Biomarkers Associated With ALT Flares and HBsAg Decline in Patients With Chronic Hepatitis B

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Introduction

- An insufficient antiviral response is associated with chronic hepatitis B (CHB) infection and therapeutics designed to promote immune responses are under clinical evaluation
- Clinical study GS-US-174-0149 (NCT01277601) evaluated pegylated interferon α-2a (PEG) alone or in combination with tenofovir disoproxil fumarate (TDF)[1,2]
- PEG triggers expression of numerous interferon-stimulated genes and downstream pathways in cells, such as hepatocytes, T cells, and natural killer (NK) cells
- On- and off-treatment alanine aminotransferase (ALT) flares are common in patients with CHB and can range in severity
- Flares are often associated with reductions in serum viral antigens

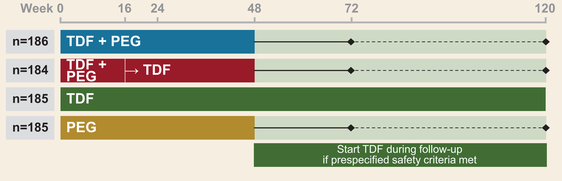
Objectives

- To characterize plasma proteins in patients with CHB treated with PEG at timepoints before and during ALT flares
- To determine differences between patients who experience hepatitis B surface antigen (HBsAg) decline or loss after flares vs those who don't experience HBsAg decline or loss
- To identify any baseline predictors of HBsAg decline or loss

Methods

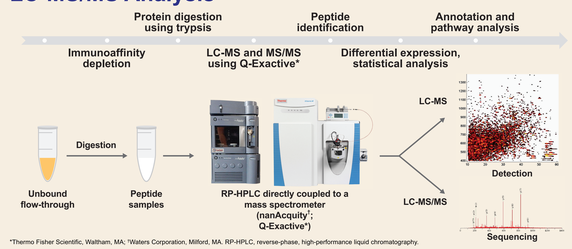
- 2 GS-US-174-0149 patient groups treated with PEG alone or in combination with TDF were included in these analyses
- Flares were defined as ALT >2× baseline, and >400 and >300 U/L for men and women, respectively
  - Group 1 (n=15): ≥1-$\log_{10}$ HBsAg decline following flare
  - Group 2 (n=7): <1-$\log_{10}$ HBsAg decline following flare
- Plasma samples collected at baseline, Week 4, just prior to flare, and during flare were evaluated by liquid chromatography–tandem mass spectrometry (LC-MS/MS)
- Protein values were used to perform a pathway analysis to identify networks differentially expressed between productive and unproductive groups
- Elastic net regularization was used to identify baseline predictive proteins and pathways of HBsAg decline/loss

GS-US-174-0149 Clinical Study Design



- Randomized, controlled, open-label study (N=740): stratified by screening hepatitis B e antigen (HBeAg) status and hepatitis B virus (HBV) genotype
- Inclusion criteria:
  - HBeAg+ and HBV DNA ≥20,000 IU/mL or HBeAg− and HBV DNA ≥2000 IU/mL
  - ALT >54 and ≤400 U/L in men, and >36 and ≤300 U/L in women
  - No bridging fibrosis or cirrhosis on liver biopsy or by transient elastography

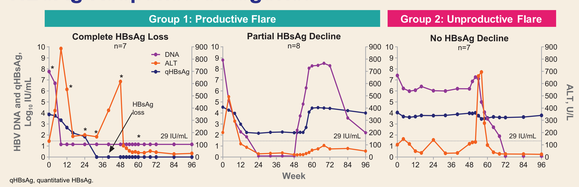
LC-MS/MS Analysis



*Thermo Fisher Scientific, Waltham, MA; †Waters Corporation, Milford, MA. RP-HPLC, reverse-phase, high-performance liquid chromatography.

Results

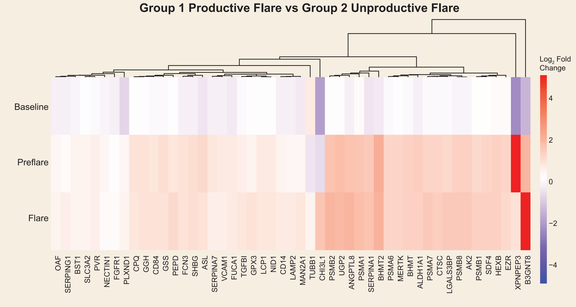
HBsAg Response Categories



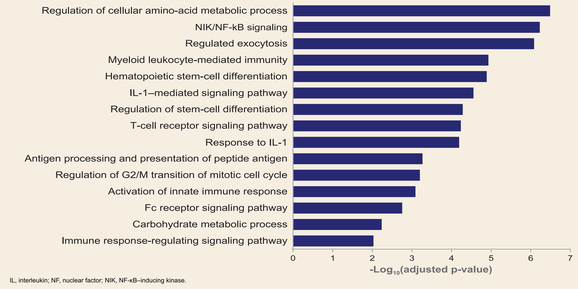
qHBsAg, quantitative HBsAg.

- ALT flares can occur on- or off-treatment

Differentially Expressed Proteins in Longitudinal Samples Between Productive and Unproductive Flare Groups

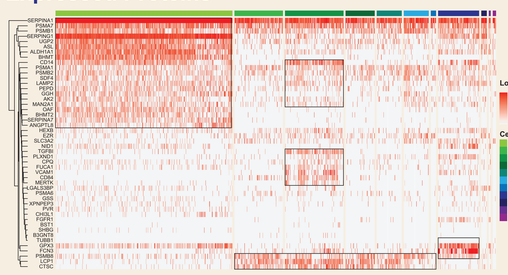


Gene Ontology Associated With HBsAg Decline or Loss in Longitudinal Samples



IL, interleukin; NF, nuclear factor; NIK, NF-κB–inducing kinase.

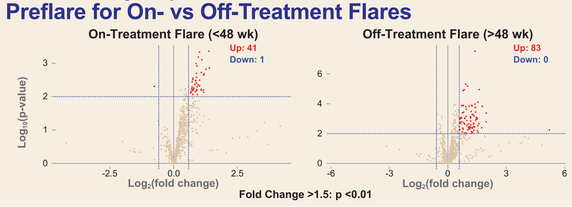
Potential Liver Cell Sources of Differentially Expressed Proteins



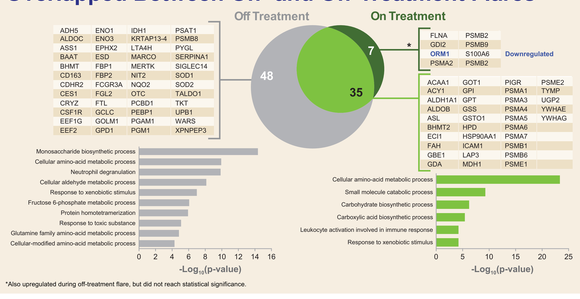
LSECs, liver sinusoidal endothelial cells; UMI, unique molecular identifier.

- Protein cell sources were determined using liver single-cell RNA sequencing data from MacParland et al[3]
- Results suggest that hepatocytes, T cells, macrophages, NK cells, and LSECs may be involved in productive flares

Differentially Expressed Proteins Between Flare and Preflare for On- vs Off-Treatment Flares

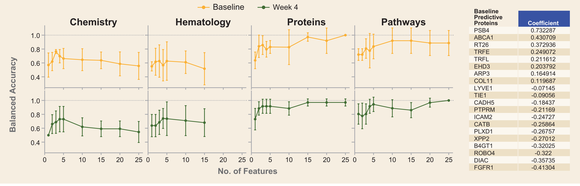


A Subset of Differentially Expressed Proteins Overlapped Between On- and Off-Treatment Flares



*Also upregulated during off-treatment flare, but did not reach statistical significance.

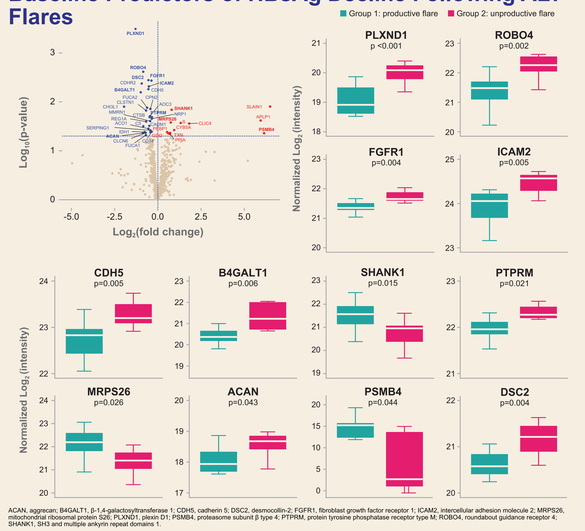
Baseline and Week 4 Proteomic (on protein or pathway level) Was More Predictive Than Clinical Characteristics for HBsAg Decline



Baseline Predictive Proteins	Coefficient
PSB4	0.732287
ABCA1	0.430709
RTZ6	0.372936
TRFE	0.249072
TRFL	0.211612
EHD3	0.203792
ARP3	0.164914
COL11	0.119687
LYVE1	-0.07145
TIE1	-0.09056
CADH5	-0.18437
PTPRM	-0.21169
ICAM2	-0.24727
CATB	-0.25864
PLXD1	-0.26757
XPP2	-0.27012
BAT1	-0.32025
ROBO4	-0.322
DAC	-0.35735
FGFR1	-0.41304

- Demographics, HBV serology, del Week 4–baseline were also analyzed and not predictive

Baseline Predictors of HBsAg Decline Following ALT Flares



ACAN, aggrecan; B4GALT1, β-1 4-galactosyltransferase 1; CDH5, cadherin 5; DSC2, desmocollin-2; FGFR1, fibroblast growth factor receptor 1; ICAM2, intercellular adhesion molecule 2; MRPS26, mitochondrial ribosomal protein S26; PLXND1, plexin D1; PSMB4, proteasome subunit β type 4; PTPRM, protein tyrosine phosphatase receptor type M; ROBO4, roundabout guidance receptor 4; SHANK1, SH3 and multiple ankyrin repeat domains 1.

Pathway Analysis Suggests Underlying Health and Immune Status May Be Important for HBsAg Decline

Features	Coefficient
PID thrombin PAR1 pathway	0.473722
PAR1-mediated thrombin signaling events	0.473722
Lectin pathway of complement activation	0.419434
IL-1 and megakaryocytes in obesity	0.354175
Negative regulation of myeloid leukocyte differentiation	0.31869
Hematopoietic progenitor cell differentiation	0.288169
Negative regulation of TNF superfamily cytokine production	0.246152
Fibrinolysis	0.102933
Semaphorin-plexin signaling pathway involved in neuron projection guidance	0.053694
Semaphorin receptor complex	0.053694
Semaphorin receptor activity	0.053694
Classical antibody-mediated complement activation	0.028367
Heterotypic cell-cell adhesion	-0.07569
Regulation of IL-1 secretion	-0.23946
Apoptotic cleavage of cellular proteins	-0.34427
Regulation of fibrinolysis	-0.35801
Proteasome core complex, β-subunit complex	-0.40206
Hallmark angiogenesis	-0.43238
Keratan sulfate metabolic process	-0.45559
Keratan sulfate keratin metabolism	-0.45559
	Innate and adaptive immune response
	Osteoarthritis, osteoporosis*

PAR1, Prader-Willi/Angelman region-1; PID, Pathway Interaction Database; TNF, tumor necrosis factor.

Conclusions

- An LC-MS/MS approach was utilized to profile productive and unproductive flares from patients with CHB treated with PEG
- Proteins associated with innate and adaptive immune pathways were found to be upregulated in productive flare groups at preflare and flare time points
- Differentially expressed proteins from productive flares may come from hepatocytes, T cells, macrophages, NK cells, and LSECs
- On- and off-treatment flares had similar differentially expressed protein profiles
- Baseline samples may be the most predictive of a productive flare, with ≥10 proteins required for high predictability
- Pathway analysis of baseline sample proteins suggests underlying health and immune status may be important predictors of HBsAg decline

References: **1.** Tan AT, et al. J Hepatol 2010;52:330-9; **2.** Tan AT, et al. J Hepatol 2014;60:54-60; **3.** MacParland SA, et al. Nat Commun 2018;9:4383; **4.** Gill US, et al. J Infect Dis 2015;211:374-82.

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