Setting the stage for cancer

By Lauren Martz, Staff Writer

Although marketed blood-based diagnostics for leukemia are sufficiently sensitive to monitor minimal residual disease, no such tests exist for solid tumors. Now, German researchers have developed a personalized diagnostic that can stage solid tumors and detect minimal residual disease following therapeutic intervention.¹ However, the approach requires the design of a unique assay for each patient, which may limit its commercial potential.

Many tumors are characterized by the presence of specific amplified genomic regions. Such regions have been reported to exist in 91% of breast cancers, 82% of glioblastomas and 75% of colon cancers.²

The sites at which single amplified genomic regions attach to each other are called amplicon fusion sites (AFSs). They are specific to individual tumors and detectable using PCR.

In a paper published in *The Journal of Clinical Investigation*, Axel Weber and colleagues at the **University of Leipzig Children's Hospital** reported a protocol that exploits the tumor specificity provided by AFS sequences to detect minimal residual disease in patients with solid tumors. The team's method involves sequencing the fusion sites from initial tumor samples and developing PCR primers to detect cells that have the same genetic characteristics as the primary tumors in unrelated organ and tissue samples from the same patient.

As proof of concept for the approach, dubbed AFS-PCR, the group took three neuroblastoma cell lines and 40 primary neuroblastoma samples with genomic amplifications near the gene *MYCN* (*v-myc myelocytomatosis viral related oncogene neuroblastoma derived*; *NMYC*). The team used high-resolution tiling arrays, which include both coding and noncoding DNA, to map and sequence AFSs.

Using the AFS sequences, Weber's group designed PCR assays specific for each sample. In culture, the assays detected 1 *MYCN*-amplified neuroblastoma cell in up to 8×10⁶ neuroblastoma cells that lacked the amplification. The assays were specific for cancer cells with the same genomic amplifications as the primary tumors and did not generate any false positives when tested on control DNA isolates from human placental tissue.

In patient samples, the assays detected tumor cells in bone mar-

row, peripheral blood and residual tumor tissue compared with no signal in control placental tissue.

"Once established from tissue of the primary tumor site, an individual AFS-PCR test offers a powerful tool to track tumor cells or tumor cell DNA in potential metastatic sites of any tissue origin as well as in bone marrow aspirates, in peripheral blood samples and even in cerebrospinal fluid, urine or any other sample of the patient," said Weber.

"AFS-PCR could significantly improve tumor staging at the time of initial diagnosis, the monitoring of a response to therapy by quantification of tumor cells in subsequent samples and the early detection of minimal residual disease or recurrent disease over time," added Weber, who is a medical doctor in the Department of Pediatric Oncology, Hematology and Hemostaseology at the hospital.

Howard Urnovitz, CEO and CSO of **Chronix Biomedical**, said diagnostics using AFS could provide more information about a given cancer than SNPs, which are germline biomarkers that might be more valuable for cancer risk assessment.

Chronix is running pilot trials of its breast and prostate cancer serum DNA tests, which detect cell-free circulating DNA associated with the cancers.

(Individual) results may vary

The AFS-PCR approach is broadly applicable to solid tumors, but two key drawbacks could impede the technology's commercialization.

First, the technique only is suitable for tumors with at least one identified amplified genomic region. Previous studies have shown that the majority of cancers contain amplified regions, but sequencing a patient's primary tumor, which is expensive, remains necessary to determine whether the regions are present.

Moreover, Weber noted that even if an amplified genomic region is present in a tumor, the ability to develop a tumor-specific assay isn't guaranteed.

"The method was described with high-copy amplified genomic regions," Weber said. While the technique should work in cancers

with low copy numbers of amplified genome regions, he said "it can be difficult to identify a PCR primer" in such cases.

The second drawback is that the individualized nature of the test means an assay needs to be developed for each patient.

"What strikes me as not very pragmatic is that this strategy requires developing individual assays, which might not be able to be clinically employed and could be very expensive," said Achim Plum, SVP of corporate development at **Epigenomics AG**. "Using tiling arrays is a pretty costly approach."

Epigenomics markets Epi proColon, a PCR diagnostic that detects plasma levels of methylated *septin 9* (*SEPT9*), a DNA methylation marker that is found in more than 90% of colon cancers and can be tested in routine blood plasma samples.

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—Axel Weber,
University of Leipzig
Children's Hospital

Weber agreed that “at the moment, the costs of our procedure might be too high for comprehensive use.” However, the cost of tiling arrays is coming down, which could make the method more affordable in the near future.

Plum suggested that there could be other ways to lower cost. “If they pinpoint the region of the cancer genome in which they are interested, it could improve the cost and ease of use compared with analyzing the whole genome in a tiling array,” he told *SciBX*.

Plum was less sanguine about the prospects for speeding up the amplified genomic region PCR tests.

“A diagnostic that can be performed on an easily accessible body fluid sample, such as blood or urine, could be of great help in cancer patient management. A drawback of the AFS-PCR strategy is that it first requires some sort of tissue sample from the tumor to develop the assay. This involves additional logistics and sometimes the samples may not be available or suitable, such as if they do not yield DNA of sufficient quality for a tiling array. The process is also very involved, and one would need to develop the assay, validate it and run a PCR for every patient.”

Amplifying potential

Despite cost and practicality issues, Weber thinks his team's strategy could offer unique benefits to patients with solid tumors.

“For neuroblastoma and many other solid tumors, no strategies exist that are routinely used like the minimal residual disease strategies for leukemia patients,” he told *SciBX*. “I think in the near future, next-generation sequencing applications could solve a lot of problems—every tumor patient will have access to his/her personal

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—Achim Plum, Epigenomics AG

minimal residual disease markers. Because no effective strategies are in routine use, we were not able to compare our strategy to others.”

Plum acknowledged the potential for addressing an unmet need.

“If they are able to prove that this provides a significant medical benefit unable to be attained by available techniques, it could be possible that the cost and time investments are warranted,” he said.

Weber said his team's next steps include an ongoing study of AFS-PCR in a larger cohort of patients and studies to show that the ability to detect minimal residual disease provides information useful to the patients and clinicians. Specifically, he said the group needs to correlate patient prognosis with the presence of minimal residual disease detected by the method and show that the technique can be used for clinical decision making.

Weber said a patent application has been filed in Germany for the method and the IP is available for licensing.

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