Looking for Cancer Clues in the Bloodstream

Next-generation sequencing has the potential to open new doors for detecting blood cancers.

Introduction

Blood cancers affect over 1 million people in the United States alone.[1] Early detection is critical for any type of cancer, but many blood disorders—such as leukemia, lymphoma, and myeloma—can be hard to diagnose. Standard tests have included phenotypic and morphological examination of cells, proteins, and chemical markers in the blood. Today, new technologies enable physicians to send suspicious samples to a laboratory for genetic and biomarker analysis. Genetic testing provides a closer look at what is occuring at the molecular level.

However, current genetic analysis techniques for cancer can be limited, leading to potential delays in diagnosis. The effectiveness of certain methods can also vary depending on the cancer being assessed and the origin of the blood sample.

Scientists at Cancer Genetics, Inc. in Rutherford, New Jersey are developing new methods to detect cancer by adding next-generation sequencing (NGS) to its collection of genomic analysis approaches. Performing targeted genomic analysis on an NGS system, they are collaborating with clinicians, pharmaceutical companies, and research labs to identify important genomic alterations in blood cancers.

iCommunity spoke with Narasimha Marella, PhD, Charles Ma, PhD, and Weiyi Chen, PhD, HCLD (ABB) at Cancer Genetics to learn how they use sequencing to detect key genomic changes.

Q: What kind of genomic assays do you run?
Weiyi Chen (WC): We use Sanger sequencing, pyrosequencing, real-time PCR-based assays, aCGH, and now have included NGS in the mix.

Q: What genes are included on your targeted genomic panel for leukemia?
WC: The panel detects genes associated with myeloid leukemia, such as acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), and myeloproliferative neoplasms (MPN). Specifically, 3 of the genes on the panel—*NPM1*, *CEBPA*, and *FLT3*—have been identified by the World Health Organization (WHO) for prognostic evaluation in AML.[2] It's a comprehensive, up-to-date panel. I compared the panel with publicly available information and it contains many of the recently identified genes, like *CALR*, and prognostic genes for MDS that have been suggested in the National Comprehensive Cancer Network (NCCN) guidelines.

The panel is also flexible. For clinical trials or research, we usually want to look at all the genes. However, depending on what a lab needs, our customers can choose a smaller set of genes or expand to the full panel.

Q: Were you part of the consortium that developed the panel?
Narasimha Marella (NM): We were part of the Myeloid Consortium and worked with a few other groups to identify genes that might be clinically relevant for the disease.

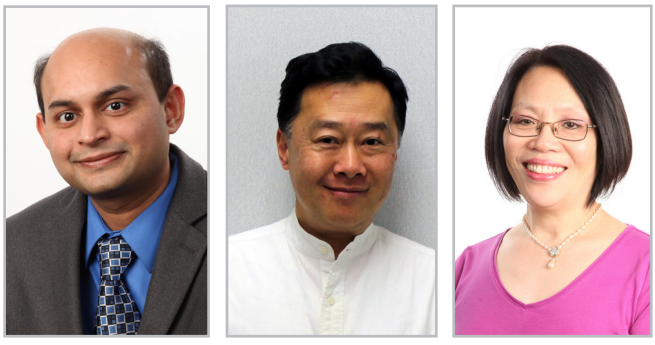
WC: We were also just getting started with sequencing. We thought it would be a great opportunity to get involved with the panel and get started with NGS.

Q: What drove your decision to start using NGS?
WC: Every week we receive samples for Sanger single-gene sequencing tests. These are low sensitivity, labor intensive, high-cost tests. By looking at mutations with targeted NGS, we can improve the detection of myeloid disease-associated gene mutations, because we're getting higher sensitivity. NGS also enables us to provide a better service, with a panel of genes that can be expanded in the future.

Cytogenetics is one of the traditional methodologies used to determine whether someone is positive or negative for the disease, but in reality, only about 50% of people with myeloid disorders have a cytogenetic abnormality. The remaining 50% are cytogenetically normal, so cytogenetic testing can't detect their disease status. However, about 80–90% of the patient population could harbor a mutation.

Today, more studies use molecular markers to stratify people into different groups for clinical trials. For example, in MDS, if a person has a 5q deletion, they might be classified into a lower-risk group. However, if they have a *TP53* mutation, that completely changes the prognosis. If you're only looking at cytogenetic markers, that can be misleading. You need a more comprehensive look at the molecular markers too.

Identifying mutations could also be important for treatment. Some mutations, like *IDH1* and *IDH2*, have been reported as indicators of response to treatment. In addition, some markers have been used to



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select people for clinical trials. By providing more information about what's happening at the molecular level, NGS goes far beyond what we see cytogenetically.

Charles Ma (CM): Sensitivity is also a concern. Traditionally, the limit of sensitivity for us is about 15–20% for mutations. Even that is a stretch, because chances are we're getting a very heterogeneous mixture of normal and tumor cells that is variable from person to person. Whenever we perform Sanger sequencing, we always have to ask, are we missing something? That's why we want to adopt NGS, where we can routinely achieve much higher sensitivity at around 5%.

> "By looking at mutations with targeted NGS, we can improve the detection of myeloid disease-associated gene mutations, because we're getting higher sensitivity."

Q: What other qualities did you look for in an NGS System?
CM: NGS system output and accuracy are important. We cannot afford to process one sample each time. We have to be able to multiplex many samples in one run and still get enough data to perform the analysis. Those attributes are important for research and development as well.

Q: What have you seen so far in your experience with NGS?
CM: It works! This is a brand new area that we moved into, and we thought the learning curve might be steep. We were fully prepared to fail a couple of times initially, but I was amazed that we got it to work the first time. We obtained useful sequence data even in our first run, so that gave us more confidence using NGS.

We chose a benchtop, walk-away system that is easy to use. You just start it and you don't have to watch it.

> "From the DNA library preparation to analyzable data, it takes only 2–3 days."

Q: What is the sample-to-results turnaround that you've experienced?
CM: From the DNA library preparation to analyzable data, it takes only 2–3 days.

Q: Do you see any trends in your field that might shape the future of genetic testing?
NM: Many companies that develop therapies are interested in biomarker data as early as Phase I trials. It enables them to personalize therapies and reduce drug development costs by identifying the appropriate population faster. NGS supports multiplexing, delivering such data from many more genes in less time. I think pharmaceutical companies are looking forward to using biomarker data routinely as they develop new therapies.

References

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2. Vardiman JW, Thiele J, Arber DA, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood*. 2009; 114: 937–951.

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