

## MURDOCK UPDATE

### Protein signature for hepatitis C treatment response replicated

By Maggie De Pano

In 2008, MURDOCK Study investigators identified a set of proteins that can predict, in nine cases out of 10, whether a patient will respond to standard therapy for hepatitis C. In November of 2009, they validated these findings in an independent cohort of 41 patients. This brings their research one step closer to the possibility of being translated to a laboratory assay.

Chronic hepatitis C affects close to 170 million people around the world. An infection acquired primarily through blood contact, it is the leading cause of liver cirrhosis, a condition characterized by tissue scarring and the eventual loss of liver function.

Few treatment options exist for hepatitis C patients. The current standard of care includes weekly injections of pegylated interferon, a drug associated with adverse side effects such as headaches, fatigue, muscle pain, anemia, and depression. Most patients need to receive therapy for almost a year, yet only about half of them respond to this course of treatment. While discernible patterns of treatment response exist, until recently clinicians still did not have a good understanding of why some patients who receive pegylated interferon clear the hepatitis C virus and others do not.

Through the MURDOCK Study, a multi-tiered, long-term genomic study funded by a \$35 million grant from real estate developer David Murdock, researchers have worked for the past two years to understand what factors are associated with patient non-response to interferon. The proteomic signature of response identified by lead investigators John McHutchison, MD, Jeanette McCarthy, PhD, and Keyur Patel, MD, and their research team was the first major new discovery to emerge from the study.

#### Major breakthrough

MURDOCK Study investigators began their experiments in 2008 by sifting through the Duke Hepatology Clinical Research Database, a biorepository that houses detailed clinical data and biospecimens including whole blood, serum, DNA, and liver tissue samples from more than 3,100 hepatitis C patients. They selected serum samples from 30 patients, including responders and non-responders to interferon therapy, and broke down proteins in the serum into peptides, the short polymers that form when amino acids link in a defined order.



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**Jeanette McCarthy, PhD**  
Associate Professor of Community and Family Medicine

The investigators then sorted the peptides according to molecular weight and charge using mass spectrometry and worked in collaboration with Will Thompson, PhD, and Arthur Moseley, PhD, in the Duke Institute for Genome Sciences & Policy (IGSP) Proteomics Core facility to identify protein profiles that predict treatment response. Using factor modeling with software designed to analyze proteomic data, IGSP computational scientist Joseph Lucas, PhD, discovered three factors representing clusters of proteins that can predict who will respond to therapy and who will not.

In 2009, the investigators expanded their sample size to 96 patients (41 of whom participated in validation experiments) and found that the technology and statistical methodology they had applied to the original cohort also performed with a high degree of accuracy in the second sample. "This tells us that our technology is robust and reproducible, and the signature identified to differentiate between responders and non-responders may indeed be clinically valid," said McCarthy.

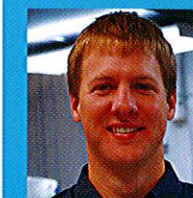
The replication of a protein signature using this type of technology represents a major breakthrough in the field of personalized medicine. "Protein mixtures are very complex. There are thousands of them and they are involved in many different types of processes," explained Thompson. "With open-platform proteomics, we do not decide ahead of time which proteins to monitor, so you can even measure different sets of proteins from the same person if you take them at different time points."

This variability makes it very challenging to validate a signature. We really took a lot of care in preparing our samples and designing our experiments to increase the chance that we could replicate our findings in an independent biological cohort, which to my knowledge has not been done before with this particular technology," Thompson continued.

The study's ultimate goal is to translate the signature into a simple blood test that can give clinicians an idea of the chances that a hepatitis C patient will respond to interferon. "With hepatitis C, it's difficult to decide whom to treat and whom not to treat," said Patel. "The treatment regimen is tedious, yet fails to clear the virus 50% of the time. A laboratory assay with strong predictive power of treatment response can give clinicians more confidence going forward with their treatment decisions."

#### Complementary discoveries

The team's findings complement another breakthrough discovery that emerged earlier from a collaboration between McHutchison and David Goldstein, PhD, director of the Center for Human Genome Variation.



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Senior Laboratory Administrator, Proteomics Core Facility

McHutchison and Goldstein recently identified the first genetic marker that can predict response to hepatitis C treatment. Their research, published in *Nature*, showed that patients who had a single letter change — a C instead of a T — in a tiny segment of their DNA near a gene called IL28B are significantly more likely to respond to interferon than those who did not have such a change in their DNA. They also found that the favorable genetic profile appeared significantly more often among white populations than it did among African populations. This explains a large part of the difference in response rates seen between African Americans and those of European ancestry.

"We now know that having this polymorphism near the IL28B gene is associated with an approximately twofold change in how patients will respond to therapy. But for patients who don't have it, there remains a significant amount of uncertainty," said McHutchison. "That's the gap we're hoping to address. The right protein signature would ideally be able to predict treatment response and therefore enhance the information provided for patients in an additive fashion to the genetic markers."

The next step is to understand the overlap that the team has encountered in validation experiments. "Sometimes you get a signature that looks like it fits into a particular protein but also overlaps with other proteins, so you need to find out if it fits into that one protein or if it represents several of them," said Patel. "We're planning to use a type of technology called multiple reaction monitoring (MRM) to target our proteins of interest and determine which among them are stable and have a high correlation with treatment response for hepatitis C." The team will also conduct further experiments to determine if their methodology and the predictive power of their technology will hold up in even larger cohorts.

