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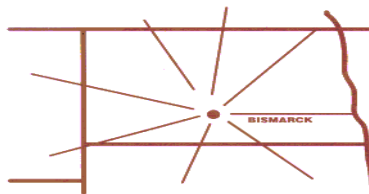
Pathology News for the Medical Community

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## **Clinical Significance of Microscopic Metastasis to Sentinel Lymph Nodes in Patients with Malignant Melanoma**

Jennifer G. Pryor, MD, CAP Residents Forum Delegate

Regional lymph node dissection improves survival in melanoma patients with nodal disease but is associated with unnecessary morbidity when used in all patients with melanoma.<sup>1</sup> Sentinel lymph node (SLN) biopsy, which is less invasive than regional lymph node dissection,<sup>2</sup> accurately predicts the status of the lymph node basin and pathologically identifies patients who do not require further treatment.<sup>3</sup> SLN biopsy can potentially improve patient survival and enable clinicians to predict prognosis, plan treatment regimens, and potentially limit disease.<sup>4</sup> Approximately twenty percent of clinically node negative melanoma patients have occult (microscopic) nodal disease,<sup>2</sup> and 15-20% of SLN positive patients will have additional metastases in non-sentinel nodes.<sup>3,5</sup> SLN biopsy followed by immediate completion lymph node dissection (CLND) in patients with microscopic nodal disease has been reported to improve survival.<sup>6</sup>

SLN biopsy has become the standard of care in the staging of melanomas  $\geq 1$  mm thick. Patients with pathologic Stage 0 (in-situ) and Stage IA (<1 mm thick nonulcerated) melanomas do not require pathologic evaluation of their lymph nodes.<sup>7</sup> A positive SLN should be followed by CLND, at least in patients whose melanomas are 1.2–3.5 mm thick.<sup>6</sup> Some data suggest that patients with small metastases (<0.1 mm) or isolated immunohistochemically positive cells are unlikely to benefit from CLND and should be treated as SLN negative for staging purposes.<sup>8</sup> However, there is contradictory evidence that these patients in fact have a significantly worse long-term prognosis.<sup>9</sup> Use of histologic characteristics of the SLN micrometastasis, such as tumor burden or location, may be used to predict the likelihood of micrometastases in non-sentinel nodes and thus help avoid CLND, but it is still in its infancy. Similarly, while molecular assays can potentially detect sub-microscopic metastases, their reliable application in a predictive fashion has not been established. Until further data is available, conservative management with CLND, regardless of quantitative sentinel node tumor burden, seems warranted.<sup>10,11</sup>

Pathologists play a critical role in staging of patients with melanoma. There are significant differences in outcome when patients without clinically apparent nodal disease are staged pathologically.<sup>12,13,14</sup> Staging is performed using the AJCC Staging System for Melanoma,<sup>7</sup> in which the number of positive lymph nodes is an important prognostic indicator.<sup>15</sup> Also of prognostic importance is whether the metastases are clinically occult (microscopic) or clinically apparent (macroscopic), discovered by palpation or radiographic studies.<sup>7</sup> Pathologists typically use hematoxylin and eosin (H&E) stained sections, augmented by immunohistochemistry to facilitate identification of micrometastases. Isolated immunohistochemically positive tumor cells must be distinguished from nodal nevus cells, macrophages, and antigen-presenting interdigitating dendritic cells; this is best achieved through the use of more than one melanocytic marker.

In summary, there is some evidence to indicate that patients with small metastases (<0.1 mm) or isolated immunohistochemically positive cells have a worse long-term

prognosis, and CLND in patients with microscopic SLN disease may improve survival. Important goals for pathologists include finding a reliable method for the rapid identification of microscopic SLN metastases and exploring alternative methods such as fineneedle aspiration for nodal staging of melanoma patients. Further research on these topics is needed in 2009, and pathologists are positioned to play a critical role in the development of these methodologies.

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## Use of Quantitative PCR in the Monitoring of Patients with Chronic Myelogenous Leukemia

Jason D. Merker, MD, PhD CAP Molecular Oncology Committee

The *BCR-ABL1* fusion, resulting from the reciprocal translocation t(9;22)(q34;q11), produces a constitutively active tyrosine kinase. In the right hematologic context, the finding of a *BCR-ABL1* fusion is diagnostic of chronic myelogenous leukemia (CML). The use of imatinib and later-generation tyrosine kinase inhibitors (TKIs) in recent years has revolutionized the treatment of patients with CML. The majority of chronic phase CML patients treated with TKIs will achieve a complete cytogenetic response (zero chromosomes with a *BCR-ABL1* fusion detected in bone marrow metaphases); therefore, more sensitive polymerase chain reaction (PCR) based methods are often used for monitoring subsequent low levels of disease.

Real-time quantitative reverse transcription PCR (RQ-PCR) is used at many centers as an adjunct to hematologic and cytogenetic monitoring. Although laboratories use a variety of methods and reagents, the underlying principles are generally the same. RNA is extracted from peripheral blood or bone marrow and reverse transcribed into cDNA. Real-time PCR with fluorescent probes is then used to quantify *BCR-ABL1* and a control gene. The results are expressed as a ratio of *BCR-ABL1* to the control gene.

RQ-PCR assays are technically challenging to perform accurately and precisely, and it is necessary to understand these limitations to use them optimally. Changes in *BCR-ABL1* levels between serial specimens may represent variation related to specimen collection, transport, or testing, rather than changes in disease burden. These seemingly minor variations in collection and testing may result in changes of *BCR-ABL1* levels of up to 0.5 log (about three-fold) or greater. Consequently, changes of less than 0.5 log are not considered significantly different in most laboratories; and for changes greater than 0.5 log, it is advisable to repeat RQ-PCR testing or confirm the results with other methods prior to changing clinical management. Furthermore, due to the variety of methods, reagents, and control genes used, results from different laboratories are generally not directly comparable; therefore, it is recommended that the same laboratory test a patient's serial specimens. Finally, some patients have significantly different levels of *BCR-ABL1* in the peripheral blood and bone marrow, so it is necessary to compare results from the same compartment. Peripheral blood is quite representative of the disease burden and is routinely used for disease monitoring.

Multiple publications have discussed how to integrate molecular studies in the monitoring of patients with CML<sup>1,2,3,4</sup>. At diagnosis, RQ-PCR studies establish a pre-treatment baseline. Subsequent serial monitoring provides a sensitive method to follow low levels of disease. Rising *BCR-ABL1* levels confirmed by repeat RQ-PCR testing, may prompt closer monitoring, further testing (bone marrow examination with cytogenetics or *ABL1* mutation analysis), or reevaluation of therapy. Finally, RQ-PCR data may provide prognostic information. Patients treated with TKIs that achieved a major molecular response (=1,000-fold or 3.0 log reduction in *BCR-ABL1*) and a complete cytogenetic response have a very low likelihood of disease progression<sup>5</sup>. How to optimally integrate RQ-PCR monitoring for patients with CML is an actively studied area, and this provides a unique opportunity for collaboration between the treating physician and the clinical laboratorian.

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## The Role of *KRAS* Mutation Testing in the Management of Colorectal Cancer

Mark D. Pool, MD, FCAP, Special Contributor

Retrospective analyses from several recent large clinical trials in metastatic colorectal cancer (mCRC) have examined the role of mutations in the *KRAS* gene with the response to treatment using the epidermal growth factor receptor (EGFR) monoclonal antibody inhibitors cetuximab and panitumumab.<sup>1-8</sup> These data show that both of these agents are ineffective in patients with *KRAS* mutation. Recently published guidelines from the National Comprehensive Cancer Network<sup>9</sup> and the American Society of Clinical Oncology<sup>10</sup> recommend *KRAS* mutation testing as part of the evaluation of mCRC patients who are being considered for anti-EGFR therapy.

EGFR is overexpressed in a large majority of colon cancers, yet there has been poor correlation between EGFR expression by immunohistochemistry and treatment response. This lack of correlation suggests that downstream effectors may be as important as EGFR expression for treatment response. Activation of EGFR triggers multiple signaling pathways resulting in promotion of tumor growth, inhibition of apoptosis, vascular proliferation, invasion, and metastasis. The most important of these pathways for CRC appears to be the Ras signaling pathway.<sup>11</sup> Ras proteins are small GTPases, and activated Ras interacts with more than 20 different effectors. *KRAS* has been long known to be involved in the development and progression of CRC<sup>12</sup> and is mutated in about 40% of CRC. The *KRAS* mutations most commonly found in CRC are at codons 12 and 13 and prevent dephosphorylation and inactivation of the protein, causing it to be permanently switched on, independent of EGFR-mediated signaling. Thus, a mutated *KRAS* protein would not likely be affected by inhibition of EGFR.

Although there is currently no FDA-approved test for the detection of *KRAS* mutations, several tests are commercially available. A recently presented abstract that examined 40 formalin-fixed paraffin embedded (FFPE) CRC tissues showed that, despite different methodologies, these four tests yielded comparable results,<sup>13</sup> although allele-specific hybridization methods (HistoGeneX and Genzyme) had the highest correlation with the reference methodology of direct sequencing. The *KRAS* mutation test may be performed on fresh, frozen, or FFPE tissue. In contrast to HER2 expression in breast cancer, *KRAS* mutation occurs early in CRC carcinogenesis and is unlikely to change during disease progression. Thus, although not confirmed by prospective studies, current practice is not to re-biopsy a tumor recurrence for *KRAS* testing if there is sufficient material available from the previous biopsy or resection.

Pathologists play an essential role in *KRAS* testing, whether or not testing is performed in their own laboratory. First, specimens must be handled with careful attention to fixation in anticipation of potential genetic testing. Second, appropriate tumor tissue for testing must be selected. Third, pathologists must be aware of which *KRAS* mutation detection methodology is utilized in their reference laboratories and evaluate the quality processes that are used to ensure reliable results. Finally, pathologists can consult with oncologists in the appropriate use of this test and interpretation of the results.

Retrospective analyses of clinical trials have consistently demonstrated that patients with mCRC and mutant *KRAS* are unlikely to benefit from the addition of cetuximab or panitumumab to a chemotherapy regimen. Testing for *KRAS* mutation in this setting is now recommended and marks a paradigm shift in the management of these patients.

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