

National Academy of Clinical Biochemistry Guidelines for the Use of Tumor Markers in Malignant Melanoma

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Abbreviations: 5-SCD = 5-S-cyteinyl-dopa; 5,8DHI = 5-8-dihydroxyindole; 6H5MI2C = 5(6)-hydroxy-6(5)-methoxyindole-2-carboxylic acid; AGP = α 1-acid glycoprotein; AJCC = American Joint Commission on Cancer; bFGF = Basic fibroblast growth factor; CI = Confidence interval; CRP = C-reactive protein; CTL = Cytotoxic T lymphocyte; DFS = Disease free survival; DOPA = 3,4-dihydroxyphenylalanine; ECM = Extracellular matrix; ELISA; Enzyme linked immunosorbent assay; GM-CSF = Granulocyte macrophage colony stimulating factor; IC = Immune complex; IFN = Interferon; IHC = Immunohistochemical; IL = Interleukin; LASA = Lipid-associated sialic acid; LDH = Lactate dehydrogenase; MIA = Melanoma inhibiting activity; MITF = Microphthalmia transcription factor; MMP-2 = Metalloproteinase-2; NACB = National Academy of Clinical Biochemistry; NSE = Neuron specific enolase; OS = Overall survival; PET = Positron emission tomography; PKC = Protein kinase C; RT-PCR = Reverse transcription-polymerase chain reaction; SD = Standard deviation; TA90 = 90kD glycoprotein tumor-associated antigen; TIMP = Tissue inhibitor of metalloproteinase; VEGF = Vascular endothelial growth factor.

INTRODUCTION

Melanoma is the deadliest form of skin cancer. The worldwide incidence of melanoma in 2000 was 132,600 with 37,000 people dying of the disease (1). There has been a worldwide increase in this incidence over the last four decades (2), the incidence of melanoma in the United States increasing by more than 125% between 1973 and 1995 (3). According to the American Cancer Society, in 2006 approximately 62,190 people in the United States will be diagnosed with melanoma and 7,910 will die as a result of the disease (4). Melanoma is now the fifth most common cancer among men and the fourth most common cancer among women in the United States.

Unfortunately, melanoma tends to strike in the prime of life (35% in individuals between the ages of 35 and 54 years), with a median age of 56 years at diagnosis (3). The lifetime risk in the United States of developing melanoma has steadily increased from 1/1,500 in 1935 to 1/74 in 2000 to a projected 1/50 in 2005. An estimated 450,000 people in this country are living with a diagnosis of melanoma; according to the National Cancer Institute, the incidence of melanoma is increasing at a rate of 3-5% per year, faster than any other cancer (5).

Melanoma is a malignant tumor of melanocytes, cells that are derived from the neural crest. Although most melanomas arise in the skin, they may also arise from mucosal surfaces or at other sites to which neural crest cells migrate. More than half of primary melanomas occur in areas of the skin often exposed to the sun. Early signs suggesting malignant change include darker or variable discoloration, itching, an increase in size, or the development of small new patches of color (satellites) around a larger lesion. Ulceration and/or bleeding are later signs. Melanoma occurs more commonly on the extremities in women and on the trunk, head and neck in men (6). Although early-stage disease is curable by surgery, the prognosis associated with metastasis to distant sites is poor; and median survival is only 4 to 6 months. With no sensitive tools available to monitor therapy and follow-up (7), these statistics reflect the unpredictable pattern of recurrence of melanoma, as well as its resistance to treatment by radiation and chemotherapy (8).

Prognosis is affected by clinical and histological factors in addition to anatomic location of the lesion. Thickness and/or level of invasion of the melanoma, mitotic index, presence of tumor-infiltrating lymphocytes, number of regional lymph nodes involved, and ulceration or bleeding at the primary site all affect the prognosis. Patients who are younger, female, and who have melanomas on the extremities generally have a better prognosis (9-11). The rate of cure is 85-95% when melanoma is diagnosed before it spreads to lymph nodes or distant body sites. Since various factors affect progression of melanoma and there is a high cure rate for early

disease, efforts to develop tumor markers that can detect melanoma in early stages of development and recurrence are important.

Evaluating the efficacy of tools for monitoring melanoma requires an understanding of the American Joint Committee on Cancer (AJCC) staging system (12). This system stages melanoma with respect to the Breslow thickness or Clark level of invasion of the primary tumor (T), metastasis to regional in-transit sites or lymph nodes (N), and metastasis beyond the regional lymph nodal basin (M) (12). The most significant prognostic tool in the melanoma staging system remains the tumor status of the regional lymph nodes. Other prognostic factors include Breslow thickness, anatomic site of the primary, patient age, and number of organs involved. However, these factors have many shortcomings: even in patients with Stage I melanomas that should be readily cured by surgery alone, the failure rate is about 12% (10 year survival). This increases to 25-30% for patients with AJCC Stage II disease and to 85-90% for patients with AJCC Stage IV disease (13).

Clinical staging is based on whether the tumor has spread to regional lymph nodes or distant sites. For disease clinically confined to the primary site, the greater the thickness and depth of local invasion of the melanoma, the higher the chance of lymph node or systemic metastases and the worse the prognosis. Melanoma can spread by local extension through the lymphatic system and/or by hematogenous routes to distant sites. Any organ may be involved, but metastases to lung and liver are most common. The risk of relapse decreases substantially over time, although late relapses are not uncommon in melanoma (14, 15).

It has been suggested that the inadequacy of current prognostic tools, especially in patients with clinically node-negative melanoma, is due to initial under staging of disease through failure to identify occult regional metastasis. To improve the sensitivity and accuracy of lymph node evaluation, Morton and his associates (16) developed a selective biopsy technique called sentinel lymphadenectomy. This minimally invasive technique begins with injection of a tracer (vital dye and/or radioisotope) in the periphery of a tumor. The tracer signal is followed along the lymphatics that drain the primary melanoma until they reach the first (sentinel) lymph node. Because this node is the most likely site of regional metastasis, it is then excised and examined for tumor cells. The sentinel node specimen is much smaller than a conventional lymphadenectomy specimen and can be quickly and cost-effectively examined with highly sensitive immunohistochemical and molecular techniques.

A changing mole is the most common symptom of melanoma. At the time of diagnosis of melanoma, variations in Asymmetry of Borders, Color and /or increase in Diameter or height of a pigmented lesion (the so-called ABCD criteria) are noted in more than 80% of patients (17).

The main presenting feature is a new or suspicious mole and the diagnosis is established by biopsy. Depending upon size and location, all or part of the mole may be biopsied for pathologic evaluation. No currently available blood-based biomarkers are sufficiently sensitive or accurate to replace biopsy diagnosis of melanoma.

Surgery is the primary mode of treatment for localized cutaneous melanoma; the intact tumor or biopsy site being removed together with a surrounding margin of normal-appearing skin and underlying subcutaneous tissue. More extensive surgical resection may be undertaken for patients with regional or distant metastatic disease (18). Post-operatively, these patients often receive immunotherapy, biotherapy, radiation therapy, or chemotherapy (19). However, primary melanoma that has not spread to other parts of the body can be cured solely by surgery all disease is excised (185). There is an urgent requirement for a biologic serum marker that could indicate whether a primary melanoma has metastasized to regional or distant sites, even in the presence of tumor-free sentinel nodes. Identification of such a marker post-surgically and/or during follow-up would indicate the presence of micrometastases and the need for more aggressive treatment

CURRENTLY AVAILABLE MARKERS FOR MELANOMA

Melanoma is a highly metabolically active tumor that expresses and releases enzymes, cytokines and other molecules into the circulation. Although a number of proteins and other factors have been proposed as blood/tissue markers for melanoma metastasis (Table 1), only a few have been rigorously investigated in retrospective and/or prospective studies (Table 2). This guideline identifies serum-based, cell-based and tissue-based melanoma markers of varying promise and examines the evidence for their proposed role in diagnosis, prognosis and/or management of melanoma.

TUMOR MARKERS IN MELANOMA: NACB RECOMMENDATIONS

Table 3 summarizes the National Academy of Clinical Biochemistry (NACB) guidelines for the use of tumor markers in melanoma. With the exception of lactate dehydrogenase (LDH) there are no published guidelines for melanoma tumor markers with which these recommendations can be compared. Below, we present a more detailed discussion of some of the markers listed in Tables 1-3.

TUMOR-ASSOCIATED GLYCOPROTEIN ANTIGEN (TA90)

A variety of tumor-associated antigens particularly common in melanoma have been identified and characterized using human antibodies. Since there are antibodies in cancer patient sera which specifically react with molecules expressed by tumor cells, it is likely that detection of circulating tumor-associated antigen specific immune complexes (the immune reaction product of immunogenic tumor-associated antigen and the antibody induced response to these antigens in the tumor bearing host) represents the most specific and sensitive approach for immunodiagnosis of human cancer. This is logical because development of such immune complexes depends upon the presence of an obvious or occult antigen source and the host immune system's ability to recognize the tumor antigen, which is truly immunogenic in man. This is particularly true with disease in the early stages of development or recurrence. During early stage disease, tumor-associated antigens would be expected to be in the form of immune complexes and (except in immunocompromised patients) their detection as free antigen by immunochemical methods would be unlikely to be feasible.

One such tumor-associated antigen is TA90, a 90-kD glycoprotein found in the serum or urine of 63% and 68% of melanoma patients, respectively (20, 22). An endogenous humoral (IgG) immune response against TA90 has been demonstrated in melanoma patients (21, 23). Because TA90 binds to endogenous anti-TA90 IgG antibody, a monoclonal antibody based enzyme-linked immunosorbent assay (ELISA) has been developed to detect specifically the TA90 immune complex (TA90-IC) in the serum of patients with melanoma (24) or other solid neoplasms (25, 26). TA90-IC status has been significantly correlated with recurrence of disease after complete surgical resection of American Joint Committee on Cancer (AJCC) Stage I/II, Stage III or Stage IV melanoma (29 - 31).

Since standard prognostic factors, including precise staging of the regional lymph nodes, cannot accurately determine which early-stage melanomas will metastasize, Chung et al (27) undertook studies to determine if TA90-IC would have any correlation with recurrence and survival in patients with Stage II thick primary melanomas. They reported that the sensitivity and specificity of the TA90-IC for predicting recurrence in these patients were 70% and 85%, respectively. Five-year disease-free survival (DFS) and overall survival (OS) rates were significantly higher for the TA90-IC-negative group than for the positive group. At a median follow-up of 25 months, multivariate analysis identified postoperative TA90-IC status and sex as significant predictors of DFS. TA90-IC status was the only independent prognostic factor in multivariate analysis. TA90-IC status after resection of thick primary melanoma was also highly

predictive of outcome (27). A positive post-operative TA90-IC level might therefore influence decisions about adjuvant therapy, regardless of regional nodal status.

Because TA90-IC status showed prognostic significance in patients with thick primary melanoma, it was also examined in those with thin primaries. Patients with primary melanomas 1.01 to 2.00 mm thick and with tumor-negative regional lymph nodes were categorized into two groups (28). Group 1 was comprised of 50 patients who died of metastases within 7 years of complete surgical treatment; Group 2 was comprised of 50 patients who were matched with Group 1 for six standard prognostic factors, but who lived for at least 10 years without recurrence. Post-operative sera from these patients were analyzed for TA90-IC. All patients in both groups underwent staging of the regional lymph nodes by complete lymph node dissection or (more recently) by lymphatic mapping and selective dissection of the sentinel lymph node. Standard microscopic examination determined the status of all excised lymph nodes. Excluded from study were those patients whose Stage I melanoma had spread to the regional nodes (Stage III disease) either at the time of lymphadenectomy or at any time before the development of distant metastases. This eliminated the possibility of studying patients with occult lymph node metastases or locoregional relapse. In addition this study excluded any patient who received any form of post-operative adjuvant therapy; thus avoiding the possible influence of such therapy on clinical outcome. Median thickness \pm SD at 95% CI of the primary melanoma was 1.40 ± 0.31 mm and 1.42 ± 0.32 mm in groups 1 and 2, respectively; median Clark's level of invasion was III in both groups, and 26 patients in each group had ulcerated primaries. Median \pm SD at 95% CI of TA90-IC level and incidence of positivity (optical density greater than 0.410) were respectively 0.557 ± 0.43 and 82% in group 1 and 0.305 ± 0.15 and 18% in Group 2. These values were statistically significantly different (t test, $p < 0.001$), and demonstrated that positive TA90-IC level correlated with distant metastasis in melanoma that was low to intermediate risk by standard prognostic factors (28).

Kelley et al (29) evaluated the efficacy of TA90-IC for the preoperative detection of subclinical metastasis in patients undergoing surgical treatment of clinically localized melanoma. Fifty-six patients had pathology-positive nodes or subsequently developed recurrence, 43 (77%) of these being positive for TA90-IC preoperatively. In contrast, only 14 of 58 patients with pathology-negative nodes who subsequently did not develop recurrence were positive for TA90-IC. The sensitivity and specificity of TA90-IC ELISA for the detection of occult metastasis were therefore 77% and 76% respectively.

A subsequent study examined the prognostic significance of post-operative TA90-IC levels in 166 patients with AJCC Stage I, II or III melanoma (30). Of the 78 patients who

experienced recurrence, 54 (77%) had positive TA90-IC values at a mean of 19 months before recurrence. TA90-IC status was an independent prognostic marker for both disease-free survival (DFS) and overall survival (OS). TA90-IC therefore appears to be the first tumor marker that accurately predicts subclinical metastatic disease and survival for patients with early stage melanoma. For this reason, it has the potential to dramatically improve the prognostic evaluation of patients with this disease.

TA90-IC is also the first serum marker shown to have importance in predicting the survival of Stage IV melanoma patients receiving adjuvant immunotherapy after complete resection of distant metastases. When TA90-IC ELISA was used to evaluate the therapeutic efficiency of post-operative adjuvant immunotherapy with a polyvalent vaccine, post-operative TA90-IC levels were strongly correlated with survival (31). Median DFS and 5-year rate of OS were 7 versus 4 months, respectively, and 49% versus 27%, respectively, in patients with negative versus positive levels of TA90-IC. In another study of patients with advanced melanoma, the TA90-IC assay was combined with positron emission tomography (PET) (32). Patients in whom both tests were positive had a higher incidence of recurrence within six months of testing than those with negative test results (32).

S100 PROTEIN

S100 is a 21kDa acidic calcium-binding protein originally isolated from bovine brain (33). It is composed of isomeric alpha and beta subunits. S100 is believed to be involved in cell cycle progression and differentiation (34). Although this protein was initially reported as a marker of human melanoma (35, 36), it is also expressed in glioma, Schwannoma and neuroblastoma (37).

S100 immunohistochemical staining is the method of choice to diagnose malignant melanoma in pathological sections (38). With the availability of two assays to detect serum S100 levels, several studies have been carried out to establish the prognostic significance of circulating levels of this protein. In the four largest studies S100 levels were found to be elevated in 4-9% of patients with Stage I, 8-19% of patients with Stage III and 48-89% of patients with Stage IV disease (39 - 42), suggesting a positive correlation with stage of disease (43 - 44).

S100 levels have also been correlated with the number of affected organs, presence of liver or bone metastasis, and response to chemotherapy or immunotherapy. In a study of 64 Stage IV patients, 78% of non-responders demonstrated elevated S100 levels at 4 weeks during therapy and 84% had elevations by the end of therapy (45). For responders, 95%

demonstrated a stable or decreased S100 expression at 4 weeks and 98% by the end of treatment. Elevated S100 levels in peripheral blood have been correlated with poor overall survival (46–48). Rising levels of S100b were specific and sensitive for tumor progression (49). While these results are promising, further longitudinal studies are needed to assess the utility of this tumor marker as a surveillance adjunct.

MELANOMA INHIBITING ACTIVITY (MIA)

Melanoma inhibiting activity (MIA) is an autocrine tumor cell growth factor (50). MIA purified from culture supernatant of melanoma cells has been shown to regulate the growth of melanoma cells (51). MIA is an 11kDa, soluble protein of 131 amino acids, with a gene locus at 19q13.32 (52, 53), and is secreted by melanoma cells and chondrocytes. Addition of MIA to cell culture is rapidly followed by clustering of melanoma cells and induction of melanoma associated genes as well as inhibition of apoptosis due to anoikis, which suggests that this protein may be involved in tumor cell metastasis and/or invasion (54-56). ELISA studies have detected a stage-related increase in MIA expression in the serum of patients with melanoma (57, 58). MIA levels normalize after resection of disease, and several investigators have reported a correlation between decreased MIA levels and stabilization or regression of disease (59, 60). Faries et al (61) recently reported a correlation between MIA level and survival of patients receiving therapeutic cancer vaccine after complete resection of AJCC Stage III melanoma.

LACTATE DEHYDROGENASE (LDH)

Lactate dehydrogenase (LDH) is found throughout the body, so nearly every type of cancer as well as many other diseases can be accompanied by elevated serum LDH levels. The marker is therefore unsuitable for use in screening or diagnosis; but can be helpful in monitoring the course of melanoma. Persistent or recurrent elevations of LDH after treatment usually indicate residual or recurrent disease. In 121 Stage II and 58 Stage III patients, Finck et al (62) reported that high levels of LDH indicated recurrence with a sensitivity and specificity of 72% and 97%, respectively. As an indicator of liver metastasis, LDH had a sensitivity and specificity of 95% and 82%, respectively, in Stage II melanoma, and 86% and 57%, respectively, in a Stage III autopsied melanoma group. An elevated LDH was the first indication of recurrent disease in 11/88 (12.5%) Stage II patients, and was almost as frequent an indicator of recurrent disease as pulmonary metastases found on chest X-ray. The mean survival following LDH

elevation was 5.9 months. It was concluded that monitoring of serum LDH can provide useful information in the postoperative follow-up of patients with melanoma.

Since then several reports have documented an association between serum levels of LDH and prognosis in patients with Stage IV melanoma (63, 64). When LDH was compared in multivariate analysis to several other tumor markers, including S100 and MIA, it was consistently the most predictive factor for a poor outcome (63). This and several other reports have led the AJCC to propose a subtype of distant metastatic disease based on an elevated LDH serum level (12). However the prognostic value of LDH in patients with Stage I-III melanoma is limited because most patients with early-stage melanoma have normal LDH levels.

MELANIN-RELATED METABOLITES

Skin pigmentation is determined by the rate of melanin synthesis in melanocytes and the rate and extent of its dispersal to epidermal keratinocytes. In malignant disease the synthesis of melanin and its precursors in the metabolic pathway is increased. During melanin synthesis, tyrosinase converts tyrosine to DOPA (3,4-dihydroxyphenylalanine) and DOPAquinone. DOPAquinone is then converted to 5-S-cysteinyldopa (5SCD) and pheomelanin, or to 5-6-dihydroxyindole (5,6DHI), which in turn is converted to 5(6)-hydroxy-6(5)-methoxyindole-2-carboxylic acid (6H5MI2C) and ultimately to eumelanin. Several investigators have investigated these melanin precursors as tumor markers (65, 66).

5SCD is a very specific marker of melanin metabolism that can be detected in urine or serum (67). Recent reports examining the utility of urine 5SCD determinations have focused primarily on advanced melanomas because earlier studies noted that less than 2% of Stage I-III melanomas were associated with elevated 5SCD urine excretion in excess of 0.4 mg per 24 hr. In contrast, 60% of IV patients had high urinary levels (67).

In simultaneous collections of urine and serum, 5SCD levels assayed by high-performance liquid chromatography were elevated earlier in serum and better-reflected melanoma recurrence or progression (68). Other investigators have demonstrated that plasma 5SCD levels were elevated on average two-fold, four-fold and 450-fold as disease progressed from Stage I/II to III to IV (69). Another study confirmed that plasma 5SCD levels of 252 patients were significantly different for clinical Stage I vs. III disease and for clinical Stage II vs. III disease (70). However, these differences carried no prognostic significance. The value of 5SCD in monitoring the response to immunochemotherapy was reviewed in a series of 11 patients (71). In this study, decreased 5SCD concentrations during therapy with interleukin-2 (IL-2), interferon (IFN)-alpha and dacarbazine were correlated with significantly longer survival.

Furthermore, 5SCD levels differentiated responders from non-responders. Thus, measurement of 5-SCD in serum was considered useful for monitoring the clinical course of melanoma patients, for discriminating between responders and non-responders to immunochemotherapy, and as a prognostic factor of survival time and death risk. However, further study with a large patient cohort is necessary to determine the role of melanin-related metabolites.

ANGIOGENESIS FACTORS

Vascularity is a requirement for tumor growth. Several studies have demonstrated that micro vessel density increases with the thickness of a primary melanoma, and that increased density correlates with decreased disease free survival (DFS) (72, 73). Vascular endothelial growth factor (VEGF) is a multifunctional homodimeric molecule. Serum ELISA revealed elevated VEGF in all patients with melanoma (74), and higher levels were observed in advanced disease (75). However, immunohistochemical evaluation of biopsy specimens revealed VEGF expression in only 42% of primary melanomas and in no benign lesions (76). In a separate study, 11 of 19 primary lesions and 15 of 20 metastatic lesions demonstrated immunohistochemical staining for VEGF (77).

Basic fibroblast growth factor (bFGF) is a heparin-binding polypeptide that is expressed by many cell types but not by melanocytes. In human skin reconstructs, bFGF is critical for transducing melanoma cells from an *in situ* phase to a vertical growth phase and thereby conferring tumorigenicity *in vivo* (78). Birck et al (76) used immunohistochemistry (IHC) to compare expression of bFGF and VEGF in paired primary and metastatic melanomas from the same patients; bFGF was expressed earlier and more often than VEGF. Although bFGF expression in melanoma tissue did not correlate with recurrence (77), elevated serum levels were correlated with advanced disease and shorter DFS and overall survival (OS).

ADHESION MOLECULES

Adhesion molecules are cell-surface proteins that mediate the recognition and adhesion of cells to a substrate and to other cells. Cell adhesion molecules belonging to the integrin, cadherin and immunoglobulin superfamilies have been implicated in progression of cutaneous melanoma.

Immunoglobulin superfamily

CD44, also known as Pgp-1 or HCAM, is a membrane glycoprotein that has a cellular receptor for hyaluronic acid. It is expressed on leukocytes and erythrocytes and enables lymphocytes to adhere to endothelial cells, so is involved in cell-cell and cell-substrate

interactions. The expression of this glycoprotein on tumor cells could potentially influence leukocyte adhesion and enhance or compromise immune surveillance. Alternatively, adherence of tumor cells to endothelial venules could encourage these cells to transmigrate and invade the microvasculature or lymphatics. CD44 exists as a standard form (CD44std) and as 10 isoforms (v1-v10) that are the result of several alternative splicings of mRNA (79). In 292 melanoma patients with clinical Stage I disease, CD44 IHC expression was significantly reduced. Reduction in CD44 expression independently predicted shorter DFS and OS (80). In a separate study of 52 melanoma patients, serum levels of CD44 were significantly decreased (81). In contrast, Dietrich et al (82) suggested that high CD44 expression significantly reduced survival. Schaidler et al (83) reported that the circulating forms of CD44std, v5, v6 and v10 were not significantly elevated in melanoma patients, although a few patients did have higher levels of circulating CD44std. The value of CD44 as a diagnostic or prognostic marker clearly needs further investigation, with special emphasis on standard vs. isoform measurements, particularly CD44v10 isoform.

Melanoma cell adhesion molecule (MCAM) is an integral membrane glycoprotein with Ca^{2+} -independent cell adhesion properties. This protein demonstrates heterophilic cell-cell interactions that result in dynamic actin-cytoskeleton rearrangements (84). Cytoskeleton rearrangements can facilitate cell detachment and migration, which are key functions in metastasis and invasion. MCAM has been mapped to chromosome 11, band q23.3 (85). Its expression depends on cAMP response element-binding protein (CREB). MCAM expression has been demonstrated by IHC in melanoma, gestational trophoblastic lesions and endothelial cells (86). MCAM is highly expressed in most melanoma cells but not in normal melanocytes (85-87). It remains a promising marker in melanoma (88), but future studies must focus on multimarker comparisons and clinical outcome correlation.

ICAM-1 (CD54) adhesion molecule is found on the surface of antigen-presenting cells (APCs), T cells and vascular endothelial cells, and its expression is a marker of poor outcome of melanoma. ICAM-1 expression plays an important role in neutrophil adhesion and transendothelial migration, the significance of which is demonstrated in ischemia-reperfusion pathology and other promoters of inflammation. ICAM-1 may be membrane-associated (mICAM-1) and/or soluble (sICAM-1) (89). mICAM-1 has been detected by IHC in 69% of primary melanomas and 89% of metastatic lesions and was associated with a shorter DFS (90). sICAM-1 has been detected in the serum of 52 individuals with primary melanoma, but serum concentrations were not significantly different from controls (81). In advanced metastatic

disease, however, pre-treatment elevation of sICAM-1 was significantly higher in patients with bone or liver metastasis and was a prognostic indicator (91, 92).

Integrin superfamily

Integrins mediate a diverse array of functions in APCs, are involved in lymphocyte migration, and modulate adherence of cells to each other and to the surrounding extracellular matrix, often through linkages to fibronectin or vitronectin. These transmembrane adhesion molecules form heterodimers composed of an alpha and a beta chain.

In vitro ligation of alphavbeta3 (a vitronectin receptor) increases protein kinase C (PKC) activity, with subsequent increased expression of urokinase plasminogen activator receptor mRNA and plasmin. The result is increased cell invasiveness (93). Other groups have noted that ligation of this integrin up-regulates the expression of matrix metalloproteinase 2 (MMP-2) and increases tumor invasiveness (94). Additional reports have attributed angiogenic properties to alphavbeta3, which may potentiate invasiveness (95, 96). IHC studies show that alphavbeta3 expression is restricted to melanoma cells but differs in extent among different histological types of melanomas (97). Thus, acral and superficial melanoma demonstrated only 50% staining whereas lymph nodes and cutaneous metastases stained 60% and 80%, respectively (98). This could be due to the fact that alphavbeta3 is first expressed when melanoma changes from a radial to vertical growth phase, its appearance perhaps heralding progression to an invasive phenotype. Several investigators have demonstrated a significant relationship between the expression of alphavbeta3 in non-acral lesions and tumor thickness, recurrence and outcome (99). Their findings suggest that this integrin may have significant prognostic potential in thicker and metastatic melanomas.

An IHC study demonstrated expression of beta1 and beta3 in 52% and 64%, respectively, of 111 intermediate-thickness melanomas (100). Beta3 expression was associated with a greater mortality (100). In another study, the expression of beta3 in 130 samples increased with tumor thickness, but its strong expression by Spitz nevi limited its diagnostic potential (101). Beta1 expression was examined in 38 metastatic melanomas from 27 patients; the 15 patients with beta1-positive tumors had significantly longer DFS and OS (102).

Cadherins are cell-surface glycoproteins that promote calcium-dependent cell-cell adhesion, and are expressed in a regulated manner (103). The major adhesion mediator between keratinocytes and normal melanocytes is E-cadherin (104). E-cadherin is found on normal melanocytes but not on nevi or melanoma cells (105). Melanoma cells express N-

cadherin rather than E-cadherin, thus allowing them to change their cellular patterns (103). However, no studies have been undertaken to utilize this molecule as a tumor marker.

CYTOKINES AND THEIR RECEPTORS

Melanoma expresses multiple cytokines that have been detected at protein and/or mRNA levels. The RT-PCR technique has identified transcripts of interleukin (IL)-1 α , IL-1 β , IL-6, IL-8, and IL-10 in cultured human melanoma cell line (106–108). The RT-PCR technique has also been used to detect expression of bFGF, IL-1 α , IL-1 β , IL-6, IL-8 and granulocyte macrophage stimulating factor (GM-CSF) in fresh biopsy specimens of primary melanoma and melanoma metastases (109). However, the complex interaction of melanoma lesions with endogenous lymphocytes that also synthesize many of these cytokines makes the RT-PCR approach difficult for analysis of serum specimens. Thus far, only serum IL-2 receptor, IL-6, IL-8 and IL-10 have been studied as tumor markers.

IL-2 has been used in the treatment of melanoma for many years and is thought to act by stimulating cytotoxic T lymphocytes (CTLs, CD8+ T-lymphocytes that are antigen-specific). Soluble IL-2 receptor (sIL-2R) is found in high levels in patients with metastatic disease and has been shown to interfere with IL-2 therapy: as many as 80% of patients with metastatic disease high levels of sIL-2R are non-responders (92, 110). In patients undergoing IL-2 treatment, elevated sIL-2 receptor expression correlated with shorter median survival and was found to be statistically significantly associated with disease progression and tumor load (92, 111).

IL-6 is elevated in Stage IV disease. In patients undergoing cisplatin/IL-2/IFN-alpha therapy, IL-6 serum levels were significantly elevated pretreatment and in non-responders, suggesting a potential mechanism for resistance to biochemotherapy (112). Endogenous IL-6 may therefore provide valuable information for monitoring the response to biotherapy in patients with metastatic malignant melanoma. The mechanism may be related to the intracellular expression of IL-6 and its receptor (IL-6R). One study of tumor cells obtained by fine-needle aspiration of lymph nodes and palpable metastatic lesions in patients with Stage IV melanoma reported a correlation between non-response to biochemotherapy and decreased intracellular IL-6 and IL-6R expression in the presence of elevated IL-6 serum levels (113). Serum IL-6 level has also been directly correlated with tumor burden in melanoma (114).

Relatively few studies have been reported on IL-8 and IL-10 in melanoma. Serum IL-8 level was found to be elevated in 21 of 56 (37.5%) metastatic melanoma patients. Elevated values were observed more frequently (62% of individuals) when tumor burden exceeded 250 cm³ than when it did not (17% of individuals) (115). The involvement of IL-10 in tumor growth

and survival is unclear. Although IL-10 has both immunosuppressive and anti-inflammatory functions, decreased serum levels of IL-10 have been associated with tumor growth (116) and poor survival of melanoma patients (117).

CELL GROWTH FACTORS

One of the initiating events in melanoma progression is dysregulated cell proliferation, which begins when the melanocyte precursor loses control over the cell cycle. Transcription factors, tumor suppressor genes, and a variety of proteins mediate the control of melanoma cell growth.

AP-2 is a 52-kDa DNA-binding transcription factor that controls gene expression in epidermal cell lineages (118). The prognostic value of IHC-detected expression of AP-2 was addressed in a study of 369 patients with clinical Stage I melanoma (119). In this group of patients, loss of AP-2 expression was predictive of decreased DFS and OS. It was also associated with reduced p21 expression and with greater Breslow thickness and Clark level.

Microphthalmia transcription factor (MITF) is a melanocyte-specific basic helix-loop-helix nuclear protein critical for melanocyte viability, maturation and regulation of melanin synthesis. MITF has demonstrated a significant pattern of positive staining in melanoma. In one study, all 76 melanoma specimens and none of 60 non-melanoma specimens were positive for MITF (120). Another study demonstrated 82% nuclear staining of intermediate-thickness cutaneous melanomas: loss of MITF expression correlated with decreased DFS and OS (121). In 266 melanoma patients, 88% had tumors that stained positive for MITF, a rate that equaled or surpassed the rate of staining for S100 or HMB45 (122). However, the specificity was poor compared to other markers such as MART-1 and MAGE-1, especially using the D5 antibody, which is considered to be superior to other antibodies to detect MITF (123).

nm23 is a metastasis suppressor gene. Originally, expression of *nm23* was detected in human melanoma cell lines that were transplanted into mice; increased *nm23* mRNA expression correlated with decreased metastatic potential (124). In an IHC study of 157 clinical melanoma specimens, strong *nm23* positivity correlated with improved survival (125). In a smaller study, *nm23* expression was lowest in the thickest melanomas and in melanomas that had metastasized to regional lymph nodes. However, *nm23* expression was not correlated with organ metastasis or subsequent 5-year survival (126).

C-myc is a nuclear oncoprotein that is over-expressed in colon and prostate cancers. One study reported c-myc expression in 96% of 48 melanoma specimens, and noted that this marker had a stronger prognostic correlation than other clinicopathological parameters, including nodal

positivity (127). However, another study of 40 patients with melanomas thicker than 1 mm demonstrated only a 47% rate of c-myc positivity and no correlation with survival (128). Over-expression was most significant in vertical-growth melanomas and in metastatic tumors (129, 130). The greatest overall expression was observed in acral lentigo melanoma, and among this variant, elevated c-myc expression predicted shorter DFS and OS (131).

Ki-67 (MIB-1 antibody) is a non-histone DNA-binding nuclear protein (132). As a diagnostic marker, Ki-67 staining using MIB-1 antibody could distinguish Spitz nevi and other benign skin lesions from melanoma (133). Because Ki-67 expression increases during a cell's transition from radial to vertical growth (134), this marker also has prognostic potential. IHC staining with MIB-1 demonstrated a progressive increase in Ki-67 expression from benign tumors to primary melanomas to metastatic lesions. The MIB-1 score of primary melanomas correlated significantly with tumor thickness and Clark level of invasion. Another study of 55 fresh samples from primary melanomas < 1.5 mm in thickness reported a significant correlation between Ki-67 positivity and tumor thickness, S phase fraction and metastasis (135). In 60 patients undergoing chemoimmunotherapy for metastatic melanoma, low Ki-67 expression was significantly associated with decreased blood vessel density (136). Because of the importance of angiogenesis in tumor growth and invasiveness, it is not surprising that low Ki-67 expression prior to treatment was an independent prognostic factor for longer DFS and OS.

IMMUNOREGULATORY MOLECULES

The best-studied immunoregulatory molecules are two melanocyte differentiation antigens, i.e. tyrosinase and melanoma antigen recognized by T cells, MART-1/melan-A (137, 138).

Expression of tyrosinase, an important enzyme in melanin synthesis, was assessed by IHC using the T311 antityrosinase monoclonal antibody. T311 was strongly reactive in 84% of paraffin-embedded metastatic melanomas, but poorly reactive in desmoplastic or spindle cell melanomas (139). In a multimarker comparison of T311, S100, HMB45 (anti-gp100) and A103 (anti-melan-A), S100 was the most sensitive and HMB45 the most specific marker (140). Although T311 did not demonstrate an advantage over A103, T311 was considered to be a reliable marker of melanocytes.

MART-1 (melan-A) is a protein of 118 amino acids and its expression is limited to melanocytes of the skin and retina. One report demonstrated IHC detection of MART-1 in 90% of primary melanomas with loss of expression increasing with Breslow thickness (141).

A significantly reduced disease-free interval and overall survival rate was observed for patients not expressing this antigen. The poor prognosis of such patients was even worse for those presenting with a primary melanoma and a Breslow thickness of ≥ 1 mm (141). Similar results were noted by de Vries et al (142), who used IHC to compare expression of MART-1, gp100, S100 and tyrosinase in 80 paraffin-embedded primary melanomas and in locoregional, lymph node and visceral metastases. Staining was negatively correlated with Clark level, and nodal metastases demonstrated less staining than primary tumors from the same patients. S100 was the most sensitive marker, and there was no significant difference among the remaining three markers. Because IHC staining for MART-1 was unable to distinguish between Spitz nevi, melanoma cells and melanocytes, this marker has limited potential to differentiate benign disease from malignant melanoma (143).

EXTRACELLULAR MATRIX-DEGRADING ENZYMES AND CATHEPSINS

A tumor cell's ability to invade and metastasize requires invasion of the basement membrane, degradation of local connective tissue, and subsequent migration into the surrounding stroma, vessels and lymphatics. The matrix is degraded by several proteinases. The two most common families of proteinases found in melanoma and other tumors are MMPs and cathepsins (144, 145).

The 26 human MMPs identified thus far have been classified into four subgroups based on their extracellular matrix substrate (ECM): interstitial collagenases, gelatinases, stromelysins and membrane-type (MT-MMP) (144). Most investigations have focused on gelatinase A (MMP-2) and gelatinase B (MMP-9), which act on type IV collagen in basement membranes. Tissue inhibitors of metalloproteinases (TIMP) act in concert with MMPs to modulate ECM degradation (146, 147). Although considered important in the late phase of invasion and metastasis, gelatinase B is reportedly expressed more strongly in melanomas < 1.6 mm (148). In one IHC study, 64% of primary paraffin-embedded melanomas stained positive for MMP-2, and over-expression of this marker (defined as $> 34\%$ positive cells) correlated with poorer 5-year survival (149).

Aspartic protease cathepsin D and cysteine proteases cathepsins B, H and L have been identified in melanoma tissues (150). In a study of 43 patients with metastatic melanoma, levels of cathepsin B above vs below the median of 4.8 $\mu\text{g/L}$ and levels of cathepsin H above vs below the median of 13.7 $\mu\text{g/L}$ correlated significantly with survival but there was no correlation for cathepsin L (151). In the same study, patients with metastatic melanoma had significantly higher

serum cathepsin B and cathepsin H levels than did those with non-metastatic melanoma and healthy volunteers. A study of cathepsin D failed to link serum levels to survival (152).

NEURON-SPECIFIC ENOLASE (NSE)

Enolase catalyzes the interconversion of 2-phosphoglycerate and phosphoenolpyruvate in the glycolytic pathway, and participates in the formation of a high-energy phosphate bond. It exists as several dimeric isoenzymes, of which the $\alpha\gamma$ and $\gamma\gamma$ dimers are known as neuron specific enolase (NSE). The name was derived from the initial observation that NSE is expressed in neurons, neuroendocrine cells and neoplasms derived from these cells. In melanoma, 10-13% of AJCC Stage III patients and 35-48% of Stage IV patients have NSE levels $>10-15 \mu\text{g/l}$ (153-155). An elevated NSE level was linked with poor prognosis in one study of 63 Stage III and IV melanoma patients (155), but a study of 282 melanoma patients did not demonstrate a correlation between NSE level and survival (184). When compared with S100, NSE has a lower diagnostic sensitivity for Stage III/IV disease than Stage I/II (156). The low sensitivity of NSE in Stage IV melanoma limits its utility for surveillance of high-risk patients.

LIPID-ASSOCIATED SIALIC ACID (LASA)

Lipid-Associated Sialic Acid (LASA) is derived from the shed lipids of cell membranes. It is present in normal cells but found at higher concentrations in tumor cells (157). Increased levels of LASA have been reported in various histologic types of cancers (158), and several methods of LASA measurements have been reported (159-160). Most of these methods measure both lipid- and glycoprotein-bound sialic acid, which includes a significant amount of an acute-phase protein, α 1-acid glycoprotein (AGP) (160). Thus, LASA levels may be elevated by an increased concentration of acute-phase proteins produced by the liver as a result of an inflammatory reaction, and not by an increased concentration of gangliosides from malignant cells. Therefore, while LASA results correlated with tumor burden in melanoma-bearing animals (161), the serum LASA level reportedly has no significant correlation with survival (157). Meaningful measurements of sialic acid await a more sensitive and specific assay technique.

C-REACTIVE PROTEIN (CRP)

C-reactive protein (CRP) is a nonspecific but sensitive marker of inflammation. IL-6, IL-1, and tumor necrosis factor alpha induce the synthesis of CRP in hepatocytes. Increased CRP level is an important risk factor for cancer recurrence and has prognostic significance in patients with multiple myeloma, melanoma, lymphoma, and ovarian, renal, pancreatic, and

gastrointestinal tumors (162). Deichmann et al (163) compared serum LDH and CRP levels in 91 consecutive melanoma patients whose disease progressed to Stage IV and 125 patients whose disease did not progress beyond Stages I, II or III. CRP but not LDH was significantly elevated in patients progressing to Stage IV. In an earlier study, Deichmann et al (164) used serum values of CRP to discriminate progressive from non-progressive disease in 74 patients with Stage IV malignant melanoma. CRP levels were significantly elevated in progressive disease, with a sensitivity and specificity of 86% and 76%, respectively. However, LDH had the highest specificity (94%). The authors concluded that LDH and CRP are useful serum markers for monitoring metastatic malignant melanoma. Tartour et al (165) determined the effect of IL-2 therapy on IL-6 and CRP levels in sera from metastatic melanoma patients. Elevated IL-6 (> 20 pg ml⁻¹) and/or CRP (> 10 mg l⁻¹) levels were associated with resistance to IL-2 therapy. A correlation between high serum IL-6 levels and a shorter median survival was also observed (165).

SENTINEL NODE AND TUMOR MARKERS

The tumor status of the regional lymph nodes remains the most significant prognostic marker in melanoma. Since the introduction of sentinel lymphadenectomy for melanoma by Morton et al (16) and its subsequent application for other solid neoplasms, it has become apparent that nodal metastasis ranges from single cells to micrometastatic to macrometastatic deposits. Sentinel lymphadenectomy allows the pathologist to focus on the regional lymph node(s) that is/are most likely to harbor any tumor cells that have spread from the primary lesion.

Wang et al (166) presented some of the earliest data on RT-PCR detection of tyrosinase in the lymph nodes of melanoma patients. In their study of 29 nodes, 11 were histologically positive whereas 19 were positive by RT-PCR. Another report noted a 21% (87/417) rate of positive findings by tyrosinase RT-PCR (167). In one study, 47 of 91 pathologically negative nodes were positive by RT-PCR for tyrosinase (168), and the rate of recurrence was statistically different according to histological/RT-PCR status: +/+ 61%, -/+ 13% and -/- 2%. Similarly, Blaheta et al (169) demonstrated recurrence rates of 67% for histopathologically positive nodes, 25% for RT-PCR positive nodes, and 6% for nodes negative by both techniques. However, the accuracy of single-marker RT-PCR is decreased by the heterogeneity of marker expression in melanoma cells (170).

Bostick et al (171) therefore described a multimarker RT-PCR assay based on MAGE-A3, MART-1, and tyrosinase. They found that no single mRNA marker was expressed by all of the

93 sentinel lymph nodes that were considered to be positive by multimarker RT-PCR. However, histologically positive sentinel nodes more often expressed at least two mRNA markers (94% patients) compared with histologically negative sentinel nodes (36% patients). Multimarker RT-PCR assay offers a selective advantage in identifying occult metastases in serum or regional lymph nodes (172). RT-PCR can, however, generate results that are false positive (e.g. augmentation of mRNA from nodal nevocytes). In addition the preparative techniques for RT-PCR destroy tissue, so it is impossible to determine the cellular source of any augmented RNA.

CONCLUSIONS

With few exceptions, none of the markers for melanoma have fulfilled early expectations. The few exceptions are TA90-IC, S100 and MIA. Of these three, only TA90 is endogenously immunogenic. S100 and MIA are late-stage serum markers that are not specific for melanoma. TA90-IC is most sensitive in early-stage disease, with sensitivity and specificity estimated at 77% and 76%, respectively (29). TA90-IC can therefore be used to identify patients who are at higher risk of developing recurrent disease after surgical treatment of primary melanoma. These patients may be candidates for trials of postoperative adjuvant therapies.

In recent years some new markers such as MIA, tyrosinase (T311), MART-1 (A103), MITF, cyclin D3 and MIB-1 appear to supplement these. The most promising of these appears to be MITF, which recently has been compared to S100, tyrosinase, MART-1 and HMB45 (122). In this multimarker IHC comparison, 266 melanoma specimens were evaluated as positive if 10% of cells were stained. Rates of positivity were 90% for S100 and tyrosinase, 88% for MITF, 78% for MART-1, and 66% for HMB45. Unfortunately, MITF failed to identify most of the desmoplastic melanomas. Additional multimarker IHC comparisons have found S100 to be the most sensitive and HMB45 to be the most specific marker, leaving T311 and A103 antibodies in the intermediate range (140, 142).

In metastatic melanoma, ICAM-1, bFGF and MMP-2 have been associated with DFS and OS. C-myc over-expression is correlated with increased visceral metastasis, and a depressed MIB-1 staining (proliferative index) is associated with longer DFS and OS. Interestingly, the marker with the greatest range and utility for melanoma appears to be LDH, which has prognostic value in Stage II, III and IV disease. In fact, LDH is so strong a predictor of poor outcome in Stage IV disease that the forthcoming revised AJCC staging for melanoma will use this tumor marker to define a separate stage subtype, M1c (12, 13).

The most significant prognostic marker in early-stage melanoma and most other cancers remains lymph node status. In this area, however, tumor markers have begun to make practical

contributions. This is especially true of RT-PCR primers for markers that are relatively melanoma-specific. As noted earlier, a significant number of pathologically negative nodes demonstrate RT-PCR positivity, some of which reflects truly occult melanoma cells. This positivity predicts a slightly higher rate of recurrence than would have been predicted by standard pathologic examination with hematoxylin and eosin staining, immunohistology, and standard sampling approaches.

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Table 1: Different categories of investigational tumor markers in melanoma

<p>Soluble Proteins</p> <ul style="list-style-type: none"> • TA-90 • S-100 • Melanoma Inhibiting Activity (MIA) • Lactic Dehydrogenase (LDH) 	<p>Cytokines</p> <ul style="list-style-type: none"> • IL-2 • IL-6 • IL-8 • IL-10 • IL-15 	<p>Immunoregulators</p> <ul style="list-style-type: none"> • MART-1 • Tyrosinase <p>Apoptosis Factors</p> <ul style="list-style-type: none"> • Fas • Bcl-2 • TRAIL/FLIP
<p>Melanin-Related Metabolites</p> <ul style="list-style-type: none"> • 5-S-Cysteinyldopa • 6H5MI2C 	<p>Cell Growth Factors</p> <ul style="list-style-type: none"> • AP-2 • MITF • nm-23 • c-myc • Ki-67 • Cell cycle regulators 	<p>Extracellular Molecules</p> <ul style="list-style-type: none"> • Matrix metalloproteinase 2 (MMP-2) • Matrix metalloproteinase 9 (MMP-9) <p>Other</p> <ul style="list-style-type: none"> • Neuron Specific Enolase (NSE) • Lipid associated sialic acid (LASA) • C- Reactive Protein
<p>Angiogenesis Factors</p> <ul style="list-style-type: none"> • Vascular Endothelial Growth Factors (VEGF) • Basic fibroblast growth factor (bFGF) 		
<p>Adhesion Molecules</p> <ul style="list-style-type: none"> • HCAM • MCAM • ICAM 		

Table 2. Useful and potentially useful tumor markers for melanoma.

a. Serum tumor markers

Cancer Marker	Proposed Use/Uses	Phase of Assay Development	Level of evidence	Reference
TA90-IC	Postoperative surveillance of patients with no evidence of disease.	Validated; in clinical use in reference labs.	II	27, 28, 30
	Monitoring therapy in advanced disease.	Used in clinical trials.	II	29 - 31
	Assessing prognosis; high preoperative levels predict adverse outcome.	Validated but not in clinical use.	II	30, 31
	Useful adjunct to positron emission tomography.	Experimental; not in clinical use.	III	32
S100	Monitoring therapeutic response in metastatic melanoma patients.	Two assays available but not in clinical use because not validated in a high-level evidence study.	V	39 - 44
	Assessing prognosis.	Not in clinical use.	V	49
	Assessing response to therapy.	Not in clinical use.	V	45 - 48
MIA	Postoperative surveillance of advanced stage melanoma patients.	Not in clinical use; not validated in a high-level evidence study.	V	57 - 60
	Monitoring therapy in AJCC stage III melanoma patients.	Not in clinical use; not validated in a high-level evidence study.	V	61
5SCD	Late stage (IV) marker.	Not in clinical use	V	67, 68
	Assessing therapeutic response.	Not in clinical use.	V	71
VEGF	Determining prognosis; high levels associated with advanced disease.	Experimental.	IV	74 -75
bFGF	Elevated serum levels correlate with advanced disease and short DFS and OS.	Experimental; limited studies.	V	77
CD44	Assessing prognosis.	Conflicting reports (various isoforms?); needs further evaluation.	V	81 - 83
sICAM	Assessing prognosis.	Not significantly useful; elevated levels in bone/liver metastases.	V	81, 91, 92
sIL-2R	Assessing prognosis; IL-2 therapy evaluation.	Experimental.	V	92, 110, 111

sIL-6	Predicting response to biotherapy; higher levels associated with higher tumor burden.	Limited studies; not in clinical use.	V	112 - 114
sIL-8	Assessing tumor burden.	Experimental; needs further investigation.	V	115
Cathepsin B/H	Assessing prognosis.	Conflicting reports; not in clinical use.	V	151, 152
Neuron-specific enolase (NSE)	Assessing prognosis; higher level in AJCC stage III/IV than stage I/II.	Less sensitive than S100; not in clinical use; limited utility for surveillance of high-risk melanoma patients.	IV, V	153 - 155
Lipid-associated sialic acid (LASA)	Higher levels associated with large tumor burden.	No correlation with survival, perhaps due to different methods of assessment; not validated and not in clinical use; levels increased by acute inflammation.	V	160, 161
C-reactive protein (CRP)	Predicting survival in advanced melanoma; disease progression marker.	Limited studies reported CRP to be superior to LDH; results controversial.	IV	163 - 165
Proteomics	Detecting early disease and monitoring.	Experimental.	V	175

b. Tissue tumor markers

Cancer Marker	Proposed Use/Uses	Phase of Assay Development	Level of evidence	Reference
S100	IHC detection of melanoma cells in biopsy specimens.	Wide clinical use.	III	37, 38
	Adds specificity when combined with HMB-45 (anti-gp100).	Clinical use.	III	140, 142
gp100 (HMB-45)	Usually combined with S100 for melanocytic lesions.	Clinical use.	III	142, 173
MART-1	Expressed by melanocytes and their tumors.	Clinical use in some centers.	III	141
VEGF	Expressed in primary melanomas and not in benign lesions; could differentiate benign and malignant lesions.	Experimental.	V	76, 77
bFGF	Associated with vertical growth phase.	Pilot study.	V	78
CD44	Adhesion molecule with several isoforms.	IHC results are conflicting.	IV-V	80, 82
MCAM	Expression depends on CREB (cAMP response element-binding) protein; expressed by several tumor types but expression is high in melanoma.	Undergoing further evaluation.	IV-V	85 - 87
ICAM (CD54)	Apparent progression marker by IHC; higher expression in metastatic lesions than primary lesions.	Experimental.	IV-V	90
Avβ3	Expression restricted to melanomas but changes from radial to vertical growth phase.	Experimental, needs further evaluation.	V	95 - 99
AP-2	Loss of AP-2 expression by IHC is associated with decreased DS and OS.	Experimental.	V	119
MITF	Nuclear staining in melanomas; expression loss correlates with decreased DFS and OS.	Prognostic value not validated.	V	120 - 123
nm23	Assessing prognosis.	Conflicting reports.	IV-V	125, 126
c-myc	Assessing prognosis.	Conflicting results.	V	127 - 131

Ki-67 (MIB-1 Ab)	Use in IHC could distinguish Spitz nevus and other benign skin lesions from melanoma; could be used as independent prognostic marker.	Under evaluation as a progression marker.	IV	134 - 136
Tyrosinase	Loss of tyrosinase is considered a progression marker.	Limited IHC studies at the experimental level	V	140
MMPs	Associated with late phase of invasion and metastasis of melanoma by IHC.	Results conflicting; undergoing further evaluation.	V	146 - 149
Gene expression microarray	For analysis of lymph nodes and assessing prognosis.	Undergoing evaluation.	IV	166 - 172

c. Tumor cell markers

Cancer Marker	Proposed Use/Uses	Phase of Assay Development	Level of evidence	Reference
Bone marrow	Assessing prognosis.	Tumor cells in bone marrow and blood predict adverse prognosis; undergoing further refinement and evaluation.	IV	170, 172, 176
Regional lymph nodes	Assessing prognosis.	Micrometastases in regional nodes predict adverse prognosis.	I-II	177 - 179
Sentinel lymph nodes	Assessing prognosis.	Prospective studies of sentinel micrometastases undergoing evaluation.	I-II	180 - 182

d. Genetic marker

Cancer Marker	Proposed Use/Uses	Phase of Assay Development	Level of evidence¹	Reference
CDKN2A (cyclin-dependent kinase inhibitor 2A)	Identifying risk of melanoma in high-risk families	Experimental; not in clinical use	V	183

Table 3. NACB Recommendations for use of markers in melanoma

	NACB, 2005
Lactate dehydrogenase (LDH) for	
Screening	No
Diagnosis / case-finding	Yes
Staging /prognosis	No
Detecting recurrence	No
Monitoring therapy	No
TA90-IC for	
Screening	No
Diagnosis / case-finding	Yes
Staging /prognosis	Yes
Detecting recurrence	Yes
Monitoring therapy	Yes
S-100 for	
Screening	No
Diagnosis / case-finding	No
Staging /prognosis	Yes
Detecting recurrence	No
Monitoring therapy	No recommendation made
Melanoma Inhibiting Activity (MIA)	
Screening	No
Diagnosis / case-finding	No recommendation made
Staging /prognosis	Yes
Detecting recurrence	Yes
Monitoring therapy	No recommendation made

Table 4. Analytical requirements and potential interfering factors for established and experimental serum markers

Marker	Sample type	Analytical requirements	Other conditions that cause elevated levels	Suggested frequency of measurement
Lactate dehydrogenase (LDH)	Serum	Collect specimen in red top tubes. Do not freeze the specimen. IQC must be applied.	Other tumor types. Non-cancerous conditions, including heart failure, hypothyroidism, anemia and lung or liver disease.	Every 6 months for Stage III and IV patients, in combination with other prognostic factors.
TA90-IC	Serum	Collect specimen in red top tube. Keep serum at -35°C or below until ready to be tested. IQC must be applied.	Other solid tumors. Liver cirrhosis.	Every 6 months post-operatively, with more frequent measurements (e.g. every 3 months) if positive.
S-100	Serum	Specimen requirement not reported.	Other tumor types. Head injury, cardiac arrest, and acute stroke. Post-exercise.	NR
Melanoma Inhibiting Activity (MIA)	Serum	Specimen requirement not reported.	Elevated levels have been reported during pregnancy, and in children; thus not recommended for individuals under age 18.	NR

IQC, Internal Quality Controls

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