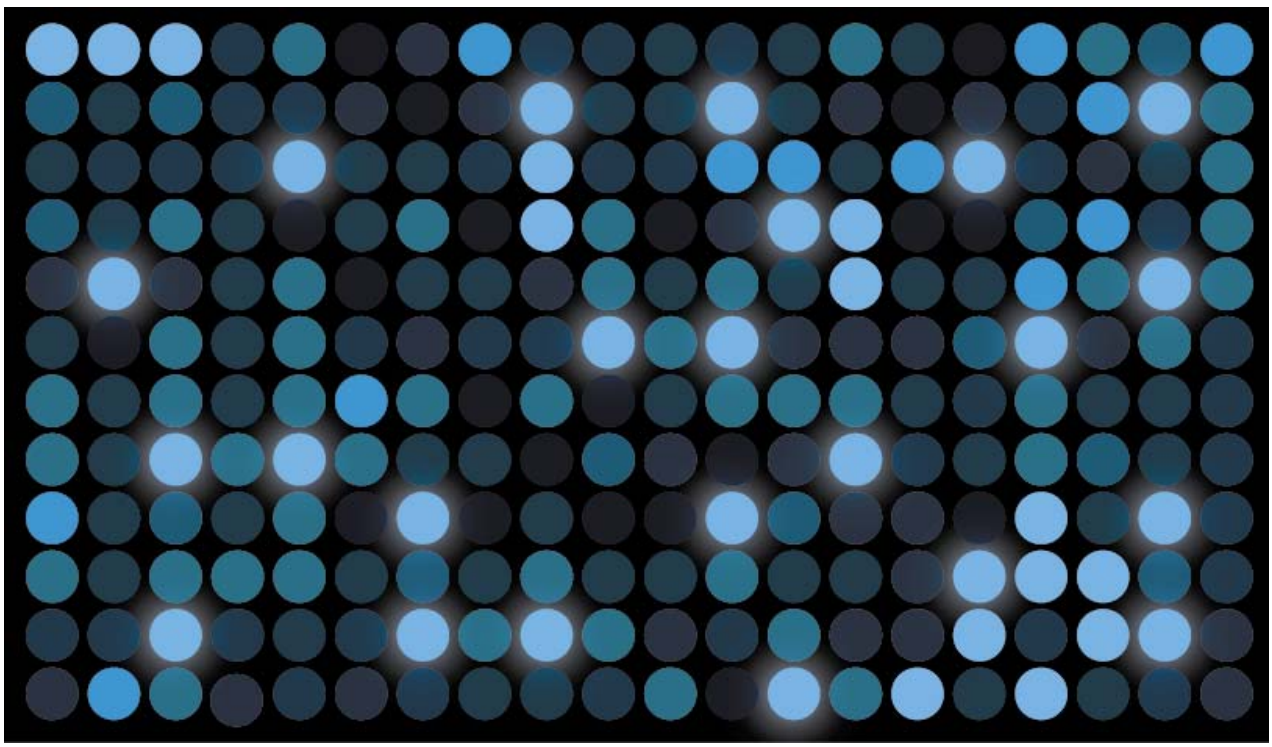
Cancer therapeutics in the 'omics' era

Moving towards practical application in the NHS

A workshop organised by the Cambridge Genetics Knowledge Park
in collaboration with the Cambridge NTRAC Centre



Workshop report and recommendations

CGKP Genetics knowledge for the benefit of society

Philippa Brice
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Cancer therapeutics in the 'omics' era:

Moving towards practical application in the NHS

Report of a workshop organised by the Cambridge Genetics Knowledge Park (CGKP), in collaboration with Cambridge National Translational Cancer Research Network (NTRAC) Centre, held in London on the 9th March 2004.

Contents

Contents **1**

Molecular genetic profiling of cancer **2**

Diagnosis and prognosis of cancers 2
Pharmacogenomics 3
Conclusions 4

Realising the potential of cancer genomics – policy issues **5**

Logistic and regulatory issues 5
Legal issues 6
Economic issues 7
Ethical Issues 7

The workshop **7**

Background 8
Presentations 8
Cancer pharmacogenetics (Professor Bruce Ponder) *8*
Microarray tumour profiling (Professor Carlos Caldas) *9*
Herceptin/HER-2 as an example of the steps and obstacles between the research finding and service implementation (Professor Mitch Dowsett) *10*
How do we go about implementing the use of new markers? (Dr Marc Van de Vijver) *11*
The role of NTRAC (Professor David Kerr) *12*

Workshop discussions **13**

Clinical implications of cancer genomics 13
Policy implications of cancer genomics 13

The way forward: recommendations **15**

References **16**

Appendix : Glossary of selected terms **18**

Cover image: US Department of Energy Genomics GTL Program, http://doegenomestolife.org

Molecular genetic profiling of cancer

DNA microarrays are systems that enable the rapid and simultaneous analysis of thousands of DNA sequences. A microarray is a solid surface chip onto which thousands of known single-stranded DNA probe sequences have been spotted in an orderly manner; microarrays may be used for a number of applications in cancer genetics including gene expression profiling and comparative genomic hybridization.

Cellular gene expression refers to the production within a cell of active, functional proteins from selected genes. Recent research has shown that particular gene expression patterns (also referred to as gene expression signatures or molecular signatures) are observed in cancerous cells and certain genes or sets of genes can be identified as biomarkers for those cancers. **Gene expression profiling** uses microarrays to identify which genes are actively being expressed within a cell; these genes are transcribed (copied) to create mRNA molecules. To analyse gene expression, mRNA from the sample cells of interest is extracted and used as a template for the production of corresponding single-stranded by a process called reverse transcription; DNA created in this manner is known as cDNA (complementary or copy DNA). A fluorescent dye is incorporated into the cDNA, which is then passed over the microarray surface. Complementary single-stranded DNA sections will bind together by forming base-pairs (a process known as DNA hybridisation), so that labelled cDNAs will become attached to corresponding probe DNAs. The presence of bound DNA is detected by fluorescence following laser excitation. Genes that are expressed in the sample cells can therefore be identified by the observation of a fluorescent light signal at the position where the known, probe DNA is bound on the microarray. The degree of fluorescence can be used to determine the relative levels of expression (abundance) of different genes. Computational analysis of microarray data can be used to identify cancer-associated genes based on gene expression patterns that show a characteristic differentiation between healthy and cancerous cells.

Comparative genomic hybridization (CGH) is a method for the identification of changes in copy-number of specific regions of DNA. Certain tumours display characteristic patterns of copy number changes (amplifications and deletions). **Array-CGH** uses hybridisation of differentially labelled tumour and reference DNA samples to whole genomic microarrays to rapidly identify copy number abnormalities, shown by changes in the ratio of tumour and reference fluorescent signal, and to map these aberrations much more precisely to the genomic sequence than is possible with standard CGH.

Diagnosis and prognosis of cancers

The appropriate diagnosis and classification of cancers is of key clinical importance, whether for selection of the most appropriate treatment regimen or for prognosis. Traditionally, cancers have been categorized based on clinical and histological signs, but the advent of gene expression profiling is revealing a new genetic taxonomy based on the molecular signatures of human tumours. Many tumours appear indistinguishable at the morphological level, but are molecularly distinct, and such molecular distinctions can be predictive of clinical outcome.

For example, gene expression profiling has been used to identify different tumour sub-types from a set of malignant breast tumours, suggesting the existence of distinct disease entities (with different associated prognoses) in what was previously supposed to be a single class of tumour (1). Microarray analysis of gene expression patterns in diffuse large B-cell lymphomas (DLBCLs) has identified disease sub-groups distinguished both by clinical outcome and by the recurrent presence of specific chromosomal abnormalities (2-4). Similarly, gene expression profiling has also been used to identify genetically distinct subgroups of acute myeloid leukaemias and prognostic sets of genes (5-7).

Prognosis is extremely important for any cancer patient; not only as a key element in selection of the most appropriate clinical response to the cancer, but also in predicting likely clinical outcome. Where prognosis is known to be good, it may be feasible to avoid unpleasant and debilitating adjuvant chemotherapy; even where the prognosis is very poor some patients will prefer to have this information. Microarray data is yielding important information about outcome in many different types

of cancer, and may allow the development of prognostic tools far superior to those currently available.

The molecular signature of metastasis, a key prognostic determinant in cancer progression, has been examined by comparison of gene expression profiles from primary and metastatic adenocarcinomas (8). Not only was a gene expression signature was found that was largely capable of distinguishing between these forms of tumour, but the presence of a similar 'metastatic signature' in primary tumours of various types was found to be associated with subsequent metastasis and poor clinical outcome.

Breast cancer outcomes are best indicated by factors such as histological tumour grade and lymph node status, but even these predictors fail to accurately classify the clinical behaviour of such tumours. Work in Amsterdam has defined gene expression profiles with reported statistically significant prognostic value (9;10); the Netherlands Cancer Institute (NKI) where these studies were carried out plans to use microarray techniques for the routine prognostic screening of cancer patients. The set of 70 genes will be used to assess the tumour profile of breast cancer patients and determine which will receive adjuvant treatment after surgery. The European Organization for Research and Treatment of Cancer is planning a large-scale trial to test the prognostic value of microarrays on lymph-node negative patients. Patients will receive adjuvant treatment based on their microarray signature, and their outcomes will be compared with patients evaluated solely according to conventional criteria. The first commercial test kits for breast cancer prognosis have appeared on the market; Oncotype DX™ (from US based company Genomic Health) was launched in January 2004 and Mammaprint (from NKI spinout company Agendia) followed two months later. The Oncotype DX test, which costs $3400 (€2870), analyse expression of a panel of 21 genes whilst the web-based Mammaprint service uses the prognostic set of 70 genes mentioned above, and costs €1650. The value of these tests has not yet been comprehensively assessed.

Although microarrays seem likely to be of critical importance in the identification of key genes associated with clinical progression and outcome, in the longer term they may not be useful in a routine clinical diagnostic setting. Once key subsets of genes have been identified and associated with the biology of a cancer, the large-scale scanning properties of microarrays may well be superfluous. Important genes may be more easily and cheaply analysed by other methods. For example, by looking at the expression levels of 36 candidate genes identified by earlier gene expression profiling studies in DLBCL patients (2-4), a later paper reported the identification of a set of just six genes whose expression was predictive of overall survival following chemotherapy, and proposed that a simple PCR-based test represented a novel diagnostic and prognostic tool suitable for routine clinical use (11).

Pharmacogenomics

Chemotherapy is an important component of treatment for cancer patients with aggressive or malignant forms of tumour. However, significant heterogeneity is observed with respect to both efficacy and toxicity; administration of the same dose of a given anticancer drug to a population of patients can result in a range of toxicity, from unaffected to lethal events (12). Only a proportion of patients respond favourably to chemotherapy, and inappropriate treatment can even select for more resistant tumour cell lines. Some patients suffer exacerbated side effects without any effective response, losing the chance to try alternative chemotherapy if their physical condition has deteriorated too far. Accurate prediction of the likely efficacy of a specific therapy is therefore an important goal in cancer research, both to improve patient care and to reduce expenditure.

It is now clear that much individuality in drug response is inherited. Polymorphisms in genes encoding drug metabolizing enzymes, drug transporters, and drug targets affect the response of an individual to a given drug. Pharmacogenomics, the study of the genetic determinants of drug response, is the basis of the ultimate goal of individualized therapy, whereby an analysis of the patient's genetic material would allow clinicians to select the most appropriate therapy and adjust dosage to maximise efficacy whilst minimising toxicity. Currently, adverse drug reactions arising from genetic polymorphisms are a serious clinical problem; one study has suggested that they may account for 1 in 15 hospital admissions in the UK (13).

Although in some cases a relatively small number of genes will have a significant effect on the response of an individual to a given drug or class of drug, in most cases drug response is likely to be a much more complex trait, controlled by polygenic determinants of drug pharmacokinetics and pharmacodynamics. Gene expression analysis using DNA microarray technology is currently being used to investigate the involvement of human genes in drug sensitivity, in the hope of finding correlations between particular gene signatures and drug response or clinical outcome suitable for predicting the most appropriate treatment options for individual cancer patients. Such studies also hope to identify potential new drug targets (14).

One small study of differences in gene expression between breast cancers samples taken prior to chemotherapy with docetaxel identified a gene signature capable of distinguishing between docetaxel sensitive and resistant tumours, distinguished by an arbitrary measure of tumour size after treatment (15). Another report of gene expression profiling in primary breast tumours before and after systemic chemotherapy identified patterns to distinguish between fully responsive and non-responsive tumours. The former group showed a much higher dynamic response of gene expression to drug treatment than the latter, leading the researchers to suggest that monitoring post-chemotherapy changes in expression profiles could be a useful predictor of response following the initial cycle of chemotherapy (16).

Almost all reports in this area cite the need for more extensive and larger-scale studies. The reliability of prediction would certainly be improved if correlation analyses incorporated more data, since it would help distinguish more clearly those markers of key importance to chemosensitivity of a tumour against a given drug or drug class.

Conclusions

Gene expression profiling is rapidly becoming an indispensable tool for cancer research, and the potential of gene signatures to guide classification and clinical management of cancer appears to be significant. However, almost all studies require further validation in independent patient data sets, although there are some cases in which independent analyses have yielded compatible data.

Much larger studies are also needed; most independent studies with compatible results have overlapping sets of genes as opposed to identical profiles. The larger the patient samples used and the more rigorous the comparative analyses, the greater the chance that the genes identified will be genuinely important biomarkers. A standardised methodology for microarray analysis would permit direct and meaningful comparisons to be made between different datasets, and wider availability (and accessibility) of databases would facilitate further analysis of study data by other researchers.

A systematic assessment of the ability of DNA microarrays to predict clinical outcomes in cancer examined 84 published gene expression profiling studies that were used to generate predictive models of outcome (30 of which included data on actual clinical outcomes), assessed the performance of the models and compared this with standard prognostic procedures based on clinical and pathological variables (17). The need for independent validation of profiling studies was emphasised as being critical to confirm proposed molecular profiles and prognostic indicators; three-quarters of the studies were found to have omitted any form of validation of their findings. The predictive performance of the prognostic molecular profiles was variable. The report proposed that larger sample sizes would be required for future confirmatory studies, along with adjustment for known predictors of outcome (such as size and stage of cancers).

To obtain reliable prognostic markers, gene expression profiling may actually need to be combined with other approaches such as proteomics. Once gene signatures are successfully validated, long-term clinical studies are also required to determine the validity of using these signatures for the prediction of patient response to chemotherapy.

Realising the potential of cancer genomics – policy issues

Logistic and regulatory issues

High-throughput gene expression analyses are accelerating the rate of discovery in cancer biology, but interpreting the mass of data they provide remains a challenge. A major pitfall is the lack of standard methodologies or vocabulary, which impedes comparison between studies. As information from gene expression profiles may ultimately affect clinical decision-making, the need for extensive validation of results is underscored.

In 2003 the National Cancer Research Institute (NCRI) launched a novel UK tumour bank, the National Cancer Tissue Resource, an initiative funded by the Department of Health, Cancer Research UK and the Medical Research Council. The aim is to develop standardization of sample and data collection and storage, to establish a network of tissue acquisition centres and to link these collection centres with processing centres where microarray data can be generated and stored (18). A central information system will track samples and provide a bioinformatics hub to link histopathological data with clinical data and research results. The NCRI Cancer Informatics network (www.cancerinformatics.org.uk) was established in 2003 to work with the international cancer research community including the US National Cancer Institute Center for Bioinformatics (NCICB) and the European Bioinformatics Institute (EBI) to encourage the use of standardised datasets and exchange mechanisms to allow the integration of data from different cancer research databases. Through the NCRI, cancer research scientists will attempt to agree on how best to record data in fields such as epidemiology, genomics and medical imaging, making it accessible to the wider research community. The NCRI have produced a strategic framework for the development of cancer research informatics in the UK. The US NCI is already developing an integrated biomedical informatics infrastructure, the cancer biomedical informatics grid (caBIG). This is intended to allow individual researchers and research groups to share collated cancer research data to facilitate knowledge sharing, reduce unnecessary duplication of experiments and drive scientific discovery in the field. The caBIG, currently being evaluated in a three-year pilot phase (2003-2006), is to provide a shared informatics platform that integrates different forms of data and supports different analytic tools.

One report observes that, since information from gene expression profiles is likely to affect clinical decision making, studies must be *"performed with statistical rigor and be reported clearly and with unbiased statistics"*. The authors recommend cross-validation with different data sets and standard methods for estimates of prediction accuracy (19). An editorial in *The Lancet* (20) points out that DNA microarray technology has yielded extensive information in oncology, including the characterisation of known cancer-related genes, and underlines the potential of these advances to bring impressive refinements to current therapeutic interventions. It cites tumour pathology as the *"best example of this unrealised potential"* with the opportunities for early detection of malignancies and the most appropriately targeted treatment for a given tumour, but notes that the corresponding clinical advances are *"frustratingly slow"* to appear. This is attributed to a lack of validation of results, due to the absence of a suitable clinical framework for testing translational discoveries. The article praises the translational initiatives of organisations such as the NCI, but warns that their efforts do not go far enough.

An article by members of the FDA on the medical applications of microarrays from a regulatory science perspective (21) also notes that in order to realise the potential of microarray technology it will be necessary to develop a cooperative framework across the scientific, clinical and regulatory communities. The authors cite the FDA's guiding principle of a risk-benefit analysis for new products. The critical issue for integration of microarray technology into medical practice is identified as the need to develop standards relevant to the reliability of microarray data, appropriate controls and references. The authors call upon members of the scientific community to demonstrate reliably the asserted linkage between genomic measurements and biological outcomes, along with a range of features relating to the reliability, accuracy and clinical relevance of each microarray application. The proposed role for regulatory scientists (in this case, the FDA) is to develop working relationships with stakeholders in order to define reasonable expectations for standards, and to produce risk-benefit analyses to assess the value and impact of new technologies on medical science.

However, the difficulty of establishing standardized regulatory guidelines for an evolving technology is noted. For instance, both microarray platforms and bioinformatics and data analysis tools continue to develop; commercial providers of microarrays aim to continually refine the quality of data (sensitivity, specificity and precision of measurements) achievable, whilst novel statistical methods for optimal data analysis are also sought. Guidelines may become easier to establish once there is a better understanding of study design and limitations, and data interpretation, but a certain level of minimal standards need to be proposed in order to allow the integrity of study data and conclusions to be evaluated. These might include standard operating procedures for performing, evaluating and interpreting gene (and protein) arrays. Databases are cited as a valuable resource via which individual microarray datasets could be usefully validated.

The International Genomics Consortium (IGC), a US-based non-profit genomics research institute, is developing a clinically annotated public gene expression database for cancer. The Expression Project for Oncology (exp*O*) aims to create a clinically annotated database of gene expression profiles of 2500 human tumour specimens and 500 normal tissues, collected with consent under standardized conditions and de-identified for public access. The database is intended to facilitate the discovery and validation of new diagnostic markers and therapeutic targets, to accelerate the development of clinical tools for improved management of cancer patients, and conforms to guidelines proposed by the Microarray Gene Expression Data Society (MGED), to allow unambiguous integration of information (22). It is hoped that this will also compel other researchers to conform to the guidelines and thereby produce more uniform, comparable data (23). Several key journals including *Nature*, *Cell* and *The Lancet* have already adopted these MIAME (Minimum Information About a Microarray Experiment) guidelines for the submission of microarray expression data.

Legal issues

The abuse of any genetic data is a possibility, especially where data includes diagnostic or prognostic information related to disease. Privacy protection is a key concern for individual genomic and pharmacogenomic data, primarily the implications of personal health data being accessed by insurers or employers. Legal protection is necessary to safeguard databases of genomic information if they become a component of healthcare provision; however, the potential benefits of DNA and cancer tissue banks may be negated by unnecessarily restrictive legislation. Tissue bank donors have rights to autonomy and privacy; the latter is a key issue because research aims to correlate clinical data with data obtained from the tissue (*eg.* biomarkers). The legal framework for tissue banking and research is still fragmentary, and there is no international consensus. Legal safeguards are currently more easily guaranteed in Europe than the United States, as insurers do not own health-care facilities (24).

The United Nations Educational, Scientific and Cultural Organization (UNESCO) has adopted an International Declaration on Human Genetic Data produced by the international bioethics committee, which calls for the protection of human rights in the collection, processing, storage and use of genetic data and the biological samples from which it is derived (25). The declaration, ratified in October 2003 by UNESCO's General Conference, also proposes governmental monitoring of the transfer of samples and data between countries and underlines the importance of proper consent procedures. It is stated that the prohibition on access to personal genetic information by third parties (*eg.* insurers or employers) may be overruled by national law 'in the public interest'. Issues of confidentiality and consent exist for genomic data from clinical trials. However, the declaration is not legally binding, and is intended as a guide for UN member states to develop their own legislation, with the awareness that the legal framework must evolve as scientific advances lead to new situations.

With a combination of commercial, government and academic researchers in the field, there are fears that valuable data may disappear into private and commercial databases. This aside, there is a danger that access to advances in microarray technology may be restricted by ownership of intellectual property. Expression data must be organized and annotated before it can be analysed and communicated to other researchers. The software necessary to perform this data processing, as well as the technology for the microarray platforms themselves, is in many cases the result of commercial research and subject to patent restrictions. The ownership of products in human genomic research (such as tissues and tissue-derived products) is another legal issue arising from the commercialization process.

If tests for the diagnosis and prognosis of cancers are developed as a result of gene expression profiling research, further legal issues may arise; lawsuits are already brought against clinicians alleging unnecessary delays in diagnosis (with or without demonstrable harm), and the public expectation of prompt diagnosis and effective treatment for cancer is likely to increase.

Economic issues

Health policy makers face a trend of increasing cancer incidence as the UK population ages, and this necessarily has a direct impact on health service costs. In particular, adjuvant chemotherapy is expensive, with results that are frequently equivocal. If gene expression profiling leads, as hoped, to more effective diagnosis and prognosis, then treatment could be targeted such that chemotherapy will only be routinely used where patients are expected to need it. By reducing unnecessary expenditure on chemotherapy and associated care, such testing could both reduce costs and improve quality-of-life for patients, although this benefit must be weighed against the costs of increased testing.

A report on economic modelling to estimate the cost-effectiveness of prognostic markers in the management of prostate cancer concludes that if novel markers can achieve specificity in excess of 80%, then a policy of radical surgery for those identified as being at high risk and conservative treatment for the remainder would be both better for patients and cost-effective (26). A preliminary report at the 2003 meeting of the American Society of Clinical Oncology on the cost-effectiveness of microarray diagnosis in the management of breast cancer in premenopausal women (27) found that the worst-case scenario was a cost approximately equivalent to that of standard chemotherapy, with data suggesting that microarray analysis could potentially save several thousand dollars per patient. However, any new prognostic tests would need to have proven high specificity if treatment is to be provided or withheld on the basis of that test result.

The potential economic benefits of pharmacogenetics stem from determining which people might benefit from a specific drug. This raises the cost-effectiveness of the drug by eliminating the cost of treating non-responders or adverse responders. Drugs previously removed from development due to toxicity in clinical trials might prove to be of benefit to selected patients. Clinical trials themselves could be designed to include only patients likely to benefit from the drug in question, based on pharmacogenetic profiling, thus reducing the risk of adverse reactions and making trials more efficient. According to one study, pharmacogenetics could reduce the cost of developing a new medicine to about 60% of present levels. Conversely, stratification of the patient population into subgroups by pharmacogenetics may produce more orphan (economically unprofitable) medicine candidates. Novel regulatory measures (legal frameworks and financial incentives) may be required to promote the development of such drugs (28). Although it is hoped that microarray technology will lead to a boom in the industry, it is worth noting that medical diagnostics is a field with much lower revenues than drug development, and it is difficult to successfully market novel diagnostic tests.

Ethical Issues

One potential ethical difficulty in pharmacogenetic research is that there is already known to be substantial variation between different ethnic groups with respect to various polymorphisms, including those involved in drug metabolism. Clinical trials therefore need to bear this in mind in order to avoid stigmatizing ethnic groups, given the potentially limited application of research to different groups. The UK Nuffield Council on Bioethics published a report, *Pharmacogenetics: ethical issues* in September 2003 (28).

The development of tissue banks and associated microarray and clinical data raises a variety of ethical concerns, including the issue of consent for future research, and how to control and monitor the use of stored tissue. Informed consent to undergo a diagnostic test requires that the patient be aware of the possibility of false-positive and false-negative findings, and that treatment options depend on the results of testing. Although the identification of cancer biomarkers may provide more information to guide treatment decisions, they may not necessarily make those decisions easier in all cases. Respect for the privacy of the individual and for the confidentiality of highly personal genetic information is also essential.

The workshop

Background

The main objective of the workshop was to bring together key stakeholders and policy makers in the heath service and research communities to review the current status of cancer genomics, to identify those areas of the field closest to clinical implementation and the necessary steps required to successfully translate research into clinical practice in the UK National Health Service.

Presentations

Cancer pharmacogenetics (Professor Bruce Ponder)

Genetic differences between individuals may affect drug responses at various levels – for example in absorption and metabolism of the drug and tissue sensitivity to drug toxicity. Analysis of genetic variation for the prediction of treatment responses (in order to select treatments and dosage to optimise efficacy and minimise toxicity) has an implicit health economics aspect.

Inherited genetic variability between patients was said to form a spectrum, as illustrated by the example of familial breast/ovarian cancer arising due to a single, rare inherited mutation conferring high disease risk as one end of the spectrum contrasted with common polygenic diseases at the other end, in which multiple common genetic variations each confer low additional disease risks. It was also noted that there is a wide range of risk between opposite ends of the spectrum for common polygenic diseases, with the combined effects of multiple genetic variants of individually minor effect able to generate a significant range of risk. It may be that the effects of genetic variation on drug dose related toxicity and outcomes generate a similarly wide spectrum of normal variation among patients, ranging from non-responders at one extreme to those who show severe adverse drug reactions at the other.

The example of 5-Fluorouracil (5-FU) was outlined; 5-FU is a drug used for the treatment of solid tumours such as breast and colorectal cancers. 5-FU metabolites inhibit DNA synthesis and slow replication of cancer cells. Most 5-FU is inactivated in the body by an enzyme called dihydropyrimidine dehydrogenase (DPD). However, DPD is subject to a common genetic polymorphism; mutations in the *DPYD* gene that inactivate or reduce DPD activity are present in around 3% of the population. Patients with low DPD activity cannot inactivate 5-FU efficiently, which increases the effective dose they receive. This is associated with improved outcomes, but also with serious gastrointestinal, haematological and neurological toxicities on treatment with the drug. It is therefore desirable to identify patients with DPD deficiency before administration of 5-FU, as they require lower doses.

The scale of the problem of adverse reactions was outlined using unpublished data on different side-effects (such as nausea, hair loss, diarrhoea and infection) from a sample of 1000 breast cancer patients receiving standard chemotherapy, noting that incidence of severe (grade 3/4) toxicities was relatively rare, generally less than 5%, but that the incidence of significant toxicities (grade 2 and above) was much higher, greater than 50%. It was proposed that the target group in terms of pharmacogenetics should be those individuals who suffer from severe reactions to drugs; not only are predictive genetic tests a more realistic goal, but also because the ability to predict these severe toxicities would be of the greatest clinical value. Intermediate toxicities may well be due to the combined effects of individual common genetic variants associated with mild toxicity; although such toxicities are clinically relevant and much more frequent than the more severe forms, genetic testing would be unlikely to have sufficient predictive power to be useful for this group.

It was emphasised that identifying relevant genetic variants is distinct from being able to utilise that information in clinical practice, primarily due to the low positive predictive values of most gene variants. For example, around 4% of the general population show reduced DPD activity, and half of these carry the most common *DPD**2A* allelic variant. 2% of cancer patients treated with 5-FU develop severe ADRs but only 25% of these are *DPD**2A* carriers. Testing for the *DPD**2A* allele prior to 5-FU treatment would therefore identify only 25% of those patients who will develop a severe reaction to the drug. A more comprehensive and useful test to predict response might be a

phenotypic assay (one that identifies individuals with reduced DPD activity, such as the ^{13}C-uracil breath test) rather than genotyping for *DPD*.

In oncology there is currently only one form genotyping used in routine clinical practice (for the *TPMT* gene; the level of thiopurine methyltransferase activity is reduced by some genetic variants, and TPMT deficient patients require much lower doses of thiopurine drugs than normal to avoid toxicity), although there are examples outside the cancer field of successful pharmacogenetic testing, notably prior to using Abacavir for the treatment of HIV. The general conclusion was that whilst pharmacogenetic data could be useful in identifying associations between genetic variants and efficacy and toxicity after drug treatment, it is unlikely to be sufficiently predictive to be of use in avoiding toxicity for such individuals. However, the more rare sub-populations of non-responders and severe adverse responders are likely to be appropriate for pharmacogenetic targeting, as genetic variants may be sufficiently predictive of response or toxicity to be clinically useful for these groups.

It was proposed that carefully planned add-ons to clinical trials to include data on DNA, phenotypes, tumours, confounding factors (*eg.* medicines and diet) and genetics and epidemiology would provide valuable pharmacogenetic data.

Microarray tumour profiling (Professor Carlos Caldas)

Principles of microarray comparative genomic hybridisation (CGH) were outlined: normal and tumour DNA are labelled with different fluorescent dyes, and images combined. Amplification of a gene is seen as an over-representation of tumour DNA (*eg.* green) whilst deletion is seen as an over-representation of normal DNA (*eg.* red). Equivalent levels of DNA are shown as yellow. The benefits of large volume, reproducible DNA arrays were discussed; it is now possible to array 30-40,000 genes per slide, a huge expansion in publication output arising from this relatively new technology is anticipated over the next few years. Tissue microarrays as a tool to allow statistically significant identification of tumour markers by simultaneous analysis of thousands of tumour samples were also discussed, and problems in data analysis and interpretation.

The requirement for large clinical databases containing data presented in a suitable format for meta-analyses and the necessity of adopting a universally accepted set of standards for performing and recording microarray experiments and associated clinical data was emphasised; although some progress has been made (for example, the adoption of MIAME or Minimum Information About a Microarray Experiment guidelines by some scientific journals) this problem was identified as hampering the transition of research findings to clinical use. Other issues identified relevant to standardisation were the choice of array platform and of reference DNA samples for the experiments. Advances in bioinformatics (including the development of novel algorithms for data analysis) and in the design of databases for microarray data were said to be needed, with a proposed model for a national relational database containing information including microarray experimental results, anonymised clinical data, epidemiology and genotyping data put forward.

The advantages of the unique healthcare infrastructure of the UK were discussed in terms of the potential to drive clinical implementation of microarray research findings. The NHS is effectively the sole provider of cancer care, and shows an increasing trend towards using evidence-based consensus recommendations for novel diagnostic and therapeutic tools. The power of the NCRI (National Cancer Research Institute) to drive research was noted, particularly the success of the NCRN (National Cancer Research Network) in increasing recruitment to clinical trials and the fact that the NTRAC centres comprise most of the molecular profiling capacity and expertise within the UK. Finally, the key role of the Cancer Research UK in funding the overwhelming majority of cancer research in the UK was emphasised.

Herceptin/HER-2 as an example of the steps and obstacles between the research finding and service implementation (Professor Mitch Dowsett)

The human epidermal growth factor receptor 2 (HER-2) is an orphan growth factor receptor with kinase and signalling activity but no known ligand. It is a member of the epidermal growth factor receptor (EGFR) or HER family of cellular receptors; unlike the other family members no ligand (binding partner) has been identified for HER-2, but it is activated by heterodimerization with other members of the HER family. Interest in HER-2 originated from a 1987 paper that presented evidence that amplification of HER-2 in primary breast cancers was significantly associated with decreased survival rates (29). HER-2 gene amplification and/or protein overexpression is seen in around 15% of breast cancers on presentation, and in around 25% of metastatic breast cancers.

Herceptin® (Trastuzumab) is a humanised anti-HER2 monoclonal antibody that targets the HER-2 oncoprotein with high affinity and specificity. Trials of Herceptin® have shown clinical benefits in terms of increased survival rates when used in combination with forms of chemotherapy such as paclitaxel; it received FDA approval in 1998 and was recommended for use in the UK by NICE in 2002.

HER-2 expression in tumour cells is a continuum as opposed to a positive/negative phenomenon. Measuring levels of HER-2 DNA, RNA or protein expression is possible using a variety of methods; the most common techniques are FISH (fluorescent *in situ* hybridisation) or CISH (comparative *in situ* hybridisation) for DNA, and IHC (immunohistochemistry) for protein. The level of HER–2 expression in a tumour is measured in the laboratory using a scale from 0 (negative) to 3+ (strongly positive). The importance of correctly determining HER-2 status was emphasised; patients with strongly positive (3+) HER–2 tumours are likely to benefit from treatment with Herceptin®, but patients with HER–2 negative (0 or +1) tumours will not. The risk of false positives is therefore substantial, since the majority of breast tumours are HER–2 negative. Current treatment guidelines consider tumours that score strongly positive (3+) using IHC as eligible for Herceptin® treatment whilst tumours showing a weak positive or negative score (+1 or 0) are not; tumours with a moderate positive score (2+) require confirmation of HER-2 expression levels using FISH.

The potential for misdiagnosis of tumour samples when using IHC was emphasised, and the relative merits of the DAKO Corporation's HercepTest™ and Clinical Trial Assay (CTA) methods for IHC of HER-2 discussed. Misdiagnosis was said to arise mainly from either visual artefacts (for instance, misleading tissue staining) or from differences in interpretation (and hence scoring) of samples. It was stated that of those samples scored as 2+ using IHC by the majority of centres, around 25% were found to be HER-2 positive using subsequent FISH analysis. Key approaches to avoid misdiagnoses were said to be quality control and on-going quality assessment measures. Carlos Caldas queried whether the new Human Tissue Bill would make some of these quality control measures illegal; Ron Zimmern said that the Bill as currently set out would not require explicit consent for quality assurance procedures if the tissue sample is from a living donor, but that consent would be required for the use of post-mortem tissue specimens.

The advantages and disadvantages of IHC and FISH as diagnostic tools were considered; notably, FISH was said to be highly reproducible but technically complex, and not feasible to perform in smaller testing centres. IHC was said to be a generally high throughput technique but one that required stringent control of testing conditions. A testing algorithm was outlined whereby therapy with Herceptin® should be recommended following a positive result with FISH, or a strongly positive (3+) result using IHC; for samples scoring moderately positive (2+) with IHC, confirmation of HER-2 status using FISH was recommended. In UK laboratories this would apply to approximately 15% of samples tested initially using IHC.

Additional key points included the observation that Herceptin® is an antibody treatment, and assays used for HER-2 expression are also antibody based, which may make the system a better one than examples where diagnostics and therapeutics are dissociated – for example, antibody diagnostics and tyrosine kinase inhibitor drugs. Estimates of cost-effectiveness for Herceptin were reportedly comparable with those for other oncology products used in NHS.

How do we go about implementing the use of new markers? (Dr Marc Van de Vijver)

In the Netherlands, as in the UK and US, the majority of breast cancer patients under the age of 50 receive adjuvant chemotherapy as well as surgical treatment, because of the important survival advantage this confers on the patient population as a whole. However, a significant proportion of these patients would be expected to survive in the absence of chemotherapy – around 70% of those with lymph node negative disease and around 40% of those with lymph node positive disease. Although chemotherapy may confer a very small additional survival advantage on these population sub-groups with good prognoses, the costs in both economic and human terms are significant. It is therefore desirable to have some means of distinguishing those patients with a poor prognosis who are likely to require chemotherapy, from those whose prognosis is good and are less likely to require it. Genetic studies have identified some useful prognostic markers, but they are not sufficiently robust to be used in treatment decisions.

Work at the Netherlands Cancer Institute (NKI) on gene expression profiles of breast cancers to identify prognostic indicators was outlined: samples from a cohort of 78 women under the age of 55 with lymph node negative tumours were used to analyse expression of 25,000 genes and correlate patterns of gene expression with known clinical outcome. 70 genes were identified as having significant prognostic value. The samples were assigned to either a 'good prognosis' or a 'bad prognosis' group, where the lower threshold value for good prognosis was a 10% risk of distant metastases after five years. A cross-validation of these data was next performed using samples from another cohort of 295 breast cancer patients, of which around half had lymph node negative and half lymph node positive tumours. Gene expression profiling assigned 180 patients to the 'bad prognosis' group and the remaining 115 to the 'good prognosis' group. Average ten-year survival rates for these subgroups were 54.6% and 94.5%, respectively. The prognostic value of this gene expression profile comprising 70 key genes was reportedly much stronger than that of other clinico-pathological factors.

It was noted that the differences in expression level between genes were in some cases only two-fold, and it was therefore very difficult to accurately identify key genes associated with prognosis. Analysis of the expression levels of a minimum set of at least 50 genes was proposed to be necessary for prognostic purposes, although the precise set of optimally prognostic genes has yet to be defined.

The NKI spin-out company (Agendia) was mentioned; it has joined with the multinational company Agilent Technologies to develop a dedicated platform for analysing expression of the 70 genes in the prognostic set identified by this research; it was reported that the US FDA (Food and Drug Adminstration) is providing input to this development process. The need for a standardisation of testing procedures (including tool, reagents and protocols for the extraction of tissue samples and subsequent RNA isolation and amplification) was stressed, as was the requirement for a single, centralised facility for storing and distributing tissue samples for such studies.

A collaborative study to be conducted with the Netherlands Insurance Council was described, to evaluate the effects of microarray testing on the outcomes of adjuvant chemotherapy. Recruitment is underway to a pilot project of 75 lymph node negative breast cancer patients under the age of 60, with plans for a second phase of the project using a further 2000 individuals. The idea is to perform randomized trials to compare the prognostic ability of microarray testing with current standard means of prognosis such as tumour grade. However, this project will take 10 years to complete, by which time it is anticipated that a more accurate and useful prognostic set of genes will have been identified.

Various reports on similar gene expression profiling studies from the San Antonio Breast Cancer Conference 2003 were outlined. One of these, looking at gene expression profiles and molecular markers to predict metastasis of early stage breast cancers identified a prognostic set of genes, of which only a single gene was also present in the prognostic set reported by Dr van de Vijver. It was noted that this group had used a different microarray platform (produced by the company Affymetrix as opposed to Agilent) and that inconsistencies such as this made useful cross-validation of results between studies difficult.

It was suggested that in the future, microarray profiling could be of value not just in determining likely prognosis for cancer patients, but also in dictating optimal treatment options from the array of

available interventions, from surgery and radiotherapy to adjuvant chemotherapy and hormone treatment. A study was reported to be underway looking at gene expression profiling to predict tumour response to alternative treatment regimens; to date, there is only one published example of this sort of study, looking at the prediction of therapeutic response to docetaxel in breast cancer patients (30).

The role of NTRAC (Professor David Kerr)

The history and structure of NTRAC was briefly outlined; the original vision was for a single centre but it was later decided to opt instead for a network of individual centres. The NTRAC Network comprises 14 Centres distributed across the UK, selected by peer-review for their excellence in translational cancer research. Each centre receives around £1.3m funding over 5 years, which is allocated in a flexible manner rather than to specific projects.

NTRAC network centres

Belfast
Birmingham
Cambridge
Edinburgh
Glasgow - Dundee
Imperial College
Leeds - Bradford
Manchester
Newcastle
Oxford
Royal Marsden
Southampton
UCL
Cardiff - Swansea

Health Ministers recognised that a knowledge-based NHS needed to be underpinned by a research network. One of the aims of the National Cancer Plan of 2000 was to foster research and integrate it into cancer care delivery within the NHS. The NTRAC Network is intended to fast-track cancer research so that NHS patients can benefit from rapid movement of advances into clinical practice, by integrating knowledge and expertise from the individual centres of excellence. The aim is to effectively add value to current research and knowledge for the NHS, by pushing the science towards the clinic and also by seeking to fill gaps in available clinical tools for cancer care.

Each of the NTRAC centres has internationally competitive areas of particular expertise in cancer research, with some overlap between the centres. Key strengths in research capabilities include immunotherapy, gene therapy, signal transduction and cell cycle inhibitors and novel diagnostic and predictive tools. The difficulty reportedly lies in inducing these ultra-competitive elite centres to work together in clinical and pre-clinical trials, since this is contrary to usual research practice. NTRAC as a whole comprises some 2000 researchers, including dedicated clinical phase I/II trial units and investigators; it has well-established links with many major pharmaceutical companies and participates in industry-sponsored early phase clinical trials. There is reportedly an increase in hypothesis-led proof-of-principle trials for novel therapeutics and diagnostics at present.

In terms of translational health research, the UK was noted to possess a valuable niche position in the form of the NHS, allowing access to a large, relatively homogeneous and largely willing population for clinical trials. Although major pharmaceutical companies have in the past tended to avoid the UK, NTRAC was reported as working with the NHS and Cancer Research UK to fast-track UK based development schemes. Examples cited included GlaxoSmithKline, AstraZeneca and two smaller biotechnology companies who have contracts with NTRAC for the development of novel therapeutics. The National Cancer Tissue Resource (NCTR) was discussed; this is a new initiative funded by the NCRI (around £1 million a year over 5 years) to be co-ordinated by NTRAC, comprising a national NHS-embedded network of tissue banks linked to clinical trials, underpinned by a central bioinformatics hub. The tissue banks will be linked to suitably anonymised clinical follow-up data and will be accessible nationally. Researchers will be able to remove tissue samples and to deposit data, to avoid unnecessary replication of trials and to maximise the power of trials by providing access to data.

The Human Tissue Bill (HTB) was also mentioned; NTRAC was said to welcome the HTB as a general response to public unease over issues surrounding organ retention, but reservations about the details of the Bill were expressed. In particular, it was considered vital that the legal framework within the HTB should be amended to ensure that research was not stifled by inappropriate criminalisation of standard collection, storage and analysis of tissue and DNA samples.

Workshop discussions

Participants were provided with a document summarising the current state of the literature with respect to gene expression analysis of cancer, and potential policy issues associated with the use of cancer genomics in the clinical setting, in advance of the workshop.

Following the presentations, delegates split into four groups to consider the clinical and policy implications of the current status of cancer genomics in the UK. Each group was asked to summarise their discussion at the final plenary session in the form of key report points from each section, and proposed action points. Feedback was delivered by representatives of the Cambridge Genetics Knowledge Park from each group: Ron Zimmern, Alison Stewart, Paul Pharoah and Philippa Brice for groups 1-4 respectively.

Clinical implications of cancer genomics

The key question under consideration was how close gene expression profiling of cancers is to implementation into clinical and public health practice. Proposed issues for consideration included validity, reliability and clinical utility of relevant techniques, and whether they could effectively add value to current clinical practice.

Group 1 considered that array technology was not ready for translation into clinical practice, but that single gene technology (such as individual genes identified as useful prognostic or diagnostic markers) might be. Group 2 agreed that array technology was not ready for use in clinical practice, notably because of the lack of validation of studies and the lack of concordance between different studies in terms of overlap of putative prognostic genes; group 4 echoed this concern, and called for more evidence. However, Group 2 did think that, if suitable validity was demonstrated, gene expression profiling might form a component of prognostic testing along with established clinical techniques, but noted that more research would be required into the attitudes of both clinicians and patients to a genomic element of diagnosis and prognosis before adoption of such methods.

Group 3 felt that although some genomic technologies and specific tests in oncology were ready to move into Phase III clinical trials, the vast majority were still in Phase II or earlier. However, this group took a more upbeat line in emphasising the enormous unrealised potential of these techniques to improve current clinical practice. Group 4 reported the belief that although current work in cancer genomics might well yield valuable information in terms of identifying novel diagnostic markers, array technologies and gene expression profiles themselves would not provide an alternative method of diagnosis or prognosis in oncology.

Policy implications of cancer genomics

Delegates were asked to consider what potential barriers to the clinical implementation of gene expression profiling in cancer therapeutics existed, in terms of policy issues including logistics, resources and ethical and legal constraints.

The need for an appropriate regulatory structure for the evaluation of these technologies for use in clinical oncology was noted by Group 1, as was the requirement for appropriate logistics in terms of organising links between research and clinical practice. Group 3 also called for the development of a formal evaluation framework for diagnostics similar to that already in place for novel therapeutics. This group further noted the need for allocation of public funding and resources for future research and development in cancer genomics, since industrial interest in diagnostic and prognostic tools was very limited due to the relatively low profit to be gained from such products. Group 4 voiced concern as to whether an appropriate infrastructure and funding would be forthcoming in the event of genomic technologies delivering effective new tools for cancer diagnosis and treatment selection, based on the experiences of some group members. Group 1 also identified the requirement for appropriate logistics in terms of organising links between research and clinical practice.

In terms of ethical, legal and social (ELSI) issues, both Groups 1 and 2 noted potential ethical issues surrounding the denial of treatment, for instance to patients who might be identified as having a high risk of adverse response to a given drug, or good prognoses (based on gene expression profiling) such that chemotherapy was withheld. Group 4 made the point that if novel genomic technologies were successfully adopted as a component of clinical practice, it might be necessary to consider the wider implications of certain diagnostic tests beyond the individual patient (*ie.* for family members), as is already the case for forms of tests related to genetics.

The workshop participants came from a range of backgrounds, including experts in clinical oncology and cancer genomics (many of them based at NTRAC Centres), representatives of major funding bodies and the Department of Health, and members of the different Genetics Knowledge Parks. They were therefore able to address the issues in question from varied perspectives, lending weight to the workshop discussions and final conclusions. Genomic approaches to developing and refining novel diagnostic and therapeutic strategies in cancer care are among the most advanced applications of the rapidly expanding knowledge base of human genetics to a major clinical field, and facilitating their transition into tangible benefits for patients may serve as a blueprint for future developments. A forward-looking strategy should take into account both the abilities and limitations of the science, and the potential for associated issues to advance or impede progress towards clinical application.

The way forward: recommendations

1) **NHS resources should be allocated to the development of an appropriate infrastructure for the storage and use of a) tissue samples and b) information, including clinical data. A national open access data repository should be established with required terms of standardisation. Selecting the most appropriate standardisation will require the evaluation of alternative microarray platforms and methodology.**

2) **The Human Tissue Bill should be appropriately modified to provide legal protection for the needs of clinicians and researchers to use tissue samples both for testing and research purposes.**

3) **NHS funding should be allocated for research and development in cancer genomics, particularly for large-scale clinical trials to validate results and to make improvements in bioinformatics to allow the optimal analysis of data from these trials.**

4) **Information Technology systems, expertise and infrastructure should be developed with a view to handling the massive increase in data that will result from large-scale microarray analysis of gene expression. Current IT capacity within the NHS is considered to be inadequate to meet the predicted demands in this area.**

5) **A robust health economic analysis of the novel technologies with respect to oncology in the NHS should be performed.**

6) **Formal guidelines for the evaluation of novel diagnostics should be developed, by the MHRA or NICE.**

7) **Potential intellectual property (IP) issues related to the use of hardware, reagents and algorithms for genomic analysis of samples should be evaluated; NTRAC was suggested as an appropriate lead for this action.**

8) **Research into the possible ethical implications of gene expression patterns for diagnosis and prognosis of cancers in clinical practice should be conducted; the likelihood that public perception of this 'genetic' technology was likely to be different from that for normal clinical methods was considered grounds for concern.**

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Appendix : Glossary of selected terms

adjuvant chemotherapy: Chemotherapy treatment given in addition to the primary cancer treatment (surgery or radiotherapy).

alleles: Variant forms of the same gene.

biomarker: A molecule with some feature that makes it useful for measuring the progress of disease or effects of treatment.

CGKP: Cambridge Genetics Knowledge Park

comparative genomic hybridization (CGH): A technique for screening tumours for genetic changes. DNA gains and losses (including mutations) are revealed in a characteristic pattern.

DNA: Deoxyribonucleic acid, a nucleic acid present in all living cells that encodes genetic information in the form of genes.

FDA: Food and Drug Administration, the US regulatory body for drugs, medical devices and biological products.

gene: Part of the DNA molecule of a chromosome which encodes (directs the synthesis of) a protein.

genotype: The specific genetic constitution of an individual.

histology: The study of cells and tissues at a microscopic level.

metabolites: Substances produced by a metabolic process (the physical and chemical reactions by which living organisms are produced and maintained).

metastasis: Process by which cancer spreads from one part of the body to another, also used to refer to the new tumour arising from the original (primary) tumour in a different location**.**

microarray platform: A flat surface support on which DNA samples have been spotted in an ordered grid.

morphology: The study of structure or configuration (*eg.* of human cells).

NCI: National Cancer Institute (USA).

phenotype: The observable traits of an organism, resulting from the combination of genetic and environmental factors.

polygenic: Involving the combined action of more than one gene.

polymorphism: Variation in a region of DNA sequence among different individuals; the variation should be present in at least 1-2% of the population to be considered a polymorphism.

proteomics: Analysis of the full set of proteins encoded by a genome (the proteome).

reverse transcription: The process of copying information found in RNA into DNA, performed by the reverse transcriptase (RT) enzyme.

RNA: Ribonucleic acid, a nucleic acid present in all living cells that is involved in the transfer of genetic information from DNA to the systems that produce proteins.

taxonomy: Theories and techniques of scientific naming, description and classification.