BioFluidica Applying Liquid Biopsy to Detect Leukemia While Reducing Biopsies

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NEW YORK (GenomeWeb) – Fresh off being awarded several grants by the National Institutes of Health, startup BioFluidica has begun three different clinical studies to detect exosomes, cell-free DNA, and rare cells in patients' bloodstreams to identify acute lymphoblastic leukemia (ALL) and other cancer-related diseases.

The San Diego, California-based startup is also exploring the use of its Liquid Scan platform in the non-invasive prenatal testing space by extracting and detecting neonatal cells from a mother's bloodstream.

In order to detect and monitor ALL, clinicians normally perform tissue biopsy extractions from a patient's bone marrow. However, the procedures are often expensive, invasive, and can only be performed up to a few times a year because of the pain they cause patients.

BioFluidica believes its liquid biopsy technology may be able to reduce the number of bone marrow biopsies and improve earlier detection of relapse in patients and overall outcome.

BioFluidica CEO and Cofounder Rolf Muller explained that the firm's technology uses microfluidic chips with sinusoidal shaped channels and flow cytometry to collect targeted cells and other molecules. Researchers can program the capture channel surface and control the flow rate using robotic software.

Each channel on the microfluidic chip also contains specific antibodies that target different types of circulating tumor cells, allowing researchers to wash and release the cells from the channel's surface. While BioFluidica's platform currently produces results in about two hours, efforts are under way to whittle the sample-to-result time to 30 minutes.

The NIH's National Cancer Institute awarded BioFluidica three separate Small Business Innovation Research grants — one Phase I grant and two Phase II grants — last summer to develop assays based on the three biomarkers.

According to Muller, BioFluidica is collaborating with up to 12 different academic groups between the three studies. He noted that the firm will also use patient samples from its own ongoing studies on rare cells types, isolating cells and exosomes using fraction extraction.

In the first study, which started in July 2018, BioFluidica is developing a microfluidic affinity purification device to isolate disease-associated exosomes for downstream molecular profiling. The NCI awarded the firm a $239,139 SBIR Phase II grant for the study, which it expects to conclude by July.

As part of the study, BioFluidica will collect blood samples from 30 healthy donors and samples from 60 breast cancer patients. The firm aims to demonstrate the microfluidic

device's ability to distinguish between cancerous and non-cancerous biological samples from the Kansas University Medical Center's biorepository.

In the second study, which began in September 2018, BioFluidica is building an automated microfluidic chip-based platform that applies solid phase extraction to isolate cell-free DNA fragments from a patient's plasma sample. The NCI awarded Biofluidica a $286,548 SBIR Phase II grant for the study, which the firm expects to complete in September.

BioFluidica's researchers plan to show the microfluidic chip's clinical utility by enriching cfDNA isolated from plasma spiked with cfDNA containing mutant alleles of several oncogenes. Muller said that the firm will also use the grant for SNP analysis and other downstream purposes.

In the third study, which also began in July 2018, BioFluidica is applying a minimal residual disease assay — previously developed to track acute myeloid leukemia (AML) patients following stem cell transplantation — to analyze peripheral blood and search for circulating leukemia cells (CLCs) in ALL patients. The NCI awarded BioFluidica an SBIR Phase II grant of $1.7 million over four years for the clinical study, with an initial grant of $348,768 for the first year of research.

For this effort, researchers will collect blood samples from 30 pediatric patients. The MRD assay will identify CD19 surface antigens expressed by B-type ALL CLCs by binding them to anti-CD19 antibodies immobilized within the microfluidic device.

As part of the study, the team will expand and develop the CLC microfluidic test to monitor MRD and potential relapse in B-ALL pediatric patients. In addition, researchers can reprogram the assay to search for other pediatric oncological diseases like T-cell ALL. According to Muller, the firm has now switched to the second part of the study, where its researchers are now screening patients with leukemia.

"Since the whole field of medicine is going toward predictive or personalized medicine, where the evaluation or disease diagnosis is being done based on molecular markers, these kinds of biomarkers become very important," Muller explained. "So all of a sudden, you have access to all three different biomarkers from the same sample prep, and that is a huge capability increase over any kind of current technology."

Ultimately, BioFluidica hopes to use the studies to develop products that will "provide high specificity and high throughput, and [act as] an automated, cost-effective, and commercially viable technology," Muller said. He envisions the firm's liquid biopsy platform initially acting as a support tool for federal research and clinical trials, where researchers can study all three biomarkers at the same time. By monitoring ALL patients at risk for relapse, Muller believes the tool could also help improve patients' overall outcomes.

In addition to circulating tumor cells, BioFluidica aims to use its Liquid Scan platform to identify rare cells such as trophoblastic fetal cells. In a preprint poster presented at the 2018 Advanced Prenatal Molecular Diagnostic Conference in November, BioFluidica demonstrated the results of a proof-of-concept study to detect and extract neonatal cells from maternal blood cells.

Jiri Sonek, a clinical professor at Wright State University and second author of the unpublished study, said that his team wanted to identify and capture cultured trophoblasts with a high degree of efficiency.

The researchers first passed 1 milliliter of preserved maternal blood from healthy donors — at concentrations of 1,500, 100, 50, and 8 trophoblastic fetal cells per milliliter — through the microfluidic chips "coated with capture antibodies targeting trophoblasts." They then

eluted the captured cells by "using an enzymatic method in an automated platform" and stained them using immunocytochemistry for the trophoblastic markers.

Overall, the researchers found the chip had at least a 75 percent capture rate among the different concentrations of trophoblasts per milliliter, and the team was able to confirm the presence of fetal cells and quickly isolate trophoblast cells from a maternal blood sample using BioFluidica's platform and a targeted capture agent.

In future studies, BioFluidica will examine additional cell surface antigens to target for microfluidic capture, optimize the system, and highlight its preclinical ability. The firm aims to develop a fully integrated and automated platform to enrich trophoblast cells with high recovery and reproducibility. According to Sonek, the team will also develop tools to help capture other types of fetal cells.

However, Sonek said that BioFluidica's biggest hurdle will be to improve the platform's ability to collect as many cells as possible. In future iterations, the team will also need to identify neonatal diseases that have a genetic mutation or abnormal number of chromosomes.

"We're first looking at more of the common chromosomal number problems, such as trisomy 21, trisomy 18, and trisomy 13, and then we can potentially diagnose single gene disorders," Sonek explained. "In the long run, when you actually have this cell, you can diagnose potentially any diseases that has a DNA abnormality."

Sonek also highlighted that his team has since used Liquid Scan to detect trophoblast cells in patients as early as nine weeks and as late as 38 weeks of pregnancy.

As BioFluidica continues to develop its liquid biopsy assays for a wide range of cell-based and cell-free applications, it will enter a busy market filled with competitors offering their own cell-free and cell-based liquid biopsy methods for early cancer detection.

In terms of the NIPT space, UK-based Angle is seeking to enter the market with its Parsortix cell-based enrichment system. The firm believes its cell separation system, currently used in oncology, can extract rare fetal cells from maternal blood to detect genetic disorders. In addition, Danish firm Arcedi Biotech published a validation study on its technology for isolating circulating fetal cells from maternal blood in 2017.

BioFluidica Chief Operating Officer Judy Muller-Cohn emphasized that BioFluidica's Liquid Scan stands out from the competition due to its ability to capture all three biomarkers on a single platform.

In addition, Muller noted that the platform minimizes all sources of biomarker loss during the sample prep stage. He said the platform can screen "magnitudes of more cells" because it saves most cells during isolation.

Muller said the platform does not require separate preprocessing steps such as adding a buffy coat or performing red blood cell lysis; instead, researchers can directly add whole-blood samples onto the chip for enrichment and downstream analysis.

"We use a proprietary preservative to be able to ship blood samples at ambient temperatures for three days, extending the reach of the technology to global applications," Muller-Cohn said. "The cells are kept alive and therefore can be used in all types of downstream applications."

BioFluidica currently has facilities in San Diego; Lawrence, Kansas; and Research Triangle Park, North Carolina. The firm is also seeking CLIA validation for its Liquid Scan platform. According to Muller, BioFluidica has clinically validated the technology for a variety of

cancers including lung cancer, breast cancer, prostate cancer, pancreatic cancer, colorectal cancer, bladder cancer, and AML.

Muller-Cohn noted that BioFluidica currently offers a fee-for-service to isolate circulating cells, and it expects to offer end users a laboratory-developed test version of the assay by 2020. In addition, the firm plans to eventually file for 510(k) approval from the US Food and Drug Administration. While Biofluidica has yet to determine the price of the different assays, Muller-Cohn believes the cost will be "definitely below the $5,000 to $16,000 that it costs to get a lung or AML biopsy."

In addition to improving its liquid biopsy platform, BioFluidica has been expanding its commercial partnerships across the US.

According to Muller, BioFluidica has signed its first contract with an undisclosed pharma partner and made its first round of sales in 2018. The firm has also established supply chain partners for both its microfluidic chip and instrument development, which Muller said will help the firm leverage clinical trial processing out of its core facilities in the US and eventually move into a decentralized supply system later this year.

Muller also noted that BioFluidica is now debating whether to open facilities in both the Asian and European markets. While the firm has decided that it would most likely open a center in Japan, Muller said that the firm's board is still considering which European country to possibly establish a regional branch.