Update on Lymphatic Mapping for Malignant Melanoma

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For most solid tumors, including melanoma, the most powerful predictor of recurrence and survival is the presence or absence of regional lymph node metastases. For patients with melanoma and stage III disease, the five-year survival decreases 40% compared to patients without nodal metastases. With the approval of interferon alfa-2b as the first effective adjuvant therapy for the high risk for recurrence melanoma population, one can argue that patients with a defined rate of nodal metastases (tumor thickness >1 mm) should have a nodal staging procedure as part of their primary therapy. Lymphatic mapping with sentinel lymph node (SLN) biopsy is the most morbid method to obtain this information.

In order for a surgeon to perform accurate lymphatic mapping, good support is needed from both the nuclear medicine department and the pathology department. Preoperative lymphoscintigraphy is used to (1) identify all the basins at risk for metastatic disease, (2) identify in-transit nodal areas, (3) estimate the number of SLNs, and (4) locate the SLN in relation to other nodes in the lymphatic basin. With a five-year follow-up on over 400 studies, there has never been a metastasis in a basin not visualized by scan in patients with clinical stage I and II disease, indicating that it is an accurate method to determine all the basins at risk for disease.

With data from the preoperative lymphoscintigraphy, the surgeon can proceed to intraoperative lymphatic mapping. Combination mapping techniques (vital dye and radiocolloid) have been found to be more effective than single-agent methods. Radiocolloid mapping is important to identify the “hot spot” of SLN location through the skin, to provide a directed dissection through the basin, and to ensure that all SLNs have been removed. The blue-dye mapping technique becomes important when primary sites are close to the regional basin. There are now 20 reports in the literature (involving a total of more than 2,000 patients) showing that the histology of the SLN, particularly if it is negative, reflects the histology of the remainder of the nodal basin. Full nodal staging can be performed with an SLN harvest.

Routine histology will identify 75% of the melanoma metastases to the regional node. The remaining 25% are identified only with a more detailed examination that typically includes serial sectioning and immunohistochemical staining. Since pathologists are given only one to two SLNs to examine in detail because of the lymphatic mapping technique, it is neither too time consuming nor expensive to perform this more detailed examination.

Lymphatic mapping and SLN biopsy will provide a more accurate staging of the patient with melanoma. In this way, morbidity is reduced, cost of care is decreased, and effective adjuvant therapy is provided only to those who will derive the most benefit.

References


Polymerase Chain Reaction of the Sentinel Lymph Node for Malignant Melanoma

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Routine pathologic examination of the lymph nodes in patients with melanoma examines less than 1% of the submitted material and will underestimate the number of patients with nodal metastases by 25%. The sensitivity of the routine examination is the ability to identify one abnormal melanoma cell in a background of 10⁹ normal lymphocytes. When used routinely, special immunohistochemical staining, such as the S-100 and HMB-45 stain, will increase the sensitivity of the occult metastases examination to identify one abnormal cell in a background of 10⁵ lymphocytes.

Molecular biology assays for submicroscopic metastases have been proposed to increase the sensitivity of the regional nodal examination and provide a more accurate staging for the melanoma patient. Lymphatic mapping protocols allow for this more detailed examination by identifying one or two nodes most likely to harbor metastases.

Reverse transcriptase polymerase chain reaction (RT-PCR) assays to detect tyrosinase mRNA have been used as markers for submicroscopic metastases to the sentinel lymph node (SLN). The sensitivity of this assay is the ability to identify one melanoma cell in a background of 10⁷ lymphocytes, two orders of magnitude greater in sensitivity than that of the routine examination.

A study conducted at our institute included 114 patients with clinical stage I and II melanoma who had their SLNs harvested (Table). They were followed for 6 to 48 months (mean = 28 months) for recurrence. Of these 114 patients, 23 (20.2%) had histologic-positive (histo+) SLNs. All of these SLNs were also PCR positive (PCR+). Of the 91 patients with histologic-negative (histo−) SLNs, 47 (51.6%) were PCR+. At follow-up of patients who were both histo+ and PCR+, 14 (60.9%) of 23 recurred. Among patients whose SLNs were histo− but PCR+, 6 (12.9%) of 47 recurred. In patients whose SLN was both histo− and PCR−, only 1 (2.3%) of 44 recurred. The difference in the rate of recurrence between the histo−/PCR+ group and the histo−/PCR− group is statistically significant (P=0.02).
This assay is now being tested in a national trial called the Sunbelt Melanoma Trial. During its first year, 350 patients have been recruited of the 1,400 patients needed to complete the trial. A four-marker RT-PCR panel is being used to determine the presence or absence of SLN metastases. Of the histo– patients, 38.5% are “upstaged” with the RT-PCR assay. On this trial, there is a correlation among Clark level of invasion, tumor thickness, and the proportion of patients who are RT-PCR positive in their SLNs. Five patients have recurred on the study, three of them having SLNs that are histo+ and RT-PCR+. Two of the patients who recurred had SLNs that were histo– but RT-PCR+.

These preliminary data suggest that missed micrometastatic disease is clinically relevant disease since the RT-PCR assay correlates with tumor thickness. If this is the case, the assay may eventually correlate with survival.

<table>
<thead>
<tr>
<th>Number of Patients</th>
<th>Histopathologic Examination</th>
<th>Recurrence</th>
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<tr>
<td>(114 total)</td>
<td>Histo+ PCR+</td>
<td>23 (20.2%)</td>
</tr>
<tr>
<td>23 (20.2%)</td>
<td>Histo- PCR+</td>
<td>47 (41.2%)</td>
</tr>
<tr>
<td>44 (38.6%)</td>
<td>Histo- PCR-</td>
<td>1 (2.2%)</td>
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The RT-PCR assay for occult metastases has been used to examine the peripheral blood of patients with melanoma. The PCR assay was negative in all 13 normal controls. Tyrosinase mRNA was detected in the peripheral blood in 1 (20%) of 5 stage I patients, in 6 (16%) of 38 stage II patients, and in 5 (20%) of 25 stage III patients. At one year of follow-up, recurrence rates are 0% for stage I, 16% for stage II, and 36% for stage III. Of patients who were PCR–, 10 (18%) of 56 patients relapsed, while 5 (42%) of 12 PCR+ patients relapsed. Other groups have used multiple marker PCR assays in peripheral blood to show that PCR positivity correlates with stage of disease and disease activity. Peripheral blood assays are also being tested as part of the Sunbelt Melanoma Trial.

RT-PCR assays for occult metastases based on tyrosinase probes improve the prediction of melanoma recurrence over routine histologic examination, suggesting clinical correlation for the assay. The value of this assay as a prognostic factor for melanoma is being tested in a national multicenter study.

References

**Adjuvant Therapy of Melanoma**

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The modified American Joint Committee of Cancer (AJCC) staging system assigns localized cutaneous melanoma of increasing thickness to stage I and II and regional lymph node involvement to stage III. Patients with AJCC stage IIB melanoma (Breslow depth >4 mm) have an estimated five-year survival rate of 47.9% to 69.3% and a 10-year survival rate of 35.5% to 54.5%. Patients with stage III melanoma have an estimated five-year survival rate of 10% to 46%. These two groups of patients have been the primary focus of a decade-long search for an effective adjuvant therapy that can improve the cure rate following surgical resection.2

**Interferon-alfa in the Adjuvant Therapy of Stage IIB and Stage III Melanoma**

**Eastern Cooperative Oncology Group (ECOG) Trial E1684**

In the ECOG trial E1684, 287 patients with deep primary disease (T4, AJCC stage IIB) or regional lymph node metastatic disease (N1, AJCC stage III) were randomized to interferon-alfa (IFN-alfa) treatment or observation between 1984 and 1990. All patients underwent wide excision of the primary tumor and therapeutic or elective lymph node dissection (ELND) prior to entry. They were identified as high-risk patients if they had microscopically positive sentinel lymph nodes. Patients were randomized to receive interferon-alfa2b (Intron A), as the first adjuvant therapy of melanoma following surgical resection, or observation.4

At 7 years of follow-up, the increases in median survival from 2.8 to 3.8 years and in relapse-free interval from 1 to 1.7 years were both statistically significant (P=0.02 and P=0.002, respectively). The benefit was most pronounced among the 249 stage III patients (89% of the study population; P=0.006 for overall survival and P=0.0006 for relapse-free survival).3 This landmark trial was the pivotal study accepted by the Food and Drug Administration’s Oncologic Drug Advisory Committee in its recommendation to approve licensure for IFN-alfa2b (Intron A), as the first adjuvant therapy of melanoma following surgical resection in patients with stage IIB or stage III melanoma.4

This treatment regimen had substantial toxicity, and grade 3 (ECOG toxicity scale) events were noted at least once in the majority of recipients. Dose modification or delays were required at least once in 50% of patients during the intravenous induction phase and in 46% of patients during the subcutaneous maintenance phase. Most treatment delays occurred in the first four months of the regimen. Concerns regarding the impact of this therapy on quality of life were evaluated in a retrospective quality-of-life (Q-TWIST) study.5 After 84 months of follow-up, patients in the IFN-alfa2b arm gained a mean of 8.9 months without relapse (P=0.03) and 7 months of overall survival time (P=0.02) compared to the observation group. The treated group experienced severe treatment-related toxicity for an average of 5.8 months. The net result was that the group treated with IFN-alfa2b had more quality-of-life-adjusted survival time than did the observation group, regardless of the relative valuations placed on time with toxicity and time without relapse.

An economic analysis of the E1684 regimen was recently published by Hiller et al.6 The projected incremental cost in 1996 US dollars per life-year gained in the IFN cohort of patients ranged from $13,700 after 35 years to $32,600 at seven years (the median follow-up of E1684). The benefits of IFN projected over a lifetime yield incremental cost–effectiveness ratios of $20,000 per quality-of-life-year gained and $25,000 per life-year gained and other accepted adjuvant therapies of breast and colorectal cancer.

**Intergroup Trial ECOG 1690**

This trial was initiated in 1990 before the results of the E1684 trial were mature. This randomized three-arm study compared the same high-dose regimen of IFN-alfa2b described above to the World Health Organization (WHO) low-dose regimen of IFN-alfa2b 3 MU subcutaneously three times per week compared to observation. Targeted accrual of 642 patients was completed in June 1995, and preliminary data analysis was performed in September 1998. A significant impact in relapse-free but not overall survival was seen for the high-dose IFN-alfa arm compared to observation. No impact in either relapse-free or overall survival was seen for the low-dose arm. Further analysis of these preliminary results is awaited.

**Other Trials**
The North Central Cancer Treatment Group (NCCTG) study 83-7052 randomized 262 patients with stage II and III melanoma to treatment with IFN-alpha2a 20 MU/m² three times per week intramuscularly or to observation. Approximately 50% of the patients had clinically node-negative (stage II) disease. Analysis at maturity in 1995 revealed no significant impact on overall or relapse-free survival, but a trend-to-benefit was noted among the patients with clinical stage III disease.7

The WHO Melanoma Programme trial 16 tested a low-dose regimen of IFN-alpha2s 3 MU given subcutaneously three times per week for three years in 444 patients with recurrent nodal disease. This study was preliminarily reported in a subset analysis at 22 months of median follow-up, suggesting a relapse-free interval prolongation among women younger than 50 years and men older than 50 years.8 However, later presentations of this study at 39 months or more of follow-up have not continued to suggest durable antitumor activity, in terms of either overall survival or relapse-free interval.9

The European Organization for Research on Treatment of Cancer (EORTC) has recently commenced a study (18-952) testing the impact on relapse-free interval and survival of intermediate dosages of IFN-alpha2b administered over one or two years compared to observation. The induction phase of this trial is a flat dose of 10 MU subcutaneously five days per week for four weeks followed by a maintenance phase of either 10 MU subcutaneously three times per week for one year or 5 MU subcutaneously three times per week for two years. This study will accrue 1,000 patients with high-risk resected melanoma, including patients with nodal disease with or without extracapsular extension.

**Interferon-alpha in the Adjuvant Therapy of Stage IIA Disease**

Patients with T3 melanoma (Breslow depth 1.5 to 4 mm, stage IIA) comprise a substantial number of individuals with five- and 10-year survivals of 72.5% and 62%, respectively, for the group as a whole. Thirty-one percent of all newly diagnosed patients with melanoma fall into this category. Given the projected 1998 melanoma incidence of 40,300 cases, approximately 12,400 new cases of stage IIA melanoma will occur and at least 3,400 of these people will die of their disease. An effective adjuvant therapy for this patient population would be of major importance, yet most adjuvant therapy trials for high-risk melanoma have excluded patients with stage IIA melanoma.

The French Cooperative Group on Melanoma recently reported the updated results of a low-dose IFN-alpha trial in stage II (A and B) patients.10 Eligible patients had surgically resected melanoma without clinical evidence of regional lymph node involvement. Patients were randomized to IFN-alpha2a 3 MU given subcutaneously three times per week for 18 months or to observation. While a preliminary report at less than two years of follow-up was encouraging, a follow-up at three years demonstrated no overall durable survival advantage.

A recently initiated study to be conducted by ECOG (E1697) and the National Cancer Institute of Canada is the only trial to specifically address the issue of adjuvant IFN-alpha2b for stage IIA patients. Patients will be randomized to either the induction phase of E1684 (20 MU/m², Monday through Friday for four weeks) or to observation. A total of 1,420 patients are expected to accrue over a two- to three-year period to detect a 7% increase in survival.

**Vaccines in the Adjuvant Therapy of Melanoma**

The application of modern molecular biologic and immunologic techniques to the serology and cellular dissection of human tumor antigens has led to an explosion in the knowledge of the basis of immune recognition of melanoma. The gangliosides G₃₈, G₃₂, G₂₂ and G₂₅ comprise a series of glycolipids that are major constituents of melanoma cells. The ganglioside G₂₅ elicits anti-G₂₅ antibodies in 5% to 10% of melanoma patients, and the presence of anti-G₂₅ antibodies may confer a survival advantage.11,12 A phase III randomized trial of vaccination with G₂₅ and bacille Calmette-Guérin (BCG) compared to BCG alone in 122 patients with surgically resected AJCC stage III melanoma.13 A nonsignificant increase in disease-free interval (18%) and overall survival (11%) was found for the G₂₅/BCG-treated group. However, exclusion of patients with preexisting anti-G₂₅ antibodies from the analysis resulted in a 23% (P = 0.02) increase in disease-free interval for this group of patients. The G₂₅ molecule has subsequently been coupled with a carrier protein derived from the keyhole limpet hemocyanin (KLH) and a new and potent immunologic adjuvant of the saponin class, QS21. Immunization with this G₂₅-KLH (GMK) vaccine has been shown to induce qualitatively improved antibody responses of the immunoglobulin-G (IgG) as well as the IgM isotype and of substantially higher titer than by G₂₅ plus BCG.13

The ECOG has undertaken a randomized phase III trial (E1694) comparing standard therapy with high-dose IFN-alpha for one year with the G₂₅/KLH vaccine for patients with stage IIB and III melanoma. Accrual of 851 patients is expected to be completed by late 1999. A combination of high-dose IFN with the GMK vaccine has been tested in a randomized phase II trial (ECOG 2696) to determine if the anti-G₂₅ antibody response diminished or enhanced when IFN-alpha2b is administered concurrent with or following initiation of vaccination with GMK. Results of this important study will be available soon and may promote future trials that combine this vaccine and IFN.

Another commercial vaccine preparation from cultured tumor cell lines (Melacine), given together with the proprietary adjuvant agent Detox (monophosphoryl lipid A), has been shown to induce antitumor responses in patients with metastatic melanoma. The Melacine vaccine has been tested with the adjuvant Detox in the US Cooperative Groups by the Southwest Oncology Group (SWOG). The trial (SWOG 9035) concluded in 1996 with more than 600 patients receiving either the vaccine with Detox or observation following surgery for T2 or T3 melanoma. Results of this trial are awaited with interest.

**References**


Treatment of Metastatic Melanoma

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Nearly 30% of patients with melanoma develop metastatic disease (stage IV) requiring systemic therapy. A complete staging is needed to determine the sites of metastases, which most frequently involve skin, lymph nodes, lung, liver, and brain. Since patients with brain metastases require specialized care and may not be candidates for systemic therapy, magnetic resonance imaging of the brain is recommended prior to initiation of systemic therapy.

Options of systemic therapy for patients with stage IV melanoma if they are not candidates for surgical resection (which may be a consideration for isolated metastases in brain, bowel, or lungs) include the following: chemotherapy, interferon alfa (IFN-alpha2a or IFN-alpha2b), Interleukin-2 (IL-2), and investigational therapy (new drugs, melanoma vaccines). Although dacarbazine (DTIC) alone has been the most commonly used chemotherapy for the treatment of melanoma, other drugs — especially cisplatin and carbustine (BCNU) — have significant activity and are increasingly used in combination. Several combination chemotherapy regimens used during the past two decades have often produced a modest increase in the response rates over that expected from DTIC, although their impact on increasing the survival is still debated. Our efforts in the development of treatment for metastatic melanoma are summarized below.

Cisplatin, Vinblastine, and DTIC

The three-drug regimen containing cisplatin, vinblastine, and DTIC (CVD) was tested in 1986 to 1987. The combination produced a response rate of 40%, which is twofold higher than that of DTIC alone and is comparable to the activity of Dartmouth regimen (cisplatin, BCNU, DTIC, and tamoxifen).

Interferon-alfa

In our experience, IFN-alpha demonstrated a modest response rate of 10% but produced some durable complete responses (CRs). Moreover, IFN-alpha was equally active in chemotherapy-resistant disease, indicating lack of cross-resistance with chemotherapy.

Interleukin-2

Interleukin-2 (IL-2) showed significant activity in metastatic melanoma in 1987. The Food and Drug Administration approved IL-2 for the treatment of stage IV melanoma in January 1998. At our institute, IL-2 demonstrated a response rate of 22% (range = 15% to 20%). The response was not influenced by prior CVD, indicating lack of cross-resistance.

Interleukin-2 and Interferon

The combination of IL-2 and IFN, initially tested by Lee and Talpaz, demonstrated a response rate of 25%, indicating some evidence of additive antitumor activity for this combination compared to the response rates due to IL-2 alone (15% to 20%).

Biochemotherapy Regimens

Based on the independent activity of biotherapy (IL-2 plus IFN-alpha) and CVD, a combined regimen of biochemotherapy was developed to investigate evidence of additive or synergistic activity of the two components. The dose and schedule of the components were as follows:

**Biotherapy:**
- IL-2: 9 x 10^6 IU/m^2/d x 4 days (continuous infusion)
- IFN-alpha: 5 x 10^6 U/m^2/d x 5 days (subcutaneously)

**Chemotherapy (CVD):**
- Cisplatin: 20 mg/m^2/d x 4 days
- Vinblastine: 1.5 mg/m^2/d x 4 days
- DTIC: 800 mg/m^2/d x 1 day

The courses of biotherapy were timed with CVD, which was repeated at 21-day intervals. Three different schedules of biochemotherapy were tested in phase II trials done sequentially (alternating, sequential, and concurrent). Alternating biotherapy and chemotherapy at three- to six-week intervals failed to produce any additive effects due to biotherapy. However, an immediate sequence of CVD followed by biotherapy (sequential regimen) or concurrent delivery of both components of CVD and biotherapy produced additive antitumor activity of both components resulting in an overall response rate of near 60% and, more importantly, a CR rate of 20%. This was the highest CR rate reported for any systemic therapy tested in the treatment of metastatic melanoma during the past two decades of efforts to develop treatment for this disease. More importantly, durable CRs occurred in more than 50% of the patients who achieved a clinical CR. The CRs were observed in all sites of metastases (including liver and bone) and occurred regardless of the volume of the metastatic tumor. Thus, biochemotherapy has produced the first clear evidence of cure of metastatic melanoma in approximately 10% of the patients treated with this regimen.

References