17 Debate: Whither Haematoxylin and Eosin?

SETTING THE SCENE

The technical advances of the past 30 years and the burgeoning scope of molecular and genetic analyses have been predicted by some to be the harbingers of the demise of pathology as a diagnostic discipline. It has been suggested that the traditional heamotoxylin and eosin stained preparation interpreted and considered by a histopathologist using a light microscope will be replaced in short order by molecular analyses and perhaps by automated and robotic procedures.

At the beginning of the second century of The Pathological Society we have challenged a leading molecular pathologist to argue the case that *H&E will be replaced by 'chips'*. Nick Lemoine has advanced this thesis with his sub-heading *Microarrays are the way: adding value to precious tissue in the molecular era*. This proposition has been rebutted by Jason Hornick and Chris Fletcher from the more traditional camp of diagnostic (or surgical) pathologists. They have responded to the progressive molecular view with the response that *H&E will hold sway*! and the sub-heading of *The invaluable role of morphology in the molecular era*. The reader will have their own view and the future will indicate where the truth lies!

The Editors

H&E Will Be Replaced by 'Chips'

Microarrays Are the Way: Adding Value to Precious Tissue in the Molecular Era

Nick R. Lemoine

INTRODUCTION: MODERNISE OR DIE!

Since the founding father of the science of histopathology, François-Xavier Bichat, worked throughout his career without a microscope 200 years ago, perhaps we can consider the subsequent focus on cells and microscopy introduced by Rudolf Virchow to be but a passing phase in the evolution of the specialty. Maybe we can now at last join the molecular revolution and move on to a new era where pathologists become central rather than peripheral to patient management, and proactive rather than reactive to scientific and clinical developments.

Medicine is generally a conservative profession, and many believe histopathology to be among the most conservative of its specialties. This might hold some superficial attractions in the rapidly changing world of healthcare: investigative fashions may come and go but clinicians have continued to recognise the gold standard of '30 years of experience and an H&E' when it comes to diagnostics for treatment decisions. However, healthcare professionals – and perhaps more importantly, patients – are now more informed than ever about the potential for individualised therapy, and managers are examining more closely the cost–benefit issue of the laboratory services. If the right information cannot be delivered from a pathology service to make resource-critical decisions on a patient's treatment pathway, then the resource will be withdrawn and invested elsewhere. It is absolutely critical that pathologists are forewarned with the right intelligence and armed with the right tools as the molecular revolution sweeps through the practice of medicine.

It is crucial that molecular pathology is recognised and encouraged as a specialty if we are to contribute to progress. The shift in thinking represented by systems biology means that we must have the expertise to analyse biological complexity and exploit it in predictive, preventive and personalised medicine. The profession must modernise or die.

APPLICABILITY IN CLINICAL PRACTICE

A criticism frequently levelled at the use of molecular approaches in the analysis of clinical material is that tissue handling, transport and storage all make a significant impact on gene and protein profiles independent of the disease process under study. Indeed, when healthy and malignant colon tissue samples were snap-frozen at various time points after colon resection, and gene and protein expression were determined by the two common platform technologies (Affymetrix microarray HG-U133A chips and surface-enhanced laser desorption/ionisation time-of-flight mass spectrometry (SELDI-ToF-MS), respectively) changes in profiles were already observed 5–8 min after colon resection (Spruessel *et al*., 2004). Fifteen minutes after surgery, 10–15%

(and, after 30 min, 20%) of all detectable genes and proteins differed significantly from the baseline values. Studies such as this have meant that control of these variables has become mandatory to obtain reliable data in screening programmes for molecular targets and diagnostic molecular patterns.

However, many of the same criticisms over the handling and storage of tissue samples apply equally to technologies that the classical histopathogist now regards as part of his standard armentarium, such as immunohistochemistry. A long delay between cutting the sections and immunohistochemical staining can decrease the immunohistochemical reaction intensity, as revealed in a recent study of the influence of slide age on the results of IHC analyses for oestrogen receptor (ER), progesterone receptor (PR), cyclin D1, HER2 (HercepTest) and E-cadherin (Mirlacher *et al*., 2004). The frequency of positivity on old sections (stored for 6 months at 4 °C) compared to freshly cut sections decreased from 65 to 46% for ER ($P < 0.0001$), from 33 to 18.5% for PR ($P < 0.0001$), from 16.3 to 9.6% for HER2 ($P = 0.0047$), from 45.1 to 37.7% for cyclin D1 ($P = 0.10$) and from 58.9 to 32.9% for E-cadherin ($P < 0.0001$). Hence both classical and molecular pathologists need to take care of their tissues if they are to have confidence in their outcomes.

More reassuringly, a recent study evaluated the validity of assessing gene expression in cervical tissues acquired in a clinical setting, investigating whether standard procedures such as the application of acetic acid and/or Lugol's iodine, employed for the visualisation of colposcopically directed biopsies, altered patterns in oligonucleotide array analysis (Wang *et al*., 2005). Microarray profiles were compared in tissues from six women, each with three adjacent samples removed from benign hysterectomy specimens and either immediately frozen or had acetic acid only or both acetic acid and Lugol's iodine applied. They found that standard precolposcopic procedures do not substantially affect the overall gene expression patterns in the normal cervix.

As clinical practice moves ever further towards minimally invasive diagnostic procedures, it is important to note that it is feasible to generate gene expression profiles from both fine-needle aspirates and core needle biopsies that yield (from breast cancers, for instance) an average of 1–2 µg of total RNA (Assersohn *et al*., 2002; Ellis *et al*., 2002; Sotiriou *et al*., 2002; Pusztai *et al*., 2003; Symmans *et al*., 2003). The rate of successful profiling is around 70–80% with both biopsy methods, and the gene expression profiles obtained from matching fine-needle aspirates and core needle biopsies of the same tumour are similar (Symmans *et al*., 2003). At least one commercial laboratory in the USA already offers comprehensive gene expression profiling of formalin-fixed, paraffin-embedded human cancer tissues, although currently for research purposes only (US Laboratories, Irvine, CA).

MOLECULAR DIAGNOSIS OF TUMOURS OF UNKNOWN PRIMARY SITE

Although classical histopathology is well equipped to make the diagnosis of malignancies in their primary site or typical metastatic locations, it is less powerful for the identification of tumours of unknown primary site and because such cases make up perhaps 5% of new cancer presentations (Briasoulis and Pavlidis, 1997) this is a significant limitation to practice. These neoplasms (often adenocarcinoma) represent a clinically diverse group, typically presenting with moderately to poorly differentiated tumours involving multiple organs, including liver, bone, lung, lymph nodes, pleura and brain (Le Chevalier *et al*., 1988). Such a diagnosis may be a potential source of financial frustration for the patient because in the USA Medicare and many private insurers will not pay their drug costs because the diagnosis of 'unknown primary site' is not listed in the indications for various drugs in the United States Pharmacopeia Dispensing Information (Reynolds, 1998). Tumours of unknown primary site also cause much clinical frustration, because patients with such disease represent a disproportionate fraction of cancer deaths due to their poor median

survival, which is typically a matter of months (Abbruzzese *et al*., 1995). This is unsatisfactory in today's world because treatments are becoming increasingly specific, with approaches varying significantly depending on the cellular origin (and molecular subtype) of the cancer. With recent advances in chemotherapy, specific regimens have led to improvements in survival and quality of life even for tumour types that traditionally have been regarded as relatively chemoresistant, such as pancreatic and non-small-cell lung cancers.

Gene expression profiling could be an important tool to identify the site of origin of these malignancies, and indeed several studies have shown that it is possible to identify correctly metastatic tumours from known primary carcinoma using this approach (Ramaswamy *et al*., 2001; Su *et al*., 2001; Bloom *et al*., 2004), although until recently no study had benchmarked the efficacy of such tests for predicting the site of origin of tumours against clinical diagnostic variables. Now a highly accurate multiclass classifier designed for clinical application to tumours of unknown primary has been reported (Tothill *et al*., 2005). A large and comprehensive data set of gene expression was obtained from microarray analysis of 229 tumour samples, representing 14 common sites of origin in the differential diagnosis of tumours of unknown primary site. A single cDNA microarray platform was used to profile 229 primary and metastatic tumours representing 14 tumour types and multiple histological subtypes (which addresses the confounding issue of molecular heterogeneity of specific tumour classes). This data set was subsequently used for training and validation of a support vector machine (SVM) classifier that demonstrated 89% accuracy using a 13-class model.

Of course an objection raised to the application of expression profiling in clinical practice is that microarray analysis typically requires as a substrate fresh-frozen tissue harvested and stored in pristine condition. However, a number of studies have shown that accurate classification of multiple cancer types can be made using a reduced number of genes – hundreds rather than thousands (Su *et al*., 2001; Giordano *et al*., 2001; Shedden *et al*., 2003; Bloom *et al*., 2004). Hence a classification could be achieved using cheaper, faster and more robust platforms for quantifying gene expression, such as quantitative polymerase chain reaction (PCR), and already several studies have demonstrated that expression analysis from fixed material using quantitative PCR is perfectly feasible (Specht *et al*., 2001; Abrahamsen *et al*., 2003; Cronin *et al*., 2004). Generating low-density quantitative-PCR arrays or using multiplex reactions therefore offers an attractive alternative to standard high-density microarrays for eventual clinical application in a conventional pathology laboratory using formalin-fixed, paraffin-embedded material. Tothill and colleagues selected 79 optimal gene markers and achieved the translation of a five-class classifier to a quantitative-PCR low-density array, allowing the assay of both fresh-frozen and formalin-fixed, paraffin-embedded tissue. Data generated using both quantitative PCR and microarray were subsequently used to train and validate a cross-platform support vector machine model with high prediction accuracy, and on prospective application to a test series the classifiers were capable of making high-confidence predictions in 11 of 13 cases of tumour of unknown primary site. Importantly, Tothill *et al*. emphasise that it is likely that there will be cost savings from the application of such a molecular genomics test because it will enable more directed clinical evaluation of patients. The average cost for diagnostic evaluation of patients with a tumour of unknown primary site at a major US cancer centre was estimated at $18 000 when a large series was considered (Schapira and Jarrett, 1995), whereas a test similar to that described here is likely to cost under $1000 (Tothill *et al*., 2005).

BIOLOGICAL INSIGHT INTO CONTROVERSIAL TUMOUR ENTITIES

There are some areas of oncology where classical light microscopy has probably gone as far as it is ever likely to go and the classification of sarcomas is a good example (as even the protagonists

for the survival of the H&E might concede). For instance, the malignant fibrous histiocytoma was for some decades regarded as the most common soft-tissue sarcoma of adult life, but almost from its first description in the early 1960s controversy has raged over its histogenesis and its validity as a clinicopathological entity (indeed the latest World Health Organization classification no longer includes malignant fibrous histiocytoma as a distinct diagnostic category but rather as subtypes of an undifferentiated pleomorphic sarcoma). Similar, if less incandescent, arguments break out over other entities in the sarcoma spectrum and some order needs to be established for clinical practice to advance.

Perhaps because it is such a fertile hunting ground for those determined to classify objectively on grounds other than individual, subjective opinion on morphology, microarray technology has been applied extensively in the analysis of sarcomas (Allander *et al*., 2001; Nielsen *et al*., 2002; Segal *et al*., 2003). Some of the most interesting reports have described expression profiles associated with poor clinical outcome in leiomyosarcoma (Lee *et al*., 2004) and Ewing's sarcoma (Ohali *et al*., 2004), and novel biomarkers in dermatofibrosarcoma protuberans (West *et al*., 2004) and clear cell sarcoma (Schaefer *et al*., 2004). Recently, a comprehensive study examined 181 tumors representing 16 classes of human bone and soft-tissue sarcomas on a 12 601-feature cDNA microarray (Baird *et al*., 2005). A set of 2766 probes differentially expressed across this sample panel clearly delineated the various tumour classes. Many genes previously associated with specific tumour types were among those most highly weighted (for example: the muscle markers *MYLK, CNN1* and *ACTG2* in leiomyosarcoma; *KIT* in gastrointestinal stromal tumours; *SSX1* in synovial sarcoma; and *PDGFB* in dermatofibrosarcoma protuberans), but each group was also associated with a highly informative list of other associated genes, including numerous genes not previously associated with sarcoma.

The group of tumours pathologically classified as malignant fibrous histiocytomas proved to be a rather complex group at the molecular level. On unsupervised clustering of the microarray data, the majority of malignant fibrous histiocytoma tumours co-clustered with the more poorly differentiated tumours, forming a large branch that included dedifferentiated and pleomorphic liposarcomas, malignant peripheral nerve sheath tumours and the sarcomas that are not otherwise specified. Interestingly, the most closely adjacent branch on the unsupervised clustering dendrogram contained the leiomyosarcomas.

Unsupervised hierarchical cluster analysis of the malignant fibrous histiocytoma tumors identified two groups of nearly equal proportions. The group of genes associated with the first group carried a muscle profile with the genes *myosin X, sarcoglycan ß* and *tenascin C* among the ten genes, with the most significant differences in expression. In contrast, the second group of tumours was characterized by a cluster of immune regulatory genes, with *HEM1, MX1, DAP10, PLCG2* and *FOLR3* constituting the five most highly weighted genes. The distinction of malignant fibrous histiocytoma with myogenic differentiation versus inflammatory characteristics could prove clinically significant. Certainly it is known that the presence of myogenic differentiation in malignant fibrous histiocytoma and undifferentiated sarcomas correlates with a poor prognosis (Fletcher *et al*., 2001), although the prevalence of such differentiation is approximately 30% on classical histological criteria rather than 50% on molecular profiling. Quite what the significance is of the inflammatory signature needs more investigation, because classical histopathologists recognise an inflammatory-type malignant fibrous histiocytoma only rarely. However, an inflammatory profile is correlated with good prognosis in other malignancies (Lotze and Rees, 2004), such as colorectal cancer with microsatellite instability (Banerjea *et al*., 2004) and follicular lymphoma (Fujii *et al*., 2005), which is considered in more detail below.

A really significant advantage that large-scale molecular analysis such as this offers over simple histopathological descriptions is the facility for other investigators to mine the data and build their own hypotheses to test in other clinical series or model systems. The complete raw data from the study are available through the Gene Expression Omnibus (GEO) data repository (GEO accession number GSE2553) and the expression profiles of the 7788 probes of highest quality from the gene

expression array can be viewed at http://watson.nhgri.nih.gov/sarcoma/. Similar facilities now exist for many different tumour panels, and the future entry of clinical trial series will increase the value of such resources.

LYMPHOMA – THE EXEMPLAR OF MOLECULAR PROGNOSTICATION

Follicular lymphoma is a disease with marked clinical heterogeneity in which some patients undergo rapid transformation to aggressive lymphoma and die, whereas others survive for years with indolent disease. Reliable prognostic markers have not been established to guide therapy and it is well recognised that pathological grading is highly subjective and the clinically based International Prognostic Index identifies relatively few high-risk patients (Decaudin *et al*., 1999). Significantly, it was in this disease that expression profiling had its first high-profile successes in prognostication (Dave *et al*., 2004; Glas *et al*., 2005), and highlighted the critical impact of the cytokine milieu in the lymphoma microenvironment (Fujii *et al*., 2005).

More recently, follicular lymphoma has been the exemplar for the identification of biologically relevant differences in protein expression using reverse-phase protein microarrays (Gulmann *et al*., 2005). By using reverse-phase protein microarrays and antibodies to proteins in the intrinsic apoptotic pathway, it was shown that high ratios of Bcl-2/Bak and Bcl-2/Bax were associated with early death from disease, with differences in median survival times of 7.3 years ($P = 0.0085$) and 3.8 years ($P = 0.018$), respectively. Such data are powerful arguments to incorporate proteomic endpoints in clinical trial protocols to validate fully their clinical utility.

A CLASSIC EXAMPLE: DUKES' STAGING ONLY TAKES US PART OF THE WAY – MOLECULAR PROFILING IS NEEDED TO COMPLETE THE JOURNEY

One of the best examples of how classical histopathology can be exploited to guide clinical practice is Dukes' staging of colorectal cancer, which has long been the gold standard for prognostication and treatment decisions. However, considerable variability exists in the long-term survival of patients within each class. Notably, around 50% of Dukes' C patients will have disease recurrence and die as a result of their disease after surgery with curative intent, whereas the other half are surgically cured, and it has not been possible by histopathological means to distinguish between these two subgroups of patients. The advent of routine adjuvant treatment for these patients over the last decade has significantly improved the survival of 10–20% of these patients, but at the cost of overtreating those who are already surgically cured. Hence, although classical histopathology has served us well, it has reached the limit of its power in this group and more sophisticated stratification is needed.

A recent study used high-density oligonucleotide microarray analysis to identify profiles of expression in tumours from Dukes' C patients who had surgery as the only form of treatment (Arango *et al*., 2005). A total of 218 genes showed a significant difference in expression in tumours from patients with good and bad outcomes. Microarray-based expression profiling outperformed other genetic markers previously investigated, such as *TP53* and *K-RAS* status or allelic imbalance in chromosome 18q. One of the genes with the most significantly reduced expression in tumours from patients with bad prognosis compared with tumours from good-prognosis patients was the RAS homologue gene *RHOA*. Using immunohistochemistry and a tissue microarray, the level of expression of *RHOA* was assessed in an independent set of 137 formalin-fixed, paraffin-embedded tumour samples from Dukes' C patients. Patients with low RHOA levels in the tumour

had significantly worse overall ($P = 0.03$) and disease-free ($P = 0.01$) survival compared with patients whose tumours had high *RHOA* protein levels. Interestingly, shorter survival of patients with low *RHOA* tumour protein levels could also be observed in those patients who received 5-fluorouracil–based adjuvant chemotherapy. Therefore, *RHOA* could be used to identify a subset of patients with a higher probability of recurrence and for whom a more aggressive treatment may be justified. Although adjuvant treatment with 5-fluorouracil has become the standard treatment of Dukes' C colorectal cancer, effective alternatives exist in irinotecan and oxaliplatin. Reduced *RHOA* levels could therefore identify a group of patients who could benefit from combined treatment with 5-fluorouracil and CPT-11 and/or oxaliplatin.

TREATMENT INDIVIDUALISATION BY MOLECULAR PROFILING

Two subgroups of patients do not get any benefit from adjuvant chemotherapy: the first one comprises patients who are already cured by locoregional treatment alone (as exemplified in the Dukes' C group reviewed above), and the second one is represented by patients who do not profit from adjuvant chemotherapy because of resistance to the regimens employed. There are two ways in which we can improve the cost–benefit issue of this treatment strategy: one is to improve the sensitivity of prognostic factors to be able to select a specific group with a good signature that does not need adjuvant treatment; the second is to identify predictive factors that may help us to select the optimal therapeutic strategy or the optimal regimen or drug for individual patients.

When chemotherapy is administered preoperatively to women with breast cancer, a complete and partial response rate assessed clinically exceeding 70% is achieved with many regimens. However, a lower percentage ranging from about 5% to 38% of patients achieve tumour eradication when response is assessed by careful pathological examination of the surgical specimen after chemotherapy (Bonadonna *et al*., 1998; Fisher *et al*., 1998; Smith *et al*., 2002; Kaufmann *et al*., 2003). Several studies have shown that, together with nodal status at surgery, pathological complete response is the most significant independent variable associated with the likelihood of benefit as measured by disease-free and overall survival. In principle, diagnostic tests that predict, before administration of neoadjuvant therapy, which patients are going to experience Pathological complete response will identify patients who are likely to benefit from treatment and will also identify individuals with low or no likelihood of benefit, sparing them the toxicity of an inactive treatment and allowing earlier application of alternative approaches. Successful prediction of a durable benefit of chemotherapy using routinely obtained fixed tumour tissue would make possible and widely applicable the tailoring of toxic chemotherapy to individual patients. The identification of a predictive multivariate marker from microarray expression data involves three main steps: invariant genes must be filtered from the data set to prevent noise obscuring true biological associations, and the remaining genes of interest are ranked by their strength of association with the outcome; a model is identified that can predict outcome using the gene expression values as input to a mathematical formula; and a prediction rule is defined that categorises the output from the model into clinically defined classes using cut-off points.

Expression of 250 candidate genes in tumour tissues from a total of 447 patients identified a 21-gene panel and a Recurrence Score (RS) algorithm. The RS was clinically validated to quantify the risk of distant recurrence in 668 women with ER-positive, node-negative breast tumours treated with adjuvant tamoxifen in a clinical trial conducted in the USA as the National Surgical Adjuvant Breast and Bowel Project (Paik *et al*., 2004). The expression of 384 genes, including the RS panel of 21 genes, was similarly quantified in fixed diagnostic biopsy specimens of women with locally advanced breast cancer who were treated with chemotherapy before surgery (Gianni *et al*., 2005). The 86 candidate genes that were significantly correlated with pathological complete response in this cohort of patients were then tested for their correlation with pathological complete

response in a separate cohort of 82 patients treated with similar neoadjuvant chemotherapy whose tumour tissue was profiled by DNA microarrays. The predictive genes included three particularly prominent co-expression clusters. Pathological complete response correlated, as expected, with higher expression of genes regulating proliferation (e.g., *CDC20*, *E2F1*, *MYBL2* and *TOPO2A*) and lower expression of genes related to expression of the ER (e.g., *ER*, *PR*, *SCUBE2* and *GATA3*). The third cluster predicting pathological complete response comprised a group of upregulated genes associated with immune response (e.g., *MCP1*, *CD68*, *CTSB*, *CD18*, ILT-2, *CD3z*, *FasL* and *HLA.DPB1*) and hence it is possible that the pretreatment host response may enhance the ability of chemotherapy to eliminate cancer cells.

Validation of the recurrence score will require a randomised phase III study such as that being planned by the US Intergroup Program for the Assessment of Clinical Cancer Tests. A large randomised multinational trial (Microarray In Node-negative Disease may Avoid Chemo Therapy, MINDACT) will compare the information obtained with genomic profiling and the classical clinicopathological index (St Gallen classification); the objective is to allow women not to be treated with adjuvant chemotherapy if their genomic signature is 'good'. Another trial (EORTC 10994) is being conducted to investigate whether, in patients with p53-mutated tumours, neoadjuvant chemotherapy with docetaxel is more efficient than an anthracycline-containing regimen (a supplementary study will evaluate gene profiles in each group). This type of treatment response prediction may be more broadly applicable: expression profiling of pancreatic cancer cells with differential resistance to gemcitabine has revealed that analysis of as few as six genes (among which downregulated *TNFSF6* – also known as Fas ligand – *BNIP3* and *AKT* may be the most informative combination) may allow the development of an algorithm for stratification of patients before treatment (Akada *et al*., 2005; Nakai *et al*., 2005). This is now being tested as part of large-scale prospective clinical trials in which the construction of tissue microarrays is an integral component of protocol design.

An important outcome of the confirmatory trials will be to determine whether the new predictors of response are specific for particular chemotherapy regimens, or whether they predict response to any cytotoxic treatment. To be most useful clinically, a pharmacogenomic predictor would need to be regimen-specific and a portfolio of such predictors developed for assisting decision points in the treatment pathway of individual patients. Multigene predictors using prediction scores and machine-learning algorithms have several features distinct from traditional single (gene) markers. The number of genes (as well as the individual sequences) included in the prediction model can change over time as more data become available, allowing the introduction of a series of sequential predictors with increasing predictive accuracy. Different mathematical methods and alternative combinations of genes could yield a number of different predictors with similar performance. The profiling methods that will be used routinely in the clinic are not yet clear. Limited profiling using real-time reverse-transcription (RT)-PCR or small custom arrays that measure only the genes that contribute to a prognostic predictive signature are already commercially available (OncotypeDX®, Genomic Health, Inc, Redwood City CA; MammaPrint®, Agendia, Amsterdam, The Netherlands). Similar products for chemotherapy prediction could soon appear on the market soon after completion of the ongoing validation studies. External validation will entail the use of any molecular test in different laboratories to show that the methods are reproducible and widely applicable to tissue in routine clinical practice.

HAS MICROARRAY TECHNOLOGY IDENTIFIED A UNIVERSAL DEATH-FROM-CANCER SIGNATURE?

A highly provocative report has suggested recently that a 'death-from-cancer' gene expression signature may be identifiable in tumour biopsies at the time of presentation, and this represents

dominance of a stem cell population in the malignancy (Glinsky, 2005; Glinsky *et al*., 2005). The hypothesis is that activation in transformed cells of normal stem cells' self-renewal pathways might contribute to the survival of cancer stem cells and promote tumour progression. The gene expression pathway driven by the BMI-1 oncogene is essential for the self-renewal of haematopoietic and neural stem cells, and now it appears that it may be a critical determinant in cancer cell fate. A mouse/human comparative translational genomics approach was used to identify an 11-gene (*GBX2*, *KI67*, *CCNB1*, *BUB1*, *KNTC2*, *USP22*, *HCFC1*, *RNF2*, *ANK3*, *FGFR2*, *CES1*) signature that consistently displays a stem-cell-resembling (BMI-1-driven) expression profile in distant metastatic lesions, as revealed by the analysis of metastases and primary tumours from cancer patients. The 11-gene set comprises two groups: those in which elevated expression levels are associated with stem cell-ness and a poor prognosis (*Ki67*, *CCNB1*, *GBX2*, *BUB1*, *KNTC2*, *USP22* and *RNF2*, in descending order of strength of association), and those for which decreased expression levels are associated with stem cell-ness and a good prognosis (*CES1*, *FGFR2* and *ANK3*).

The prognostic power of the 11-gene signature was validated in several independent therapy-outcome sets of clinical samples obtained from 1153 cancer patients diagnosed with 11 different types of cancer, including five common epithelial malignancies (prostate, breast, lung, ovarian and bladder cancers) and five non-epithelial malignancies (lymphoma, mesothelioma, medulloblastoma, glioma and acute myeloid leukaemia). Kaplan-Meier analysis demonstrated that a stem cell-like expression profile of the 11-gene signature in primary tumours is a consistently powerful predictor of a short interval to disease recurrence, distant metastasis and death after therapy in cancer patients diagnosed with any of these distinct types of cancer. These data suggest the presence of a conserved BMI-1-driven pathway, which is similarly engaged in both normal stem cells and a highly malignant subset of human cancers diagnosed in a wide range of organs and uniformly exhibiting a marked propensity towards metastatic dissemination as well as a high probability of unfavourable therapy outcome. Other authors (Lahad *et al*., 2005) have already replicated part of the analysis of the stem-cell-like phenotype association index (SPAI) in a lung cancer study that included survival data on 125 patients and microarray data from Affymetrix U95Av2 GeneChips, and so more universal application may be possible.

TISSUE MICROARRAYS: WHERE CLASSICAL HISTOPATHOLOGY HANDS THE BATON ON TO MOLECULAR PATHOLOGY

The validation required for translation of tissue biomarkers from the research laboratory to the clinical setting will depend on the combination of tissue microarray (TMA) technology with automated quantitative analysis. Tissue microarrays offer molecular information for both RNA and protein within the context of cellular morphology and tissue architecture. Using conventional histopathology slides, a study of 20 biomarkers in a 400-sample cohort would take weeks to assemble and months to process. Tissue microarrays allow processing of the biomarkers on serial sections of the array on the same day, and at a fraction of the cost. Classical histopathologists are clearly required for initial selection of representative areas for harvesting donor cores, but much of the remaining processing and analysis can be automated. The high-density format of TMAs allows variables such as antigen retrieval, temperature, washing time and reagent concentration to be standardised for the entire cohort, and combination with fully automated systems such as the Ventana Discovery platform (www.ventanadiscovery.com/) adds further quality control. A number of commercial software packages have been produced for the automated analysis of TMAs based on the optical density of chromagen-detected antigens, resulting in objective scores on a continuous scale. Some systems (e.g. Chromavision ACIS,® which combines both image acquisition and quantitative interpretation) achieve higher accuracy than microscopic analysis by classical histopathologists and better agreement with the standard FISH assay for the evaluation of HER-2/*neu*

overexpression by chromagen-linked immunohistochemistry (Wang *et al*., 2001). Similarly promising results have been reported for quantitative analysis of ER and p21 in tumours.

Immunofluorescence-based antigen detection has the well-established advantages of being more sensitive, more linear and having a wider dynamic range. An automated quantitative analysis system that measures protein expression in subcellular compartments on a continuous scale – including in arrayed specimens – has been validated for several biomarkers (Camp *et al*., 2002). An alternative immunofluorescence technique using laser imaging has been shown to give a good correlation to well-studied biomarkers in colon cancer and can be applied to nucleic acid detection by mRNA *in situ* hybridisation (Jubb *et al*., 2003). Using such technology, molecular definition of subcellular compartments beyond the range of classical histopathology is possible: fluorescence-based systems allow co-localisation with markers of specific subcellular compartments, such as the Golgi, mitochondria or endoplasmic reticulum, or even virtual compartments, such as components of signal transduction pathways.

Bioinformatics tools developed for the analysis of gene expression arrays are not suitable for TMA analysis, because the TMA data structure has absolute rather than relational values and missing data preclude the use of Euclidean distance algorithms. New methods that are specific to the problems of TMAs but include elements of microarray annotation and epidemiological analysis are now being developed. Advances in web-based browser systems that allow archiving and annotation of large sets of tissue microarray data (Kim *et al*., 2005) will speed the process of validating individual or clustered markers while maintaining the quality assurance of pathological expertise.

CONCLUSION

So much is now possible in the molecular analysis of clinical material that it is important for pathologists to develop *modi operandi* that enhance their chances of survival in the evolving molecular world. Understanding the technology and embracing the informatics can be facilitated by specialist training. Adopting the new way of thinking encapsulated by systems biology should be mandatory, as should an open attitude to professional networks and the warehousing and mining of data (perhaps, controversially, including the biological data represented by tissue).

The future for molecular medicine is bright; it is up to us to ensure that pathology is part of it.

REFERENCES

Abbruzzese, J.L., Abbruzzese, M.C., Lenzi, R, Hess, K.R. and Raber, M.N. (1995) Analysis of a diagnostic strategy for patients with suspected tumors of unknown origin. *J. Clin. Oncol.* **13**: 2094–2103.

Abrahamsen, H.N., Steiniche, T., Nexo, E., Hamilton-Dutoit, S.J. and Sorensen, B.S. (2003) Towards quantitative mRNA analysis in paraffin-embedded tissues using real-time reverse transcriptase-polymerase chain reaction: a methodological study on lymph nodes from melanoma patients. *J. Mol. Diagn.* **5**: 34–41.

Akada, M., Crnogorac-Jurcevic, T., Lattimore, S., Mahon, P., Lopes, R., Sunamura, M., *et al.* (2005) Intrinsic chemoresistance to gemcitabine is associated with decreased expression of BNIP3 in pancreatic cancer. *Clin. Cancer Res.* **11**: 3094–3101.

Allander, S.V., Nupponen, N.N., Ringner, M., Hostetter, G., Maher, G.W., Goldberger, N., *et al.* (2001) Gastrointestinal stromal tumors with KIT mutations exhibit a remarkably homogeneous gene expression profile. *Cancer Res.* **61**: 8624–8628.

Arango, D., Laiho, P., Kokko, A., Alhopuro, P., Sammalkorpi, H., Salovaara, R., *et al.* (2005) Gene-expression profiling predicts recurrence in Dukes' C colorectal cancer. *Gastroenterology* **129**: 874–84.

Assersohn, L., Gangi, L., Zhao, Y., Dowsett, M., Simon, R., Powles, T.J. and Liu, E.T. (2002) The feasibility of using fine needle aspiration from primary breast cancers for cDNA microarray analyses. *Clin. Cancer Res.* **8**: 794–801.

Baird, K., Davis, S., Antonescu, C.R., Harper, U.L., Walker, R.L., Chen, Y., *et al.* (2005) Gene expression profiling of human sarcomas: insights into sarcoma biology. *Cancer Res.* **65**: 9226–9235.

Banerjea, A., Ahmed, S., Hands, R.E., Huang, F., Han, X., Shaw, P.M., *et al.* (2004) Colorectal cancers with microsatellite instability display mRNA expression signatures characteristic of increased immunogenicity. *Mol. Cancer* **3**: 21.

Bloom, G., Yang, I.V., Boulware, D., Kwong, K.Y., Coppola, D., Eschrich, S., *et al.* (2004) Multi-platform, multi-site, microarray-based human tumor classification. *Am. J. Pathol.* **164**: 9–16.

Bonadonna, G., Valagussa, P., Brambilla, C., Ferrari, L., Moliterni, A., Terenziani, M., *et al.* (1998) Primary chemotherapy in operable breast cancer: eight-year experience at the Milan Cancer Institute. *J. Clin. Oncol.* **16**: 93–100.

Briasoulis, E. and Pavlidis, N. (1997) Cancer of unknown primary origin. *Oncologist* **2**: 142–152.

Camp, R.L., Chung, GG and Rimm, D.L. (2002) Automated subcellular localization and quantification of protein expression in tissue microarrays. *Nat. Med.* **8**: 1323–1327.

Cronin, M., Pho, M., Dutta, D., Stephans, J.C., Shak, S., Kiefer, M.C., *et al.* (2004) Measurement of gene expression in archival paraffin-embedded tissues: development and performance of a 92-gene reverse transcriptase-polymerase chain reaction assay. *Am. J. Pathol.* **164**: 35–42.

Dave, S.S., Wright, G., Tan, B., Rosenwald, A., Gascoyne, R.D., Chan, W.C., *et al.* (2004) Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells. *N. Engl. J. Med.* **351**: 2159–2169.

Decaudin, D., Lepage, E., Brousse, N., Brice, P., Harousseau, J.L., Belhadj, K., *et al.* (1999) Low-grade stage III-IV follicular lymphoma: multivariate analysis of prognostic factors in 484 patients – a study of the groupe d'Etude des lymphomes de l'Adulte. *J. Clin. Oncol.* **17**: 2499–2505.

Ellis, M., Davis, N., Coop, A., Liu, M., Schumaker, L., Lee, R.Y., *et al.* (2002) Development and validation of a method for using breast core needle biopsies for gene expression microarray analyses. *Clin. Cancer Res.* **8**: 1155–1166.

Fisher, B., Bryant, J., Wolmark, N., Mamounas, E., Brown, A., Fisher, E.R., *et al.* (1998) Effect of preoperative chemotherapy on the outcome of women with operable breast cancer. *J. Clin. Oncol.* **16**: 2672–2685.

Fletcher, C.D., Gustafson, P., Rydholm, A., Willen, H. and Akerman, M. (2001) Clinicopathologic re-evaluation of 100 malignant fibrous histiocytomas: prognostic relevance of subclassification. *J. Clin. Oncol.* **19**: 3045–3050.

Fujii, A., Oshima, K., Hamasaki, M., Utsunomiya, H., Okazaki, M., Kagami, Y., *et al.* (2005) Differential expression of cytokines, chemokines and their receptors in follicular lymphoma and reactive follicular hyperplasia: assessment by complementary DNA microarray. *Oncol. Rep.* **13**: 819–824.

Gianni, L., Zambetti, M., Clark, K., Baker, J., Cronin, M., Wu, J., *et al.* (2005) Gene expression profiles in paraffin-embedded core biopsy tissue predict response to chemotherapy in women with locally advanced breast cancer. *J. Clin. Oncol.* **23**: 7265–7277.

Giordano, T.J., Shedden, K.A., Schwartz, D.R., Kuick, R., Taylor, J.M., Lee, N., *et al.* (2001) Organ-specific molecular classification of primary lung, colon, and ovarian adenocarcinomas using gene expression profiles. *Am. J. Pathol.* **159**: 1231–1238.

Glas, A.M., Kersten, M.J., Delahaye, L.J., Witteveen, A.T., Kibbelaar, R.E., Velds, A., *et al.* (2005) Gene expression profiling in follicular lymphoma to assess clinical aggressiveness and to guide the choice of treatment. *Blood* **105**: 301–307.

Glinsky, G.V. (2005) Death-from-cancer signatures and stem cell contribution to metastatic cancer. *Cell Cycle* **4**: 1171–1175.

Glinsky, G.V., Berezovska, O. and Glinskii, A.B. (2005) Microarray analysis identifies a death-from-cancer signature predicting therapy failure in patients with multiple types of cancer. *J. Clin. Invest.* **115**: 1503–1521.

Gulmann, C., Espina, V., Petricoin, E., III, Longo, D.L., Santi, M., Knutsen, T., *et al.* (2005) Proteomic analysis of apoptotic pathways reveals prognostic factors in follicular lymphoma. *Clin. Cancer Res.* **11**: 5847–5855.

Jubb, A.M., Landon, T.H., Burwick, J., Pham, T.Q., Frantz, G.D., Cairns, B., *et al.* (2003) Quantitative analysis of colorectal tissue microarrays by immunofluorescence and *in situ* hybridization. *J. Pathol.* **200**: 577–588.

Kaufmann, M., von Minckwitz, G., Smith, R., Valero, V., Gianni, L., Eiermann, W., *et al.* (2003) International expert panel on the use of primary (preoperative) systemic treatment of operable breast cancer: review and recommendations. *J. Clin. Oncol.* **21**: 2600–2608.

Kim, R., Demichelis, F., Tang, J., Riva, A., Shen, R., Gibbs, D.F., *et al.* (2005) Internet-based profiler system as integrative framework to support translational research. *BMC Bioinformatics* **6**: 304.

Lahad, J.P., Mills, G.B. and Coombes, K.R. (2005) Stem cell-ness: a 'magic marker' for cancer. *J. Clin. Invest.* **115**: 1463–1467.

Le Chevalier, T., Cvitkovic, E., Caille, P., Harvey, J., Contesso, G., Spielmann, M., *et al.* (1988) Early metastatic cancer of unknown primary origin at presentation. A clinical study of 302 consecutive autopsied patients. *Arch. Intern. Med.* **148**: 2035–2039.

Lee, Y.F., John, M., Falconer, A., Edwards, S., Clark, J., Flohr, P., *et al.* (2004) A gene expression signature associated with metastatic outcome in human leiomyosarcomas. *Cancer Res.* **64**: 7201–7204.

Lotze, M.T. and Rees, R.C. (2004) Identifying biomarkers and surrogates of tumors (cancer biometrics): correlation with immunotherapies and immune cells. *Cancer Immunol. Immunother.* **53**: 256–261.

Mirlacher, M., Kasper, M., Storz, M., Knecht, Y., Durmuller, U., Simon, R., *et al.* (2004) Influence of slide aging on results of translational research studies using immunohistochemistry. *Mod. Pathol.* **17**: 1414–1420.

Nakai, Y., Otsuka, M., Hoshida, Y., Tada, M., Komatsu, Y., Kawabe, T., *et al.* (2005) Identifying genes with differential expression in gemcitabine-resistant pancreatic cancer cells using comprehensive transcriptome analysis. *Oncol. Rep.* **14**: 1263–1267.

Nielsen, T.O., West, R.B., Linn, S.C., Alter, O., Knowling, M.A., O'Connell, J.X., *et al.* (2002) Molecular characterisation of soft tissue tumours: a gene expression study. *Lancet* **359**: 1301–1307.

Ohali, A., Avigad, S., Zaizov, R., Ophir, R., Horn-Saban, S., Cohen, I.J., *et al.* (2004) Prediction of high risk Ewing's sarcoma by gene expression profiling. *Oncogene* **23**: 8997–9006.

Paik, S., Shak, S., Tang, G., Kim, C., Baker, J., Cronin, M., *et al.* (2004) A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N. Engl. J. Med.* **351**: 2817–2826.

Pusztai, L., Ayers, M., Stec, J., Clark, E., Hess, K., Stivers, D., *et al.* (2003) Gene expression profiles obtained from fine-needle aspirations of breast cancer reliably identify routine prognostic markers and reveal large-scale molecular differences between estrogen-negative and estrogen-positive tumors. *Clin. Cancer Res.* **9**: 2406–2415.

Ramaswamy, S., Tamayo, P., Rifkin, R., Mukherjee, S., Yeang, C.H., Angelo, M., *et al.* (2001) Multiclass cancer diagnosis using tumor gene expression signatures. *Proc. Natl. Acad. Sci. USA* **98**: 15149–15154.

Reynolds, T. (1998) Patients with unknown primaries face unusual insurance problems. *J. Natl. Cancer Inst.* **90**: 269.

Schaefer, K.L., Brachwitz, K., Wai, D.H., Braun, Y., Diallo, R., Korsching, E., *et al.* (2004) Expression profiling of t(12;22) positive clear cell sarcoma of soft tissue cell lines reveals characteristic up-regulation of potential new marker genes including ERBB3. *Cancer Res.* **64**: 3395–3405.

Schapira, D.V. and Jarrett, A.R. (1995) The need to consider survival, outcome, and expense when evaluating and treating patients with unknown primary carcinoma. *Arch. Intern. Med.* **155**: 2050–2054.

Segal, N.H., Pavlidis, P., Antonescu, C.R., Maki, R.G., Noble, W.S., DeSantis, D., *et al.* (2003) Classification and subtype prediction of adult soft tissue sarcoma by functional genomics. *Am. J. Pathol.* **163**: 691–700.

Shedden, K.A., Taylor, J.M., Giordano, T.J., Kuick, R., Misek, D.E., Rennert, G., *et al.* (2003) Accurate molecular classification of human cancers based on gene expression using a simple classifier with a pathological tree-based framework. *Am. J. Pathol.* **163**: 1985–1995.

Smith, I.C., Heys, S.D., Hutcheon, A.W., Miller, I.D., Payne, S., Gilbert, F.J., *et al.* (2002) Neoadjuvant chemotherapy in breast cancer: significantly enhanced response with docetaxel. *J. Clin. Oncol.* **20**: 1456–1466.

Sotiriou, C., Powles, T.J., Dowsett, M., Jazaeri, A.A., Feldman, A.L., Assersohn, L., *et al.* (2002) Gene expression profiles derived from fine needle aspiration correlate with response to systemic chemotherapy in breast cancer. *Breast Cancer Res.* **4**: R3.

Specht, K., Richter, T., Muller, U., Walch, A., Werner, M., and Hofler, H. (2001) Quantitative gene expression analysis in microdissected archival formalin-fixed and paraffin-embedded tumor tissue. *Am. J. Pathol.* **158**: 419–429.

Spruessel, A., Steimann, G., Jung, M., Lee, S.A., Carr, T., Fentz, A.K., *et al.* (2004) Tissue ischemia time affects gene and protein expression patterns within minutes following surgical tumor excision. *Biotechniques* **36**: 1030–1037.

Su, A.I., Welsh, J.B., Sapinoso, L.M., Kern, S.G., Dimitrov, P., Lapp, H., *et al.* (2001) Molecular classification of human carcinomas by use of gene expression signatures. *Cancer Res.* **61**: 7388–7393.

Symmans, W.F., Ayers, M., Clark, E.A., Stec, J., Hess, K.R., Sneige, N., *et al.* (2003) Total RNA yield and microarray gene expression profiles from fine-needle aspiration biopsy and core-needle biopsy samples of breast carcinoma. *Cancer* **97**: 2960–2971.

Tothill, R.W., Kowalczyk, A., Rischin, D., Bousioutas, A., Haviv, I., van Laar, R.K., *et al.* (2005) An expression-based site of origin diagnostic method designed for clinical application to cancer of unknown origin. *Cancer Res.* **65**: 4031–4040.

Wang, S., Saboorian, M.H., Frenkel, E.P., Haley, B.B., Siddiqui, M.T., Gokaslan, S., *et al.* (2001) Assessment of HER-2/neu status in breast cancer. Automated Cellular Imaging System (ACIS)-assisted quantitation of immunohistochemical assay achieves high accuracy in comparison with fluorescence *in situ* hybridization assay as the standard. *Am. J. Clin. Pathol.* **116**: 495–503.

Wang, S.S., Dasgupta, A., Sherman, M.E., Walker, J.L., Gold, M.A., Zuna, R., *et al.* (2005) Towards improved biomarker studies of cervical neoplasia: effects of precolposcopic procedures on gene expression patterns. *Diagn. Mol. Pathol.* **14**: 59–64.

West, R.B., Harvell, J., Linn, S.C., Liu, C.L., Prapong, W., Hernandez-Boussard, T., *et al.* (2004) Apo D in soft tissue tumors: a novel marker for dermatofibrosarcoma protuberans. *Am. J. Surg. Pathol.* **28**: 1063–1069.

H&E Will Hold Sway

The Invaluable Role of Morphology in the Molecular Era

Jason L. Hornick and Christopher D.M. Fletcher

INTRODUCTION – THE DEATH OF MORPHOLOGY?

Despite remarkable (and rapid) advances in understanding the molecular genetic basis of human malignancies over the past several decades, recently coupled with the accumulation of vast and often overwhelmingly complex expression profiling data, traditional surgical pathology employing conventional haematoxylin and eosin (H&E)-stained slides remains the cornerstone of tumour classification and prognostication. It is indeed surprising how little these molecular developments have altered current clinical practice. This overview explores the ways in which ancillary molecular methodologies have already affected diagnostic histopathology, and emphasises the central role that 'old-school' light microscopic evaluation will continue to play in tumour diagnosis and in guiding emerging applications of molecular findings for prognostication and targeted therapeutics. Although conventional histopathological examination will also continue to dominate the evaluation of most other (non-neoplastic) diseases, because cancer classification and prognostication have been the most hotly debated areas in which molecular genetics might replace morphology, tumour pathology will be the focus of this discussion.

For decades, new biomedical scientific discoveries have led to increasingly frequent proclamations that H&E would soon be obsolete. Such pronouncements often criticise the lack of objectivity of the technique and praise an imminent 'scientific' tumour classification that will replace 'old-fashioned' surgical pathology. However, existing (and evolving) diagnostic categories, created in large part by meticulous attention to cytological and architectural findings, as well as careful correlation with clinical features and patient outcome, are (to employ an overused adjective!) 'robust' to say the least – a sea change in the diagnosis of cancer has yet to occur, despite the great advances in molecular genetics. Nonetheless, new techniques have refined the practice of surgical pathology and have added useful prognostic and therapeutic information in selected cases.

ROLE OF IMMUNOHISTOCHEMISTRY

Immunophenotypic evaluation is now the most widespread and routine ancillary approach to tumour classification (see Chapter 15 of this volume), which arguably plays its most prominent role in haematopathology. For example, in most centres, flow cytometric phenotyping is an integral part of the assessment of virtually all haematolymphoid malignancies. Similarly, in soft-tissue pathology, immunohistochemical findings also provide key support for the diagnosis of some of these rare tumour types. In perhaps its most widely used application outside of haematopathology and soft-tissue pathology, immunohistochemistry can help to suggest possible primary sites in

many cases of metastatic carcinoma in which the primary site is clinically occult. In the uncommon cases of undifferentiated malignancies that show no specific morphological features to aid in classification, immunohistochemical evidence for a specific line of differentiation (e.g. carcinoma versus lymphoma) can be helpful to guide subsequent therapy. Immunohistochemistry has largely supplanted electron microscopy in this regard. In the case of pleomorphic ('MFH'-like) sarcomas, immunohistochemistry can help to support subclassification as leiomyosarcoma or rhabdomyosarcoma, which show significantly higher rates of metastasis than other pleomorphic sarcomas (Gaffney *et al*., 1993; Fletcher *et al*., 2001), such as dedifferentiated liposarcoma (McCormick *et al*., 1994; Henricks *et al*., 1997) or myxofibrosarcoma (Merck *et al*., 1983; Mentzel *et al*., 1996). Even in cases where subclassification is not possible, recent studies have suggested that myogenic differentiation *per se* (defined by immunohistochemistry) is a significant indicator of more aggressive behaviour in pleomorphic sarcomas (Fletcher *et al*., 2001; Deyrup *et al*., 2003; Massi *et al*., 2004). This type of subclassification may in the future guide the development of specific or more intensive therapies exploiting these differences in clinical behaviour.

ROLE OF GENETICS

The earliest contribution that genetics made to tumour classification was the identification, nearly 50 years ago, of the Philadelphia chromosome (Nowell and Hungerford, 1960), resulting from the chromosomal translocation t(9;22) (Rowley, 1973), the defining feature of chronic myelogenous leukaemia (CML). Several decades later, this translocation was shown to result in the juxtaposition of *BCR* and *ABL* (Bartram *et al*., 1983; Groffen *et al*., 1984), the fusion transcript of which encodes a receptor tyrosine kinase, constitutive activation of which is believed to be the central pathogenetic event in CML (Shtivelman *et al*., 1985). In the past decade, the first targeted therapy directed against a receptor tyrosine kinase, imatinib mesylate (Gleevec or Glivec), developed to inhibit the BCR–ABL fusion protein, has shown considerable clinical efficacy in CML (Druker *et al*., 2001). This type of rational targeted molecular therapy has become a new model for the development of pharmacological reagents in oncology (see below).

Reciprocal chromosomal translocations and other karyotypic abnormalities are a recurring theme in haematological malignancies, including other myeloid disorders and lymphomas, the identification of which has, in most cases, confirmed existing diagnostic categories and occasionally has defined entities that were difficult to tease out. For example, mantle cell lymphoma is now recognised as a specific clinicopathological entity chiefly following identification of the t(11;14) chromosomal translocation, which results in fusion of the immunoglobulin heavy chain gene promoter to *BCL-1*, which drives overexpression of cyclin D1 (Williams *et al*., 1990; Rosenberg *et al*., 1991; Vandenberghe *et al*., 1991). Since this discovery, an immunohistochemical assay for cyclin D1 has been developed and is now widely available (de Boer *et al*., 1995; Swerdlow *et al*., 1995; Zukerberg *et al*., 1995), which can allow confirmation of the diagnosis of mantle cell lymphoma in the absence of cytogenetic (or other molecular) analysis. This paradigm, namely the development of simple immunohistochemical tests to demonstrate overexpression of proteins of pathogenetic importance, is widely applicable not only in the subclassification of tumours (numerous examples of which are already used clinically), but also will likely aid in prognostication and the identification of targets for specific cancer therapies (see below).

Cytogenetics is now an invaluable adjunct to the morphological classification and prognostic assessment of myeloid stem cell disorders. Specifically, defined chromosomal abnormalities predict outcome in myelodysplastic syndromes (Greenberg *et al*., 1997), and cytogenetic findings are strong predictors of behaviour in acute myeloid leukaemias (AML) (Bloomfield *et al*., 1998; Grimwade *et al*., 1998; Slovak *et al*., 2000). In fact, the presence of such recurrent chromosomal abnormalities has largely replaced the traditional morphological/histochemical FAB classification

as being the more clinically meaningful distinguishing features among these leukaemias and this is reflected in the current World Health Organisation classification. Nonetheless, in the majority of cases, the traditional classification of AML subtypes correlates remarkably well with cytogenetics, underscoring the power of careful morphological assessment and validating pre-existing classification schemes.

There are also examples of chromosomal translocations that distinguish different prognostic groups among non-Hodgkin lymphomas that are not discernible by routine histological examination. For example, patients with anaplastic large-cell lymphoma showing translocations that result in rearrangement of the *ALK* gene (most frequently t(2;5)) have significantly better survival than those whose tumours lack such translocations. Moreover, immunohistochemical detection of the ALK protein serves as a clinically valuable surrogate for such translocations and has prognostic value (Shiota *et al*., 1995; Falini *et al*., 1999; Gascoyne *et al*., 1999). As another example, in the case of gastric extranodal marginal zone B-cell lymphoma (MALT lymphoma), in which most patients experience sustained remission following eradication of *Helicobacter pylori* infection, the presence of the t(11;18) translocation correlates with resistance to *Helicobacter* eradication and a worse outcome (Alpen *et al*., 2000; Liu *et al*., 2001; Levy *et al*., 2005).

Simple recurrent chromosomal translocations are also characteristic of some types of soft-tissue sarcomas, the presence of which, in the majority of cases, has served to validate existing morphological classification schemes rather than defining new entities. For example, the t(X;18) translocation resulting in the fusion of the *SYT* and *SSX1* or *SSX2* genes is found in essentially all cases of synovial sarcoma (Clark *et al*., 1994; de Leeuw *et al*., 1995), and translocations of *EWS* (most often t(11;22) involving *EWS* and *FLI1*) characterize the Ewing sarcoma/peripheral neuroectodermal tumour (PNET) family of tumours (Delattre *et al*., 1992; Zucman *et al*., 1992). These tumour types have sufficiently distinctive morphological and clinical features that the diagnosis can usually be based simply upon conventional H&E examination, supported by immunohistochemistry, and cytogenetic or molecular genetic analysis is generally reserved for cases with atypical histological or clinical features. However, recent clinical studies have suggested that the specific fusion types (or breakpoint sites) may alter the prognosis for patients with these sarcomas, but the results are contradictory. For instance, in synovial sarcoma, several studies have shown that tumours harbouring the *SYT–SSX1* gene fusion behave in a more aggressive fashion than those with the *SYT–SSX2* gene fusion (Kawai *et al*., 1998; Nilsson *et al*., 1999; Inagaki *et al*., 2000; Panagopoulos *et al*., 2001; Ladanyi *et al*.; 2002), but a recent large study found no survival difference between fusion types (Guillou *et al*., 2004). Similar survival benefits for specific fusion sites have been suggested in Ewing sarcoma/PNET (Zoubek *et al*., 1996; de Alava *et al*., 1998; Ginsberg *et al*., 1999) and alveolar rhabdomyosarcoma (Kelly *et al*., 1997; Sorensen *et al*., 2002), but the results of these studies are preliminary and also somewhat contradictory. Nonetheless, these types of molecular analyses may ultimately prove useful in adding prognostic information beyond that which is available from current histological and clinical parameters. It should be emphasised, however, that the apparent prognostic information gained from these expensive molecular tests (which can only be undertaken in specialised, usually academic, centres) is at best modest, and widespread application may not be a reasonable expectation nor cost-effective (Hahn and Fletcher, 2005).

In carcinomas, which constitute the overwhelming majority of human cancers, molecular genetics has had surprisingly little impact on tumour classification and prognostication. The conventional H&E-based assessment of tumour grade and the determination of tumour stage remain the most important predictors of outcome for carcinomas arising at most anatomic sites. One notable, but pretty much isolated, exception, which exemplifies an emerging role for molecular genetics, is in colorectal carcinomas (CRC). In these tumours, deficiencies in components of the mismatch repair (MMR) machinery correlate with improved survival (Sankila *et al*., 1996; Gryfe *et al*., 2000; Guidoboni *et al*., 2001; Popat *et al*., 2005), and, at the same time, decreased

susceptibility to conventional 5-fluorouracil-based chemotherapy (Ribic *et al*., 2003; Carethers *et al*., 2004). As a consequence of MMR deficiency, these CRCs show high levels of microsatellite instability (MSI). Approximately 15% of all CRCs show MSI, most of which arise sporadically secondary to promoter methylation and transcriptional silencing of the *MLH1* gene (Herman *et al*., 1998). In a relatively small proportion of tumours, MSI is caused by inherited mutations (most often in the *MSH2* or *MLH1* genes) (Peltomaki and Vasen, 1997), in patients with the hereditary non-polyposis colorectal carcinoma (HNPCC) syndrome. These patients also have an increased risk of developing carcinomas at other anatomic sites, most often the endometrium, renal pelvis, ureter and small bowel. Interestingly, there are some histological correlates to MMR deficiency. Specifically, right-sided mucinous CRCs and those showing poor differentiation and numerous tumour-infiltrating lymphocytes are more likely to show MSI (Jass *et al*., 1998; Shashidharan *et al*., 1999). However, although such features may suggest MSI, this correlation is not very strong. As described above in other tumour types, recent studies have shown that immunohistochemistry is a good surrogate for assessment of MSI (Marcus *et al*., 1999; Chaves *et al*., 2000; Lindor *et al*., 2002; Shia *et al*., 2005): loss of expression of either the MLH1 or MSH2 protein can serve as a good initial screening test, followed by polymerase chain reaction (PCR)-based assessment of microsatellite repeats to confirm MSI. Although MSI determination is not yet standard practice and is generally reserved for patients in whom there is a suspicion for HNPCC, it seems likely that this type of analysis, whether by immunohistochemistry or formal microsatellite testing, will in time be applied widely, given its significant prognostic and therapeutic implications.

GENE EXPRESSION PROFILING: CLINICAL APPLICATIONS?

Recently, there has been a remarkably rapid emergence of vast amounts of gene expression data for countless human tumours, mainly in attempts to identify genes or groups of genes, the expression of which correlates with either diagnosis or clinical outcome. Virtually all attempts at 'diagnostic' classification by this rather expensive route have done no more than validate long-standing (and much cheaper!) light microscopic classifications. Nevertheless, perhaps the best example thus far of the potential for this approach is in the case of the most common type of lymphoma, diffuse large B-cell lymphoma (DLBCL), which is clinically heterogeneous, with nearly half of the affected patients entering remission following combination chemotherapy, while the remainder die of progressive disease. Morphology alone cannot predict these differences in outcome. In 2000, a gene expression profiling study using DNA microarrays suggested two distinct subgroups of DLBCL, which have different expression patterns, that appeared to correlate remarkably well with outcome: a 'germinal centre B cell-like' group and an 'activated B cell-like' group (Alizadeh *et al*., 2000). Patients in this study whose tumours showed the former expression profile had a significantly better overall survival than the latter. Because such a complex (and expensive) analysis is not currently feasible in routine clinical practice (and is unlikely to be so in the foreseeable future), expression array studies have in some cases stimulated follow-up studies utilising immunohistochemical analysis of the most promising markers that had been identified. Along these lines, several studies of DLBCL have attempted to recapitulate these data with a small group of tissue-based markers (Chang *et al*., 2004; Hans *et al*., 2004; Berglund *et al*., 2005; Biasoli *et al*., 2005). For example, detection of the 'germinal centre' markers CD10 and bcl-6 by immunohistochemistry appears to predict good outcome, whereas the 'activation' marker MUM1/IRF4 appears to correlate with poor outcome in patients with DLBCL (Chang *et al*., 2004; Hans *et al*., 2004). Although these studies are preliminary, they provide a model for the translation of gene expression profiling data to a much more widely applicable technology such as immunohistochemistry. It is critical that such immunohistochemical analyses of potential prognostic markers be performed rigorously in clinical trials to prove significance prior to introduction into the routine clinical arena.

In this regard, it is important to remember that the results of expression profiling studies are only as good as the initial diagnostic assignment of the tumour categories or subtypes being examined – invaluable annotation that can be provided only by a trained anatomic pathologist. For this reason, it is essential that a morphologist be directly involved in such studies to verify and specify diagnoses prior to analysis, not to mention confirm that the sample analysed is in fact lesional (and not adjacent non-neoplastic or necrotic tissue). Unfortunately, some high-profile expression microarray studies have not been appropriately meticulous; in fact, pathologists are frequently absent from such studies when they should be playing a central and critical role and such large-scale (and expensive) experiments run the significant risk of yielding data that are of dubious (if any) significance. At the same time, with few notable exceptions (such as those described above), there has generally been insufficient effort to validate the results of these molecular studies by tissue-based clinical trials using selected potentially prognostic markers, including critical multivariate analyses for comparison with conventional histological (and clinical) predictors of outcome. Again, this major gap in bridging bench-top research and clinical medicine stems in part from the lack of involvement in many such studies by histopathologists, who are uniquely qualified to assist in the development and evaluation of such tissue-based markers for appropriate translation to patient care.

TARGETED THERAPEUTICS

Another promising application of molecular genetics to cancer is the development of targeted therapeutics. One of the first examples of such a targeted therapy is trastuzumab (herceptin), a monoclonal antibody directed against the HER-2/neu oncoprotein in breast cancer. Approximately 30% of breast cancers show amplification (and consequent overexpression) of HER-2/neu, which not only serves as a therapeutic target but also correlates with poor prognosis (Slamon *et al*., 1987, 1989). In most centres (and in most, but not all, cases), HER-2/neu testing is assessed by immunohistochemistry and light microscopy. Initial studies of patients with metastatic breast cancer overexpressing HER-2/neu showed a modest survival benefit with trastuzumab therapy (Baselga *et al*., 1996; Cobleigh *et al*., 1999), strengthened by the addition of chemotherapy (Slamon *et al*., 2001), and very recent studies demonstrated a dramatic survival benefit for patients with early stage HER-2/neu-overexpressing breast cancers who were treated with adjuvant trastuzumab combined with chemotherapy following surgery (Piccart-Gebhart *et al*., 2005; Romond *et al*., 2005). In a second recent example of a targeted molecular therapy, a small subgroup of patients with non-small-cell lung cancer (NSCLC) respond dramatically to the epidermal growth factor receptor (EGFR) inhibitor gefitinib (Fukuoka *et al*., 2003; Kris *et al*., 2003). Very recently, activating mutations in *EGFR* were shown to predict gefitinib response (Lynch *et al*., 2004; Paez *et al*., 2004). Patients who respond to such therapy are also more likely to have adenocarcinomas (particularly those with a bronchioloalveolar component) (Miller *et al*., 2004; Kim *et al*., 2005; Yatabe *et al*.; 2005). Similarly, and as mentioned previously, the small-molecule tyrosine kinase inhibitor imatinib mesylate, directed against BCR–ABL, has clinical efficacy in CML (Druker *et al*., 2001). This same drug is also effective in the treatment of metastatic gastrointestinal stromal tumours (GISTs) (Demetri *et al*., 2002), due to its activity against the tyrosine kinase receptor *c*-kit (Heinrich *et al*., 2000; Tuveson *et al*., 2001), which is mutated in approximately 85% of GISTs (Hirota *et al*., 1998), leading to constitutive kinase activity. Most of the remaining GISTs harbour analogous activating mutations in the tyrosine kinase platelet-derived growth factor receptor-alpha gene (*PDGFRA*) (Heinrich *et al*., 2003b). Immunohistochemical detection of *c*-kit is invaluable to confirm the diagnosis of GIST, although a small subset of GISTs are *c*-kit-negative (Debiec-Rychter *et al*., 2004; Medeiros *et al*., 2004); most of these latter tumours have activating mutations in *PDGFRA*. Nonetheless, tyrosine kinase inhibitors such as imatinib may also be efficacious in some of these tumours by inhibiting PDGFRA. Interestingly, recent studies have indicated that the specific site of

the *c-kit* or *PDGFRA* gene mutation in GISTs predicts the clinical response to imatinib (Heinrich *et al*., 2003a; Corless *et al*., 2005). The role of DNA sequence analysis for prognostication remains uncertain, however, because at present such an approach is time-consuming, expensive and not widely available. The imminent possibility of different targeted therapies, most effective against specific types of mutation, may well influence this situation.

LESS AND LESS TISSUE

Increasingly, radiology-guided core-needle biopsies and aspirations are replacing surgical (incisional) biopsies as alternative approaches to tumour sampling for diagnosis. Although such procedures may allow for decreased hospital stays and (arguably) less morbidity, the yield of diagnostic material is lower and, even if lesional tissue is obtained, the scant nature of the specimen may not allow for a specific (morphological) diagnosis. In this context, in at least some cases molecular genetics may provide useful and, at times, essential contributions to tumour classification. Appropriate examination of immunophenotype (e.g. using flow cytometry of a lymphoid neoplasm), detection of a fusion product of a chromosomal translocation (as described above, using fluorescence *in situ* hybridisation (FISH) or reverse-transcription PCR) and/or evaluation for an immunoglobulin or T-cell receptor gene rearrangement (to support clonality in the case of a low-grade lymphoproliferative disorder) may then lead to a specific diagnosis. However, even in this context, skilled morphological assessment is absolutely critical for guiding selection of the appropriate molecular investigation that should be undertaken.

CONCLUSION

In summary, light microscopic examination of tissue specimens by surgical pathologists will continue to remain central to tumour classification, at least for the foreseeable future. Without question, morphology remains the gold standard against which all newer technologies have to be validated and assessed. Expression profiling of tumours, instead of replacing conventional H&E-based morphological assessment, will likely aid in the identification of prognostic markers and targets for therapeutic intervention. Such an approach will likely require the development of immunohistochemical reagents for rapid and inexpensive assessment of protein overexpression in order for these discoveries to translate into routine clinical practice. In the future, it seems likely that a panel of markers will be evaluated in many human cancers as adjuncts to the standard prognostic parameters established by routine morphological assessment. The panel of markers investigated will, of course, be determined by the specific tumour type, as established by the surgical pathologist. Therapeutic targets can then be evaluated in a similar fashion. As we move into this new era of rational targeted molecular therapies, meticulous attention to precise tumour classification remains critical for the development of such therapies, and surgical pathology will undoubtedly continue to occupy a central role.

We should also remember that any new technologies must be both affordable and as widely available as possible to ensure maximum benefit for society, otherwise there will continue to be increasingly wide disparities in the distribution and quality of healthcare between the extremely wealthy and the remainder of society. Even now, 25 years after its introduction, immunohistochemistry is not used in many parts of the world because of its expense and a related lack of both technical training and interpretative expertise. If many of the world's healthcare systems cannot afford such established technology, it is difficult to justify the widespread dissemination of newer, much more expensive and technically challenging molecular diagnostic approaches to tumour classification and prognostication until broader and more basic needs are met.

REFERENCES

Alizadeh, A. A., Eisen, M. B., Davis, R. E., *et al.* (2000) Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* **403**: 503–511.

Alpen, B., Neubauer, A., Dierlamm, J., *et al.* (2000) Translocation t(11;18) absent in early gastric marginal zone B-cell lymphoma of MALT type responding to eradication of Helicobacter pylori infection. *Blood* **95**: 4014–4015.

Bartram, C. R., de Klein, A., Hagemeijer, A., *et al.* (1983) Translocation of c-ab1 oncogene correlates with the presence of a Philadelphia chromosome in chronic myelocytic leukaemia. *Nature* **306**: 277–280.

Baselga, J., Tripathy, D., Mendelsohn, J., *et al.* (1996) Phase II study of weekly intravenous recombinant humanized anti-p185HER2 monoclonal antibody in patients with HER2/neu-overexpressing metastatic breast cancer. *J. Clin. Oncol.* **14**: 737–744.

Berglund, M., Thunberg, U., Amini, R. M., *et al.* (2005) Evaluation of immunophenotype in diffuse large B-cell lymphoma and its impact on prognosis. *Mod. Pathol.* **18**: 1113–1120.

Biasoli, I., Morais, J. C., Scheliga, A., *et al.* (2005) CD10 and Bcl-2 expression combined with the International Prognostic Index can identify subgroups of patients with diffuse large-cell lymphoma with very good or very poor prognoses. *Histopathology* **46**: 328–333.

Bloomfield, C. D., Lawrence, D., Byrd, J.C., *et al.* (1998) Frequency of prolonged remission duration after high-dose cytarabine intensification in acute myeloid leukemia varies by cytogenetic subtype. *Cancer Res.* **58**: 4173–4179.

Carethers, J. M., Smith, E. J., Behling, C. A., *et al.* (2004) Use of 5-fluorouracil and survival in patients with microsatellite-unstable colorectal cancer. *Gastroenterology* **126**: 394–401.

Chang, C. C., McClintock, S., Cleveland, R. P., *et al.* (2004) Immunohistochemical expression patterns of germinal center and activation B-cell markers correlate with prognosis in diffuse large B-cell lymphoma. *Am. J. Surg. Pathol.* **28**: 464–470.

Chaves, P., Cruz, C., Lage, P., *et al.* (2000) Immunohistochemical detection of mismatch repair gene proteins as a useful tool for the identification of colorectal carcinoma with the mutator phenotype. *J. Pathol.* **191**: 355–360.

Clark, J., Rocques, P. J., Crew, A. J., *et al.* (1994) Identification of novel genes, SYT and SSX, involved in the t(X;18)(p11.2;q11.2) translocation found in human synovial sarcoma. *Nat. Genet.* **7**: 502–508.

Cobleigh, M. A., Vogel, C. L., Tripathy, D., *et al.* (1999) Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. *J. Clin. Oncol.* **17**: 2639–2648.

Corless, C. L., Schroeder, A., Griffith, D., *et al.* (2005) PDGFRA mutations in gastrointestinal stromal tumours: frequency, spectrum and in vitro sensitivity to imatinib. *J. Clin. Oncol.* **23**: 5357–5364.

de Alava, E., Kawai, A., Healey, J. H., *et al.* (1998) EWS-FLI1 fusion transcript structure is an independent determinant of prognosis in Ewing's sarcoma. *J. Clin. Oncol.* **16**: 1248–1255.

de Boer, C. J., Schuuring, E., Dreef, E., *et al.* (1995) Cyclin D1 protein analysis in the diagnosis of mantle cell lymphoma. *Blood* **86**: 2715–2723.

de Leeuw, B., Balemans, M., Olde Weghuis, D., *et al.* (1995) Identification of two alternative fusion genes, SYT-SSX1 and SYT-SSX2, in t(X;18)(p11.2;q11.2)-positive synovial sarcomas. *Hum. Mol. Genet.* **4**: 1097–1099.

Debiec-Rychter, M., Wasag, B., Stul, M., *et al.* (2004) Gastrointestinal stromal tumours (GISTs) negative for KIT (CD117 antigen) immunoreactivity. *J. Pathol.* **202**: 430–438.

Delattre, O., Zucman, J., Plougastel, B., *et al.* (1992) Gene fusion with an ETS DNA-binding domain caused by chromosome translocation in human tumours. *Nature* **359**: 162–165.

Demetri, G. D., von Mehren, M., Blanke, C. D., *et al.* (2002) Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumours. *N. Engl. J. Med.* **347**: 472–480.

Deyrup, A. T., Haydon, R. C., Huo, D., *et al.* (2003) Myoid differentiation and prognosis in adult pleomorphic sarcomas of the extremity: an analysis of 92 cases. *Cancer* **98**: 805–813.

Druker, B. J., Talpaz, M., Resta, D. J., *et al.* (2001) Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N. Engl. J. Med.* **344**: 1031–1037.

Falini, B., Pileri, S., Zinzani, P. L., *et al.* (1999) ALK+ lymphoma: clinico-pathological findings and outcome. *Blood* **93**: 2697–2706.

Fletcher, C. D., Gustafson, P., Rydholm, A., *et al.* (2001) Clinicopathologic re-evaluation of 100 malignant fibrous histiocytomas: prognostic relevance of subclassification. *J. Clin. Oncol.* **19**: 3045–3050.

Fukuoka, M., Yano, S., Giaccone, G., *et al.* (2003) Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer (The IDEAL 1 Trial). *J. Clin. Oncol.* **21**: 2237–2246.

Gaffney, E. F., Dervan, P. A. and Fletcher, C. D. (1993) Pleomorphic rhabdomyosarcoma in adulthood. Analysis of 11 cases with definition of diagnostic criteria. *Am. J. Surg. Pathol.* **17**: 601–609.

Gascoyne, R. D., Aoun, P., Wu, D., *et al.* (1999) Prognostic significance of anaplastic lymphoma kinase (ALK) protein expression in adults with anaplastic large cell lymphoma. *Blood* **93**: 3913–3921.

Ginsberg, J. P., de Alava, E., Ladanyi, M., *et al.* (1999) EWS-FLI1 and EWS-ERG gene fusions are associated with similar clinical phenotypes in Ewing's sarcoma. *J. Clin. Oncol.* **17**: 1809–1814.

Greenberg, P., Cox, C., LeBeau, M. M., *et al.* (1997) International scoring system for evaluating prognosis in myelodysplastic syndromes. *Blood* **89**: 2079–2088.

Grimwade, D., Walker, H., Oliver, F., *et al.* (1998) The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial. The Medical Research Council Adult and Children's Leukaemia Working Parties. *Blood* **92**: 2322–2333.

Groffen, J., Stephenson, J. R., Heisterkamp, N., *et al.* (1984) Philadelphia chromosomal breakpoints are clustered within a limited region, bcr, on chromosome 22. *Cell* **36**: 93–99.

Gryfe, R., Kim, H., Hsieh, E. T., *et al.* (2000) Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. *N. Engl. J. Med.* **342**: 69–77.

Guidoboni, M., Gafa, R., Viel, A., *et al.* (2001) Microsatellite instability and high content of activated cytotoxic lymphocytes identify colon cancer patients with a favorable prognosis. *Am. J. Pathol.* **159**: 297–304.

Guillou, L., Benhattar, J., Bonichon, F., *et al.* (2004) Histologic grade, but not SYT-SSX fusion type, is an important prognostic factor in patients with synovial sarcoma: a multicenter, retrospective analysis. *J. Clin. Oncol.* **22**: 4040–4050.

Hahn, H. P. and Fletcher, C. D. M. (2005) The role of cytogenetics and molecular genetics in soft tissue tumour diagnosis – a realistic appraisal. *Curr. Diagn. Pathol.* **11**: 361–370.

Hans, C. P., Weisenburger, D. D., Greiner, T. C., *et al.* (2004) Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood* **103**: 275–282.

Heinrich, M. C., Corless, C. L., Demetri, G. D., *et al.* (2003a) Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumour. *J. Clin. Oncol.* **21**: 4342–4349.

Heinrich, M. C., Corless, C. L., Duensing, A., *et al.* (2003b) PDGFRA activating mutations in gastrointestinal stromal tumours. *Science* **299**: 708–710.

Heinrich, M. C., Griffith, D. J., Druker, B. J., *et al.* (2000) Inhibition of c-kit receptor tyrosine kinase activity by STI 571, a selective tyrosine kinase inhibitor. *Blood* **96**: 925–932.

Henricks, W. H., Chu, Y. C., Goldblum, J. R, *et al.* (1997) Dedifferentiated liposarcoma: a clinicopathological analysis of 155 cases with a proposal for an expanded definition of dedifferentiation. *Am. J. Surg. Pathol.* **21**: 271–281.

Herman, J. G., Umar, A., Polyak, K., *et al.* (1998) Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. *Proc. Natl. Acad. Sci. USA* **95**: 6870–6875.

Hirota, S., Isozaki, K., Moriyama, Y., *et al.* (1998) Gain-of-function mutations of c-kit in human gastrointestinal stromal tumours. *Science* **279**: 577–580.

Inagaki, H., Nagasaka, T., Otsuka, T., *et al.* (2000) Association of SYT-SSX fusion types with proliferative activity and prognosis in synovial sarcoma. *Mod. Pathol.* **13**: 482–488.

Jass, J. R., Do, K. A., Simms, L. A., *et al.* (1998) Morphology of sporadic colorectal cancer with DNA replication errors. *Gut* **42**: 673–679.

Kawai, A., Woodruff, J., Healey, J. H., *et al.* (1998) SYT-SSX gene fusion as a determinant of morphology and prognosis in synovial sarcoma. *N. Engl. J. Med.* **338**: 153–160.

Kelly, K. M., Womer, R. B., Sorensen, P. H., *et al.* (1997) Common and variant gene fusions predict distinct clinical phenotypes in rhabdomyosarcoma. *J. Clin. Oncol.* **15**: 1831–1836.

Kim, K. S., Jeong, J. Y., Kim, Y. C., *et al.* (2005) Predictors of the response to gefitinib in refractory non-small cell lung cancer. *Clin. Cancer Res.* **11**: 2244–2251.

Kris, M. G., Natale, R. B., Herbst, R. S., *et al.* (2003) Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. *JAMA* **290**: 2149–2158.

Ladanyi, M., Antonescu, C. R., Leung, D. H., *et al.* (2002) Impact of SYT-SSX fusion type on the clinical behavior of synovial sarcoma: a multi-institutional retrospective study of 243 patients. *Cancer Res.* **62**: 135–140.

Levy, M., Copie-Bergman, C., Gameiro, C., *et al.* (2005) Prognostic value of translocation t(11;18) in tumoural response of low-grade gastric lymphoma of mucosa-associated lymphoid tissue type to oral chemotherapy. *J. Clin. Oncol.* **23**: 5061–5066.

Lindor, N. M., Burgart, L. J., Leontovich, O., *et al.* (2002) Immunohistochemistry versus microsatellite instability testing in phenotyping colorectal tumours. *J. Clin. Oncol.* **20**: 1043–1048.

Liu, H., Ruskon-Fourmestraux, A., Lavergne-Slove, A., *et al.* (2001) Resistance of t(11;18) positive gastric mucosa-associated lymphoid tissue lymphoma to Helicobacter pylori eradication therapy. *Lancet* **357**: 39–40.

Lynch, T. J., Bell, D. W., Sordella, R., *et al.* (2004) Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N. Engl. J. Med.* **350**: 2129–2139.

Marcus, V. A., Madlensky, L., Gryfe, R., *et al.* (1999) Immunohistochemistry for hMLH1 and hMSH2: a practical test for DNA mismatch repair-deficient tumours. *Am. J. Surg. Pathol.* **23**: 1248–1255.

Massi, D., Beltrami, G., Capanna, R., *et al.* (2004) Histopathological re-classification of extremity plemorphic soft tissue sarcoma has clinical relevance. *Eur. J. Surg. Oncol.* **30**: 1131–1136.

McCormick, D., Mentzel, T., Beham, A., *et al.* (1994) Dedifferentiated liposarcoma. Clinicopathologic analysis of 32 cases suggesting a better prognostic subgroup among pleomorphic sarcomas. *Am. J. Surg. Pathol.* **18**: 1213–1223.

Medeiros, F., Corless, C. L., Duensing, A., *et al.* (2004) KIT-negative gastrointestinal stromal tumours: proof of concept and therapeutic implications. *Am. J. Surg. Pathol.* **28**: 889–894.

Mentzel, T., Calonje, E., Wadden, C., *et al.* (1996) Myxofibrosarcoma. Clinicopathologic analysis of 75 cases with emphasis on the low-grade variant. *Am. J. Surg. Pathol.* **20**: 391–405.

Merck, C., Angervall, L., Kindblom, L. G., *et al.* (1983) Myxofibrosarcoma. A malignant soft tissue tumour of fibroblastic-histiocytic origin. A clinicopathologic and prognostic study of 110 cases using multivariate analysis. *Acta Pathol. Microbiol. Immunol. Scand. Suppl.* **282**: 1–40.

Miller, V. A., Kris, M. G., Shah, N., *et al.* (2004) Bronchioloalveolar pathologic subtype and smoking history predict sensitivity to gefitinib in advanced non-small-cell lung cancer. *J. Clin. Oncol.* **22**: 1103–1109.

Nilsson, G., Skytting, B., Xie, Y., *et al.* (1999) The SYT-SSX1 variant of synovial sarcoma is associated with a high rate of tumour cell proliferation and poor clinical outcome. *Cancer Res.* **59**: 3180–3184.

Nowell, P. C. and Hungerford, D. A. (1960) A minute chromosome in human chronic granulocytic leukemia. *Science* **132**: 1497–1500.

Paez, J. G., Janne, P. A., Lee, J. C., *et al.* (2004) EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* **304**: 1497–1500.

Panagopoulos, I., Mertens, F., Isaksson, M., *et al.* (2001) Clinical impact of molecular and cytogenetic findings in synovial sarcoma. *Genes Chromosomes Cancer* **31**: 362–372.

Peltomaki, P. and Vasen, H. F. (1997) Mutations predisposing to hereditary nonpolyposis colorectal cancer: database and results of a collaborative study. The International Collaborative Group on Hereditary Nonpolyposis Colorectal Cancer. *Gastroenterology* **113**: 1146–1158.

Piccart-Gebhart, M. J., Procter, M., Leyland-Jones, B., *et al.* (2005) Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. *N. Engl. J. Med.* **353**: 1659–1672.

Popat, S., Hubner, R. and Houlston, R. S. (2005) Systematic review of microsatellite instability and colorectal cancer prognosis. *J. Clin. Oncol.* **23**: 609–618.

Ribic, C. M., Sargent, D. J., Moore, M. J., *et al.* (2003) Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *N. Engl. J. Med.* **349**: 247–257.

Romond, E. H., Perez, E. A., Bryant, J., *et al.* (2005) Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *N. Engl. J. Med.* **353**: 1673–1684.

Rosenberg, C. L., Wong, E., Petty, E. M., *et al.* (1991) PRAD1, a candidate BCL1 oncogene: mapping and expression in centrocytic lymphoma. *Proc. Natl. Acad. Sci. USA* **88**: 9638–9642.

Rowley, J. D. (1973) A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature* **243**: 290–293.

Sankila, R., Aaltonen, L. A., Jarvinen, H. J., *et al*. (1996) Better survival rates in patients with MLH1-associated hereditary colorectal cancer. *Gastroenterology* **110**: 682–687.

Shashidharan, M., Smyrk, T., Lin, K. M., *et al*. (1999) Histologic comparison of hereditary nonpolyposis colorectal cancer associated with MSH2 and MLH1 and colorectal cancer from the general population. *Dis. Colon Rectum* **42**: 722–726.

Shia, J., Klimstra, D. S., Nafa, K., *et al*. (2005) Value of immunohistochemical detection of DNA mismatch repair proteins in predicting germline mutation in hereditary colorectal neoplasms. *Am. J. Surg. Pathol.* **29**: 96–104.

Shiota, M., Nakamura, S., Ichinohasama, R., *et al*. (1995) Anaplastic large cell lymphomas expressing the novel chimeric protein p80NPM/ALK: a distinct clinicopathologic entity. *Blood* **86**: 1954–1960.

Shtivelman, E., Lifshitz, B., Gale, R. P., *et al*. (1985) Fused transcript of abl and bcr genes in chronic myelogenous leukaemia. *Nature* **315**: 550–554.

Slamon, D. J., Clark, G. M., Wong, S. G., *et al*. (1987) Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* **235**: 177–182.

Slamon, D. J., Godolphin, W., Jones, L. A., *et al*. (1989) Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* **244**: 707–712.

Slamon, D. J., Leyland-Jones, B., Shak, S., *et al*. (2001) Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N. Engl. J. Med*. **344**: 783–792.

Slovak, M. L., Kopecky, K. J., Cassileth, P. A., *et al*. (2000) Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/Eastern Cooperative Oncology Group Study. *Blood* **96**: 4075–4083.

Sorensen, P. H., Lynch, J. C., Qualman, S. J., *et al*. (2002) PAX3-FKHR and PAX7-FKHR gene fusions are prognostic indicators in alveolar rhabdomyosarcoma: a report from the children's oncology group. *J. Clin. Oncol*. **20**: 2672–2679.

Swerdlow, S. H., Yang, W. I., Zukerberg, L. R., *et al*. (1995) Expression of cyclin D1 protein in centrocytic/mantle cell lymphomas with and without rearrangement of the BCL1/cyclin D1 gene. *Hum. Pathol*. **26**: 999–1004.

Tuveson, D. A., Willis, N. A., Jacks, T., *et al*. (2001) STI571 inactivation of the gastrointestinal stromal tumour c-KIT oncoprotein: biological and clinical implications. *Oncogene* **20**: 5054–5058.

Vandenberghe, E., De Wolf-Peeters, C., van den Oord, J., *et al*. (1991) Translocation (11;14): a cytogenetic anomaly associated with B-cell lymphomas of non-follicle centre cell lineage. *J. Pathol*. **163**: 13–18.

Williams, M. E., Westermann, C. D. and Swerdlow, S. H. (1990) Genotypic characterization of centrocytic lymphoma: frequent rearrangement of the chromosome 11 bcl-1 locus. *Blood* **76**: 1387–1391.

Yatabe, Y., Kosaka, T., Takahashi, T., *et al*. (2005) EGFR mutation is specific for terminal respiratory unit type adenocarcinoma. *Am. J. Surg. Pathol*. **29**: 633–639.

Zoubek, A., Dockhorn-Dworniczak, B., Delattre, O., *et al*. (1996) Does expression of different EWS chimeric transcripts define clinically distinct risk groups of Ewing tumour patients?. *J. Clin. Oncol*. **14**: 1245–1251.

Zucman, J., Delattre, O., Desmaze, C., *et al*. (1992) Cloning and characterization of the Ewing's sarcoma and peripheral neuroepithelioma t(11;22) translocation breakpoints. *Genes Chromosomes Cancer* **5**: 271–277.

Zukerberg, L. R., Yang, W. I., Arnold, A., *et al*. (1995) Cyclin D1 expression in non-Hodgkin's lymphomas. Detection by immunohistochemistry. *Am. J. Clin. Pathol*. **103**: 756–760.