Update on Molecular Testing for Melanoma

BY MARK FUERST

SAN FRANCISCO—Large-scale next-generation sequencing may soon become available for melanoma, a prospect that several speakers here at the American Academy of Dermatology Annual Meeting said they were very much looking forward to.

Next-generation sequencing has the potential to revolutionize oncology through the classification of tumors and identification of biomarkers that can predict response to individualized therapy. A few molecular biomarker-based therapies are now available, including vemurafenib in BRAF-mutated melanoma.

However, the implementation of genomic medicine in melanoma is not that simple. As tumors are treated, a variety of acquired genomic alterations may emerge. Melanoma treated with BRAF or MEK inhibitors has been shown to acquire BRAF amplifications and downstream alterations that lead to reactivation of the MAP kinase pathway, an essential driver of melanoma mutations.

Jeffrey North, MD, Assistant Professor of Dermatology at the University of California at San Francisco School of Medicine, noted that cancer-promoting events in melanoma include mutations, epigenetic modifiers, and chromosome instability. "We are able to sequence genes for melanoma mutations and provide targets for therapies," he said.

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"At UCSF, we now use a panel of more than 300 genes on any tumor, including BRAF and other melanoma genes, as a therapeutic guideline. Many genes have drugs that target them. With the whole panel, we now may pick up five actionable melanoma targets," he said.

Although the patients with melanoma that most oncologists see already have an established diagnosis, it is still important to confirm the diagnosis before starting treatment, he said. Many oncologists use sequencing panels to look for BRAF mutations, and with a wide genomic diversity in melanoma,

Broad-Spectrum Analysis

The new trend is to use broad-spectrum analysis of the whole genome in next-generation sequencing. At the moment, UCSF researchers primarily use next-generation sequencing for gastrointestinal biopsies to detect colon cancer, but will begin to

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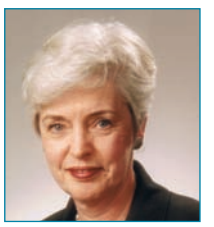
member of the MSKCC Genitourinary Oncology faculty and Director of MSKCC's Medical Oncology/Hematology Fellowship Program and the Advanced Oncology Fellowship Program. His research focus is on the development of novel treatments for patients with genitourinary cancers.


JOSE ANGEL SANCHEZ, MD

Jose Angel Sanchez, MD, hematologic oncologist at Hospital Escuela at the University of Honduras, will receive the Humanitarian Award. He is being recognized for personifying ASCO's mission and values by going above and beyond the call of duty in providing outstanding patient care through exceptional service and leadership. He has volunteered for the International Cancer Corps/Health Volunteer Overseas, which partners with oncologists and oncology nurses to discuss the needs of patients and health care providers in Honduras and ultimately improve patient care.

Mary Lou Smith, MPH, MBA, JD, longtime cancer patient advocate, will receive the Partners in Progress Award. In 2003, Smith co-founded the Research Advocacy Network (RAN) to bring patients and researchers together to ensure that patients' voices are part of clinical research. Smith is also Co-chair of the ECOG-ACRIN Cancer Research Group's Cancer Research Advocates Committee and a member of the National Cancer Institute Board of Scientific Advisors.


MARY LOU SMITH, MPH, MBA, JD

And the following ASCO members will be recognized with the Fellow of the American Cancer Society of Clinical Oncology (FASCO) distinction for extraordinary volunteer service, dedication, and commitment to ASCO:

- **Kathy S. Albain, MD**, Professor of Medicine at Loyola University Chicago Stritch School of Medicine and Director of the Breast Clinical Research and the Thoracic Oncology Program at Loyola's Cardinal Bernadin Cancer Center;
- **Craig Earle, MD**, Professor of Medicine at the University of Toronto, Director of the Health Services Research Program for Cancer Care Ontario and the Ontario Institute for Cancer Research; Senior Scientist at the Institute for Clinical Evaluative Sciences in Toronto; and Scientist at the Odette Cancer Centre at Sunnybrook Health Sciences Centre;
- **Roscoe F. Morton, MD, FACP**, partner at Medical Oncology and Hematology Associates of Iowa, Clinical Assistant Professor at the University of Iowa College of Medicine-Des Moines;
- **Lori J. Pierce, MD**, Vice Provost for Academic and Faculty Affairs and Professor of Radiation Oncology at the University of Michigan Medical School;
- **Lillian L. Siu, MD, FRCPC**, Senior Medical Oncologist at Princess Margaret Cancer Centre and Professor of Medicine at University of Toronto;
- **Eric J. Small, MD**, Professor of Medicine and Urology, Chief of the Division of Hematology and Oncology, and Director of Clinical Sciences at Helen Diller Family Comprehensive Cancer Center at the University of California, San Francisco; and
- **Sandra M. Swain, MD, FACP**, Medical Director of the Washington Cancer Institute at the MedStar Washington Hospital Center, a Professor of Medicine at Georgetown University, and Adjunct Professor of Medicine at F. Edward Hebert School of Medicine.

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sequence other tumors later this year, North said.

"Although the cost is expensive—from $2,000 to $3,000—we could use next-generation sequencing for any melanoma patient with advanced, stage 4 cancer, or possibly stage 3 disease. It expands the range of treatment of cancers, including melanoma, that can have multiple mutations."

Another speaker, Klaus Busam, MD, Director of the Dermatopathology Service at Memorial Sloan-Kettering Cancer Center (MSKCC), noted that other institutions, such as the University of Texas MD Anderson Cancer Center and MSKCC, are also investigating next-generation sequencing. "At Memorial Sloan-Kettering, we use next-generation sequencing routinely for mutation analysis of tumors for targeted therapies. This is standard not just for melanoma but for other cancers as well."

For some melanomas, physicians cannot tell what type of mutation the patient has. "This is a view into the future for the impact of analysis. If next-generation sequencing is developed further, it could be a nice way to get a look at both mutations and genomic aberrations," he said.

Types of Molecular Diagnosis

North presented an overview of the available types of molecular diagnosis for melanoma, including:

- **Next-generation sequencing:** DNA sequencing and mutation analysis for BRAF, NRAS, GNAQ/GNA11, and C-KIT genes can guide therapeutic decision-making, but it is not helpful as a diagnostic test. The cost for next-generation sequencing is $500 to $1,000 per gene.
- **Fluorescence in Situ Hybridization (FISH)**—an easy, quick test, but requiring pre-knowledge of targets. It has a sensitivity of 80 to 90 percent and a specificity of 90 percent. Typically, four to six probes are used, with a price of $280 to $550 per probe, for a total cost of $1,500 to $3,000. "Depending on the type of tumor, FISH helps clear the waters, but it has a false-negative rate of about 15 percent. FISH does not exclude melanoma from the differential diagnosis."
- **Comparative Genomic Hybridization (CGH)**, which in melanocytic melanomas shows that virtually all (96%) of melanomas have genomic aberrations. In addition, 13 percent of nevi have genomic aberrations. The cost of CGH is approximately $1,800 to $3,500.
- **Gene-Expression Profiling (GEP)**: Myriad Genetics' myPath Melanoma GEP test uses 23 gene sequencing, with a 90 percent sensitivity and 91 percent specificity. The test identifies the primary tumor only and is available only through an early-access program. The price is $1,500 to $7,000. "MyPath is purely a diagnostic test. We need to see more data on ambiguous tumors," North said.

Molecular tests are warranted for melanocytic tumors with histopathologic ambiguity. So which test should clinicians order? "Array CGH provides a representative view of all chromosomes and is the first choice for most ambiguous cases," he said.

"FISH targets foci on the genome with single-cell resolution and is good for small samples or heterogeneous tumors—for example, a melanoma arising in a nevus. The value of GEP is yet to be determined. FISH and CGH have true data behind them. We need to figure out how to incorporate all of these tests into our clinical practice. Results do affect management with diagnostic and therapeutic testing."

'Paradigm Shift'

Mohammed Kashari-Sabet, MD Professor in the Department of Dermatology at UCSF, pointed to the "paradigm shift" in the therapy of metastatic melanoma: "Novel immune and targeted therapies have revolutionized therapy, and are actively being investigated in the adjuvant setting. Immunotherapy produces significant prolongation of survival with acceptable toxicity. In the frontline setting, there is rapid tumor regression. This is no longer reserved for low-burden disease. We have shifted treatment from IL-2 and interferon to ipilimumab and PD-1 inhibitors."

Targeted therapies produce rapid responses, but acquired drug resistance limits its long-term efficacy. "Combinatorial therapy is the way to go for targeted therapy, and possibly for immunotherapy," he said.

"We need to tie diagnostics tests with therapy. The value of a prognostic test is minimal. With the availability of new immunotherapy approaches, we need tests to go from prognostic to predictive. A great majority of melanoma patients benefit from therapy with BRAF inhibitors. If we find a mutation, we need to tie it to clinical benefit. It is critical for tests to identify patients who can benefit from therapy."

He added: "Incorporating GEP could transform therapy in advanced melanoma. We eagerly await the results of GEP tests in the adjuvant setting."

Copy Number Changes

Another speaker, Iwei Yeh, MD, Assistant Professor of Dermatology at UCSF, explained that tumors have genomic instability and accumulate mutations, which translates into copy number changes.

"We can detect copy number changes by FISH or CGH, and 95 percent of melanomas have copy number changes. We use CGH to assess genomic stability of tumors in melanoma. Next-generation sequencing can identify BRAF fusion proteins in melanocytic tumors. Patients with BRAF fusion proteins respond to sorafenib."

She added: "We are not using next-generation sequencing for diagnosis. Potentially, in the next year or two, we may move in that direction. We may be able to identify copy number alternations as a platform for diagnosis to identify mutations."

Busam questioned the extent of copy number gains detected with next-generation sequencing: "Is our testing sensitive enough?" he asked. "For problematic cases, what do we do? I had a FISH-negative melanoma patient, and sent biopsy material to Myriad for GEP analysis. The report came back that the biopsy was normal, but the lesion still looked atypical. So what is the best test? GEP is not as developed as cytogenetic testing at this point."

He added: "GEP tests for diagnosis correlate with consensus diagnosis in 90 percent of cases. These tests have not yet been adequately proven for outcomes prediction."

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> —*Mohammed Kashari-Sabet, MD*