Predicting cancer relapse: Study finds high-throughput sequencing bests flow cytometry

May 16 2012

A study led by researchers at Fred Hutchinson Cancer Research Center has found that a next-generation, high-speed DNA-decoding technology called high-throughput sequencing can detect the earliest signs of potential relapse in nearly twice the number of leukemia patients as compared to flow cytometry, the current gold standard for detecting minimal residual disease. The results of the study, led by Hutchinson Center computational biologist Harlan Robins, Ph.D., are reported in the May 16 issue of *Science Translational Medicine*.

"The ability to predict disease relapse sooner with high-throughput sequencing would give hematologists the option to treat cancer recurrence earlier, offering a greater chance of survival. Longer term, this technology potentially also could be used to initially diagnose leukemia and lymphoma much earlier than we can today," said Robins, an associate member of the Hutchinson Center's Public Health Sciences Division and corresponding author of the paper.

For the study, Robins and colleagues compared the effectiveness of high-throughput sequencing versus flow cytometry to detect minimal residual disease in 43 patients diagnosed with acute T lymphoblastic leukemia, a type of blood cancer that is most common in children under age 7. By sequencing the patients' T-cell receptor genes before and 29 days after chemotherapy, the researchers were able to precisely measure their presence in the blood and provide a more accurate prediction of leukemia relapse.

"The high-throughput sequencing detected minimal residual disease in nearly double the number of patients than flow cytometry – 22 versus 12 patients, respectively," Robins said.

Minimal residual disease, or MRD, a major predictor of cancer relapse, is when a small number of cancer cells survive treatment and persist in patients. Until recently, MRD was undetectable.

Flow cytometry, the primary method for detecting MRD in the United States, counts the number of cells in the blood with cancer-specific protein markers on their surface. While it is considered the gold standard, flow cytometry comes with a number of limitations:

- it has been difficult to standardize across different labs because there is no standard protocol;
- the antibodies used to tag the cancerous cells are expensive;
- every cancer type requires a different test, because each malignancy is associated with a different protein marker; and
- the sensitivity of the test is low, which means it sometimes fails to recognize the presence of cancer cells.

This study – the first use of high-throughput sequencing, or HTS, to detect minimal-residual disease in a clinical trial setting – found it to be at least 20 times more sensitive than flow cytometry in detecting MRD.

"Our research indicates that HTS offers many advantages over flow cytometry," Robins said. "Since HTS can detect any pre-identified clone and is performed in a centralized lab, it consistently generates reproducible and reliable results regardless of cancer type, using the same process for disease detection and tracking. Furthermore, HTS is highly automated, cost-effective and objective, whereas flow cytometry is more time consuming, relies on the skill of the operator and is

therefore subject to human error."

The Hutchinson Center has patents pending on core technologies employed by Robins and colleagues in conjunction with high-throughput DNA-sequencing used for this study. These core technologies have been licensed exclusively to Adaptive Biotechnologies, a Seattle biotechnology company Robins co-founded that offers commercial DNA sequencing and analysis.

Robins and colleagues discovered how to adapt traditional high-throughput technology to sequence only variable regions of the human genetic code: the T- and B-cell receptors – a critical component of the human adaptive immune system. These receptors are short strands of DNA that constantly rearrange to allow the immune system to fight viruses, infection or disease.

"This discovery was critical to our understanding of how patients mount immunological responses against cancer, autoimmune disorders and infectious diseases," said Robins, whose Hutchinson Center research focuses on the genetics of the immune system – particularly how it responds to pathogens and the aging process.

Provided by Fred Hutchinson Cancer Research Center