REVIEW

Detection and Clinical Importance of Micrometastatic Disease

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Metastatic relapse in patients with solid tumors is caused by systemic preoperative or perioperative dissemination of tumor cells. The presence of individual tumor cells in bone marrow and in peripheral blood can be detected by immunologic or molecular methods and is being regarded increasingly as a clinically relevant prognostic factor. Because the goal of adjuvant therapy is the eradication of occult micrometastatic tumor cells before metastatic disease becomes clinically evident, the early detection of micrometastases could identify the patients who are most (and least) likely to benefit from adjuvant therapy. In addition, more sensitive methods for detecting such cells should increase knowledge about the biologic mechanisms of metastasis and improve the diagnosis and treatment of micrometastatic disease. In contrast to solid metastatic tumors, micrometastatic tumor cells are appropriate targets for intravenously applied agents because macromolecules and immunocompetent effector cells should have access to the tumor cells. Because the majority of micrometastatic tumor cells may be nonproliferative (G_0 phase), standard cytotoxic chemotherapies aimed at proliferating cells may be less effective, which might explain, in part, the failure of chemotherapy. Thus, adjuvant therapies that are aimed at dividing and quiescent cells, such as antibody-based therapies, are of considerable interest. From a literature search that used the databases MEDLINE®, CANCERLIT®, Biosis®, Embase®, and SciSearch®, we discuss the current state of research on minimal residual cancer in patients with epithelial tumors and the diagnostic and clinical implications of these findings. [J Natl Cancer Inst 1999;91:1113–24]

Malignant tumors of epithelial tissues are the most common form of cancer and are responsible for the majority of cancer-related deaths in Western industrialized countries. Because of progress in the surgical treatment of these tumors, mortality is linked increasingly to early metastasis, which is often occult at the time of primary diagnosis *(1).* For patients with no evidence of systemic metastases when the primary tumor is resected, traditional staging parameters of the tumor (e.g., tumor size and lymph node status) are determined; with this information and a statistical assessment of the risk of disease recurrence, the decision is made as to whether to give systemic adjuvant therapy to prevent metastatic relapse. Undetected micrometastases can contribute to the failure of primary treatment. Therefore, the identification of occult metastases in patients with early stage cancer could have a substantial clinical impact on the prognosis and optimal therapy for patients with cancer. For this reason, improved direct identification of minimal residual cancer is particularly important. At later stages of the disease, it may be useful to determine the presence of and change in the number of residual malignant cells so that the therapies selected can be monitored and adjusted to the changing needs of the patient.

Research into the molecular basis of tumor metastasis has identified numerous proteins that influence this process (Fig. 1). Conditions that allow growth of epithelial cells at metastatic sites are largely unknown but undoubtedly include the appropriate microenvironment for tumor cell growth (e.g., hormonal milieu, oxygenation, nutrients, or growth factors) and an environment for the formation of new blood vessels (angiogenesis). The factors determining the length of the period from the dissemination of tumor cells until the appearance of clinically manifest metastases are also unclear. From analyses of single cells, most disseminated tumor cells in bone marrow do not appear to be proliferating at the time of primary surgery *(2,3).* For this reason, it may be important to use adjuvant therapies that are aimed at both proliferating and nonproliferating cells.

This review was based on a literature search that used the databases MEDLINE®, CANCERLIT®, Biosis®, Embase®, and SciSearch®. We discuss the use of immunologic and molecular analyses in the diagnosis and characterization of minimal residual cancer. Available methods give access to this critical stage of tumor progression and can lead to the development of new therapeutic approaches that are aimed at preventing manifest metastasis.

Diagnosis and Prognostic Relevance

Tumor Cell Dissemination by the Circulatory System

Although micrometastatic tumor cell aggregates may be identified by conventional histopathologic methods *(4),* individual disseminated carcinoma cells in bone marrow have generally resisted clear cytologic identification *(5).* In fact, the standard method rarely detects such cells in the bone marrow of patients with early stage operable cancer *(6,7).* During the past decade, more sensitive immunologic and molecular procedures have been developed that permit the identification of individual tumor cells in organs remote from the primary tumor *(1,8).* For epithelial tumors that tend to have skeletal metastases, individual tumor cells are easily detected among bone marrow cells aspirated from the iliac crest. The medullary space of the iliac crest

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See "Note" following "References."

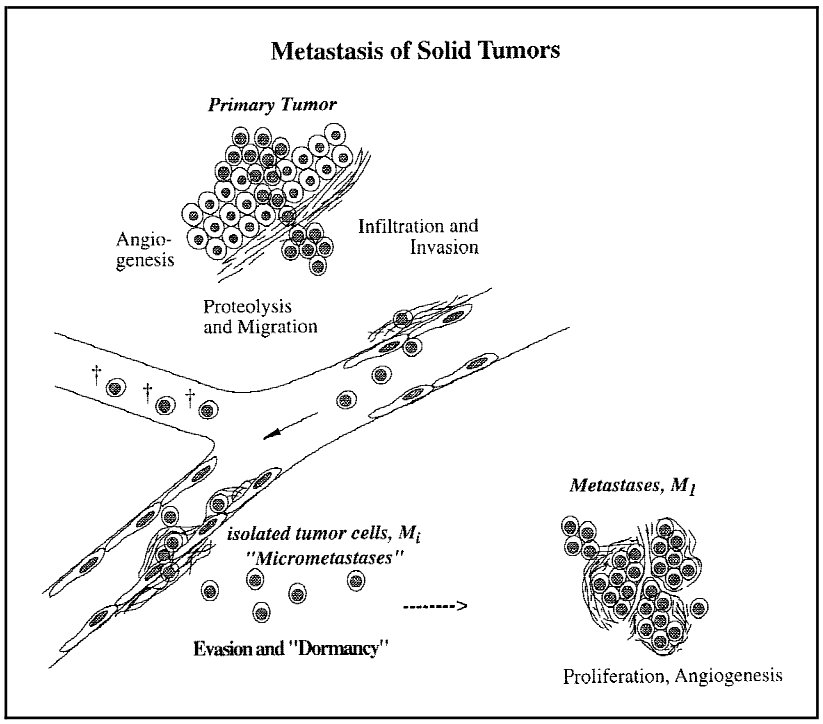

Fig. 1. Diagram of tumor cell metastasis. Cells from the primary tumor leave the tumor as a result of proteolysis, infiltrate and invade the circulatory system, and migrate to a new site where they adhere to the walls of the capillary and invade a new organ. At this site, micrometastases (i.e., isolated tumor cells or small clusters of tumor cells) that are undetectable by conventional tumor staging procedures can survive for several years. The cells can evade chemotherapy because they are in a dormant state. We propose that this stage be called "M_i." Later, the cells become proliferative, stimulate angiogenesis, and begin to form a metastatic tumor (stage M_1, tumor–lymph node–metastasis [TNM] classification system). † = Apoptotic tumor cells.

Table 1. Incidence of CK18-positive cells in bone marrow: tumor histology and clinical metastasis status*

Tumor stage†	No. of patients examined	No. of patients with CK18-positive cells (%)
Mammary carcinoma (two-sided $P<.001$, χ^2 test)		
M_0	116	35 (30.2)
M_1	19	14 (73.7)
Gastric carcinoma (two-sided $P = .02$, χ^2 test)		
M_0	80	25 (31.3)
M_1	40	21 (52.5)
Colorectal carcinoma (two-sided $P = .05$, χ^2 test)		
M_0	195	53 (27.2)
M_1	82	32 (39.0)

*CK = cytokeratin. CK18-positive cells were identified with monoclonal antibody CK2 (alkaline phosphatase anti-alkaline phosphatase-staining technique). Data are from *(177)*.

†Tumor–lymph node–metastasis (TNM) classification system.

is a site of a particularly intensive exchange of cells between blood and the mesenchymal interstitium. Occult tumor cells are even detected in the bone marrow of patients who have cancers that generally do not metastasize to the bone (e.g., colon cancer), indicating that the bone marrow is a particularly good site for the detection of occult tumor cells. In contrast, detection of occult tumor cells in the peripheral blood of patients with early stage cancer is much more difficult because of the low frequency of these cells, and the clinical relevance of circulating tumor cells remains questionable *(9–12)*. Thus, blood may be a suboptimal source for the detection of occult tumor cells (despite its obvious ease of sampling). In the future, it may be possible to enrich the tumor cell population in blood, which would improve its utility.

Detection of occult epithelial tumor cells in the bone marrow relies on methods that distinguish cells with different origins (e.g., hematopoietic cells versus epithelial cells), a concept introduced by investigators at the Royal Marsden Hospital and the Ludwig Institute nearly 20 years ago *(13)*. Currently, most studies *(5–7,13–22)* that show an association between the success of a cancer patient's treatment and the presence of occult micrometastases have used immunocytochemical methods (specific monoclonal antibodies) to detect extrinsic epithelial tumor cells. Several studies *(17,21,22)* have also shown that detection of occult tumor cells with monoclonal antibodies is clinically useful. However, caution needs to be exercised in the choice of marker antigens used; some marker antigens, such as epithelial membrane antigen or mucin-1, may not be suited for routine use because they also appear to be expressed on a subset of hematopoietic cells. Many studies *(23,24)* have used cytokeratins (CKs) as marker antigens; these proteins are stably and abundantly expressed in a majority of epithelial tumors and in a majority of the cells in these tumors. Although ectopic or illegitimate CK messenger RNA (mRNA) expression cannot be ruled out *(25–29)*, many studies *(16,30–33)* have now shown that CK antigens are rarely detected in hematopoietic cells. Finally, a combination of several antibodies to various CK antigens or a broad spectrum of anti-CK antibodies has been used because of the antigenic heterogeneity of tumor cells *(7,30,31,33)*.

When an antibody against CK18 was used as a probe, individual epithelial cells were found in the bone marrow aspirates from 20% to 30% of the patients examined who had primary carcinomas at different stages but no evidence of metastases (stage M_0, tumor–lymph node–metastasis [TNM] classification system; Table 1). However, most of the patients had fewer than 10 CK18-positive tumor cells per 8×10^5 mononuclear bone marrow cells. Thus, techniques for detecting occult tumor cells must be extremely sensitive, well beyond the limits of sensitivity of standard histopathologic analysis. Immunocytochemical methods are exquisitely sensitive and can detect as few as one to two tumor cells in 1×10^6 bone marrow mononuclear cells *(30,33,34)*. Whether this level of sensitivity is adequate remains to be determined, but enrichment methods are now available that can increase the sensitivity by at least one order of magnitude *(20,35–38)*.

When bone marrow aspirates from patients who had tumors in various stages were analyzed immunocytochemically (Table 1), the rate of early dissemination of tumor cells was similar for different types of carcinoma. When mammary carcinoma and colorectal carcinoma were compared, equivalent incidences of positive findings from both types of tumor were found in the bone marrow until the more advanced

stage M_1, when the incidence of finding mammary carcinoma cells in the bone marrow was statistically significantly higher than the incidence of finding colorectal carcinoma cells (Table 1). The appearance of this difference at such an advanced tumor stage might be explained by a specific growth and/or survival advantage for the disseminated mammary carcinoma cells in bone marrow, in agreement with the "seed and soil" theory that Paget *(39)* proposed in 1889 [*see also (40)*] and with the presence of microenvironmental factors that support the growth of mammary tumor cells *(41–43)* but not the growth of colorectal tumor cells. This hypothesis could explain why clinically manifest skeletal metastasis from colorectal carcinoma is rare, despite the presence of disseminated colorectal tumor cells in bone marrow *(44–47)*. Besides the interaction of tumor cells with the surrounding organ milieu, the path that cells must follow in the circulatory system also plays a role in tumor dissemination. Colon carcinoma cells, for example, must first pass through the capillary bed of the liver, which traps many of the cells and enhances the chances of metastasis of colon carcinoma to the liver *(44)*.

The prognostic importance of the immunocytochemical identification of occult tumor cells in bone marrow has been confirmed in various prospective clinical studies (Table 2), several of which have shown that the presence of occult tumor cells in bone marrow is an independent risk factor *(6,7,16,17,21,47–49)*. Because this is true even for tumors that rarely produce clinically important skeletal metastases, the appearance of epithelial cells in the bone marrow of patients with early stage cancer might indicate that tumor cells have also spread to other secondary sites, including the site of final metastatic deposit. However, not all studies have confirmed the prognostic relevance of occult tumor cell detection *(50)*. It is possible that certain technical factors may account for these discrepancies. For early stage breast cancer, occult tumor cell detection rates of 4%–45% have been reported *(18)*. The choice of antibodies and immunologic methods and the technical skills involved in performing the procedures and interpreting the results may introduce variation, and there is a certain risk of false-positive identification of nonepithelial cells *(51–53)*. For example, prostate-specific antigen (PSA)-positive cells in the blood could be monocytes that have pinocytosed free PSA in serum or phagocytosized PSA-positive tumor cells *(51,52)*. Thus, it will be important to define the critical variables in the methods and to introduce at least some level of standardization to allow more reliable and reproducible results *(30,33,53)*. Finally, differences in study design may account for some of the discrepancies; some studies have not been designed to allow evaluation of the association of occult tumor detection with clinical outcome.

Table 2. Immunocytochemical studies of the prognostic relevance of disseminated tumor cells in bone marrow

Type of tumor	Marker protein(s)*	Detection rate (%)	Prognostic value*	Reference No.
Mammary carcinoma	EMA	89/350 (25)	DFS, OS	*(22)*
	EMA, TAG12, CK	38/100 (38)	DFS, OS†	*(21)*
	CK	18/49 (37)	DFS†	*(6)*
	TAG12	315/727 (43)	DFS, OS†	*(17)*
Colorectal carcinoma	CK18	28/88 (32)	DFS†	*(47)*
Gastric carcinoma	CK18	34/97 (35)	DFS	*(175)*
	CK18	47/78 (60)	DFS	*(141)*
	CK18	95/180 (53)	DFS†	*(48)*
Esophagus carcinoma	CK	37/90 (41)	DFS, OS	*(176)*
Bronchial carcinoma	CK	17/43 (40)	DFS	*(7)*
NSCLC*	CK18	83/139 (60)	DFS†	*(16)*

*EMA = epithelial membrane antigen; CK = cytokeratin; TAG12 = tumor-associated glycoprotein 12; DFS = disease-free survival; OS = overall survival; and NSCLC = non-small-cell lung cancer.

†Prognostic value as an independent parameter was confirmed through multivariate analysis.

In addition to the presence or absence of occult tumor cells, it now appears that the number of tumor cells detected may be clinically relevant. Preliminary work by Cote et al. *(6)* indicates that there is an increased risk of relapse for patients with mammary carcinoma who have more disseminated cells. By quantitatively transferring cells to slides by cytocentrifugation, Jauch et al. *(48)* showed that the occult tumor cell count is associated with the rate of relapse in patients with gastric carcinoma. Thus, the number of occult disseminated tumor cells detected may reflect the tumor cell burden, and this number may be an important clinical variable. This result again highlights the importance of reliable and reproducible techniques to detect such cells.

In studies of patients who had either prostate or colorectal carcinoma or who had melanoma, perioperatively obtained peripheral blood samples were examined by molecular methods (*see below*). Results showed that a temporary intraoperative dissemination of tumor cells into circulation can occur *(54–57)*. It is not known whether these cells reach, survive, and form detectable metastases in secondary organs.

Tumor Cell Dissemination Through Lymph Nodes

The presence or absence of metastases to regional lymph nodes is the single most important standard risk factor for patients with the most solid tumors when no evidence of systemic metastases is present. However, routine histopathologic examination of lymph nodes will underestimate the prevalence of such metastases; in fact, it has been calculated that a pathologist has only a 1% chance of identifying a small (three-cell diameters) metastatic focus of cancer *(58)*.

The detection of occult metastases in the lymph nodes of patients with node-negative cancer is being shown to be prognostically important in an increasing number of studies on many types of cancers, including breast cancer *(59–62)*, colon cancer *(63,64)*, gastric cancer *(65)*, non-small-cell lung cancer *(66–68)*, esophageal cancer *(69)*, prostate cancer *(70,71)*, and melanoma *(72)*. These results emphasize the importance of verifying the lymph node status, which may improve tumor staging and may provide additional criteria for administering adjuvant therapy. Although it seems obvious that regional tumor spread is clinically important, several investigations [reviewed in *(73–75)*] have found that such tumor deposits are not associated with clinical outcome. This appears to be largely the result of study design; most of the negative studies involved too few patients to address the issue with sufficient statistical power. In addition, technical issues may account for discrepancies in some studies. For example, the analysis of a few sections, 5–6 μm thick, represents a relatively small random sample of a lymph node. Furthermore, in general, the use of anti-CK antibodies appears to be a reliable and an effective method for tumor cell detection, although normal lymph node (reticulum) cells can express CKs (e.g., CK19) *(23)*. However, antibodies against other epithelial

antigens that are not present on normal lymph node cells have been used, including BerEP4, an antibody that recognizes two glycoproteins of 34 and 49 kd present on the cell surface *(66,67,69)*, and an antibody against carcinoembryonic antigen (CEA) *(76)*.

In addition to immunohistochemistry, molecular methods based on the polymerase chain reaction (PCR)-mediated amplification of tumor cell DNA or of complementary DNA reverse transcribed from mRNA have been used to detect tumor cells in lymph nodes. However, the specificity of RNA-based markers, such as CEA mRNA, recently used for the analysis of lymph nodes in patients with colon cancer *(64)*, is not absolute because of the low-level illegitimate expression of the marker gene in the surrounding lymph node cells *(77)*. Better alternatives are DNA-based markers, such as mutations in the p53 gene or Ki-ras gene, that have been used in patients with colorectal cancer, lung cancer, or head and neck cancer to detect single tumor cells in a background of thousands of lymph node cells *(78–80)*.

An important advance in the evaluation of regional lymph nodes has been the development of a more limited dissection, the sentinel lymph node dissection, that is based on the identification, with dyes or radioactivity, of the specific lymph node that drains the tumor and the removal of this lymph node for analysis. This approach was pioneered by Morton et al. *(81)* and Giuliano et al. *(82,83)* and has been extensively evaluated in patients with melanoma and breast cancer *(81–85)*. Although the advantages of a more limited lymph node dissection are clear (in particular, the potential for decreasing the rate of postoperative complications), there is less material available for staging evaluation. The use of sensitive methods to detect micrometastasis may allow the identification of metastases in more limited amounts of material, such as lymph nodes, and thus may influence the subsequent therapeutic approach (e.g., more extensive lymph node dissection and the administration of adjuvant therapy). The use of immunohistochemistry can change the status of a negative lymph node to a positive lymph node in 5%–20% of the sample tested, and thus inclusion of an immunohistochemical evaluation may reduce the false-negative rate of the sentinal lymph node technique to almost zero *(86)*. Thus, detection of occult tumor cells may be an important adjunct to the use of limited lymph node dissection for staging and for therapy *(87,88)*.

New Techniques for Detection of Disseminated Tumor Cells

The immunocytochemical detection of micrometastases has been developed during the last 10 years, and its clinical relevance has been validated. This method is currently the standard method for the early detection of occult tumor cells disseminated from solid tumors. Microscopic analysis of many cytologic samples is, however, time consuming and requires considerable expertise. A new method of cytocentrifugation (Hettich, Tuttlingen, Germany) that permits the analysis of larger sample volumes *(89)* should address the first problem. The microscopic screening of large numbers of cytologic samples could be automated by the use of an image-analysis system (scanner). Systems of this type are currently being developed that have high sensitivity and specificity and can be used for screening occult metastases in patients enrolled in clinical trials *(90,91)*. One way to increase the sensitivity of tumor cell detection in bone marrow and blood is to selectively enrich for tumor cells. This enrichment is in addition to the standard density gradient procedure used to isolate the mononuclear cell fraction *(30,33)*. Several selective enrichment methods are currently being tested. By use of various density gradients and antibody-coupled magnetic particles, tumor cells (from cell lines) have been enriched by several orders of magnitude in model tests *(35–38,92)*. Enrichment can be achieved by positive or negative selection. Tumor cells can be selected with beads coated with antibodies against tumor-associated antigens, or normal blood cells in the preparation can be depleted by use of beads coated with antibodies against hematopoietic cell antigens *(35–38,92)*. These selection strategies have the additional advantage that the tumor cells are still viable and can be used for additional studies, including the propagation of malignant cells *in vitro (19)*. However, testing by clinical trials will be required to determine whether these enrichment techniques are superior to "standard" methods.

Immunocytochemical methods relying on monoclonal antibodies against various epithelium-specific cytoskeletal and membrane antigens have been used to detect individual disseminated carcinoma cells in mesenchymal organs (Tables 1 and 2). Previous methodologic studies have used surrogate model systems of bone marrow samples to which cells from cell lines have been added. These studies have demonstrated that the technique can detect two to four cells in 10×10^6 bone marrow cells and, by extrapolation, has a 95% chance of detecting one cancer cell in 2×10^6 bone marrow cells *(93)*. Methodologic studies based on surrogate model systems consisting of bone marrow samples to which cancer cells from cell lines have been added have demonstrated that immunocytologic techniques are superior to conventional histopathologic examinations. When we compared immunocytochemistry and flow cytometry studies, we found that the results of the published studies are heterogeneous, depending on the method used to detect tumor cells *(94–97)*. Molino et al. *(94)* and Vredenburgh et al. *(96)* claimed that immunocytochemistry was superior to flow cytometry. In contrast, Gross et al. *(97)* developed a flow cytometric assay with comparably high sensitivity; however, to reach the sensitivity reported, 40 hours was required to analyze one sample, which is not acceptable for analyzing large numbers of samples. Although flow cytometry is a good method for detecting occult metastases in patients with lymphoma and leukemia *(98,99)*, no study using patient samples has shown that flow cytometry is more sensitive than immunocytochemistry for the detection of micrometastases in patients with epithelial tumors. Some discrepancies may be due to the characteristics of the model cell lines used as surrogates for micrometastases *(100)*. For example, if CK antibodies were used to detect epithelial tumor cells, the loss of CKs would render the cells undetectable. In breast cancer cells, studied with multiparameter DNA flow cytometry, the loss of CKs has been shown to be a function of the cellular factors present and the preparation procedure used *(95)*. Thus, the clinical relevance of these methods remains in dispute, because tumor cells selected *in vitro* may display different characteristics than cancer cells *in vivo*.

More recently, molecular detection procedures have been used extensively to identify residual tumor cells in bone marrow; for example, follicular lymphomas have been detected by specific genetic changes (bcl-2 translocation and immunoglobulin gene rearrangements) *(101,102)*. In principle, the DNA of disseminated tumor cells can be amplified by the PCR, so that very small numbers of tumor cells can be detected in a heterogeneous

population of cells *(8)*. However, the tumor cell must have specific changes in its genome or mRNA expression pattern that distinguish it from the surrounding hematopoietic cells. At the DNA level, this criterion is difficult for most solid tumors to meet because the cells are quite genetically heterogeneous. Screening for genomic changes requires the molecular analysis of the primary tumor from each patient to determine the individual genomic alterations of that tumor. Exceptions are colon and pancreatic carcinomas, which commonly harbor distinct mutations of the Ki-ras oncogene that has been targeted for the detection of occult tumor cells in lymph nodes *(78,103)*, blood *(104,105)*, bone marrow *(106)*, and liver *(107)*. In tumors with a virus-associated oncogenesis, such as cervical cancer, screening of lymph nodes for human papillomavirus DNA and mRNA may be a fruitful approach *(108)*. In light of earlier studies *(109–112)* indicating that cancer patients have larger amounts of circulating DNA in serum or plasma, blood samples from patients with head and neck tumors or lung cancer have been analyzed for microsatellite alterations *(113,114)*. With the rapid advancement of new technologies that allow the profiling of individual tumors *(115)*, the development of methods for patient-specific tumor cell detection may be possible. Another interesting application of DNA-based markers is the analysis of the p53 [also known as TP53] gene in cells of the resection margins, which are called tumor free by conventional histopathologic examination. This type of analysis has been shown to provide clinically important data for patients with squamous cell carcinomas of the head and neck *(80)*.

The detection of differentially expressed mRNA species, on the other hand, appears to present fewer obstacles to more widespread use of the PCR method *(73)*, in which the cell's mRNAs are transcribed into complementary DNAs by reverse transcription (RT), and the complementary DNAs are amplified in a subsequent PCR (RT–PCR). In this method, mRNAs that are differentially expressed in epithelial cells (i.e., occult tumor) compared with hematopoietic cells are amplified, exactly analogous to the immunohistochemical detection of occult tumor cells with antibodies specific for epithelial cell antigens. Because CKs are highly expressed in epithelial tumors, they have frequently been targets, particularly CK19 and CK20 *(9,116–120)*, although many epithelial markers have been evaluated (Table 3). Other transcripts used as markers include CEA *(64,121)*, epidermal growth factor receptor *(122,123)*, mucin-1 *(119)*, human chorionic gonadotropin-β *(124)*, and α-fetoprotein *(125)*. In prostate cancer, prostate-specific marker transcripts are available, including PSA, prostate-specific membrane antigen, and human kallikrein-2 [reviewed in *(73)*]. However, it is now becoming clear that many of these targets may not have the requisite specificity to distinguish epithelial tumor cells from hematopoietic cells. Several studies *(25–29,118,126–129)* have shown that these transcripts are consistently identified in normal bone marrow, blood, and lymph node tissue. There are several possible reasons for this lack of specificity, including the presence of pseudogenes and low-level transcription of epithelium-specific mRNA by hematopoietic cells. Besides the choice of the appropriate marker transcript, the specificity of the RT–PCR assay largely depends on the method of sample preparation and on the assay conditions *(73,130,131)*. For example, false-positive findings of RT–PCR assays for CK20 can be avoided by analysis of mononuclear cells instead of whole blood preparations, because normal granulocytes express CK20 *(9,132)*. Another limiting factor is the deficient expression of the marker gene (e.g., PSA) in micrometastatic tumor cells *(27,133)*. To overcome this problem, a multimarker RT–PCR assay can be established, as was done for melanoma-associated antigens *(134,135)*. However, the increased sensitivity of the assay may be achieved by a loss of specificity, unless the selected marker genes are expressed exclusively in tumor cells. In addition, RT–PCR needs to be quantitative if RT–PCR determination of tumor cell numbers (burden) is to become an important component of the detection of occult metastasis. Nevertheless, the use of RT–PCR for the detection of occult tumor remains an interesting possibility, and the prognostic importance of the RT–PCR results should be examined in future clinical studies to compare RT–PCR and immunocytochemical analysis.

Table 3. Detection of disseminated epithelial tumor cells by molecular methods

Tissue	Tumor organ	Messenger RNA/DNA marker(s)* (reference Nos.)
Bone marrow	Breast	CK19, CEA *(116,121)*
	Colorectum	Ki-ras mutations, CEA, CK19, CK20 *(9,106,118,121)*
	Stomach	CEA, CK20 *(9,121)*
	Pancreas	CEA *(121)*
	Prostate	PSA, CK19, hK2 *(73)*
	Head and neck	E48 antigen *(178)*
Lymph nodes	Breast	CK19, MUC1, β-hCG *(119,120,124)*
	Lung	p53, Ki-ras mutations *(79)*
	Cervix	HPV16, E6/E7 *(108)*
	Colorectum	p53 and Ki-ras mutations, CEA, CK19, CK20 *(64,78,118)*
	Pancreas	Ki-ras mutations *(103)*
	Prostate	PSA, PSM, hK2 *(73)*
	Head and neck	p53 mutations *(80)*
Blood	Breast	CK19, EGF receptor, β-hCG *(122–124)*
	Lung	Microsatellite alterations *(114)*
	Colorectum	CK20, Ki-ras mutations *(9,104,117)*
	Stomach	CK20 *(9)*
	Pancreas	Ki-ras mutations *(105)*
	Liver	α-Fetoprotein *(125)*
	Prostate	PSA, PSM, hK2 *(73)*
	Head and neck	Microsatellite alterations *(113)*
Liver	Pancreas	Ki-ras mutations *(107)*
Tumor resection margins	Head and neck	p53 mutations *(80)*

*CK = cytokeratin; CEA = carcinoembryonic antigen; PSA = prostate-specific antigen; MUC = mucin; HPV = human papillomavirus; EGF = epidermal growth factor; PSM = prostate-specific membrane antigen; β-hCG = β subunit of human chorionic gonadotropin; and hK2 = human kallikrein-2.

Biologic Characteristics of Disseminated Cancer Cells

The detection of disseminated tumor cells has introduced a new opportunity to evaluate which of the diverse biologic characteristics of the primary tumor might favor the early dissemination of its cells. Two groups *(136,137)* have recently reported an association of tumor angiogenesis with bone marrow micrometastases for breast and gastric cancers. In addition, Choy and McCulloch *(138)* and McCulloch et al. *(139)* found an association between tumor angiogenesis and tumor cell shedding into

effluent venous blood during breast cancer surgery. The metastatic potential to bone marrow was not associated with the expression of p53 and RB genes or the proliferative activity of the primary lesion of gastric cancer *(140)*. In view of the malignant potential of CK-positive cells, a number of tumor-associated characteristics have been identified in CK-positive cells with immunocytochemical double-staining methods, including expression of urokinase plasminogen activator receptor, overexpression of the erbB2 oncogene, and deficient expression of major histocompatibility complex (MHC) class I molecules (Table 4).

Urokinase plasminogen activator receptor expression on disseminated tumor cells in bone marrow of patients with gastric cancer was associated with increasing tumor cell counts and poor clinical prognosis *(141,142)*. This finding suggests that expression of the urokinase plasminogen activator receptor not only is involved in tumor invasion but also influences the survival and/or growth of disseminated tumor cells in bone marrow, which is consistent with the currently accepted role of proteinases in metastasis *(143)*. Another selection criterion for tumor cell dissemination might be overexpression of the erbB2 oncogene, which was frequently observed on bone marrow micrometastases *(2,3)*. It is interesting that patients with breast cancer exhibited distinctly higher incidences of $p185^{erbB2}$ expression on micrometastases (60%–70%) compared with their primary tumors (20%–30%), indicating that erbB2 overexpression might be a positive selection criterion for disseminated tumor cells. All breast carcinoma patients analyzed who had distant metastases (stage M_1) had $p185^{erbB2}$ on CK-positive cells compared with about 50% of the patients who had regional disease (stage M_0; TNM classification system). More recently, Brandt et al. *(144)* suggested that blood-borne c-erbB2-positive CK-positive clustered cells are the possible precursors of distant metastases. These findings might explain why antibody therapy directed against erbB2-expressing cancer cells appears to be successful in patients with metastatic breast cancer who are receiving additional chemotherapy *(145,146)*.

Table 4. Phenotype of cytokeratin (CK)-positive tumor cells in bone marrow

Marker	Tumor origin	Marker-positive or CK-positive cells.* No. of patients with marker/No. of total patients (%)
Growth factor receptor		
erbB2	Breast	48/71 (67.6)
	Colorectum	8/28 (28.6)
	Stomach	6/22 (27.3)
	Lung	5/6 (83.3)
Transferrin receptor	Breast	17/59 (28.8)
	Colorectum	7/17 (41.1)
MHC class I antigen†		
	Breast	9/26 (34.6)
	Colorectum	12/17 (70.6)
	Stomach	8/11 (72.7)
Adhesion molecule		
17-1A (EpCAM)†	Breast	20/31 (64.5)
ICAM-1†	Lung (NSCLC†)	13/31 (41.9)
Plakoglobin	Lung (NSCLC†)	4/12 (33.3)
	Colorectum	4/13 (30.8)
Proliferation-associated protein		
Ki-67	Breast	1/12 (8.3)
	Colorectum	0/13
	Stomach	0/8
	Lung	0/7
p120	Breast	1/11 (9.1)
	Colorectum	5/12 (41.7)
	Stomach	4/13 (30.8)
	Lung	3/10 (30)
Protease uPA† receptor	Stomach	20/44 (45)

*From *(2,3,141,142,147,148,179,180)*.

†uPA = urokinase plasminogen activator; MHC = major histocompatibility complex; ICAM-1 = intercellular adhesion molecule-1; NSCLC = non-small-cell lung cancer; and EpCAM = epithelial cell adhesion molecule.

The low frequency of epithelial tumor cells in bone marrow and the localization of epithelial cells in an organ that has such a good blood supply offer ideal conditions for the elimination of epithelial cells by immunocompetent cells. The clinical history of epithelial tumor cells shows, however, that micrometastatic tumor cells can be ignored for many years by the immune system. In this context, the deficient expression of MHC class I molecules *(147,148)*, which, as restrictive elements, participate in T-lymphocyte-mediated tumor cell recognition, may be an important survival feature (Table 4). The underexpression of MHC class I molecules could limit the prospects for success of tumor cell vaccines *(149)*. Antibody-mediated tumor cell killing, on the other hand, is independent of tumor-cell MHC expression.

The malignant nature of CK-positive cells in bone marrow has been further confirmed through genomic analysis by the fluorescence *in situ* hybridization, in which many aberrations in chromosomes 7, 8, and 18 and the amplification of the erbB2 gene were observed in these cells *(150,151)*. The sensitivity of this procedure for detecting cells with amplified erbB2 and Int2 genes was increased by including an immunomagnetic enrichment step for CK-positive cells before the test *(152)*. Extensive cell culture experiments have also shown that cells disseminating into the bone marrow have a time-limited proliferative potential *(153)*. Thus, these cells apparently cannot yet proliferate autonomously and may be dormant *(154)*. This assumption has been corroborated by double-staining studies *(2,3)*, in which the fraction of disseminated tumor cells in bone marrow that express a proliferation marker (Ki-67 or p120) appears to be small. The dormant state of these cells may be one explanation of the relative resistance of micrometastatic tumor cells to chemotherapy and would confirm the appropriateness of therapies that are independent of the proliferative status of the cells targeted. Multiple labeling experiments [e.g., using antibody-coupled fluorescent particles *(12)*] may allow various tumor cell characteristics to be ranked according to their utility as therapeutic targets. Moreover, cell lines established from bone marrow micrometastases of cancer patients are now available to evaluate new anticancer agents *(155,156)*.

THERAPY

Adjuvant therapy for patients with early stage operable disease has been shown to be an important component in the management of many cancers. Conventional adjuvant chemotherapy has been modified and improved in various ways *(157,158)*. For colorectal cancer, it has been shown that adjuvant chemotherapy is effective in some patients and generally well tolerated *(159)*; the efficacy threshold in chemotherapy protocols published to date is an approximately 30% reduction in mortality *(159,160)*. The success of adjuvant therapy is assumed to stem from its ability to eradicate occult metastases before they become clini-

cally evident *(161)*. However, the success of standard adjuvant chemotherapy, particularly chemotherapy aimed at proliferating cell populations, may be limited by the fact that many of the residual systemic tumor cells present after primary resection may be nonproliferative or dormant *(2,3)*.

The basic idea proposed by Paul Ehrlich of treating tumors with specific antibodies ("magic bullets") is more than 100 years old. The hybridoma technique for making monoclonal antibodies in large quantities *(162)* was described in 1975 and has presented a vast array of potential therapeutic options (i.e., specific targets expressed by the cancer cells). Although monoclonal antibody therapy has been effective in various experimental systems, the clinical experience has been disappointing for patients with advanced stage solid tumors [reviewed in *(1,146)*], probably as a result of the large tumor cell burden and the lack of access that macromolecules have to cells in large tumors *(163)*. Complete remission has nevertheless been induced in some patients with metastatic colorectal carcinoma through a combination therapy for monoclonal antibody 17-1A and granulocyte–macrophage colony-stimulating factor *(164)*. Another approach is the use of antibody–toxin conjugates, or immunotoxins *(165,166)*, and promising effects with such agents, even in advanced disease, have been reported *(167)*.

Despite these results, however, micrometastatic or isolated tumor cells should in theory be much more promising targets for antibody-based therapy *(1,146)*. The incidence of micrometastatic cells in mesenchymal tissue such as bone marrow makes them easily accessible for intravenously applied macromolecules, a vital prerequisite for the effectiveness of these forms of therapy. For free antibodies, this therapeutic rationale has been examined in a randomized study of patients with colorectal carcinoma of International Union Against Cancer stage III after complete resection of the primary tumor. The patients were given five postoperative infusions of the monoclonal antibody edrecolomab against the 17-1A antigen (Panorex^{R}; GlaxoWellcome, Hamburg, Germany) as adjuvant therapy. During a 7-year period, the test group showed a substantial reduction in mortality and, in particular, a reduction in remote metastasis formation compared with the control group *(168)*.

The heterogeneity of solid tumors poses a problem for all types of therapy and limits the chances of complete elimination of all residual tumor cells. Although expression of the 17-1A antigen is relatively homogeneous in colon carcinoma cells, it is more heterogeneous in disseminated mammary carcinoma cells (Table 4). This highlights the value of characterizing the micrometastatic cells in individual patients before antibody-based therapy is initiated. Multiple analyses of tumor cells isolated from bone marrow or peripheral blood could characterize the cells and guide the choice of antibody and/or conjugate for individual patients. Because of the heterogeneity of the residual carcinoma cells (Table 4), it might also be possible to use a mixture of antibodies and/or immunotoxins that target different membrane proteins expressed by the tumor cells to achieve the greatest possible therapeutic effect.

Occult tumor cells in the bone marrow of patients with early stage cancer have been the target of another class of therapy, where it has recently been shown that the bisphosphonate clodronate (Ostac^{R}; Boehringer Mannheim GmbH, Mannheim, Germany) can reduce the incidence of developing overt metastases in patients with early stage breast cancer who have occult metastases detected in their bone marrow *(169)*. Thus, this therapy aimed at a population of patients at risk for developing metastases (i.e., those with occult systemic disease at the time of presentation) has been shown to be beneficial.

Conclusions and Outlook

Despite the progress made in clinical oncology in recent decades, the presence of minimal residual cancer has limited the prospects for further improvements in lethality rates.

Although conventional tumor-staging parameters can provide reliable information about the proportion of a population of patients who will experience a recurrence of the disease, these measures cannot predict which individuals will have a recurrence of disease after primary therapy, particularly if the patient has early stage disease. Thus, new parameters need to be defined that better identify those patients at the greatest (and at the least) risk of relapse, because this would provide information critical to the subsequent management of the patient. The detection of the earliest manifestations of tumor dissemination is an extremely promising approach that should improve risk assessment and the identification of specific patients who would benefit from adjuvant treatment. During the last 10 years, new immunologic and molecular analytic procedures have been developed to diagnose and characterize minimal residual cancer. Studies are currently in progress to evaluate and standardize these procedures for clinical use. The encouraging results to date from studies on the prognostic relevance of disseminated tumor cells in bone marrow should be standardized, categorized, and incorporated into the staging nomenclature of the International Union Against Cancer. As part of the pathologic assessment process, additional tumor-staging information could be provided by including micrometastases in the TNM classification system *(170)*. Improved methods for genomic analysis of single tumor cells *(106,171,172)* and for assessment of target molecule expression may increase the diagnostic precision of current detection techniques and optimize the therapy for individual patients.

As far as adjuvant therapy is concerned, success or failure can be assessed only after an observation period of several years. The availability of a surrogate marker for monitoring the effectiveness of a treatment should speed the evaluation and development of new adjuvant therapies. Periodic examination of bone marrow and peripheral blood during therapy could indicate whether the therapeutic approach being used was effective. Monitoring procedures of this type would be of considerable value. Because of their accessibility, bone marrow or peripheral blood samples would be logical contenders for monitoring minimal residual cancer at the subclinical stage. Our experience to date indicates that immunologic or molecular monitoring of disseminated cells is in principle possible for individual patients and that a step involving the reproducible enrichment of rare tumor cells for these tests would be desirable to improve the chances of detecting tumor cells over the course of longitudinal studies *(173,174)*. In therapeutic studies, long-term observations are still required to establish whether the therapy-associated reduction in individual disseminated cells is associated with improved prognosis. In conclusion, we believe that there is an increasing body of evidence demonstrating that detection and characterization of tumor cells disseminated in bone marrow or peripheral blood can provide clinically important data that are of value for tumor staging and for prognostication and that can identify surrogate markers for early assessment of the effectiveness of adjuvant therapy. Thus, these data would have a sub-

stantial influence on future oncologic diagnosis and treatment. At the very least, examination for occult metastases should be incorporated into future clinical trials to evaluate cancer treatments. In the future, adjuvant therapy, specifically tailored to the disease in subgroups of patients or individual patients with residual disease, may represent a substantial advance.

REFERENCES

(1) Pantel K, Riethmuller G. Micrometastasis detection and treatment with monoclonal antibodies. Curr Top Microbiol Immunol 1996;213(Pt 3): 1–18.

(2) Pantel K, Schlimok G, Braun S, Kutter D, Schaller G, Funke I, et al. Differential expression of proliferation-associated molecules in individual micrometastatic carcinoma cells. J Natl Cancer Inst 1993;85:1419–24.

(3) Pantel K, Izbicki JR, Angstwurm M, Braun S, Passlick B, Karg O, et al. Immunocytological detection of bone marrow micrometastasis in operable non-small cell lung cancer. Cancer Res 1993;53:1027–31.

(4) Burkhardt R, Frisch B, Kettner G. The clinical study of micro-metastatic cancer by bone biopsy. Bull Cancer 1980;67:291–305.

(5) Schlimok G, Funke I, Holzmann B, Gottlinger G, Schmidt G, Hauser H, et al. Micrometastatic cancer cells in bone marrow: *in vitro* detection with anti-cytokeratin and *in vivo* labeling with anti-17-1A monoclonal antibodies. Proc Natl Acad Sci U S A 1987;84:8672–6.

(6) Cote RJ, Rosen PP, Lesser ML, Old LJ, Osborne MP. Prediction of early relapse in patients with operable breast cancer by detection of occult bone marrow micrometastases. J Clin Oncol 1991;9:1749–56.

(7) Cote RJ, Beattie EJ, Chaiwun B, Shi SR, Harvey J, Chen SC, et al. Detection of occult bone marrow micrometastases in patients with operable lung carcinoma. Ann Surg 1995;222:415–23; discussion 423–5.

(8) Sidransky D. Nucleic acid-based methods for the detection of cancer. Science 1997;278:1054–9.

(9) Soeth E, Vogel I, Roder C, Juhl H, Marxsen J, Kruger U, et al. Comparative analysis of bone marrow and venous blood isolates from gastrointestinal cancer patients for the detection of disseminated tumor cells using reverse transcription PCR. Cancer Res 1997;57:3106–10.

(10) Wyld DK, Selby P, Perren TJ, Jonas SK, Allen-Mersh TG, Wheeldon J, et al. Detection of colorectal cancer cells in peripheral blood by reverse-transcriptase polymerase chain reaction for cytokeratin 20. Int J Cancer 1998;79:288–93.

(11) Redding WH, Coombes RC, Monaghan P, Clink HM, Imrie SF, Dearnaley DP, et al. Detection of micrometastases in patients with primary breast cancer. Lancet 1983;2:1271–4.

(12) Fodstad O, Overli GE, Hovland B, Andresen M, Olsen OE, Hoifodt HK. New method for phenotypic characterization of micrometastatic cancer cells [abstract]. Proc Am Assoc Cancer Res 1998;39:436 [abstract 2969].

(13) Dearnaley DP, Sloane JP, Ormerod MG, Steele K, Coombes RC, Clink HM, et al. Increased detection of mammary carcinoma cells in marrow smears using antisera to epithelial membrane antigen. Br J Cancer 1981; 44:85–90.

(14) Beiske K, Myklebust AT, Aamdal S, Langholm R, Jakobsen E, Fodstad O. Detection of bone marrow metastases in small cell lung cancer patients. Comparison of immunologic and morphologic methods. Am J Pathol 1992;141:531–8.

(15) Porro G, Menard S, Tagliabue E, Orefice S, Salvadori B, Squicciarini P, et al. Monoclonal antibody detection of carcinoma cells in bone marrow biopsy specimens from breast cancer patients. Cancer 1988;61:2407–11.

(16) Pantel K, Izbicki J, Passlick B, Angstwurm M, Haussinger K, Thetter O, et al. Frequency and prognostic significance of isolated tumour cells in bone marrow of patients with non-small-cell lung cancer without overt metastases. Lancet 1996;347:649–53.

(17) Diel IJ, Kaufmann M, Costa SD, Holle R, von Minckwitz G, Solomayer EF, et al. Micrometastatic breast cancer cells in bone marrow at primary surgery: prognostic value in comparison with nodal status. J Natl Cancer Inst 1996;88:1652–8.

(18) Osborne MP, Rosen PP. Detection and management of bone marrow micrometastases in breast cancer. Oncology (Huntingt) 1994;8:25–31; discussion 35–6, 39–42.

(19) Rye PD, Hoifodt HK, Overli GE, Fodstad O. Immunobead filtration: a novel approach for the isolation and propagation of tumor cells. Am J Pathol 1997;150:99–106.

(20) Myklebust AT, Pharo A, Fodstad O. Effective removal of SCLC cells from human bone marrow. Use of four monoclonal antibodies and immunomagnetic beads. Br J Cancer 1993;67:1331–6.

(21) Harbeck N, Untch M, Pache L, Eiermann W. Tumour cell detection in the bone marrow of breast cancer patients at primary therapy: results of a 3-year median follow-up. Br J Cancer 1994;69:566–71.

(22) Mansi JL, Easton D, Berger U, Gazet JC, Ford HT, Dearnaley D, et al. Bone marrow micrometastases in primary breast cancer: prognostic significance after 6 years' follow-up. Eur J Cancer 1991;27:1552–5.

(23) Moll R, Franke WW, Schiller DL, Geiger B, Krepler R. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. Cell 1982;31:11–24.

(24) Fuchs E, Cleveland DW. A structural scaffolding of intermediate filaments in health and disease. Science 1998;279:514–9.

(25) Krismann M, Todt B, Schroder J, Gareis D, Muller KM, Seeber S, et al. Low specificity of cytokeratin 19 reverse transcriptase–polymerase chain reaction analyses for detection of hematogenous lung cancer dissemination. J Clin Oncol 1995;13:2769–75.

(26) Traweek ST, Liu J, Battifora H. Keratin gene expression in non-epithelial tissues. Detection with polymerase chain reaction. Am J Pathol 1993;142: 1111–8.

(27) Zippelius A, Kufer P, Honold G, Kollermann MW, Oberneder R, Schlimok G, et al. Limitations of reverse-transcriptase polymerase chain reaction analyses for detection of micrometastatic epithelial cancer cells in bone marrow. J Clin Oncol 1997;15:2701–8.

(28) Bostick PJ, Chatterjee S, Chi DD, Huynh KT, Giuliano AE, Cote R, et al. Limitations of specific reverse-transcriptase polymerase chain reaction markers in the detection of metastases in the lymph nodes and blood of breast cancer patients. J Clin Oncol 1998;16:2632–40.

(29) Ruud P, Fodstad O, Hovig E. Identification of a novel cytokeratin 19 pseudogene that may interfere with reverse transcriptase–polymerase chain reaction assays used to detect micrometastatic tumor cells. Int J Cancer 1999;80:119–25.

(30) Pantel K, Schlimok G, Angstwurm M, Weckermann D, Schmaus W, Gath H, et al. Methodological analysis of immunocytochemical screening for disseminated epithelial tumor cells in bone marrow. J Hematother 1994; 3:165–73.

(31) Braun S, Muller M, Hepp F, Schlimok G, Riethmuller G, Pantel K. Re: Micrometastatic breast cancer cells in bone marrow at primary surgery: prognostic value in comparison with nodal status [letter]. J Natl Cancer Inst 1998;90:1099–101.

(32) Cote RJ, Rosen PP, Hakes TB, Sedira M, Bazinet M, Kinne DW, et al. Monoclonal antibodies detect occult breast carcinoma metastases in the bone marrow of patients with early stage disease. Am J Surg Pathol 1988;12:333–40.

(33) Chaiwun B, Saad AD, Groshen S, Chen SC, Mazumder A, Imam A, et al. Immunohistochemical detection of occult carcinoma in bone marrow and blood: critical analysis of efficiency of separation, preparation and limits of detection in a model system. Diagn Oncol 1992;2:267–76.

(34) Osborne MP, Asina S, Wong GY, Old LJ, Cote RJ. Immunofluorescent monoclonal antibody detection of breast cancer in bone marrow: sensitivity in a model system. Cancer Res 1989;49:2510–3.

(35) Naume B, Borgen E, Beiske K, Herstad TK, Ravnas G, Renolen A, et al. Immunomagnetic techniques for the enrichment and detection of isolated breast carcinoma cells in bone marrow and peripheral blood. J Hematother 1997;6:103–14.

(36) Martin VM, Siewert C, Scharl A, Harms T, Heinze R, Ohl S, et al. Immunomagnetic enrichment of disseminated epithelial tumor cells from peripheral blood by MACS. Exp Hematol 1998;26:252–64.

(37) Naume B, Borgen E, Nesland JM, Beiske K, Gilen E, Renolen A, et al. Increased sensitivity for detection of micrometastases in bone-marrow/peripheral-blood stem-cell products from breast-cancer patients by negative immunomagnetic separation. Int J Cancer 1998;78:556–60.

(38) Racila E, Euhus D, Weiss AJ, Rao C, McConnell J, Terstappen LW, et al. Detection and characterization of carcinoma cells in the blood. Proc Natl Acad Sci U S A 1998;95:4589–94.

(39) Paget S. The distribution of secondary growth in cancer of the breast. Lancet 1889;1:571–3.

(40) International symposium. Critical determinants in cancer progression and metastasis. A centennial celebration of Dr. Stephen Paget's 'seed and soil' hypothesis. March 6–10, 1989, Houston, Texas. Abstracts. Cancer Metastasis Rev 1989;8:93–197.
(41) Kjonniksen I, Breistol K, Fodstad O. Site-dependent differences in sensitivity of LOX human melanoma tumors in nude rats to dacarbazine and mitozolomide, but not to doxorubicin and cisplatin. Cancer Res 1992;52: 1347–51.
(42) Dong Z, Radinsky R, Fan D, Tsan R, Bucana CD, Wilmanns C, et al. Organ-specific modulation of steady-state mdr gene expression and drug resistance in murine colon cancer cells. J Natl Cancer Inst 1994;86: 913–20.
(43) Fodstad O, Kjonniksen I. Microenvironment revisited: time for reappraisal of some prevailing concepts of cancer metastasis. J Cell Biochem 1994;56:23–8.
(44) Weiss L, Grundmann E, Torhorst J, Hartveit F, Moberg I, Eder M, et al. Haematogenous metastatic patterns in colonic carcinoma: an analysis of 1541 necropsies. J Pathol 1986;150:195–203.
(45) Welch JP, Donaldson GA. The clinical correlation of an autopsy study of recurrent colorectal cancer. Ann Surg 1979;189:496–502.
(46) Bonnheim DC, Petrelli NJ, Herrera L, Walsh D, Mittelman A. Osseus metastases from colorectal carcinoma. Am J Surg 1986;151:457–9.
(47) Lindemann F, Schlimok G, Dirschedl P, Witte J, Riethmuller G. Prognostic significance of micrometastatic tumour cells in bone marrow of colorectal cancer patients. Lancet 1992;340:685–9.
(48) Jauch KW, Heiss MM, Gruetzner U, Funke I, Pantel K, Babic R, et al. Prognostic significance of bone marrow micrometastases in patients with gastric cancer. J Clin Oncol 1996;14:1810–7.
(49) Mansi JL, Berger U, Easton D, McDonnell T, Redding WH, Gazet JC, et al. Micrometastases in bone marrow in patients with primary breast cancer: evaluation as an early predictor of bone metastases. Br Med J (Clin Res Ed) 1987;295:1093–6.
(50) Funke I, Schraut W. Meta-analyses of studies on bone marrow micrometastases: an independent prognostic impact remains to be substantiated. J Clin Oncol 1998;16:557–66.
(51) Fadlon EJ, Rees RC, McIntyre C, Sharrard RM, Lawry J, Hamdy FC. Detection of circulating prostate-specific antigen-positive cells in patients with prostate cancer by flow cytometry and reverse transcription polymerase chain reaction. Br J Cancer 1996;74:400–5.
(52) Brandt B, Junker R, Griwatz C, Heidl S, Brinkmann O, Semjonow A, et al. Isolation of prostate-derived single cells and cell clusters from human peripheral blood. Cancer Res 1996;56:4556–61.
(53) Borgen E, Beiske K, Trachsel S, Nesland JM, Kvalheim G, Herstad TK, et al. Immunocytochemical detection of isolated epithelial cells in bone marrow: non-specific staining and contribution by plasma cells directly reactive to alkaline phosphatase. J Pathol 1998;185:427–34.
(54) Denis MG, Tessier MH, Dreno B, Lustenberger P. Circulating micrometastases following oncological surgery [letter]. Lancet 1996;347:913.
(55) Eschwege P, Dumas F, Blanchet P, Le Maire V, Benoit G, Jardin A, et al. Haematogenous dissemination of prostatic epithelial cells during radical prostatectomy. Lancet 1995;346:1528–30.
(56) Hansen E, Wolff N, Knuechel R, Ruschoff J, Hofstaedter F, Taeger K. Tumor cells in blood shed from the surgical field. Arch Surg 1995;130: 387–93.
(57) Weitz J, Kienle P, Lacroix J, Willeke F, Benner A, Lehnert T, et al. Dissemination of tumor cells in patients undergoing surgery for colorectal cancer. Clin Cancer Res 1998;4:343–8.
(58) Gusterson B, Ott R. Occult axillary lymph-node micrometastases in breast cancer [letter]. Lancet 1990;336:434–5.
(59) International (Ludwig) Breast Cancer Study Group. Prognostic importance of occult axillary lymph node micrometastases from breast cancers. Lancet 1990;335:1565–8.
(60) McGuckin MA, Cummings MC, Walsh MD, Hohn BG, Bennett IC, Wright RG. Occult axillary node metastases in breast cancer: their detection and prognostic significance. Br J Cancer 1996;73:88–95.
(61) de Mascarel I, Bonichon F, Coindre JM, Trojani M. Prognostic significance of breast cancer axillary lymph node micrometastases assessed by two special techniques: reevaluation with longer follow-up. Br J Cancer 1992;66:523–7.
(62) Cote JR, Peterson HF, Chalwun B, Gelber RD, Goldhirsch R, Castiglione-Gertsch M, et al. The role of the immunohistochemical detection of lymph node metastases in the management of breast cancer. Lancet. In press 1999.
(63) Greenson JK, Isenhart CE, Rice R, Mojzisik C, Houchens D, Martin EW Jr. Identification of occult micrometastases in pericolic lymph nodes of Duke's B colorectal cancer patients using monoclonal antibodies against cytokeratin and CC49. Correlation with long-term survival. Cancer 1994; 73:563–9.
(64) Liefers GJ, Cleton-Jansen AM, van de Velde CJ, Hermans J, van Krieken JH, Cornelisse CJ, et al. Micrometastases and survival in stage II colorectal cancer. N Engl J Med 1998;339:223–8.
(65) Maehara Y, Oshiro T, Endo K, Baba H, Oda S, Ichiyoshi Y, et al. Clinical significance of occult micrometastasis lymph nodes from patients with early gastric cancer who died of recurrence. Surgery 1996;119:397–402.
(66) Passlick B, Izbicki JR, Kubuschok B, Nathrath W, Thetter O, Pichlmeier U, et al. Immunohistochemical assessment of individual tumor cells in lymph nodes of patients with non-small-cell lung cancer. J Clin Oncol 1994;12:1827–32.
(67) Kubuschok B, Passlick B, Izbicki JR, Thetter O, Pantel K. Disseminated tumor cells in lymph nodes as a determinant for survival in surgically resected non-small-cell lung cancer. J Clin Oncol 1999;17:19–24.
(68) Cote RJ, Hawes D, Chaiwun B, Beattie EJ Jr. Detection of occult metastases in lung carcinomas: progress and implications for lung cancer staging. J Surg Oncol 1998;69:265–74.
(69) Izbicki JR, Hosch SB, Pichlmeier U, Rehders A, Busch C, Niendorf A, et al. Prognostic value of immunohistochemically identifiable tumor cells in lymph nodes of patients with completely resected esophageal cancer. N Engl J Med 1997;337:1188–94.
(70) Freeman JA, Esrig D, Grossfeld GD, Stein JP, Chen SC, Young LL, et al. Incidence of occult lymph node metastases in pathological stage C (pT3NO) prostate cancer. J Urol 1995;154:474–8.
(71) Edelstein RA, Zietman AL, de las Morenas A, Krane RJ, Babayan RK, Dallow KC, et al. Implications of prostate micrometastases in pelvic lymph nodes: an archival tissue study. Urology 1996;47:370–5.
(72) Cochran AJ, Wen DR, Morton DL. Occult tumor cells in the lymph nodes of patients with pathological stage I malignant melanoma. An immunohistological study. Am J Surg Pathol 1988;12:612–8.
(73) Corey E, Corey MJ. Detection of disseminated prostate cells by reverse transcription–polymerase chain reaction (RT–PCR): technical and clinical aspects. Int J Cancer 1998;77:655–73.
(74) Calaluce R, Miedema BW, Yesus YW. Micrometastasis in colorectal carcinoma: a review. J Surg Oncol 1998;67:194–202.
(75) Taylor CR, Cote RJ. Immunohistochemical markers of prognostic value in surgical pathology. Histol Histopathol 1997;12:1039–55.
(76) Hitchcock CL, Arnold MW, Young DC, Schneebaum S, Martin EW Jr. TAG-72 expression in lymph nodes and RIGS [letter]. Dis Colon Rectum 1996;39:473–5.
(77) Bostick PJ, Hoon DS, Cote RJ. Detection of carcinoembryonic antigen messenger RNA in lymph nodes from patients with colorectal cancer [letter]. N Engl J Med 1998;339:1643–4.
(78) Hayashi N, Ito I, Yanagisawa A, Kato Y, Nakamori S, Imaoka S, et al. Genetic diagnosis of lymph-node metastasis in colorectal cancer. Lancet 1995;345:1257–9.
(79) Ahrendt SA, Yang SC, Wu L, Westra WH, Jen J, Califano JA, et al. Comparison of oncogene mutation detection and telomerase activity for the molecular staging of non-small cell lung cancer. Clin Cancer Res 1997;3:1207–14.
(80) Brennan JA, Mao L, Hruban RH, Boyle JO, Eby YJ, Koch WM, et al. Molecular assessment of histopathological staging in squamous-cell carcinoma of the head and neck. N Engl J Med 1995;332:429–35.
(81) Morton DL, Wen DR, Wong JH, Economou JS, Cagle LA, Storm FK, et al. Technical details of intraoperative lymphatic mapping for early stage melanoma. Arch Surg 1992;127:392–9.
(82) Giuliano AE, Kirgan DM, Guenther JM, Morton DL. Lymphatic mapping and sentinel lymphadenectomy for breast cancer. Ann Surg 1994;220: 391–8; discussion 398–401.
(83) Giuliano AE, Dale PS, Turner RR, Morton DL, Evans SW, Krasne DL. Improved axillary staging of breast cancer with sentinel lymphadenectomy. Ann Surg 1995;222:394–9; discussion 399–401.
(84) Veronesi U, Paganelli G, Galimberti V, Viale G, Zurrida S, Bedoni M, et

al. Sentinel-node biopsy to avoid axillary dissection in breast cancer with clinically negative lymph-nodes. Lancet 1997;349:1864–7.
(85) Krag D, Weaver D, Ashikaga T, Moffat F, Klimberg VS, Shriver C, et al. The sentinel node in breast cancer—a multicenter validation study. N Engl J Med 1998;339:941–6.
(86) Giuliano AE, Jones RC, Brennan M, Statman R. Sentinel lymphadenectomy in breast cancer. J Clin Oncol 1997;15:2345–50.
(87) Van der Velde-Zimmermann D, Roijers JF, Bouwens-Rombouts A, De Weger RA, De Graaf PW, Tilanus MG, et al. Molecular test for the detection of tumor cells in blood and sentinel nodes of melanoma patients. Am J Pathol 1996;149:759–64.
(88) Min CJ, Tafra L, Verbanac KM. Identification of superior markers for polymerase chain reaction detection of breast cancer metastases in sentinel lymph nodes. Cancer Res 1998;58:4581–4.
(89) Schwarz G. Cytomorphology and cell yield in a new cytocentrifugal technique allowing the collection of the cell-free supernatant. Lab Med 1991; 15:45–50.
(90) Cote RJ, Shi SR, Beattie EJ, Makarewicz B, Chaiwun B. Automated detection of occult bone marrow micrometastases in patients with operable lung carcinoma [abstract]. Proc ASCO 1997;16:458a.
(91) Makarewicz K, McDuffie L, Shi SR, Catterjee S, Yang C, Taylor C, et al. Immunohistochemical detection of occult micrometastases using an automated intelligent microscopy system [abstract]. Proc Am Assoc Cancer Res 1997;38:269 [abstract 1805].
(92) Fodstad O, Trones GE, Forus A, Rye PD, Beiske K, Aamdal S, et al. Improved immunomagnetic method for detection and characterization of cancer cells in blood and bone marrow [abstract]. Proc Am Assoc Cancer Res 1997;38:26 [abstract 172].
(93) Osborne MP, Wong GY, Asina S, Old LJ, Cote RJ, Rosen PP. Sensitivity of immunocytochemical detection of breast cancer cells in human bone marrow. Cancer Res 1991;51:2706–9.
(94) Molino A, Colombatti M, Bonetti F, Zardini M, Pasini F, Perini A, et al. A comparative analysis of three different techniques for the detection of breast cancer cells in bone marrow. Cancer 1991;67:1033–6.
(95) Wingren S, Guerrieri C, Franlund B, Stal O. Loss of cytokeratins in breast cancer cells using multiparameter DNA flow cytometry is related to both cellular factors and preparation procedure. Anal Cell Pathol 1995;9: 229–33.
(96) Vredenburgh JJ, Silva O, Tyer C, DeSombre K, Abou-Ghalia A, Cook M, et al. A comparison of immunohistochemistry, two-color immunofluorescence, and flow cytometry with cell sorting for the detection of micrometastatic breast cancer in the bone marrow. J Hematother 1996;5:57–62.
(97) Gross HJ, Verwer B, Houck D, Hoffman RA, Recktenwald D. Model study detecting breast cancer cells in peripheral blood mononuclear cells at frequencies as low as 10(–7). Proc Natl Acad Sci U S A 1995;92: 537–41.
(98) Jennings CD, Foon KA. Recent advances in flow cytometry: application to the diagnosis of hematologic malignancy. Blood 1997;90:2863–92.
(99) Ciudad J, San Miguel JF, Lopez-Berges MC, Vidriales B, Valverde B, Ocqueteau M, et al. Prognostic value of immunophenotypic detection of minimal residual disease in acute lymphoblastic leukemia. J Clin Oncol 1998;16:3774–81.
(100) Simpson SJ, Vachula M, Kennedy MJ, Kaizer H, Coon JS, Ghalie R, et al. Detection of tumor cells in the bone marrow, peripheral blood, and apheresis products of breast cancer patients using flow cytometry. Exp Hematol 1995;23:1062–8.
(101) Gribben JG, Freedman AS, Neuberg D, Roy DC, Blake KW, Woo SD, et al. Immunologic purging of marrow assessed by PCR before autologous bone marrow transplantation for B-cell lymphoma. N Engl J Med 1991; 325:1525–33.
(102) Zwicky CS, Maddocks AB, Andersen N, Gribben JG. Eradication of polymerase chain reaction detectable immunoglobulin gene rearrangement in non-Hodgkin's lymphoma is associated with decreased relapse after autologous bone marrow transplantation. Blood 1996;88:3314–22.
(103) Tamagawa E, Ueda M, Takahashi S, Sugano K, Uematsu S, Mukai M, et al. Pancreatic lymph nodal and plexus micrometastases detected by enriched polymerase chain reaction and nonradioisotopic single-strand conformation polymorphism analysis: a new predictive factor for recurrent pancreatic carcinoma. Clin Cancer Res 1997;3:2143–9.
(104) Hardingham JE, Kotasek D, Sage RE, Eaton MC, Pascoe VH, Dobrovic A. Detection of circulating tumor cells in colorectal cancer by immunobead-PCR is a sensitive prognostic marker for relapse of disease. Mol Med 1995;1:789–94.
(105) Tada M, Omata M, Kawai S, Saisho H, Ohto M, Saiki RK, et al. Detection of ras gene mutations in pancreatic juice and peripheral blood of patients with pancreatic adenocarcinoma. Cancer Res 1993;53:2472–4.
(106) Dietmaier W, Hartmann A, Wallinger S, Heinmoller E, Kerner T, Endl E, et al. Multiple mutation analyses in single tumor cells with improved whole genome amplification. Am J Pathol 1999;154:83–95.
(107) Inoue S, Nakao A, Kasai Y, Harada A, Nonami T, Takagi H. Detection of hepatic micrometastasis in pancreatic adenocarcinoma patients by two-stage polymerase chain reaction/restriction fragment length polymorphism analysis. Jpn J Cancer Res 1995;86:626–30.
(108) Czegledy J, Iosif C, Hansson BG, Evander M, Gergely L, Wadell G. Can a test for E6/E7 transcripts of human papillomavirus type 16 serve as a diagnostic tool for the detection of micrometastasis in cervical cancer? Int J Cancer 1995;64:211–5.
(109) Leon SA, Shapiro B, Sklaroff DM, Yaros MJ. Free DNA in the serum of cancer patients and the effect of therapy. Cancer Res 1977;37:646–50.
(110) Shapiro B, Chakrabarty M, Cohn EM, Leon SA. Determination of circulating DNA levels in patients with benign or malignant gastrointestinal disease. Cancer 1983;51:2116–20.
(111) Stroun M, Anker P, Lyautey J, Lederrey C, Maurice PA. Isolation and characterization of DNA from the plasma of cancer patients. Eur J Cancer Clin Oncol 1987;23:707–12.
(112) Stroun M, Anker P, Maurice P, Lyautey J, Lederrey C, Beljanski M. Neoplastic characteristics of the DNA found in the plasma of cancer patients. Oncology 1989;46:318–22.
(113) Nawroz H, Koch W, Anker P, Stroun M, Sidransky D. Microsatellite alterations in serum DNA of head and neck cancer patients. Nat Med 1996;2:1035–7.
(114) Chen XQ, Stroun M, Magnenat JL, Nicod LP, Kurt AM, Lyautey J, et al. Microsatellite alterations in plasma DNA of small cell lung cancer patients. Nat Med 1996;2:1033–5.
(115) Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Schraml P, Leighton S, et al. Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med 1998;4:844–7.
(116) Fields KK, Elfenbein GJ, Trudeau WL, Perkins JB, Janssen WE, Moscinski LC. Clinical significance of bone marrow metastases as detected using the polymerase chain reaction in patients with breast cancer undergoing high-dose chemotherapy and autologous bone marrow transplantation. J Clin Oncol 1996;14:1868–76.
(117) Burchill SA, Bradbury MF, Pittman K, Southgate J, Smith B, Selby P. Detection of epithelial cancer cells in peripheral blood by reverse transcriptase–polymerase chain reaction. Br J Cancer 1995;71:278–81.
(118) Gunn J, McCall JL, Yun K, Wright PA. Detection of micrometastases in colorectal cancer patients by K19 and K20 reverse-transcription polymerase chain reaction. Lab Invest 1996;75:611–6.
(119) Noguchi S, Aihara T, Motomura K, Inaji H, Imaoka S, Koyama H. Detection of breast cancer micrometastases in axillary lymph nodes by means of reverse transcriptase–polymerase chain reaction. Comparison between MUC1 mRNA and keratin 19 mRNA amplification. Am J Pathol 1996;148:649–56.
(120) Schoenfeld A, Luqmani Y, Sinnett HD, Shousha S, Coombes RC. Keratin 19 mRNA measurement to detect micrometastases in lymph nodes in breast cancer patients. Br J Cancer 1996;74:1639–42.
(121) Gerhard M, Juhl H, Kalthoff H, Schreiber HW, Wagener C, Neumaier M. Specific detection of carcinoembryonic antigen-expressing tumor cells in bone marrow aspirates by polymerase chain reaction. J Clin Oncol 1994; 12:725–9.
(122) Hildebrandt M, Mapara MY, Korner IJ, Bargou RC, Moldenhauer G, Dorken B. Reverse transcriptase–polymerase chain reaction (RT–PCR)-controlled immunomagnetic purging of breast cancer cells using the magnetic cell separation (MACS) system: a sensitive method for monitoring purging efficiency. Exp Hematol 1997;25:57–65.
(123) Mapara MY, Korner IJ, Hildebrandt M, Bargou R, Krahl D, Reichardt P, et al. Monitoring of tumor cell purging after highly efficient immunomagnetic selection of CD34 cells from leukapheresis products in breast cancer patients: comparison of immunocytochemical tumor cell staining and reverse transcriptase–polymerase chain reaction. Blood 1997;89:337–44.

(124) Hoon DS, Sarantou T, Doi F, Chi DD, Kuo C, Conrad AJ, et al. Detection of metastatic breast cancer by beta-hCG polymerase chain reaction. Int J Cancer 1996;69:369–74.
(125) Matsumura M, Niwa Y, Kato N, Komatsu Y, Shiina S, Kawabe T, et al. Detection of alpha-fetoprotein mRNA, an indicator of hematogenous spreading hepatocellular carcinoma, in the circulation: a possible predictor of metastatic hepatocellular carcinoma. Hepatology 1994;20:1418–25.
(126) Ko Y, Klinz M, Totzke G, Gouni-Berthold I, Sachinidis A, Vetter H. Limitations of the reverse transcription–polymerase chain reaction method for the detection of carcinoembryonic antigen-positive tumor cells in peripheral blood. Clin Cancer Res 1998;4:2141–6.
(127) Smith MR, Biggar S, Hussain M. Prostate-specific antigen messenger RNA is expressed in non-prostate cells: implications for detection of micrometastases. Cancer Res 1995;55:2640–4.
(128) Henke W, Jung M, Jung K, Lein M, Schlechte H, Berndt C, et al. Increased analytical sensitivity of RT–PCR of PSA mRNA decreases diagnostic specificity of detection of prostatic cells in blood. Int J Cancer 1997;70:52–6.
(129) de Graaf H, Maelandsmo GM, Ruud P, Forus A, Oyjord T, Fodstad O, et al. Ectopic expression of target genes may represent an inherent limitation of RT–PCR assays used for micrometastasis detection: studies on the epithelial glycoprotein gene EGP-2. Int J Cancer 1997;72:191–6.
(130) Traystman MD, Cochran GT, Hake SJ, Kuszynski CA, Mann SL, Murphy BJ, et al. Comparison of molecular cytokeratin 19 reverse transcriptase polymerase chain reaction (CK19 RT–PCR) and immunocytochemical detection of micrometastatic breast cancer cells in hematopoietic harvests. J Hematother 1997;6:551–61.
(131) Neumaier M, Gerhard M, Wagener C. Diagnosis of micrometastases by the amplification of tissue-specific genes. Gene 1995;159:43–7.
(132) Jung R, Kruger W, Hosch S, Holweg M, Kroger N, Gutensohn K, et al. Specificity of reverse transcriptase polymerase chain reaction assays designed for the detection of circulating cancer cells is influenced by cytokines *in vivo* and *in vitro.* Br J Cancer 1998;78:1194–8.
(133) Ferrari AC, Stone NN, Eyler JN, Gao M, Mandeli J, Unger P, et al. Prospective analysis of prostate-specific markers in pelvic lymph nodes of patients with high-risk prostate cancer. J Natl Cancer Inst 1997;89: 1498–504.
(134) Hoon DS, Wang Y, Dale PS, Conrad AJ, Schmid P, Garrison D, et al. Detection of occult melanoma cells in blood with a multiple-marker polymerase chain reaction assay. J Clin Oncol 1995;13:2109–16.
(135) Sarantou T, Chi DD, Garrison DA, Conrad AJ, Schmid P, Morton DL, et al. Melanoma-associated antigens as messenger RNA detection markers for melanoma. Cancer Res 1997;57:1371–6.
(136) Fox SB, Leek RD, Bliss J, Mansi JL, Gusterson B, Gatter KC, et al. Association of tumor angiogenesis with bone marrow micrometastases in breast cancer patients. J Natl Cancer Inst 1997;89:1044–9.
(137) Maehara Y, Hasuda S, Abe T, Oki E, Kakeji Y, Ohno S, et al. Tumor angiogenesis and micrometastasis in bone marrow of patients with early gastric cancer. Clin Cancer Res 1998;4:2129–34.
(138) Choy A, McCulloch P. Induction of tumour cell shedding into effluent venous blood breast cancer surgery. Br J Cancer 1996;73:79–82.
(139) McCulloch P, Choy A, Martin L. Association between tumour angiogenesis and tumour cell shedding into effluent venous blood during breast cancer surgery. Lancet 1995;346:1334–5.
(140) Maehara Y, Yamamoto M, Oda S, Baba H, Kusumoto T, Ohno S, et al. Cytokeratin-positive cells in bone marrow for identifying distant micrometastasis of gastric cancer. Br J Cancer 1996;73:83–7.
(141) Heiss MM, Allgayer H, Gruetzner KU, Funke I, Babic R, Jauch KW, et al. Individual development and uPA-receptor expression of disseminated tumour cells in bone marrow: a reference to early systemic disease in solid cancer. Nat Med 1995;1:1035–9.
(142) Allgayer H, Heiss MM, Riesenberg R, Grutzner KU, Tarabichi A, Babic R, et al. Urokinase plasminogen activator receptor (uPA-R): one potential characteristic of metastatic phenotypes in minimal residual tumor disease. Cancer Res 1997;57:1394–9.
(143) Stephens RW, Brunner N, Janicke F, Schmitt M. The Urokinase plasminogen activator system as a target for prognostic studies in breast cancer. Breast Cancer Res Treat 1998;52:99–111.
(144) Brandt B, Roetger A, Heidl S, Jackisch C, Lelle RJ, Assmann G, et al. Isolation of blood-borne epithelium-derived c-erbB-2 oncoprotein-positive clustered cells from the peripheral blood of breast cancer patients. Int J Cancer 1998;76:824–8.
(145) Baselga J, Tripathy D, Mendelsohn J, Baughman S, Benz CC, Dantis L, et al. Phase II study of weekly intravenous recombinant humanized anti-p185HER2 monoclonal antibody in patients with HER2/neu-overexpressing metastatic breast cancer. J Clin Oncol 1996;14:737–44.
(146) Scott AM, Welt S. Antibody-based immunological therapies. Curr Opin Immunol 1997;9:717–22.
(147) Schlimok G, Funke I, Bock B, Schweiberer B, Witte J, Riethmuller G. Epithelial tumor cells in bone marrow of patients with colorectal cancer: immunocytochemical detection, phenotypic characterization, and prognostic significance. J Clin Oncol 1990;8:831–7.
(148) Pantel K, Schlimok G, Kutter D, Schaller G, Genz T, Wiebecke B, et al. Frequent down-regulation of major histocompatibility class I antigen expression on individual micrometastatic carcinoma cells. Cancer Res 1991; 51:4712–5.
(149) Pardoll DM. Cancer vaccines. Immunol Today 1993;14:310–6.
(150) Muller P, Weckermann D, Riethmuller G, Schlimok G. Detection of genetic alterations in micrometastatic cells in bone marrow of cancer patients by fluorescence *in situ* hybridization. Cancer Genet Cytogenet 1996;88:8–16.
(151) Litle VR, Lockett SJ, Pallavicini MG. Genotype/phenotype analyses of low frequency tumor cells using computerize image microscopy. Cytometry 1996;23:344–9.
(152) Forus A, Hoifodt HK, Overli GE, Myklebost O, Fodstad O. Sensitive method for FISH characterisation of breast cancer cells in bone marrow aspirates. Mol Pathol 1999;52:68–74.
(153) Pantel K, Dickmanns A, Zippelius A, Klein C, Shi J, Hoechtlen-Vollmar W, et al. Establishment of micrometastatic carcinoma cell lines: a novel source of tumor cell vaccines. J Natl Cancer Inst 1995;87:1162–8.
(154) Uhr JW, Scheuermann RH, Street NE, Vitetta ES. Cancer dormancy: opportunities for new therapeutic approaches. Nat Med 1997;3:505–9.
(155) Rye PD, Norum L, Olsen DR, Garman-Vik S, Kaul S, Fodstad O. Brain metastasis model in athymic nude mice using a novel MUC1-secreting human breast-cancer cell line, MA11. Int J Cancer 1996;68:682–7.
(156) Putz E, Witter K, Offner S, Stosiek P, Zippelius A, Johnson J, et al. Phenotypic characteristics of cell lines derived from disseminated cancer cells in bone marrow of patients with solid epithelial tumors: establishment of working models for human micrometastases. Cancer Res 1999; 59:241–8.
(157) Connors TA. Is there a future for cancer chemotherapy? The Michel Clavel Lecture. Ann Oncol 1996;7:445–52.
(158) van Triest B, van Groeningen CJ, Pinedo HM. Current chemotherapeutic possibilities in the treatment of colorectal cancer. Eur J Cancer 1995;31A: 1193–7.
(159) Haller DG. An overview of adjuvant therapy for colorectal cancer. Eur J Cancer 1995;31A:1255–63.
(160) Monson J, Pazdur R. Adjuvant therapy for colorectal cancer. Inpharma 1996;Suppl 4:3–11.
(161) Schabel FM Jr. Rationale for adjuvant chemotherapy. Cancer 1977;39(6 Suppl):2875–82.
(162) Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 1975;256:495–7.
(163) Jain RK. Physiological barriers to delivery of monoclonal antibodies and other macromolecules in tumors. Cancer Res 1990;50(3 Suppl): 814s–819s.
(164) Ragnhammar P, Fagerberg J, Frodin JE, Hjelm AL, Lindemalm C, Magnusson I, et al. Effect of monoclonal antibody 17-1A and GM-CSF in patients with advanced colorectal carcinoma—long-lasting, complete remissions can be induced. Int J Cancer 1993;53:751–8.
(165) McNeil C. A new generation of monoclonal antibodies arrives at the clinic [news]. J Natl Cancer Inst 1995;87:1658–60.
(166) Vitetta ES, Thorpe PE, Uhr JW. Immunotoxins: magic bullets or misguided missiles? Immunol Today 1993;14:252–9.
(167) Pai LH, Wittes R, Setser A, Willingham MC, Pastan I. Treatment of advanced solid tumors with immunotoxin LMB-1: an antibody linked to *Pseudomonas exotoxin.* Nat Med 1996;2:350–3.
(168) Riethmuller G, Holz E, Schlimok G, Schmiegel W, Raab R, Hoffken K, et al. Monoclonal antibody therapy for resected Dukes' C colorectal can-

cer: seven-year outcome of a multicenter randomized trial. J Clin Oncol 1998;16:1788–94.
(169) Diel IJ, Solomayer EF, Costa SD, Gollan C, Goerner R, Wallwiener D, et al. Reduction in new metastases in breast cancer with adjuvant clodronate treatment. N Engl J Med 1998;339:357–63.
(170) Hermanek P. What's new in TNM? Path Res Pract 1994;190:97–102.
(171) Schutze K, Lahr G. Identification of expressed genes by laser-mediated manipulation of single cells. Nat Biotechnol 1998;16:737–42.
(172) Klein CA, Schmidt-Kittler O, Schardt JA, Pantel K, Speicher MR, Riethmuller G. Comparative genomic hybridization, loss of heterozygosity, and DNA sequence analysis of single cells. Proc Natl Acad Sci U S A 1999; 96:4494–9.
(173) Schlimok G, Pantel K, Loibner H, Fackler-Schwalbe I, Riethmuller G. Reduction of metastatic carcinoma cells in bone marrow by intravenously administered monoclonal antibody: towards a novel surrogate test to monitor adjuvant therapies of solid tumours. Eur J Cancer 1995;31A: 1799–803.
(174) Pantel K, Enzmann T, Kollermann J, Caprano J, Riethmuller G, Kollermann MW. Immunocytochemical monitoring of micrometastatic disease: reduction of prostate cancer cells in bone marrow by androgen deprivation. Int J Cancer 1997;71:521–5.
(175) Schlimok G, Funke I, Pantel K, Strobel F, Lindemann F, Witte J, et al. Micrometastatic tumour cells in bone marrow of patients with gastric cancer: methodological aspects of detection and prognostic significance. Eur J Cancer 1991;27:1461–5.
(176) Thorban S, Roder JD, Pantel K, Siewert JR. Epithelial tumour cells in bone marrow of patients with pancreatic carcinoma detected by immunocytochemical staining. Eur J Cancer 1996;32A:363–5.
(177) Pantel K, von Knebel Doeberitz M, Izbicki JR, Riethmuller G. Disseminated tumor cells: diagnosis, prognostic relevance, phenotyping and therapeutic strategies. Chirurg 1997;68:1241–50
(178) Brakenhoff RH, Stroomer J, De Bree R, Ten Brink CB, Weima S, Snow GB, et al. Sensitive detection of squamous cells in bone marrow and blood of head and neck cancer patients by E48 reverse transcriptase–polymerase chain reaction. Clin Cancer Res 1999;5:725–32.
(179) Braun S, Pantel K Prognostic significance of micrometastatic bone marrow involvement. Breast Cancer Res Treat 1998;52:201–16.
(180) Pantel K, Braun S, Passlick B, Schlimok G. Minimal residual epithelial cancer: diagnostic approaches and prognostic relevance. Prog Histochem Cytochem 1996;30:1–60.

NOTE

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