

## mRNA Profile of *Ex Vivo* Interferon- $\alpha$ Response of Interferon- $\alpha$ Treated Hepatitis C

### Patients

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The standard therapy for chronic hepatitis C is a 24- or 48-week course of the combination of pegylated interferon- $\alpha$  (IFN- $\alpha$ ) and ribavirin. Both viral factors, including viral load prior to treatment and HCV genotype, and host factors, age, sex, and ethnicity of the patient, affect the outcome of IFN- $\alpha$  treatment. Additional measures that are even more predictive of treatment response would be a valuable addition to clinical practice.

We have developed an *ex vivo* interferon treatment system to investigate the difference in response to IFN- $\alpha$  of peripheral blood mononuclear cells (PBMCs) of 27 IFN- $\alpha$  non-responders and 20 sustained responders of previous interferon treatment. Blood samples were collected in BD Vacutainer™ CPT™ tubes; PBMCs in patient's plasma were prepared and treated with 1,000 IU/ml of Intron A (Schering Corp.) for 2 hours and 6 hours at 37°C. Total RNA was extracted from each timepoint, and the expression level of 54 genes that are involved in the interferon signaling pathway and immune response were profiled using quantitative kinetic RT-PCR. Quantitative kinetic RT-PCR was employed because of greater dynamic range and improved sensitivity relative to microarray platform as well as to interrogate multiple splice variants. Candidate genes were selected from previous studies representing the IFN regulated pathways. The JAK-STAT pathway, induced by IFN binding via the cell surface receptor, has been studied extensively; multiple IFN-inducible proteins, including 2',5'-oligoadenylate synthetase, double-stranded RNA-dependent protein kinase, and Mx proteins have well-documented anti-viral activities. Most importantly, the mechanism of resistance to IFN- $\alpha$  treatment is not clear, and as a result immune response genes, such as CCL4 and CCL9, were also studied.

Using unsupervised hierarchical clustering to analyze the expression profiles in untreated PBMCs; 14/20 of sustained responders formed a cluster distinct from the cluster formed by 21/27 of the non-responders. In general, the expression levels of IFN inducible genes in sustained responders were lower than those in non-responders, perhaps due to the on-going infection in the non-responders. After *ex vivo* IFN- $\alpha$  treatment, approximately 17/20 sustained responders and approximately 18/27 non-responders, formed distinct clusters. Confounding variables for interpretation include on-going HCV infection and differential and less effective IFN- $\alpha$  treatment protocols. Additional supervised clustering analyses and statistical analyses of the correlation of mRNA profiles and clinical information will be presented. This *ex vivo* IFN- $\alpha$  treatment system may provide an opportunity to identify HCV patients who differentially respond to IFN- $\alpha$  therapy.