JOURNAL OF PROTEOMICS 116 (2015) 59–67

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jprot

# A signature for immune response correlates with HCV treatment outcome in Caucasian subjects

*Brian J. Hare*[a,*], *Eric Haseltine*[a], *Mark Fleming*[a], *Daniel Chelsky*[b], *Laura McIntosh*[b], *Rene Allard*[b], *Martyn Botfield*[a]

[a]*Vertex Pharmaceuticals Incorporated, Boston, MA, USA*
[b]*Caprion Proteomics, Montreal, Quebec H2X 3Y7, Canada*

ARTICLE INFO

*Article history:*
Received 2 September 2014
Accepted 30 December 2014
Available online 8 January 2015

*Keywords:*
Focal adhesion
Response predictor
Direct-acting antiviral
Telaprevir
Proteomic profiling

ABSTRACT

Broad proteomic profiling was performed on serum samples of phase 2 studies (PROVE1, PROVE2, and PROVE3) of the direct-acting antiviral drug telaprevir in combination with peg-interferon and ribavirin in subjects with HCV. Using only profiling data from subjects treated with peg-interferon and ribavirin, a signature composed of pretreatment levels of 13 components was identified that correlated well ($R^2$ = 0.68) with subjects' underlying immune response as measured by week 4 viral decline and was highly predictive of sustained virologic response in non-African American subjects (AUC = 0.99). The signature was validated by predicting in an independent cohort of non-African American subjects treated with telaprevir, peg-interferon and ribavirin (AUC = 0.854). Samples from extreme responders were over-represented in these analyses. Proteins identified as differentially-expressed between responders and non-responders to HCV treatment were quantified using multiple reaction monitoring in samples from all Caucasian subjects in the peg-interferon and ribavirin arms of PROVE1 and PROVE2, revealing 15 proteins that were significantly differentially expressed between treatment responders and non-responders. Seven of the proteins are part of focal adhesions or other macromolecular assemblies that form structural links between integrins and the actin cytoskeleton and are involved in antiviral response.

Biological significance
HCV is a significant health problem. We describe a novel approach for identifying markers that predicts HCV treatment response different treatment regimens and use this approach to identify a novel HCV treatment response signature. The signature has potential to guide optimization of HCV treatment regimens.



## 1. Introduction

Hepatitis is a chronic inflammatory condition of the liver caused by hepatitis C virus (HCV) infection. Approximately 170 million people worldwide are chronically infected with HCV [1]. HCV infection is one of the leading causes of both liver transplant and cancer-related death in the United States because it is a major risk factor for cirrhosis and hepatocellular carcinoma [2,3]. The goal of HCV treatment is eradication of the virus as determined by achievement of a sustained virologic response (SVR).

Molecular biomarkers have long been sought to guide clinical care for subjects infected with the HCV. HCV genotype was identified more than a decade ago as a strong predictor of

* *Corresponding author at: Vertex Pharmaceuticals Incorporated, 50 Northern Avenue, Boston, MA 02210, USA.*

http://dx.doi.org/10.1016/j.jprot.2014.12.015

treatment outcome [4] and is still used to determine the best treatment regimen for a subject [5]. Circulating markers of liver damage are indicators of severe fibrosis in HCV subjects [6] and are used clinically to predict prognosis and to trigger monitoring for hepatocellular carcinoma [7].

The introduction of broad molecular profiling greatly accelerated the discovery of novel predictive biomarkers of response for HCV. A genome-wide association study found that genetic polymorphisms near the IL28B gene were a major determinant of SVR to interferon-based treatments [8]. However, IL28B genotype explains only about 15% of the variability in response to treatment, implying the involvement of other factors [9]. Genome-wide expression profiling of liver samples from HCV-infected subjects identified strong predictors of SVR for PR treatment [10], but a liver biopsy would be required to use these biomarkers clinically. A proteomics-based approach employing liquid chromotagraphy mass spectrometry (LC–MS) identified pretreatment serum markers of SVR for interferon-based treatment [11]. The common theme between each of these elegant works is that the identified biomarkers predict SVR for a specific interferon-based regimen.

However, standard treatment for HCV infection is rapidly transitioning away from interferon-based regimens. In order to improve response rates and shorten treatment duration, the cornerstone of HCV treatment for many years, pegylated interferon with ribavirin (PR), has been augmented with direct-acting antivirals such as the protease inhibitors telaprevir, boceprevir, and simeprevir. These protease inhibitors specifically bind to the HCV nonstructural 3/4A serine protease [12]. The direct-acting antiviral sofosbuvir was recently approved as part of all-oral or interferon-containing combination regimens [13], and other all-oral combinations are progressing in the clinic.

Markers for interferon response may predict outcome to HCV treatment regardless of regimen. Interferon response has been shown to affect the treatment outcome for both interferon-based [14,15] and interferon-free regimens [16,17], suggesting that the variability observed in interferon-based regimens may reflect underlying variation in subjects' immune response to HCV rather than interferon-specific variation. Consequently, identifying predictors of interferon response may have broad utility in predicting SVR for HCV treatment regimens.

Here, we report the discovery of a novel immune response signature in HCV using data solely from PR treatment. The signature accurately predicted SVR for the PR regimen. Furthermore, we independently validated the signature by demonstrating that it predicted SVR for PR augmented with telaprevir (T/PR), thereby providing further evidence that interferon response underlies response to direct-acting antivirals. To better understand the biology underlying the PR signature, differentially-expressed components were identified using LC–MS resulting in the identification of 71 proteins. These proteins were quantified in a broad set of subjects with HCV treated with PR, resulting in the identification of 15 proteins that were differentially expressed between treatment responders and non-responders. The differentially-expressed proteins revealed a host response to HCV infection that was not previously known to affect treatment outcome. Finally, literature data reporting SVR rates for various regimens was used to provide quantitative evidence that the SVR rates for both interferon-based and interferon-free regimens are correlated in subject populations with different interferon responses, suggesting that markers correlating with interferon response are broadly useful for predicting response to HCV treatment.

## 2. Methods

Methods are described in detail in a Data in Brief article [44].

All subjects from which samples used in this study were collected were enrolled in The Protease Inhibition for Viral Evaluation trials (PROVE 1, 2 and 3) [14,18,19]. The PROVE 1 and 2 trials enrolled treatment naïve subjects and the PROVE 3 trial enrolled subjects who previously failed PR treatment. Demographic information for all the subjects from which profiling samples were obtained is in Table 1 in [44]. The protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, and was approved by the institutional review board at each participating site. All patients provided written informed consent.

### 2.1. Broad proteomic profiling (discovery stage)

#### 2.1.1. Sample selection and mass spectrometric analysis

Pre-treatment serum samples were analyzed for 50 subjects in the PR arms of the PROVE 1 and PROVE 2 trials. Among the 50 subjects, 25 subjects achieved SVR and 25 failed to achieve SVR in the clinical trial. Samples from subjects with the best response to PR (undetectable virus or lowest viral titer at week 4) were selected for profiling in the discovery stage. The samples from the 25 non-responder subjects were chosen to match demographic characteristics of the 25 responders. Only samples from non-responders who were adherent to treatment, defined as completing dosing, or stopping treatment based on pre-defined stopping rules for virologic failure, were selected for profiling.

The remaining samples were from subjects in the T/PR arms of the PROVE 1, PROVE 2 and PROVE 3 trials. T/PR treatment non-responders were defined as subjects who completed at least four weeks of T/PR dosing and failed to achieve undetectable virus at any time-point during the study. Samples from a total of 38 Caucasian treatment non-responders were profiled in the discovery stage. Additionally, samples from 49 Caucasian subjects who achieved SVR in the study were chosen to match demographic characteristics of the T/PR non-responders. Finally, all 35 pretreatment samples from African Americans enrolled in T/PR arms of the PROVE 1, PROVE 2 and PROVE 3 studies were profiled in the discovery stage. Seven samples were omitted from the statistical analysis because they appeared to contain very high abundance of proteins that were not completely removed by the immunoaffinity depletion. Demographics for the subjects used in statistical analyses are summarized in Table 1 in [44].

Samples were depleted of abundant proteins, digested with trypsin and analyzed by liquid chromatography/mass spectrometry (LC–MS). Detected ions were matched across samples and compared for relative peak intensity.

#### 2.1.2. Predictive model

The decline in viral titer at week 4, rather than SVR, was used as a continuous metric to quantify interferon response in the predictive model. By using a continuous metric for interferon