

Clinical Perspectives™

Highlights from the Perspectives in Melanoma XII Conference

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IMPACT OF EMERGING TECHNOLOGIES FOR BIOMARKER DEVELOPMENT

New technology for identifying circulating tumor cells: Reliable at last?

Previous studies using quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) showed MelanA/MART1 and tyrosinase expression is increased in later stages of cutaneous or ocular melanoma,¹ and that MelanA/MART1 and tyrosinase transcripts are independent prognostic factors for metastases or distant metastasis-free survival (DMFS) in patients with primary uveal melanoma.² The PCR results did not correlate with clinical characteristics. **Ulrich Keilholz**, of Charite University in Berlin, Germany, discussed initial findings from a study examining circulating melanoma cells and DMFS in stage III melanoma patients, with and without adjuvant interferon-alpha (IFN- α) treatment.

In this study, which represented a subset of patients enrolled in the EORTC 18991 phase 3 trial, peripheral blood samples from 299 patients were depleted of leukocytes and remaining cells were stained for melanoma markers and evaluated by flow cytometry. Approximately a third (109/299; 36.5%) had at least 1 sample by RT-PCR analysis, 5.6% at randomization and 30.7% subsequently. A time-dependent Cox model (corrected for lead-time bias) showed RT-PCR positivity versus negativity was a significant predictor of shortened DMFS (HR, 2.23; 95% CI, 1.40-3.55; $P < .001$). However, these results were comparable in the 2 treatment groups, indicating that RT-PCR assessment was not predictive of treatment outcome. The identity of the circulating melanoma cells is unclear at this time, but initial analyses indicate the circulating cells express stem-cell associated genes and can be differentiated into at least groups by differences in CD133 expression.

Taken together, Dr. Keilholz said, these studies demonstrated the utility of RT-PCR for the identification of circulating melanoma cells, and suggest expression of tyrosinase and Mart-1/Melan-A is a prognostic factor for subsequent development of distant metastases in stage III patients. Eventual characterization of the circulating cells should help advance understanding of the biology of this process.

References

1. Gudbjartsson DF, Sulem P, Stacey SN, et al. ASIP and TYR pigmentation variants associate with cutaneous melanoma and basal cell carcinoma. *Nat Genet.* 2008;40:886-891.
2. Schuster R, Bechrakis NE, Stroux A, et al. Circulating tumor cells as prognostic factor for distant metastases and survival in patients with primary uveal melanoma. *Clin Cancer Res.* 2007;13:1171-1178.

Tumor infiltrating lymphocytes

Alan Spatz, of McGill University in Montreal, Quebec, described the results from 2 studies looking at the prognostic value of lymphocyte infiltration and the clinical relevance of dendritic cells (DCs) at the tumor site in melanoma patients, respectively. The first study included 1,171 patients with cutaneous melanoma in vertical growth phase, with available slides for review and at least 3 years of follow-up. The tumor infiltrating lymphocyte (TIL) response was categorized by pattern (brisk, non-brisk, absent), intensity (scanty, moderate, dense), and topography (peripheral, central, or both). A brisk versus absent TIL response was associated with reduced risk of melanoma-related death (HR, 0.43; 95% CI, 0.28-0.68). Furthermore, a Cox multivariate analysis controlling for thickness, ulceration, mitotic rate, age, and site, identified TIL response as a significant independent predictor of melanoma-related death ($P < .001$). Dense and diffuse TIL responses were observed in 5.5% of melanoma patients, and none of these patients exhibited distant metastases or died during the course of the study.

The other study Dr. Spatz discussed looked at whether the accumulation of antigen-presenting cells in melanoma sentinel lymph nodes (SLNs) is beneficial. A prior study showed that accumulation of DC-Lamp⁺ DCs in SLNs was associated with absence of metastasis in downstream non-SLNs.¹ In the pilot study Dr. Spatz described, CD123⁺ pDC density was decreased in melanoma-positive SLNs versus negative SLNs ($P = .003$), the density of mature DC-Lamp⁺ DCs was significantly increased in melanoma-positive SLNs without versus with distant metastases at 3 years ($P = .001$). The SLN is a window to scrutinize the immune response of the patient, Dr. Spatz said, and therefore can be used to evaluate patient's immune response and, perhaps, to predict response to interferon- α or other immunotherapies. In this study, which represented a subset of patients enrolled in the EORTC 18991 phase 3 trial, peripheral blood samples from 299 patients were depleted of leukocytes and remaining cells were stained for melanoma markers and evaluated by flow cytometry. Approximately a third (109/299; 36.5%) had at least 1 sample by RT-PCR analysis, 5.6% at randomization and 30.7% subsequently. A time-dependent Cox model (corrected for lead-time bias) showed RT-PCR positivity versus negativity was a significant predictor of shortened DMFS (HR, 2.23; 95% CI, 1.40-3.55; $P < .001$). However, these results were comparable in the 2 treatment groups, indicating that RT-PCR assessment was not predictive of treatment outcome. The identity of the circulating melanoma cells is unclear at this time, but initial analyses indicate the circulating cells express stem-cell associated genes and can be differentiated into at least groups by differences in CD133 expression.

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Reference

1. Movassagh M, Spatz A, Davoust J, et al. Selective accumulation of mature DC-Lamp⁺ dendritic cells in tumor sites is associated with efficient T-cell-mediated antitumor response and control of metastatic dissemination in melanoma. *Cancer Res.* 2004;64:2192-2198.