. The mass spectrometric profiles of serum samples from individuals each show about 30,000 components. Several hundred statistically significant changes are seen with a p-value less than 0.05. Six of these changes have been linked to three different peptides from lumican, a small proteoglycan found in connective tissue. The +3 molecular ion for the peptide RAFNALQYLR is shown in the figure (with an asterisk), from one patient with RA and one normal individual. The fold-change observed for this molecular ion is 1.8 (p=0.0012) and average CVs of the measurement are 29% for the RA cohort and 19% for the normal

SELDI and MALDI-MS data. The reproducible detection of low signal-to-noise features and overlapping ions in MS profile requires sophisticated algorithms. These low-abundance ions may correspond to the most interesting proteins, but if the signal-to-noise is too low, their detection may fall outside the dynamic range of the mass spectrometer (which is already only about two orders of magnitude), or may register as false negatives in one sample set because of the unreliable detection. Sophisticated algorithms for detecting low-abundance ions have been developed independently by each of the above-mentioned companies, but are not commercially available, raising the bar for others wishing to utilise these approaches in their own research

programmes. Even with reliable detection, the next challenge is identification; the software tools for accurate identification of the differentially expressed ions from tandem mass spectrometry, although advanced, are still evolving[7].

The empirical approach is still developing and has not completely replaced hypothesis-driven research. Molecular Staging Inc (New Haven, CT, USA) focuses on levels of proteins that are linked to the disease or treatment in question. The output of each experiment is lower, but the relevance of each datum is much higher. The company validated its protein biochip platform with 75 sandwich antibody assays on two biochips[8]. The company has since added additional antibody pairs based on relevance for cancer and inflammatory diseases and now has 133 sandwich pairs on five biochips[9]. The set of biochips includes cytokines, chemokines, growth factors, apoptosis markers and coagulation proteins. Molecular Staging reports preliminary success in finding potential protein biomarkers in oncology, sepsis and Alzheimer's disease and has published biomarkers of cerebral palsy identified in cord blood samples (**Figure 3**)[10]. The company has six ongoing projects with major pharmaceutical companies and has analysed more than 2,000 clinical samples. Moreover, the tradeoff between output and relevance will be narrowed in the future. Molecular Staging plans to extend the set to 160 and 170 sandwich pairs, which includes the addition of another biochip to the set. The goal for the next generation biochip is to be able to examine approximately 500 proteins, of which approximately 200 would be specific markers for cell types and reveal the presence and source of tissue damage. By continuing to ask well-posed questions, the company expects to continue to generate high-quality data. The final assessment of success awaits validation of the potential biomarkers (see below), but the optimism is supported by the early successes of DNA microarrays. The interrogation of a limited number of gene products (as all had yet to be discovered) by microarrays proved immensely useful to begin to ask biological questions on a larger scale than previously possible.

Single biomarkers versus patterns

Potential biomarkers are falling out of proteomics research at such a high rate that they can be assembled into groups. The concept is straightforward: the more partially predictive or partially diagnostic elements that are combined, the better the performance of the assay. The reality, of course, is not quite so simple, because each biomarker has inherent variability among individuals; one person could have a higher level of a particular biomarker with no disease, or *vice versa*1. If finding a cut-off value between normal and disease can be difficult for a single biomarker (due to genetic variation between individuals) the process only becomes more difficult in the case of patterns[1]. On the positive side, however, biomarkers are not inherently fraught with noise. The level of a given protein can correlate well with a particular disease in an individual, and the level of a given protein has been shown to be very stable in an individual over time[1]. Another challenge for potential biomarkers is patentability. Biotechnology and pharmaceutical companies often terminate the pursuit or commercialisation of potential biomarkers if they are unable to establish a patent position. The intellectual property minefield becomes much more daunting for patterns of biomarkers, although similar concerns about gene patents have yet to derail the development of diagnostic patterns of gene expression.

Despite the challenges, the value of patterns has been established with genes and metabolites (although interestingly, methods such as clustering algorithms and multivariate statistics were developed in the context of 2-D gel image analysis more than two decades ago[11]). Patrick Brown at Stanford University, for example, has used DNA microarrays to classify breast, gastric and pancreatic tumours. Moreover, DNA microarrays have been used to generate profiles of efficacy, toxicity and mechanisms of action of potential drugs. CuraGen Corporation (New Haven, CT, USA) has identified expression profiles of 500-600 marker genes – selected from initial screens of 22,000 genes – that are predictive for 10 liver pathologies, ranging from apoptosis to cholestasis. The company has tested several thousand

Biomarker glossary[28]

- **Power**
  The ability of the study to detect a postulated difference between treatment groups if it in fact exists.
- **Sensitivity**
  The proportion of specimens from the affected patients where the test is positive.
- **Specificity**
  The proportion of specimens from the non-affected patients where the test is negative.

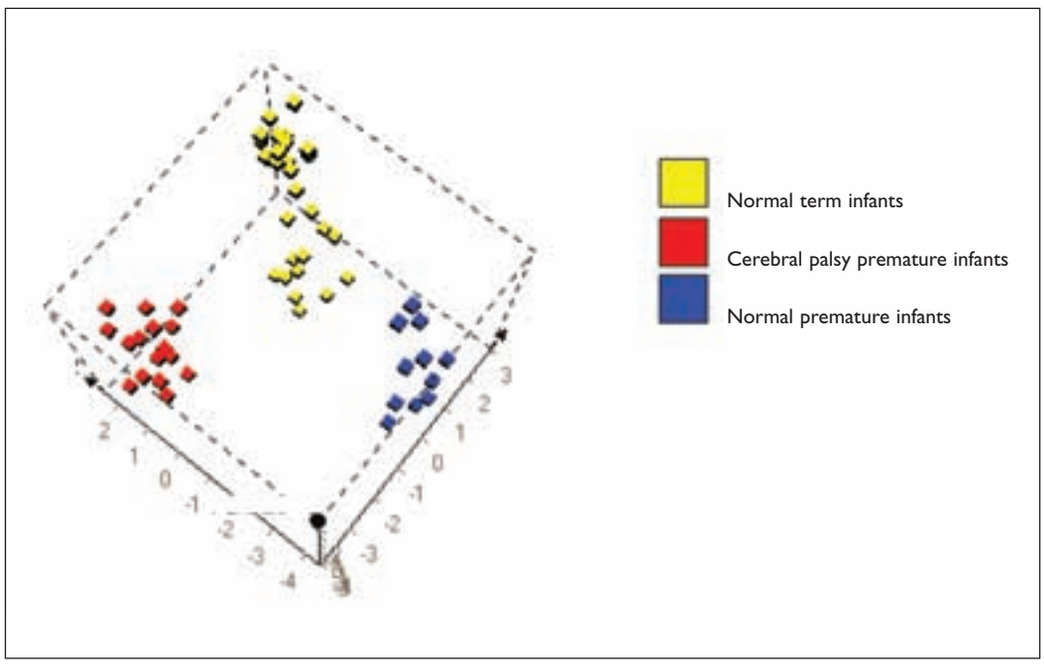

Figure 3
Principal component analysis of cytokine levels in cord blood from new born infants enables prediction of cerebral palsy[10]. Molecular Staging compared cerebral palsy (CP) babies, born prematurely, with premature normals and full-term normals. The company used linear discriminant analysis and other similar techniques to analyse the data from its protein biochip platform. They were able to distinguish babies who would go on to develop CP from those who would not with 68% accuracy (as measured by positive predictive value or cross validation) based on 2 cytokines, with 94% accuracy based on 16 cytokines, and 96% based on 22 cytokines

compounds, and the success rate is 80% for true hepatotoxic positives and 90% for true hepatotoxic negatives. Gene Logic Inc (Gaithersburg, MD, USA) examines differential gene expression in more than 300 pathways. In August 2003, the company entered into an agreement to work with the US Food and Drug Administration (FDA) Center for Drug Evaluation and Research (CDER) to contribute to the evaluation of RNA performance standards for toxicogenomics data. Iconix Pharmaceuticals, Inc (Mountain View, CA, USA) has identified 300 gene expression signatures that correspond to specific toxicological or mechanism of action endpoints. For example, the company can distinguish toxicity in the liver between necrosis and proliferative hyperplasia, and the company can distinguish mechanisms of action in the liver between acetylcholinesterase inhibition and DNA alkylation. PHASE-1 Molecular Toxicology, Inc (Santa Fe, NM, USA) has identified specific sets of gene expression biomarkers for specific pathology endpoints in liver and kidney; the company reports that the markers are more than 90% accurate in studies of predictive performance. In addition to genes, patterns of metabolites in biological fluids can provide profiles of disease and toxicity[12]. For example, a combination of changes in the urinary levels of trimethylamine-N-oxide, N,N-dimethylglycine, dimethylamine and succinate, indicate renal papillary damage, for which no biochemical markers existed previously[13].

If patterns of changes in genes and metabolites are useful biomarkers, then it stands to reason that patterns of changes in proteins would be as well. A highly publicised report came from Emanuel Petricoin, of the FDA's Center for Biologics Evaluation, and his colleagues who discovered a pattern of protein markers in serum that has a 95% success rate for identifying women with ovarian cancer[14]. Petricoin relied entirely on empiricism, surveying proteins with SELDI and using statistical analysis to find a reproducible pattern of biomarkers. Subsequent analysis of the same data, however, has raised questions about the conclusions[15]. Many potentially diagnostic ions had m/z values less than 500, which would correspond to small molecules or peptides of only 3-5 residues, indicating that the ions may not have been related to the disease process under study, but rather the sample collection or handling. Cancer and control samples could be distinguished, for example, on the basis of ions having m/z values of 2.79 (note, carbon has a mass of 12) and 245.53, which is well into the noise and represents a bias of the mass spectrometer. (The re-evaluation of the data, however, did not refute all of the original conclusions. Both studies agree, for example, that specific ions (Rule 3) greater than m/z 2000 were found that distinguished cancer versus controls with 100% sensitivity and 97.8% specificity in the test set[14].) Because of the possibility of experimental bias, the ions of interest should be identified to ensure that they are related

both to a relevant molecular species and to the disease or disease process.

In fact, given all of the possible sources of experimental bias, determining the relevance of the potential biomarkers is essential. Sample collection, sample processing and data analysis are all possible sources of bias. Moreover, each of these has numerous subpoints that need to be carefully considered in constructing a biomarker discovery study. Just taking sample collection as an example, the following points needs to be considered (and this is not an exhaustive list): patient recruitment procedures, patient demographics, patient and specimen inclusion/exclusion criteria, specimen collection procedures (ie patient standing or lying), time of specimen collection and testing (ie fasting or otherwise), duration of specimen collection (ie if blood draw, whether first or last tube collected), types of specimens collected, number of specimens collected and tested, number of specimens included in final data analysis, specimen collection devices (if applicable), and specimen storage and handling procedures[1,16].

The concept of patterns of biomarkers, however, is valid and may yet prove its value. The above-mentioned studies with SELDI often combine small groups of biomarkers, typically less than 10. Many of these patterns are currently undergoing validation, and if successful, would markedly advance the concept. Larger numbers of elements are also possible, as demonstrated by Molecular Staging (**Figure 3**) and a publication by Richard Caprioli and his research group at Vanderbilt University. The research group analysed patterns of protein biomarkers by a method referred to as imaging-MS, and mapped proteins from specific regions of 50 tissue sections (42 surgically resected lung tumours and normal normal controls) by interrogation with MALDI-MS[17]. Using this method, the group was able to identify a proteomic pattern comprised of 15 distinct mass spectrometry peaks that distinguished between patients with resected non-small-cell lung cancer who had poor prognosis (median survival six months, n=25) and those who had good prognosis (median survival 33 months, n=41, $p<0.0001$). The research group has not identified all the proteins in the pattern, but three of them are known tumour markers, providing a preliminary level of validation. Moreover, the proteomic pattern appears be associated with nodal involvement at the time of surgical lymph node dissection. The accuracy of this pattern is, in fact, comparable to standard staging techniques, and better than any other molecular markers identified to date. Since nodal involvement is one of the most important factors in determining therapeutic strategies, if this accuracy could be confirmed and improved through analysis of a larger cohort, the potential clinical usefulness of this profile could be great. As with all of the discovery methods to date the profile has enormous promise, but needs to be fully validated.

Currently available versus emerging methods for validation

Potential biomarkers are falling out of proteomics research, but their harvest is at least partially due to the inherent variability of biological systems. A change in protein levels may not be reproducible across experiments or generalisable across subjects. The only way to know is to test the potential biomarker across enough subjects to get a statistically significant result, but the high-output empirical proteomics methods that produce large volumes of data – mass spectrometry, chromatography and 2D gel electrophoresis – are low-throughput with respect to how many samples can be analysed per unit time. Clearly the high rate of candidate discovery needs to be coupled with an increase in the rate of validation. Currently the best fit for this assignment is the new, parallelised screening methods, such as beads and biochips. The challenge for these methods is the limited availability of high-quality antibodies or other affinity reagents with sufficient selectivity to be used in combination on the surface of a bead or biochip[18]. It is also essential that these reagents be generated in a timescale compatible with the discovery rate.

As a result, a decision is required. One option is to make do with existing content. Molecular Staging has demonstrated the power of this approach, although the company turned the paradigm upside down by moving protein biochips upstream into biomarker discovery. Other companies also use focused content. Zyomyx, Inc (Hayward, CA, USA) sells protein biochips to study classes of plasma proteins. BD Biosciences (Becton, Dickinson and Company), Bio-Rad Laboratories, Inc, Linco Research, Inc and Upstate Group, Inc sell kits for use with capture bead-based systems. Corning Incorporated Life Sciences (Acton, MA, USA), Hypromatrix, Inc (Worchester, MA, USA), NextGen Sciences Ltd (Cambridgeshire, United Kingdom), ProteinOne Inc (College Park, MD, USA), SomaLogic, and TeleChem International, Inc (Sunnyvale, CA,

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USA) are developing disease-specific content for their protein biochip-based approaches.

If these examples of focused content do not match up with the potential biomarkers, then the next option is to turn to emerging technologies. Multiple companies are dedicated to solving the content problem by developing platforms for large-scale, rapid production of high-quality capture agents. Affibody AB (Bromma, Sweden), BioInvent International AB (Lund, Sweden), Cambridge Antibody Technology Group plc (Cambridgeshire, United Kingdom), and Domantis Limited (Cambridge, United Kingdom) have developed recombinant methods for producing antibodies and/or antibody fragments, but have yet to implement large-scale production. Archemix Corporation (Cambridge, MA, USA), Aspira Biosystems, Inc, (South San Francisco, CA, USA) Phylos, Inc (Lexington, MA, USA), and SomaLogic, Inc (Boulder, CO, USA) are developing novel capture agents that can readily be produced in large scale, but have yet to consistently match the affinity of antibodies.

Parallelised screening methods are not the only screening tool. Ruedi Aebersold of the Institute for Systems Biology has proposed the development of a serial method using specific peptide reference reagents in mass spectrometry to increase the speed and accuracy of protein identification, and to enable absolute quantification[19]. This method would not have the same throughput as bead-based and protein biochip technologies, but could circumvent the content problem.

Diagnostics versus drug development market

The diagnostics market is established and has significant unmet needs, but is also competitive and has several significant roadblocks to success. In 2001, the worldwide *in vitro* diagnostic (IVD) market was approximately $20 billion[20]. The largest segment is immunodiagnostics, consisting of approximately 27% of the market or $4 billion. Immunodiagnostics represent the dominant technique for protein diagnostics, but the segment is languishing, with projected declines of 1% per year through 2004[21]. The declines, however, are not the result of market saturation, but rather the lack of new products (**Figure 1**). The market awaits new diagnostics. Earlier detection of cancer, for example, could dramatically improve the lives of hundreds of thousands of patients. Patients diagnosed with localised non small-cell lung cancer, for example, are approximately 20 times more likely to survive five years than patients whose disease is diagnosed with distant metastases, but fewer than 20% of cases are diagnosed at the localised stage[22]. Moreover, fine-tuning the diagnosis, such as determining nodal involvement, would guide physicians to more aggressive treatments for the patients who need them, and prevent unnecessary suffering for those who do not[17]. Fine-tuned diagnoses create the foundation for personalised medicine, hence the excitement of the data published recently by Caprioli and described above[17].

The market also awaits less expensive diagnostics, especially for patterns of protein biomarkers. The current cost is approximately $100/protein measurement, and a 15-marker panel, such as the one described above, would cost approximately $1,500[1]. This expense would generate considerable resistance from payers. The miniaturisation capabilities of biochip- and bead-based systems, as originally described by Roger Ekins, would greatly increase sensitivity and thereby reduce reagent costs[23]. Furthermore, the parallelisation capabilities would increase the number of analytes measured for the same sample volume, thereby decreasing the incremental cost of additional measurements in a panel. Moreover, more efficient production of capture agents could reduce costs to as little as $0.10/protein measurement, although a significant proportion of the savings would come from multiplexing hundreds of measurements[24].

Despite these opportunities, the diagnostics market is a challenging one. To enter the general market, diagnostics need to be approved by the FDA (or appropriate regulatory agencies in other countries), which requires the time and expense of clinical trials. Clinical laboratories also implement their own 'home brew' or analyte-specific reagent (ASR) tests, which may provide the initial clinical evaluations of biomarker assays, but these tests represent a small percentage of clinical diagnostics and must be labelled "not cleared or approved by the FDA"[1]. To be successful in the marketplace, diagnostics need to provide demonstrable clinical decision points to physicians. The risk for Alzheimer's disease, for example, can be assessed by examining the alleles for apoE, but with no specific preventive measures available, the diagnostic is not yet especially useful. In addition, the competition is intense. The top 10 diagnostics manufacturers control 80% of the IVD market in the US, and Roche Diagnostics (Basel, Switzerland) and Abbott Laboratories (Abbott Park, IL, USA) together have more than 35%[21]. This market

dominance extends into sales and distribution channels, making it difficult for new products to enter the market. Moreover, these companies are not sitting back and watching the emergence of new technologies. Roche and Abbott have made significant inroads into the rapidly growing nucleic acids market. In October 2001, Abbott acquired Vysis, which had developed several nucleic acid diagnostics, including a test for her-2/neu (see below), and was close to profitability. In January 2003, Roche licensed non-exclusive rights to Affymetrix (Santa Clara, CA, USA) GeneChip® (DNA microarray) technology for use in diagnostics, resulting in the first microarray-based genotyping kit (AmpliChip™ CYP450 Array) to enter the market, launched in June of this year.

The challenges and risks of the diagnostics market may not be commensurate with the rewards. Typical diagnostic assays only generate sales of tens of millions of dollars, whereas therapeutics can generate hundreds of millions or more[25]. The diagnostic assays for her-2/neu receptor, for example, sold by multiple vendors, generated total revenues of $22 million in 2002, compared with $385 million for Herceptin. Moreover, the entry into the diagnostics market for panels or patterns of biomarkers may be more difficult than for singlets. Roche launched the AmpliChip CYP450 as an ASR, but the FDA is questioning this status because the DNA microarray measures more than one analyte and uses a relatively complicated device[26]. The FDA may require a clinical demonstration of safety, but as yet has only requested Roche's assistance to develop a new classification. The results from this trial case will no doubt affect the classification of panels of protein analytes, which will also use new measurement technology. This risk-reward analysis changed the strategy of Millennium Predictive Medicine (Cambridge, MA, USA). The company originally intended to develop and market diagnostics, but now is using molecular diagnostics for the drug development process. The two strategies, however, are not mutually exclusive.

The use of biomarkers for the drug development market has not been established, but has significant potential. Definitive proof that protein biomarkers can improve the efficiency of the drug discovery process is probably two to three years away, after validation is completed. Then drug developers will be able to generate metrics about the time saved in animal and human testing or the number of compounds removed from the developmental pipeline due to poor efficacy or toxicity. The size of this opportunity is significant. Of the $32 billion spent on drug development by the major pharmaceutical companies in 2002, approximately two-thirds, or $21 billion was spent on testing of potential therapeutics[27].

Compared with the diagnostics market, the use of protein biomarkers for the drug development market has several advantages. From the business point of view, regulatory approval is not required for the biomarkers to be of value. In fact, the search for biomarkers can generate revenues, as evidenced by most of the business models of the above-mentioned biotechnology companies. From the scientific point of view, tying the validation process to a specific therapeutic reduces the above-mentioned content problem in parallelised screening assays by significantly constraining the relevant protein biomarkers to the therapeutic target and related pathways. If the therapeutic reduces inflammation, for example, then the most important set of biomarkers are most likely those related to the inflammatory response. In addition, from the application point of view, the drug development market has a broader range of useful markers. Measurements of on- or off-target effects could determine the efficacy and/or toxicity of a compound in both preclinical and clinical studies.

Moreover, starting out in the drug development market provides an opportunity to break into the diagnostics market as either a 'home brew' kit/ASR or FDA-regulated IVD. (However, as discussed above, the FDA may not allow a pattern of protein biomarkers to be classified as an ASR, especially if the measurement relies on new technology.) The potential protein biomarker can be initially tested with clinical samples, providing an assessment of the probability of clinical success without the expense of a dedicated trial. (However, if the developer of the therapeutic plans to use the diagnostic as a surrogate marker in clinical trials to support or demonstrate efficacy, regulatory approval is required in advance. In addition, the effectiveness of the surrogate marker would need to be confirmed in a different population to avoid circular results.) Also, by providing information about the best and worst responders, the protein biomarkers provide a clinical decision point for physicians, demonstrating clear utility and value for prevention, management, and/or treatment of disease. Furthermore, if the biomarkers are linked to a targeted therapeutic that is approved by the FDA, the diagnostic assay would be a required part of the treatment, allowing it to piggy-back on the marketing of the therapeutic,

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such as the above-mentioned case with the her-2/neu diagnostic and Herceptin.

Conclusion

The optimal strategy for generating value from protein biomarkers is not yet clear, and likely will not be for at least two or three years. Fortunately, companies can hedge against the uncertainty by combining strategic decisions. The results of empirical surveys can drive hypothesis-driven research, and hypothesis-driven research can direct empirical surveys to the most useful tissues and conditions. Groups of potential biomarkers can be validated in parallel, and the analysis can reveal whether single biomarkers or patterns have the best performance. The validation process can start with currently available technology, but maintain the flexibility to incorporate new content as it becomes available. The initial target market (or application) can be either in drug discovery, with the goal of also producing a diagnostic product that enables physicians to optimise treatments on an individual basis, or in the diagnosis of disease (where many studies are currently focused). No matter which approach is pursued and no matter how extensive the validation, however, differentially expressed proteins will not be of value without a demonstrable clinical benefit. Planning for the future of the emerging protein biomarkers industry is difficult, but the potential rewards are great. The right decisions will provide a significant competitive advantage. DDW

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