Aspiring cancer diagnostic

By Michael J. Haas, Senior Writer

Massachusetts researchers have developed portable NMR technology that uses fine needle aspirates to help scientists diagnose cancer more quickly and accurately than is possible with conventional tumor biopsies.¹ Although the technology could confirm the results of a biopsy or provide preliminary evidence that a biopsy is warranted, it is unlikely to replace histological analysis of biopsies as the diagnostic standard because it does not involve visual inspection of the sample to confirm the presence of cancer cells.

Biopsies rely on histological analysis and microscopic inspection of a sample of suspect tissue. The tissue sample is taken percutaneously with a large-gauge needle in core biopsy procedures or with a fine-gauge needle in a less invasive procedure called fine needle aspiration. The latter has a lower risk of complications, such as excessive bleeding, but yields a smaller amount of tissue that limits the number and total amount of markers available for histological analysis.

In addition, fine needle aspirates have inconclusive or false-negative results in up to 25% of cases. Furthermore, methods of analyzing core biopsies or fine needle aspirates can take days to complete and thus cannot be performed at the point of care.

To overcome these limitations, a team led by Ralph Weissleder and Cesar Castro sought to develop technology that could use fine needle aspirates to diagnose cancer rapidly and accurately in the clinic.

Weissleder is a professor of systems biology and radiology at **Harvard Medical School** and director of the Center for Systems Biology (CSB) at **Massachusetts General Hospital** (MGH). Castro is a physician at Massachusetts General Hospital Cancer Center and a member of Weissleder's CSB lab.

The group's technology involves incubating fine needle aspirates with magnetic nanoparticles covalently linked to antibodies² to tag selected marker proteins in the tissue sample. Next, the tagged sample is put into a portable NMR (microNMR) analyzer for quantitative detection of the markers. The 10 cm by 10 cm analyzer weighs about as much as a laptop and can be controlled with a smartphone.

In 2008, the team described a prototype of the microNMR technology that distinguished breast cancer cell lines expressing epidermal growth factor receptor 1 (EGFR1; HER1; ERBB1) and HER2 (EGFR2; ERBB2; neu) from normal fibroblasts.³ Further refinements of the technology allowed detection of up to three cancer markers in fine needle aspirates from mice with xenograft breast or prostate tumors.⁴

In a new paper published in *Science Translational Medicine*, the team showed that a third generation of the technology could detect multiple

markers in fine needle aspirates to diagnose cancer in humans.

The team used microNMR to detect 11 cancer markers in 50 patients who had suspected breast, lung, pancreatic and other abdominal tumors and determined which combination of markers gave the most accurate diagnoses. A 4-protein signature consisting of mucin 1 (MUC1; CD227), EGFR1, HER2 and epithelial cell adhesion molecule (EpCAM) diagnosed patients with 96% accuracy and a turnaround time of about 1 hour per patient. By comparison, core biopsies in the same patients had 84% accuracy and a mean turnaround of 3 days.

The team, which included researchers from the **Harvard School of Public Health** and **Massachusetts Institute of Technology**, validated its findings by using the 4-protein signature to diagnose an independent cohort of 20 patients with 100% accuracy.

"We're excited that the third iteration of the device maintained the same level of accuracy and speed that we found in cell lines and mouse cancer models," Weissleder told *SciBX*.

"I like the underlying concept of the technology—combining an ELISA-like assay with analysis of multiple markers—and it has some interesting potential uses in the clinic," said David Rimm, professor of pathology, director of medical studies and director of pathology tissue services at **Yale School of Medicine**.

"Ultimately this is ultrasensitive protein detection technology" that works with small tumor samples, said Douglas Clark, professor of pathology and oncology at **The Johns Hopkins University School of Medicine**. The ability to get information from small samples is important because "imaging technology lets us detect lesions when they are early and small, and we don't want tumors to grow too large before we can sample them," he said.

Snap decisions

Rimm thinks the microNMR technology could be used "on top of the standard histological and morphological evaluation to provide added confidence in the diagnosis."

Clark, who is also founder and CSO of **BioMarker Strategies LLC**, added that the technology could be used for triage in point-of-care settings.

"For example, if a patient comes into the emergency room with a lump, you would want to know whether it's a tumor or an abscess," he said. "The microNMR technology would have enough accuracy in that setting to help you decide whether to schedule a core biopsy or begin antibiotic therapy."

Clark said he was also interested to know whether the technology would work in conjunction with BioMarker's SnapPath platform. SnapPath utilizes live tumor cells from fine needle aspirates to identify the dominant signaling pathways in an individual tumor and thus predict which targeted therapy will be most effective in that tumor.

The microNMR technology could serve as an ultrasensitive detector of the phosphoproteins that SnapPath uses as a readout of signaling pathway activity, he said. BioMarker plans to begin a pilot study of SnapPath in patients with solid tumors later this year.

But Rimm said the variability inherent in fine needle aspiration would make it challenging to develop the microNMR technology as a primary cancer diagnostic.

“Aspirates essentially use a needle to scrape some sample from the suspect site and you hope you’re getting tumor cells,” he said. “But you can only confirm whether you have tumor cells or normal cells by examining part of the sample under a microscope” during histological analysis—which the Weissleder-Castro team did not do.

“They seem to be suggesting that they can use this technology to make the primary diagnosis without ever seeing the cells,” which would present a huge barrier to clinical development, he said.

Rimm also said that although the small patient cohort the team used to evaluate the markers and select the four-protein signature was acceptable, the number of patients in the team’s validation study was too small for the results to be meaningful.

“The validation group should be at least twice the size of the training group—preferably three or four times larger,” he said. “Instead, their validation group was half the size of the training group,” which made it difficult to compare their technology with conventional methods.

Clark also pointed out that the four proteins in the signature “are not cancer-specific molecules but instead epithelium-specific molecules” that are indicative of cancer when they occur out of place—such as in the abdominal cavity or liver. But because the four proteins also occur in normal tissue, he cautioned against “assuming their signature is a litmus test for cancer.”

Future aspirations

Castro expects microNMR to complement rather than replace biopsy.

For instance, in the earliest stages of diagnosis—such as when imaging shows a suspicious result—the technology could help determine whether performing a core biopsy by taking a larger tissue sample with a more invasive procedure is warranted, he said.

The technology could also confirm the results of a core biopsy, Castro said.

Additionally, “we envision using this device to interrogate how relevant cancer markers change over the course of treatment or to

steer patients toward clinical trials of therapies that target a marker that may be elevated in their tumors,” he said.

The Weissleder-Castro team plans to begin testing the microNMR technology in a larger patient population this year. “We will work with our statisticians to arrive at an ideal study size based on our previous findings,” said Castro.

Weissleder added that the group is also working on “customization of the technology and selected markers to query specific subsets of cancer such as ovarian cancer.”

MGH has patented the technology. That IP is unlicensed, and Weissleder said the team has sufficient funding to proceed with future testing.

In addition to developing the microNMR technology as a diagnostic, the team is investigating undisclosed applications of the magnetic nanoparticle–antibody affinity ligands² that the microNMR uses to detect protein markers, Castro said.

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