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Perspectives in Cancer Research

The Pathologist and the Clinical Oncologist: a New Effective Partnership in Assessing Tumor Prognosis

GLORIA SACCANI JOTTI* and GIANNI BONADONNA†

**Institute of Pathology, University of Parma, School of Medicine, Parma, Italy and †Division of Medical Oncology, National Tumor Institute, Milan, Italy*

Abstract—*During the past 20 years, we have witnessed a progressive radical change in the role of the pathologist vis à vis several aspects of clinical oncology. From the traditional description by light microscopy of resected specimens and study of tumor classifications, the modern pathologist has expanded his domain of action which now results in deeper and more effective interactions with the surgeon, radiotherapist and medical oncologist. The wide application of fine-needle biopsy has substantially improved early diagnosis of primary neoplasms and local and distant recurrences. The histogenetic classification based on type of tissue formed by the tumor rather than type of tissue from which tumor arises, the results of pathologic staging as well as the degree of tumor cell necrosis following primary chemotherapy are now being correlated with treatment outcome. The assessment of tumor grade and ploidy can allow more accurate selection of patient subgroups at high risk of early relapse. Last but not least, the morphologic recognition of certain types of iatrogenic morbidity helps in the reassessment of given drug combinations. During the past decade, pathologists have contributed to the development of several new investigative techniques whose first applications, in most instances, were in laboratories dedicated to basic research. More recently, some pathologists have begun to explore the feasibility of applying these same techniques to clinical cancer research. Modern sophisticated technology, including flow cytometry, immunohistochemistry and monoclonal antibodies, can now provide research physicians with important prognostic indicators such as tumor cell proliferative activity, steroid receptor status, occult distant micrometastases, immunologic phenotypes and gene amplification. By fulfilling several new tasks, which have contributed to the knowledge of the natural history of many tumors, the pathologist has become an integral part of the team planning new treatment strategies and evaluating their final outcome.*

INTRODUCTION

FROM the early seventies, progress in treatment strategy and results for many tumors have more deeply involved the pathologist in the clinical setting. Almost at the same time, the beginning of large scale randomized clinical trials required a number of morphological prognostic indicators for stratifying patients, including tumor subtype and grade, as well as the exact number of histologically involved regional nodes. The retrospective evaluation of successes and failures in treatment studies has stimulated clinicians to become more demanding about the identification of new prognostic discriminants; furthermore, both pathologists and research physicians began to question the definite limitation of tumor diagnosis, using the light microscope as research tool. In fact, what can be done has been done in the past. Because of this, some pathologists have begun to consider new ways of using their discipline's special talents to participate in clinical cancer research.

Today, advances in molecular biology and immunology have provided the pathologist with powerful new tools to enhance morphological and clinical laboratory diagnosis. The objective of this review is not to summarize all the complicated and esoteric tests which can be available in a specialized reference laboratory [1–5]. Our intent is to illustrate the modern tasks of the pathologist involved with neoplastic diseases including the essential information about new laboratory technologies which

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Address correspondence and reprint requests to Dr. Gloria Saccani Jotti, Istituto di Anatomia e Istologia Patologica, University of Parma, Via Gramsci, 14, 43100 Parma, Italy.

can have a distinct, specific clinical focus. The results of these technologies should now be available in most medical communities and can be transferred into clinical practice to improve prognosis and treatment decision making. Molecular biology, which will become crucial in the near future to every aspect of patient management, should at present be confined to research centers.

THE PATHOLOGIST'S RESPONSIBILITIES AS A FUNCTION OF PROGNOSIS

In the field of modern oncology, the pathologist must fulfill the tasks listed in Table 1 to provide clinicians with morphological elements useful for identifying prognostic subsets. The key points of new responsibilities will be briefly discussed in the following paragraphs.

Tumor subtypes

Although it has been clear for a long time that the microscopic diagnosis of cancer is frequently not a black and white decision and the classification of certain malignancies is particularly difficult, it is still important to make all possible efforts to identify tumor subtypes because this is often an important prognostic factor influencing treatment strategy. Just as an example, we would stress the complex group of non-Hodgkin's lymphomas, where cell type indicates to clinicians the tumors which should be aggressively treated with potential for cure [6, 7]. Among the solid tumors, a good example is provided by lung cancer since patients with the small cell subtype are not candidates for primary surgery and 10–15% of cases are now potentially curable with modern intensive chemotherapy [8]. The role of immunohistochemistry in improving diagnostic accuracy will be discussed in the second part of this review.

Tumor grade

Pathologists have known for about a century that the more mitotic figures and degree of anaplasia in a surgical specimen, the more likely the tumor is to exhibit aggressive behavior. For this reason, over the years several types of tumor grading have been proposed and utilized. Tumor grade consists of three major components, i.e. histologic grade, nuclear grade and mitotic grade. The final grade is determined by adding points for each of the three parameters. A major criticism of tumor grading systems has always been the issue of interobserver reproducibility. However, besides its inherent subjectivity, the limit of tumor grading may be due to a number of factors, such as lack of a considerable degree of practice on large sample size, the use of different grading systems and the fact that other variables not assessed by grading contribute independent prognostic information.

Table 1. The pathologist's classical tasks in assessing tumor prognosis

Establish definitive diagnosis and classify tumor subtype
Assign a tumor grade
Evaluate the local and regional spread within resection specimens
Establish the presence or absence of residual neoplastic disease following invasive methods
Establish definitive diagnosis of local and distant recurrence
Evaluate type and degree of iatrogenic morbidity

During the past decade, tumor grade has been documented as of particular prognostic importance in soft tissue sarcomas, osteosarcoma, breast and endometrial cancers. In soft tissue sarcomas, the results of the National Cancer Institute (NCI) led to the establishment of a grading system based on histologic type and extent of necrosis. Determination of the histologic type allows identification of a group of lesions with minimal metastatic potential (grade 1), and assessment of the extent of necrosis allows discrimination of the aggressive lesions into two groups: one with good patient survival (grade 2) and one with poor patient survival. The NCI grading of the primary tumor appears to be valuable in predicting the prognosis after the first recurrence (local or distant) independently of tumor size, age, sex or the effectiveness of therapy to render the patient free of disease [9]. Osteosarcoma (OS) is not a uniform or single entity; rather, it is a term that identifies a group of lesions having different natural histories [9]. Most cases of conventional intramedullary OS are grade 4 lesions; hence, there is little utility in cytologic grading. However, the definition of prognostically significant varieties includes the notion of cytologic grade, e.g. low-grade parosteal OS or high-grade surface OS. After the patient has received preoperative chemotherapy, the pathologist evaluates the response of the tumor in a resected specimen by estimating the proportion of tumor that has undergone necrosis. Patients are placed in three categories that represent degree of response (>90% tumor necrosis, 90–60% and <60%).

The 3-year relapse free survival in the three histopathologic categories is >60%, 40–60% and <40%, respectively. A lack of significant induced tumor necrosis is interpreted as drug resistance of most tumor cells and indicates the need to use different chemotherapeutic agents in the postoperative phase.

In breast cancer, a number of pathologic studies revealed a significantly greater number of well differentiated tumors in older (≥50 years) than in younger (<49 years) patients. This finding correlates with the well recognized observation of a greater incidence of estrogen receptor (ER)-positive

tumors in older women. Indeed, a strong association between ER status and degree of tumor differentiation has been observed. As reported in a recent review paper [10], in spite of the different methods utilized in assessing tubule structures of the neoplastic cells, irregularity of the cancer cell nuclei and the number of cells in mitosis, the common finding reported by all authors is that the degree of tumor differentiation also significantly affects the 5- and 10-year relapse-free and total survival rates in women subjected to adjuvant chemotherapy or endocrine therapy. The histologic grade of endometrial carcinoma was shown to influence clinical outcome in a number of clinical studies. Recently, Khushbakat *et al.* [11] have reported that both the FIGO (International Federation of Gynecology and Obstetrics) and nuclear grading systems correlated with the 5-year mortality rate from cancer: nuclear grade 3 proved to be a superior predictor of fatal outcome (69%) over FIGO grade 3 (31%).

Staging and restaging

From the clinical point of view, staging systems are the means to accurately document the extent of anatomic disease to indicate the prognosis of individual patient subsets and to facilitate the comparison of results between treatment methods and between centers. From the scientific point of view, staging systems are the means of stratifying patients in a study to provide a proper balance of known prognostic factors when two different treatments are compared in a prospective randomized trial. Staging systems are not static: as knowledge is accrued on the natural history, patterns of extension and response to treatment, they require revision. No clinically practical staging system can be applied easily in every tumor because of the unique features of the malignant process in the individual patient.

Thanks to the progress made with radiotherapy and chemotherapy, clinical staging has been replaced by pathologic staging in given tumors. Through invasive procedures (fine needle aspiration biopsy, laparoscopy, and laparotomy) prior to treatment decision making, clinicians provide the pathologist with tissue samples from anatomic areas at high risk of micrometastatic involvement. The most classical examples are represented by malignant lymphomas, small cell carcinoma of the lung and ovarian cancer. In the first two malignancies the most important high risk organs to be explored prior to treatment are liver and bone marrow while in ovarian cancer prognosis is influenced by the amount of tumor spread within the abdominal cavity including the liver. It is well known that in malignant lymphomas, particularly Hodgkin's disease, treatment strategy and prognosis are different between stage I–II and stage III–IV disease [12] while the 3-year median survival in small cell lung cancer varies from 17 months for intrathoracic disease to 5 months for patients with disseminated tumors [8]. In both diseases, if patients are considered to be in clinical complete remission, pathologic restaging is highly recommended by repeating the biopsy of anatomic sites which were histologically positive prior to treatment to ensure that complete response has been attained. Restaging (second look) laparotomy is particularly important in ovarian cancer to surgically document complete remissions, for both success of drug therapy and prognosis are largely influenced by the size of residual tumor masses, i.e. <2 cm vs. $\geqslant 2$ cm [13].

In the above mentioned malignancies the task of the pathologist is to carefully evaluate whether the surgical specimens contain tumor cells or are disease free. Diagnosis can at times be difficult because specimens are often very small, tumor and non-neoplastic lesions are intermixed and the lymph node pattern following lymphography may be distorted by the dye. Typical is the case of patients with Hodgkin's disease undergoing biopsy because of stable residual masses following intensive treatment. In most patients the histologic appearance is characterized by acellular to oligocellular hyalinized masses with a nodular configuration without evidence of Hodgkin's disease. The nodular pattern is believed to reflect the remaining sclerotic framework of nodular sclerosis subtype showing the effects of therapy [14].

The thorough pathological evaluation of the surgically removed specimen remains very important in assessing prognosis and helps in the selection of patients for systemic adjuvant therapy. Well-known examples are provided by breast cancer, malignant melanoma, testicular tumors, gastric and colo-rectal cancers [15–17]. Pathological discriminants are intra- and peritumoral vascular infiltration, the number of positive regional lymph nodes, the depth of tumor invasion and the characteristics of the invasive margins. Worthy of note are two recent publications on prognostic discriminants for stage I testicular cancer [18] and malignant melanoma [19]. In non-seminomatous germ-cell testicular cancer treated by orchidectomy alone, four histopathologic features independently predicted relapses: invasion of testicular veins, invasion of testicular lymphatics, absence of yolk-sac elements and presence of undifferentiated tumors. In cutaneous malignant melanoma, the prognostic value of the histologic criteria introduced by Clark [20] and Breslow [21] remain the basis for the current trend towards more conservative surgery: in patients with primary lesions thinner than 1 mm the narrow excision is a safe and effective procedure.

Iatrogenic morbidity

The widespread use of intensive drug treatment alone or combined with radiation is gradually revealing some important types of organ damage

Table 2. Examples of malignancies in which tumor subtype and/or tumor grade influence prognosis and/or treatment strategy

Acute leukemias
Non-Hodgkin's lymphomas
Soft tissue sarcomas
Osteosarcoma
Wilms' tumor
Brain tumors
Testicular tumors
Lung carcinoma
Breast carcinoma
Ovarian carcinoma
Endometrial carcinoma
Bladder carcinoma
Thyroid carcinoma

which are listed in part in Tables 3 and 4 [22, 30]. Most of the findings related to delayed toxicity, including the onset of second neoplasms, were documented in patients with Hodgkin's disease [31] because they represent successfully treated patients followed for long periods of time. In given situations the contribution of the pathologist can be of great help to clinicians. A detailed report about treatment effects on various organs and tissues is out of the context of the present review. Depending on the anatomic site of the target organ, the morphologic damage can be identified *ante mortem* through biopsy, thus indicating immediate discontinuation of the offending drug. In many situations the degree of toxic findings can be documented only at autopsy and their retrospective association to specific drugs, doses or schedules may allow clinicians to anticipate and prevent them.

The problem of treatment-induced second neoplasms deserves a brief comment. The increased risk of developing acute non-lymphocytic leukemia (ANLL) following treatment for Hodgkin's disease was observed early in the intensive treatment era [31]. The precise actuarial risk was defined about 10 years ago and has since been documented in several large series [32–34]. At the completion of combination chemotherapy alone, the overall risk within 10 years is 3–4%, but the risk increases considerably (≥15%) when patients are subjected to intensive large-field irradiation plus chemotherapy (particularly salvage chemotherapy in patients relapsing from primary radiotherapy) or in subjects older than 40 years at the time of drug treatment. Secondary malignancy after cytotoxic treatment is now presenting an evolving picture. First of all, high-grade non-Hodgkin's lymphomas have been described in 1–5% of patients with Hodgkin's disease [33, 34], ANLL was reported to occur in neoplasms other than Hodgkins's disease [35–38] and various types of solid tumors, particularly lung cancer, carcinoma of urinary bladder, bone and soft tissue sarcomas are being progressively identified in patients cured of their malignant lymphoma [39–41]. It is important to point out that the actuarial risk of solid tumors at 10 years exceeds that of leukemia and the risk of developing a secondary solid tumor is continuing to increase beyond 10 years (a finding not seen in ANLL), and the highest risk is in older patients. Following modern adjuvant chemotherapy for high-risk breast cancer, the incidence at 10 years of acute leukemia is, at present, minimal [38] or nil [42]. The drugs most frequently incriminated in the development of second leukemias and lymphomas are alkylating agents and procarbazine and nitrosourea derivatives. Two-thirds of solid tumors have occurred so far in the radiation field [42, 43].

Since the diagnosis of a second malignancy adversely affects the overall outcome (virtually none of the second acute leukemias appear curable), early detection appears important to assess prognosis. In about two-thirds of patients overt leukemia is preceeded for a few months (median 7 months) by a preleukemic phase consisting of various degrees of myelodysplastic and chromosomal changes [44]. Patients who appear cured of their primary malignancy should be carefully monitored for years and suspicious signs of 'recurrence' should be documented histologically to rule out or confirm the presence of a second malignancy.

Autopsy studies performed at Stanford University [45] and at the National Cancer Institute [46] have documented two important findings. The first concerned the altered histologic appearance of Hodgkin's disease in the curative era, i.e. curative therapy so altered the histologic appearance of lymphoma that many cases were not recognized as typical forms of Hodgkin's disease at autopsy. This observation is of practical importance when the pathologist has to decide about the diagnosis of a recurrent lesion. The same consideration applies to fibrous nodules, interpreted as sites of eradicated Hodgkin's disease, found in many organs, most commonly lymphoreticular. Furthermore, nearly one-third of the patients died without evidence of Hodgkin's disease. At autopsy infection was the most common form of death but a significant number of patients died of complications of therapy, both benign and malignant including *de novo* lymphoid malignancies. Non-fatal histopathologic effects of therapy were common and specifically assessed in thyroid and gonads.

MODERN TECHNOLOGIES TO IMPROVE PROGNOSTIC ASSESSMENT

Steroid receptors

The determination of steroid receptors is of primary importance to assess the prognosis of primary breast cancer [47]. Although biochemical assays

Table 3. Pathological findings in some complications associated with antineoplastic agents

Complication	Agents most frequently implicated	Most representative pathological findings
Myocardial damage	Anthracyclines, mitoxantrone Cyclophosphamide Vinca alkaloids, etoposide, cisplatin Radiotherapy	Myocyte degeneration, dilated mitochondria Hemorrhagic necrosis Acute myocardial infarction Fibrosis of myocardium and of coronary arteries
Pericarditis	Radiotherapy	Fibrosis
Veno-occlusive disease		
Pulmonary	Bleomycin, mitomycin	Occlusion or narrowing of pulmonary veins and venules
Hepatic	High-dose cyclophosphamide and cytosine arabinoside, cisplatin, total body irradiation	Non-thrombotic obliteration of small intrahepatic branches of the hepatic veins
Budd–Chiary syndrome	Dacarbazine, thioguanine, cytosine arabinoside, methotrexate	Thrombotic occlusion of large hepatic veins
Cerebrovascular	Cisplatin-based chemotherapy	Cerebral ischemia
Thrombotic microangiopathy	Mitomycin, cisplatin	Fibrin deposition and endothelial proliferation in glomeruli and arterioles
Venous thrombosis	CMFVP chemotherapy	Predominant deep vein thrombosis
Retinal toxicity	BCNU,* cisplatin,* tamoxifen	Fundal hemorrhages and exudates, macular retinopathy

*Intracarotid infusion.

Table 4. Pathological findings in some complications associated with antineoplastic agents

Complication	Agents most frequently implicated	Most representative pathological findings
Pulmonary	Bleomycin, mitomycin, BCNU, busulfan, cyclophosphamide, methotrexate, radiotherapy	Thickening of alveolar septa, deposition of collagen, hyperplasia of type II alveolar lining cells leading to fibrosis
Gastrointestinal	Fluorouracil, methotrexate, anthracyclines, actinomycin D	Mucositis, frequently ulcerative; candidiasis, villous athrophy
Hepatic	Methotrexate 6-Mercaptopurine, azathioprine Mithramycin Fluorodeoxyuridine* L-Asparaginase	Fibrosis, cirrhosis Cholestasis, necrosis Acute necrosis Biliary sclerosis Fatty metamorphosis
Renal	Cisplatin, methotrexate, streptozotocin, BCNU, CCNU, mitomycin, radiotherapy	Tubulointerstitial nephritis and atrophy; endothelial proliferation in glomeruli and small arterioles
Gonadal	Alkylating agents, procarbazine, BCNU, CCNU, radiotherapy	Azoospermia, testicular atrophy; loss of primordial follicles, ovarian fibrosis
Neurologic		
Central	Methotrexate	Arachnoiditis, leukoencephalopathy
Peripheral	Vinca alkaloids Cisplatin	Axonal degeneration Demyelination
Skeletal	Corticosteroids	Avascular necrosis
Carcinogenesis	Alkylating agents, procarbazine, BCNU, CCNU, radiotherapy	Acute leukemia, high-grade lymphomas, increasing variety of solid tumors

*Intra-arterial infusion.

are reliable, reproducible and correlate reasonably well with response to endocrine therapy and with patient prognosis, they have several drawbacks: (a) 40% of ER-positive tumors fail to respond, indicating the need for improvements in the assays or the development of alternative predictive assays; (b) biochemical assays can only be done on relatively large tumor samples, precluding the use of malignant effusions or needle biopsy specimens for routine assay; (c) these assays do not recognize receptors bound to estradiol *in vivo*, which may lead to inaccurate estimates of receptor content in premenopausal women or women on estrogen therapy; (d) these assays do not permit assessment of receptor status in individual tumor cells and do not assess the problem of tumor cellularity [48].

In an attempt to overcome these problems, new assays have been developed for the presence of ER with an immunocytochemical method (ERICA). The immunoperoxidase technique using a monoclonal antibody recognizing ER also makes it possible to visualize the receptor in a small amount of tumor tissue. Available results indicate that the ERICA technique offers a good, though not optimal, alternative to the dextran-coated charcoal procedure and can be used to select patients with hormone-sensitive breast cancer. There is direct correlation of ERICA staining with response to endocrine therapy in women with advanced disease [49, 50]. Furthermore, significant positive correlations were found between ER status and various pathologic features, including better histologic degrees of differentiation, smaller tumor cell size, and lower levels of either tumor necrosis or lymphocytic differentiation [51]. Last but not least, the immunocytochemical technique detects ER in samples obtained by fine-needle aspiration of primary or secondary breast carcinoma tissue thus avoiding the need for surgical biopsy [52].

Tumor cell proliferative activity and ploidy

In recent years, first thymidine labeling index and then flow cytometry have been used to measure quantitatively tumor cells engaged in DNA synthesis. Results from separate studies have confirmed that the higher the proliferative rate of a tumor, as measured on fresh tissue by the thymidine labeling index (TLI), the more likely a patient is to have a breast cancer recurrence after local-regional therapy [53–55]. The above-mentioned studies have demonstrated that the TLI is a strong prognostic indicator independent of tumor size, lymph node involvement and receptor status. These findings have stimulated interest in proliferative measurements of breast carcinoma.

Today, measurement of DNA content can be made by the new, automated method of flow cytometry (FC) rather than static cytophotometry. FC methods on fresh pulverized tissue, and more recently also on fine needle aspirates, have been utilized to measure the percentage of tumor cells in S-phase and also to determine ploidy [56–58]. DNA content abnormality or aneuploidy in FC is usually expressed as the DNA index, the ratio of DNA content of the G_0/G_1 cells of a normal diploid population. A close association has been demonstrated between S-phase calculated by TLI and by FC using selected software programs. FC analysis of DNA content can provide important prognostic information in a variety of malignancies. High- and low-risk groups of patients with early breast cancer and bladder carcinomas, non-small cell lung cancer, as well as colorectal, ovarian and cervical carcinomas, can be identified on the basis of abnormal stemline DNA content. In several hematologic and common pediatric malignancies, the prognostic relevance of DNA content by FC has been similarly established. In particular, breast cancer receptor-negative tumors are much more likely to be aneuploid and to have a high median S-phase than receptor-positive tumors [56–59]. In the future, FC is likely to become even more useful to the clinician. In fact, other variables can be examined simultaneously with DNA content, providing additional prognostic information and a more quantitative understanding of tumor cell heterogeneity. Multiparametric studies such as hormone receptors, acid phosphatase, carcinoembryonic antigens, various nuclear antigens and oncogene protein products may be assayed simultaneously with DNA ploidy and cell cycle distribution. All the above mentioned parameters could be used for better selection of high-risk patients who are candidates for adjuvant and neoadjuvant (primary) drug therapy and as a variable for stratification in therapeutic trials.

In recent years, monoclonal antibodies were studied as markers of tumor cell proliferation. A good example is the Ki-67 monoclonal antibody which reacts with a human nuclear antigen associated with cell proliferation that is expressed only in continuously proliferating cycling cells, and therefore offers a simple opportunity to determine the growth fraction of tumors by immunostaining of

Table 5. Laboratory technologies that can allow the pathologist to better contribute in the assessment of tumor prognosis

Steroid receptors
Tumor cell kinetics
Tumor DNA content and ploidy
Cytogenetics
Oncogene amplification
Clonal rearrangements in cancer cells
Immunologic phenothypes
Monoclonal antibodies to detect occult metastases
Morphologic and circulating tumor markers

fresh tissue. The interesting results of the study of Lellé *et al.* [60], however, do not yet provide evidence whether Ki-67 may represent a potentially useful predictor of the clinical course of breast cancer. In contrast, the neoplasm's high growth fraction determined by this monoclonal antibody was reported to be a significant independent predictor of survival in diffuse large cell lymphomas [61].

Cytogenetics

The cytogenetic aspects of human malignancies, their clinical meaning and their relationship to molecular events thought to play a key role in given diseases have been actively investigated during the past decade [62, 63]. Chromosome studies in leukemias, lymphomas as well as in given solid tumors have proven to be an important tool in demonstrating that neoplasms can be dissected, defined and further subdivided cytogenetically into a number of subtypes. More recent studies have shown that beyond visual cytogenetics and its limitations lie the promising vistas of molecular cytogenetics for further defining and extending the cytogenetics of human malignancies to the application in prognosis and treatment. It is now well established that the type and extent of chromosomal abnormalities, rather than their presence, determine prognosis [64].

The biological implications of consistent chromosome rearrangements in leukemia and lymphoma were recently discussed by Rowley [62]. The true Ph^1 chromosome defect in chronic myeloid leukemia (CML) was shown by Rowley to be a translocation involving numbers 9 and 22. Variant translocations have been discovered, however, in addition to the typical t(9;22), and our present evidence suggests that, with rare exceptions, Ph^1-negative CML does not exist. One of the most exciting revelations of the past few years has involved the cellular oncogenes and their chromosome location since many oncogenes are located in the bands that are involved in consistent translocations. The characterization of the precise breakpoints in chromosomal translocations in CML, the fusion of *abl* proto-oncogene on chromosome 9 with the breakpoint cluster region (*bcr*) on chromosome 22 involves a 5800 nucleotide base pair region in the *bcr* gene. Determination of the precise localization of the breakpoint in any individual patient may predict whether the patient has a long stable phase of CML or a rapid progression to blast crisis [65].

Among acute non-lymphocytic leukemias (ANLL), the consistent chromosome changes which can be identified in tumor cells pinpoint the location of genes whose functions are critical in the growth potential of that particular cell type [62]. Of these changes, the 15;17 translocation in acute promyelocytic leukemia has proved to be the most specific and has never been seen in any other tumor. Another important morphological subgroup that was defined by cytogeneticists is characterized by abnormalities of chromosome 16 in acute myelomonocytic leukemia. The data on Burkitt's lymphoma indicate that the consistent translocations involve number 8 which is therefore the consistent chromosome.

Recent studies begin to reveal cytogenetic findings that are predictive of clinical outcome in non-Hodgkin's lymphomas [66–68]. In particular, Levine *et al.* [66] have noticed that normal metaphases in tumor material were associated with a higher complete remission rate and longer survival. In particular, among patients with follicular lymphomas, those with structural abnormalities of chromosome 17 had a shorter survival than patients without these abnormalities. Among patients with high-grade lymphomas, those with breaks in the short arm of chromosome 2 had a longer survival than those without these breaks.

In solid tumors, the number of entities in which a specific chromosomal pattern has been defined is still very small and is often restricted to rare tumors. The contribution of cancer cytogenetics is, at present, related to the refinement of diagnosis and histogenetic classification of tumors, malignant as well as benign [63]. The best examples include Ewing's sarcoma, other sarcomas and lipoma. The translocation t(11;22)(q24;q12) has been observed in the typical skeletal Ewing's sarcoma as well as in the unusual skeletal subtype. Since this chromosome abnormality has not been described in other tumors of the small cell round group, it represents an example of the practical application of chromosome findings in the selection of the therapeutic approach in neoplasms where the differential diagnosis may be impossible to establish.

Molecular biology

Recent advances in molecular biology hold promise to reveal the molecular basis of human neoplasia. Human genes have been identified which function in the control of growth and differentiation of normal cells and in the suppression of malignancy [3, 4, 68, 69]. The aberrant expression of these genes resulting from genetic mutation, chromosomal translocation, deletion and amplification has been linked to the development and progression of many human tumors. Therefore, if a molecular probe or partial DNA sequence is available for a gene of interest, then the structure and expression of this gene may be studied in any tissue sample. The use of standard molecular techniques to study gene structure and expression, and the development of new highly sensitive molecular assays will revolutionize the pathological diagnosis of human tumors

and is likely to result in new disease classification systems.

Analysis of abnormal structure and expression of particular proto-oncogenes in certain human tumors has distinguished different prognostic groups of cancer patients. The prognosis of any individual patient may be determined by analysis of gene structure through Southern blots of biopsy samples. We have previously mentioned prediction of prognosis in patients with CML [65]. Gene amplification (increased number of copies of a gene in the DNA of a tumor cell) appears to be among the new promising predictive factors. Amplification of the HER-2/*neu* oncogene has indeed been shown to predict early recurrence and poor survival in breast cancer [71]. However, this kind of analysis requires a relatively large amount of tumor tissue (100 mg). Recent studies [72] have measured the protein coded by HER-2/*neu* in 305 breast cancer patients with tumor in the axillary lymph nodes. For the measurement of this protein it takes only 10 mg of the tumor sample, i.e. one tenth of what is needed for the measurement of gene amplification. Therefore, this analysis can be done on very small biopsy specimens. Excellent correlation was found between the abnormal production of the HER-2/*neu* protein and the disease outcome; patients with overexpression of this protein tend to have quicker recurrence and shorter survival. Other investigators [73–75] have reported a good correlation between given oncogene amplification and/or expression and the course of breast cancer. All these observations, along with the established predictive factors should help select high-risk patients who are candidates to receive systemic adjuvant therapy.

A major problem in the management of cancer patients is the detection of minimal residual disease and early relapse. Molecular biology has developed the technical ability to detect clonal rearrangements in cancer cells (immunoglobulin genes, T-cell receptor genes) and specific chromosomal translocations [76–79]. However, utilizing standard Southern hybridization assays, cells with these abnormalities can be reliably and consistently detected if they comprise greater than 2–5% of the cells in heterogeneous cell populations. An exciting new technical development, the polymerase chain reaction, allows amplification of an abnormal sequence by a factor of 10^6, greatly enhancing the ability to detect rare cells with specific molecular abnormality [80, 81]. Its use has recently allowed detection of early relapse and residual bone marrow involvement by follicular lymphoma cells carrying a t(14;18). Detection of specific point mutations of the *ras* oncogene family in cancer of the colon has also been performed utilizing this technology [82]. The fusion of polymerase chain reaction technology with molecular probes specific for particular genetic abnormalities will be a powerful tool in the near future in detecting minimal disease and in monitoring the effectiveness of cancer therapy.

Monoclonal antibodies for occult metastases

Monoclonal antibodies have also been actively investigated in the detection of occult tumor cells [5, 89]. Most pertinent to the subject of this review is the recent update by British investigators [84] on the detection of occult tumor cells in the bone marrow by immunocytochemistry using an antiserum to epithelial membrane antigen. After multiple bone marrow aspirates taken prior to surgery, micrometastases were detected in 81 of 307 (26.4%) women with primary breast cancer, and their presence was related to various poor prognostic factors, i.e. spread to lymph nodes, vascular invasion, T stage and pathological size. With a median follow-up time of 28 months, 75 patients relapsed and 60 in distant sites; of these 60 patients, 26 had micrometastases detected at presentation. Both relapse-free and total survival were significantly shorter for patients with micrometastases. The test, however, predicted bone metastases only and did not appear to be an independent prognostic factor.

Immunologic phenotypes

Immunologic phenotyping of leukemia and lymphomas has revealed heterogeneity not predicted by morphologic studies [85]. It is now possible to define stages of human lymphocyte and granulocyte differentiation precisely using highly specific monoclonal antibodies that define cell surface antigens and molecular probes that identify rearrangement of immunoglobulin and T cell receptor genes [3, 76, 86]. These can be combined with more traditional cell markers such as surface membrane (Smlg) and cytoplasmic immunoglobulin (Clg) on B lymphocytes, sheep erythrocyte receptors on T lymphocytes and cytochemical stains.

There have been a number of studies attempting to make clinical correlations with cell surface phenotyping, most of which have not provided useful information for clinical decision making [87, 88]. In leukemia, the importance of immunophenotype as an independent prognostic factor has been questioned except for B acute lymphoblastic leukemia. The dismal prognosis of this particular subset is well known, and therefore most study groups have excluded these patients from their standard leukemia treatment protocols. In non-Hodgkin's lymphomas the disparate results of past studies may be due to small numbers of patients studied and comparisons between different histologic subtypes. To obviate these problems Miller *et al.* [89] have studied 115 consecutive patients with a single mor-

phologic subtype, diffuse large cell lymphoma, accrued over a 10-year period. Utilizing a panel of 40 monoclonal antibodies on thin tissue sections, these investigators documented that T-cell phenotype predicted a shorter relapse free survival (median 11 months) compared to B-cell phenotype (median 43 months). The independent prognostic significance of immunophenotype was confirmed through multivariate analysis. Clearly, prospective clinical trials are needed before adapting therapy in lymphoid malignancies to immune phenotype [90].

New markers of cytodifferentiation

Morphological features of certain tumors are augmented by available information from other diagnostic techniques, especially immunohistochemistry, which is now acknowledged as being one of the most helpful ancillary diagnostic procedures in anatomic pathology. In great part, enhanced diagnostic accuracy is due to introduction of the hybridoma technique to prepare monoclonal antibodies as well as to the discovery and characterization of a growing number of novel, clinically meaningful, molecular markers of cell differentiation. We shall briefly mention the impact of monoclonal antibodies on markers of cell lineage or differentiation in the so-called undifferentiated malignant neoplasms.

Most useful among the markers of cell lineage are the intermediate filaments (IF), the fibrous proteins which form the internal skeleton of most mammalian cells [91, 92]. The principal IF are: the keratins, expressed nearly exclusively by epithelial cells; vimentin, predominantly expressed by cells of mesenchymal derivation; desmin, the IF of muscle cells; neurofilament, expressed by neurons and neuroendocrine cells; and glial fibrillary acid protein, the IF of glial cells. Since most neoplasms derived from those tissues display a conserved IF phenotype, the prognostic implications of correct tumor diagnosis on treatment strategy is obvious. Thus, the demonstration of keratins within the cells of an undifferentiated malignant neoplasm is sufficient evidence, in the proper clinical and histopathological context, to distinguish anaplastic carcinoma from melanoma, lymphoma and sarcomas, which express other intermediate filaments: desmin, the IF of muscle, is expressed by the majority of sarcomas of muscle derivation; vimentin, the IF of mesenchymal cells, is expressed in abundance by melanoma, sarcoma and with a high degree of predictability by some types of epithelial tumors where it is coexpressed with keratins [92]. Current immunohistochemical diagnosis utilizes a panel of monoclonal antibodies directed to a variety of markers of cell differentiation, the composition of the panel depending on the differential diagnosis [93].

There has been significant progress in another frequent problem of histopathological diagnosis: the metastatic carcinoma, primary site unknown. An expanding number of new markers of cytodifferentiation are now available which enable the pathologist to pinpoint accurately the origin of the metastatic neoplasm or, at least, significantly narrow the possibilities, thus focusing the clinician's attention to the most likely site of origin. For example, there are specific or relatively specific marker substances for carcinoma of the prostate, thyroid, breast, kidney, large intestine, liver and several endocrine organs. However, some of these require the availability of frozen tissue because the antigen(s) does not survive the tissue processing.

CONCLUSIONS

No one denies the key role played by classical histopathology in the diagnosis and management of human cancer. Taken in conjunction with the clinical background, the histological characterization of a tumor and its assignment to a defined entity often allow prediction of its biological behavior. Current treatment protocols are usually based on these criteria. The more accurate the histological diagnosis, the more optimized will be patient selection and management of the tumor. Progress in the treatment of various malignancies, the new multidisciplinary strategy for given patient subsets as well as the importance of randomized clinical trials to assess the relative merits of novel approaches, have considerably increased the input of clinicians on pathologists to better identify sophisticated prognostic discriminants. On the other hand, advances in cellular and molecular biology, genetics and immunology have provided the pathologists with a unique opportunity to coordinate, implement and led efforts utilizing new methods to study the pathobiology of human malignancies and influence patient assessment. The new biology requires from both clinicians and pathologists the acquisition of a new language, education in theoretical backgrounds and access to appropriate technical expertise. This desirable partnership in resolving specific clinical problems should be based on mutual knowledge and conversation on technical aspects, potential applications, limitations and relative costs of the sophisticated technologies.

To date, while the standard of surgical pathology, as discussed in the first part of this review, has considerably improved, new technologies which can further refine the prognostic discriminants are still confined within specific laboratories of research centers. However, the labeling index technique, flow cytometric and immunohistochemistry methods are now becoming widely available. Even some DNA probes and the advent of the technique of *in situ* hybridization are all becoming sufficiently common

that these techniques are beginning to move into the surgical pathology armamentarium [3, 4, 76].

Clearly, we are moving quickly from the age of morphological pathology to one in which precursor lesions, presence or absence of neoplasia, and prognosis will be based as much on biological and molecular parameters as on morphological ones. When appropriately applied, new biological approaches will hopefully influence the management of patients.

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REFERENCES

1. O'Sullivan MB. Changing technologies in the reference laboratory. *Arch Pathol Lab Med* 1987, **111**, 657–661.
2. Colvin RB, Preffer FI. New technologies in cell analysis by flow cytometry. *Arch Pathol Lab Med* 1987, **111**, 628–632.
3. Fenoglio-Preiser CM, Willman CL. Molecular biology and the pathologist. *Arch Pathol Lab Med* 1987, **111**, 601–619.
4. Barton SA. Oncogenes and surgical pathology. *Sem Diagn Pathol* 1987, **4**, 194–199.
5. Sell S, Reisfeld RA, ed. *Monoclonal Antibodies in Cancer*. Humana Press, Clifton, 1985.
6. Non-Hodgkin's lymphoma pathologic classification project: National Cancer Institute sponsored study of classification of non-Hodgkin's lymphomas: summary and description of a working formulation for clinical usage. *Cancer* 1982, **49**, 2112–2135.
7. Bonadonna G, Saccani Jotti G. Prognostic factors and response to treatment in non-Hodgkin's lymphomas (review). *Anticancer Res* 1987, **7**, 685–694.
8. Bunn PA, Lichter AS, Makuch RW *et al.* Chemotherapy alone or chemotherapy with chest radiation therapy in limited stage small cell lung cancer. *Ann Intern Med* 1987, **106**, 655–662.
9. Proceedings of the National Institutes of Health Consensus Development Conference on Limb-Sparing Treatment of Adult Soft Tissue Sarcomas and Osteosarcomas. *Cancer Treat Symp* 1985, **3**, 1–159.
10. Contesso G, Saccani Jotti G, Bonadonna G. Tumor grade as prognostic factor in primary breast cancer. *Eur J Cancer Clin Oncol* 1989, **25**, 403–409.
11. Khushbakhat RM, Schwartz PE, Barwick KW. Architectural (FIGO) grading, nuclear grading, and other prognostic indicators in stage I endometrial carcinoma with identification of high-risk and low-risk groups. *Cancer* 1988, **61**, 538–545.
12. Saccani Jotti G, Bonadonna G. Prognostic factors in Hodgkin's disease: implications for modern therapy. *Anticancer Res* 1988, **8**, in press.
13. Young RC (Ed). Ovarian cancer. *Semin Oncol* 1984, **11**, 209–332.
14. Chen JL, Osborn BM, Butler JJ. Residual fibrous masses in treated Hodgkin's disease. *Cancer* 1987, **60**, 407–413.
15. Fisher B, Wolmark N, Baver M *et al.* The accuracy of clinical nodal staging and limited axillary dissection as a determinant of histologic nodal status in carcinoma of the breast. *Surg Gynecol Obst* 1981, **152**, 765–772.
16. Cascinelli N, Vaglini M, Nava M *et al.* Prognosis of skin melanoma with regional node metastases (Stage II). *J Surg Oncol* 1984, **25**, 240–247.
17. Jass JR, Love SB, Northover JMA. A new prognostic classification of rectal cancer. *Lancet* 1987, **1**, 1303–1306.
18. Freedman LS, Jones WG, Peckham MJ *et al.* Histopathology in the prediction of relapse of patients with stage I testicular teratoma treated by orchidectomy alone. *Lancet* 1987, **2**, 294–298.
19. Veronesi U, Cascinelli N, Adamus J *et al.* The stage I primary cutaneous malignant melanoma. Comparison of excision with margins of 1 or 3 cm. *N Engl J Med* 1988, **318**, 1159–1162.
20. Clark WH Jr, From L, Bernardino EA *et al.* The histogenesis and biologic behavior of primary human malignant melanoma of the skin. *Cancer Res* 1969, **29**, 705–727.
21. Breslow A. Tumor thickness, level of invasion and node dissection in stage I cutaneous melanoma. *Ann Surg* 1975, **182**, 572–575.
22. Perry MC, Yarbro JW, ed. *Toxicity of Chemotherapy*. Clinical Oncology Monographs. Grune and Stratton, Orlando, 1984.
23. Doll DC, Ringenberg QS, Yarbro JW. Vascular toxicity associated with antineoplastic agents. *J Clin Oncol* 1986, **4**, 1405–1417.
24. Kinsella TJ, Fraass BA, Glatstein E. Late effects of radiation therapy in the treatment of Hodgkin's disease. *Cancer Treat Rep* 1982, **66**, 991–1001.
25. McReynolds RA, Gold GL, Roberts WC. Coronary heart disease after mediastinal irradiation for Hodgkin's disease. *Am J Med* 1976, **60**, 39–44.
26. Jackson AM, Rose BD, Graff LG *et al.* Thrombotic microangiopathy and renal failure associated with antineoplastic chemotherapy. *Ann Intern Med* 1984, **101**, 41–44.
27. Weiss RB, Torney DC, Holland JF *et al.* Venous thrombosis during multimodal treatment of primary breast carcinoma. *Cancer Treat Rep* 1981, **65**, 677–679.

28. Levine M, Gent M, Hirsh J *et al.* The thrombogenic effect of anticancer drug therapy in women with stage II breast cancer. *N Engl J Med* 1988, **318**, 404–408.
29. Ries F. Nephrotoxicity of chemotherapy. *Eur J Cancer Clin Oncol* 1988, **24**, 951–953.
30. Hohn D, Melnick J, Stagg R *et al.* Biliary sclerosis in patients receiving hepatic arterial infusions of floxuridine. *J Clin Oncol* 1985, **3**, 98–102.
31. Bookmann MA, Longo DL. Concomitant illness in patients treated for Hodgkin's disease. *Cancer Treat Rev* 1986, **13**, 77–111.
32. Tester WJ, Kinsella TJ, Waller B *et al.* Second malignant neoplasms complicating Hodgkin's disease. The National Cancer Institute experience. *J Clin Oncol* 1984, **2**, 762–769.
33. Valagussa P, Santoro A, Fossati-Bellani F *et al.* Second acute leukemia and other malignancies following treatment for Hodgkin's disease. *J Clin Oncol* 1986, **4**, 830–837.
34. Tucker MA, Coleman CR, Cox RS *et al.* Risks of second cancers after treatment for Hodgkin's disease. *N Engl J Med* 1988, **318**, 76–81.
35. Reimer RR, Hoover R, Fraumeni JF *et al.* Acute leukemia after alkylating agent therapy of ovarian cancer. *N Engl J Med* 1977, **297**, 177–181.
36. Chak LY, Sikic BI, Tucker MA *et al.* Increased incidence of acute nonlymphocytic leukemia following therapy in patients with small cell carcinoma of the lung. *J Clin Oncol* 1984, **2**, 385–390.
37. Boice JD Jr, Greene MH, Killen JY Jr *et al.* Leukemia and preleukemia after adjuvant treatment of gastro intestinal cancer with semustine (methyl-CCNU). *N Engl J Med* 1983, **309**, 1079–1084.
38. Fisher B, Rockette H, Fisher ER *et al.* Leukemia in breast cancer patients following adjuvant chemotherapy or post-operative irradiation: the NSABP experience. *J Clin Oncol* 1985, **3**, 1640–1658.
39. Pedersen-Bjergaard J, Ersboll J, Hansen VL *et al.* Carcinoma of the urinary bladder after treatment with cyclophosphamide for non-Hodgkin's lymphomas. *N Engl J Med* 1988, **318**, 1028–1032.
40. List AF, Doll DC, Greco A. Lung cancer in Hodgkin's disease: association with previous radiotherapy. *J Clin Oncol* 1985, **3**, 215–221.
41. Halperin EC, Greenberg MS, Suit HD. Sarcoma of bone and soft tissue following treatment of Hodgkin's disease. *Cancer* 1984, **53**, 232–236.
42. Valagussa P, Tancini G, Bonadonna G. Second malignancies after CMF for resectable breast cancer. *J Clin Oncol* 1987, **5**, 1138–1142.
43. Robinson E, Neugut AI, Wylie P. Clinical aspects of post-irradiation sarcomas. *J Natl Cancer Inst* 1988, **80**, 233–240.
44. Brusamolino E, Papa G, Valagussa P *et al.* Treatment related leukemia in Hodgkin's disease: a multi-institution study on 75 cases. *Hematol Oncol* 1987, **5**, 83–98.
45. Colby TV, Hoppe R, Warnke RA. Hodgkin's disease at autopsy: 1972–1977. *Cancer* 1981, **47**, 1852–1862.
46. Grogan TM, Berard CW, Steinhorn SC *et al.* Changing patterns of Hodgkin's disease at autopsy: a 25-year experience at the National Cancer Institute, 1953–1978. *Cancer Treat Rep* 1982, **66**, 653–665.
47. McGuire WL. Prognostic factors for recurrence and survival in breast cancer. *Breast Cancer Res Treat* 1987, **10**, 5–9.
48. Osborne K. Heterogeneity in hormone receptor status in primary and metastatic breast cancer. *Sem Oncol* 1987, **12**, 317–326.
49. McClellan RA, Berger U, Miller LS *et al.* Immunocytochemical assay for estrogen receptor in patients with breast cancer: relationship to outcome of therapy. *J Clin Oncol* 1986, **4**, 1171–1176.
50. Pertschuck LP, Eisenberg KB, Carter AC *et al.* Immunohistologic localization of estrogen receptors in breast cancer with monoclonal antibodies. Correlation with biochemistry and clinical endocrine response. *Cancer* 1985, **55**, 1513–1518.
51. Berger V, Wilson P, McClellan RA *et al.* Correlation of immunocytochemically demonstrated distribution and histopathologic features in primary breast cancer. *Hum Pathol* 1987, **18**, 1263–1267.
52. Coombes RC, Berger U, McClellan RA *et al.* Prediction of endocrine response in breast cancer by immunocytochemical detection of oestrogen receptor in fine-needle aspirates. *Lancet* 1987, **8561**, 701–703.
53. Tubiana M, Pejovic MH, Chavaudra N *et al.* The long-term prognostic significance of the thymidine labelling index in breast cancer. *Int J Cancer* 1984, **33**, 441–445.
54. Silvestrini R, Daidone MG, Di Fronzo G *et al.* Prognostic implication of labeling index versus estrogen receptors and tumor size in node negative breast cancer. *Breast Cancer Res Treat* 1986, **7**, 161–169.
55. Meyer JS. Cell kinetics in selection and stratification of patients for adjuvant therapy of breast carcinoma. *NCI Monogr* 1986, **1**, 25–26.
56. Merkel DE, Dressler LG, McGuire WL. Flow cytometry, cellular DNA content and prognosis in human malignancy. *J Clin Oncol* 1987, **5**, 1690–1703.
57. Dressler LG, Seamer LC, Owens MA *et al.* DNA flow cytometry and prognostic factors in 1331 frozen breast cancer specimens. *Cancer* 1988, **61**, 420–427.

58. Levak PA, Mullen P, Anderson TJ *et al.* DNA analysis of breast tumour fine needle aspirates using flow cytometry. *Br J Cancer* 1987, **56**, 643–646.
59. Kallioniemi OP, Blanco G, Alavaikko M *et al.* Tumor DNA ploidy as an independent prognostic factor in breast cancer. *Br J Cancer* 1987, **56**, 637–642.
60. Lellé RJ, Heidenreich, Stauch G *et al.* The correlation of growth fractions with histologic grading and lymph node status in human mammary carcinoma. *Cancer* 1987, **59**, 83–88.
61. Lippman SM, Grogan TM, Spier CM *et al.* High growth fraction (determined by the monoclonal antibody Ki-67) predicts poor survival in diffuse large cell lymphoma. *Proc Am Soc Clin Oncol* 1988, **7**, 223 (Abstr 861).
62. Rowley JD. Chromosome abnormalities in leukemia. *J Clin Oncol* 1988, **6**, 194–202.
63. Sandberg AA, Turc-Carel C, Gemmill RM. Chromosome in solid tumors and beyond. *Cancer Res* 1988, **48**, 1049–1059.
64. Gyger M, Infante-Rivard C, D'Angelo G *et al.* Prognostic value of clonal chromosomal abnormalities in patients with primary myelodysplastic syndromes. *Am J Hematol* 1988, **28**, 13–20.
65. Schaefer-Rego K, Dudek H, Arlin Z *et al.* CML patients in blast crisis have breakpoints localized to a specific region of BCR. *Blood* 1987, **70**, 448–455.
66. Levine EG, Arthur DC, Frizzera G *et al.* Cytogenetic abnormalities predict clinical outcome in non-Hodgkin's lymphoma. *Ann Intern Med* 1988, **108**, 14–20.
67. Yunis JJ, Frizzera G, Oken MM. Multiple recurrent genomic defects in follicular lymphomas: a possible model for cancer. *N Engl J Med* 1987, **316**, 79–84.
68. Juliusson G. Immunologic and cytogenetic studies improve prognosis prediction in chronic B-lymphocytic leukemia: a multivariate analysis of 24 variables. *Cancer* 1986, **58**, 688–693.
69. Friend SH, Dryja TP, Weinberg RA. Oncogenes and tumor suppressing genes. *N Engl J Med* 1988, **318**, 618–622.
70. Willman CL, Fenoglio-Preiser CM. Oncogenes, suppressor genes, and carcinogenesis. *Human Pathol* 1987, **18**, 895–902.
71. Slamon DJ, Clark GM, Wong SG *et al.* Human breast cancer: correlation of relapse and surival with amplification of the HER-2/*neu* oncogene. *Science* 1987, **235**, 177–182.
72. Tandon A, Clark G, Ullrich A *et al.* Overexpression of the HER-E/*neu* oncogene predicts relapse and survival in stage II human breast cancer. *Proc Am Soc Clin Oncol* 1988, **7**, 14 (Abstr. 51).
73. Cline MJ, Battifora H, Yokota J. Proto-oncogene abnormalities in human breast cancer: correlations with anatomic features and clinical course of disease. *J Clin Oncol* 1987, **5**, 999–1006.
74. Fromowitz FB, Viola MV, Chao S *et al.* Ras p21 expression in the progression of breast cancer. *Hum Pathol* 1987, **18**, 1268–1275.
75. Clair T, Miller WR, Cho-Chung YS. Prognostic significance of the expression of a *ras* protein with a molecular weight of 21,000 by human breast cancer. *Cancer Res* 1987, **47**, 5290–5293.
76. Cossman J, Uppenkamp M, Sundeen J *et al.* Molecular genetics and the diagnosis of lymphoma. *Arch Pathol Lab Med* 1988, **112**, 117–127.
77. Wright JJ, Polack DG, Bakhshi A *et al.* Gene rearrangements as markers of clonal variation and minimal residual disease in acute lymphoblastic leukemia. *J Clin Oncol* 1987, **5**, 735–741.
78. Horning SJ, Galili N, Cleary M *et al.* Incidence of gene rearrangements in the peripheral blood in malignant lymphomas. *Proc Am Soc Clin Oncol* 1988, **7**, 228 (Abstr 880).
79. Raffeld M, Wright JJ, Lipford E *et al.* Clonal evolution of t(14;18) follicular lymphomas demonstrated by the 18q21 major breakpoint region. *Cancer Res* 1987, **47**, 2537–2542.
80. Ming-Sheng L, Chang K-S, Cabanillas F *et al.* Detection of minimal residual cells carrying the t(14;18) by DNA sequence amplification. *Science* 1987, **237**, 175–178.
81. Saiki RK, Gelfand DH, Stoffel S *et al.* Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 1988, **239**, 487–491.
82. Bos JL, Fearon ER, Hamilton SR *et al.* Prevalence of *ras* gene mutations in human colorectal cancers. *Nature* 1987, **327**, 293–297.
83. Schlom J. Basic principles and applications of monoclonal antibodies in the management of carcinomas: the Richard and Hinda Rosenthal Foundation Award Lecture. *Cancer Res* 1986, **46**, 3225–3238.
84. Mansi JL, Berger U, Easton D *et al.* Micrometastases in bone marrow in patients with breast cancer: evolution as an early predictor of bone metastases. *Br Med J* 1987, **295**, 1093–1096.
85. Foon KA, Schroff RW, Gale RP. Surface markers on leukemia and lymphoma cells: recent advances. *Blood* 1982, **60**, 1–19.
86. Foon KA, Todd RF. Immunologic classification of leukemia and lymphoma. *Blood* 1988, **68**, 1–31.
87. Urba WJ, Longo DL. Cytologic, immunologic, and clinical diversity in non-Hodgkin's lymphoma: therapeutic implications. *Sem Oncol* 1985, **12**, 250–267.
88. Kamps WA, Humphrey GB. Heterogeneity of childhood acute lymphoblastic leukemia. Impact on prognosis and therapy. *Sem Oncol* 1985, **12**, 268–280.
89. Miller TP, Lippman CM, Spier CM *et al.* The immunophenotype of diffuse large cell

lymphoma predicts survival. *Proc Am Soc Clin Oncol* 1988, **7**, 224 (Abstr. 865).
90. Amylon M, Murphy S, Pullen J *et al.* Treatment of lymphoid malignancies according to immune phenotype: preliminary results in T-cell disease. *Proc Am Soc Clin Oncol* 1988, **7**, 225 (Abstr. 871).
91. Triche TJ. Morphologic tumor markers. *Sem Oncol* 1987, **14**, 139–172.
92. Battifora H. Recent progress in the immunochemistry of solid tumors. *Sem Diagn Pathol* 1984, **1**, 251–269.
93. Battifora H. Diagnostic uses of antibodies to keratins. A review of immunohistochemical comparison of seven monoclonal and three polyclonal antibodies. In: Fenoglio-Preiser CM, Wolff M, Rilke F, eds. *Progress in Surgical Pathology*. New York, Masson, 1987, Vol. 8, 1–15.