

Results. The in vitro drug potency to inhibit wt HBV replication, whatever the strain origin, was ranked in the following order: ETV>LAM>PMEO>ADV≈TDF. Regarding laboratory strains and from patient 2, PME0 inhibits similarly the replication of wt and mutant HBV strains whereas the activity of ADV/TDF is decreased against ADV-R and LAM+ADV-R mutants. The mutation M204V leads to a loss of susceptibility to ETV and a resistance to LAM that inhibit efficiently only the ADV-R mutant. For patient 1, all mutant strains are resistant to LAM, sensitive to TDF and retain susceptibility to PME0, the V173L/L180M/A181V/N236T mutant displaying the highest fold resistance to PME0 (FR=5.1, IC50= 28.6±5.34 μM). Conclusions. The antiviral potency and the cross-resistance profile of PME0 suggest that it represents a new candidate for the treatment of chronic HBV carriers who have developed resistance to currently approved drug regimen, and for the design of combination therapy to delay the emergence of resistance. PME0 warrants further evaluation in animal models and clinical trials.

#### Disclosures:

The following people have nothing to disclose: Marie-Noelle Brunelle, Julie Lucifora, Stephanie Villet, Johan Neyts, Christian Trepo, Fabien Zoulim

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**PF-03491390, (FORMERLY IDN-6556) A PANCASPASE INHIBITOR, IS WELL-TOLERATED AND EFFECTIVELY REDUCES RAISED AMINOTRANSFERASES (ALT AND AST) IN CHRONIC ACTIVE HEPATITIS C (HCV) PATIENTS (PTS)**  
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**Background:** Increased rates of hepatocyte apoptosis and activated caspases have been observed in viral hepatitis. Elevated serum levels of ALT and AST reflect hepatocellular damage. This study examined the efficacy and safety of the pancaspase inhibitor PF-03491390 in reducing elevated ALT and AST in pts with chronic HCV infection. **Methods:** In this multicenter, placebo-controlled, double-blind, parallel-group, dose-ranging study 204 pts with documented chronic HCV and liver fibrosis were randomized to receive either placebo or PF-03491390 5mg, 25mg, or 50mg orally bid for 12 weeks. If ALT and AST were still elevated at week (Wk) 10, the dose of study drug was doubled to Wk 12. Co-primary endpoints were absolute change from baseline in ALT and AST at Wk 10, the last visit of the initial randomized dose. **Results:** Median absolute ALT and AST values decreased from Baseline to Wk 10 in all treatment groups; reductions were statistically significant compared to placebo at all doses studied (Table 1;  $P < 0.0001$ ). <insert table> Highly significant reductions in serum AST and ALT were observed for each dose of PF-03491390 versus placebo. Reductions in ALT and AST were observed starting at Wk 1, were maintained throughout the study, and returned to baseline levels when PF-03491390 was discontinued. The degree of reductions in AST and ALT were similar across all treatment doses. Most adverse events were of mild or moderate severity; the most frequently reported treatment-emergent events were headache in 24 pts and fatigue in 22 pts. No change in mean log HCV RNA was observed in any of the treatment groups. **Conclusions:** PF-03491390 was well tolerated and effectively reduced ALT and AST in pts with chronic HCV hepatitis during a 12-Wk treatment period. Further randomized trials are necessary to determine whether PF-03491390 may influence liver histology in patients with liver fibrosis.

**Table 1.** PF-03491390 Reduced Serum AST and ALT by Wk 10 in the Intent-to-Treat Population

|   | Placebo | PF-03491390 5 mg bid | PF-03491390 25 mg bid | PF-03491390 50 mg bid |
|---|---------|----------------------|-----------------------|-----------------------|
| No. pts                                   | 51      | 55                   | 50                    | 48                    |
| Baseline AST IU/L                         | 60      | 69                   | 58                    | 73                    |
| AST IU/L Reduction from Baseline at Wk 10 | -2      | -17 <sup>†</sup>     | -23 <sup>†</sup>      | -25 <sup>†</sup>      |
| Baseline ALT IU/L                         | 101     | 103                  | 98                    | 115                   |
| ALT IU/L Reduction from Baseline at Wk 10 | -2      | -34 <sup>†</sup>     | -41 <sup>†</sup>      | -49 <sup>†</sup>      |

<sup>†</sup> $P < 0.0001$  versus placebo

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Paul Pockros - Received research grant support: IDUN Pharmaceuticals; Received research grant support: Pfizer Inc

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Jacqui Spanton - Employee: Pfizer Ltd

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**EARLY VIRAL RESPONSE AND ON TREATMENT RESPONSE TO CPG 10101 (ACTILON™), IN COMBINATION WITH PEGYLATED INTERFERON AND/OR RIBAVIRIN, IN CHRONIC HCV GENOTYPE 1 INFECTED PATIENTS WITH PRIOR RELAPSE RESPONSE** *Ira M. Jacobson<sup>1</sup>, Reem Ghalib<sup>2</sup>, Eric Lawitz<sup>3</sup>, Bradley Freilich<sup>4</sup>, Stuart C. Gordon<sup>5</sup>, Paul Kwo<sup>6</sup>, Thomas R. Riley<sup>7</sup>, Diane Bright<sup>8</sup>, Mary L. Morris<sup>8</sup>, Mohammed Al-Adhami<sup>8</sup>, Julie L. Himes<sup>8</sup>, Ferdinand E. Massari<sup>8</sup>, John G. McHutchison<sup>9</sup>; <sup>1</sup>Weill Medical College of Cornell University, New York, NY; <sup>2</sup>Liver Institute at Methodist Dallas, Dallas, TX; <sup>3</sup>Alamo Medical Research, San Antonio, TX; <sup>4</sup>Freilich & Brand LLC, Kansas City, MO; <sup>5</sup>Henry Ford Hospital, Detroit, MI; <sup>6</sup>Indiana University Medical Center, Indianapolis, IN; <sup>7</sup>Penn State Hershey Medical Center, Hershey, PA; <sup>8</sup>Coley Pharmaceutical Group, Inc., Wellesley, MA; <sup>9</sup>Duke University School of Medicine, Durham, NC*

**Background:** CPG 10101 (CPG) is an investigational Toll-like receptor 9 (TLR9) agonist that activates plasmacytoid dendritic cells and B cells directly and NK/NKT cells indirectly, initiating and enhancing antiviral mechanisms mediated by both innate (antiviral cytokines including IFN-α) and adaptive immunity. **Methods:** 74 HCV genotype 1 infected treatment-refractory Relapsed Responders (RR) who previously received ≥24 weeks PEG-IFN+RBV treatment were randomized and treated initially for 12 weeks in 1 of 5 arms: P+R, C+P+R, C+P, C+R, or C alone [CPG (C)=0.2 mg/kg SC weekly; PEG-IFN (P)=1.5 μg/kg SC weekly; RBV (R)=800-1400 mg PO daily]. Patients in CPG-containing arms who achieved ≥2 log<sub>10</sub> reduction at Week 12 (EVR) were eligible to continue treatment for up to 48 weeks. All P+R patients were eligible to roll-over to C+P+R after completing the initial 12 weeks of treatment, regardless of viral level. **Results:** The mean log<sub>10</sub> HCV RNA reduction at Week 12 was significantly greater in the C+P+R arm vs. P+R. A greater proportion of patients receiving C+P+R achieved RVR, EVR, and HCV RNA undetectability (<50 IU/mL) than those receiving P+R. In CPG-containing arms 20 of 24 EVR patients elected to continue. An additional 2 C+P+R and 2 C+P patients became undetectable during continuation (2 C+P+R & 1 C+P undetectable patient stopped prior to Week 24). On Treatment Responses (OTR; undetectable at Week 24) were achieved by 7 of 14 (50%) C+P+R and 3 of 16 (19%) C+P patients. 14 of 15 P+R patients rolled over to treatment with C+P+R. During rollover treatment 5 patients who remained positive throughout 12 weeks of P+R treatment became undetectable. 2 rollover patients achieved a first reduction of >2 log<sub>10</sub> HCV RNA. AEs were predominantly of mild/moderate intensity and consisted of headache, flu-like symptoms, nausea, and injection site reactions. **Conclusions:** In this study CPG improved early antiviral

activity of P+R in treatment-refractory (RR) patients. CPG in combination with P±R was generally well tolerated. Continuation treatment with C+P±R has led to additional HCV RNA undetectable responses beyond Week 12. Patients will be followed for End of Treatment Response (ETR) and Sustained Viral Response (SVR).

|                 | Wk 12 HCV RNA<br>log <sub>10</sub> Reduction<br>(Mean ± SEM) | Wk 12 HCV<br>RNA <50<br>IU/mL | Wk 12 EVR, ≥2<br>log <sub>10</sub><br>Reduction | EVR Patients<br>Continuing | Wk 24 OTR,<br>HCV RNA<br><50 IU/mL |
|-----------------|--|-------------------------------|---|----------------------------|------------------------------------|
| P+R<br>(N=15)   | 2.33 ± 0.38 (N=15)   | 2 (13%)                       | 9 (60%)   | NA                         | NA                                 |
| C+P+R<br>(N=14) | 3.26 ± 0.26* (N=14)  | 7 (50%)**                     | 12 (86%)  | 11                         | 7 (50%)                            |
| C+P<br>(N=16)   | 2.37 ± 0.37 (N=13)   | 2 (13%)                       | 9 (56%)   | 6                          | 3 (19%)                            |
| C+R<br>(N=15)   | 1.42 ± 0.16 (N=13)   | 0                             | 3 (20%)   | 3                          | 0                                  |
| C (N=14)        | 0.12 ± 0.08 (N=13)   | 0                             | 0   | NA                         | NA                                 |

#### Disclosures:

Ira M. Jacobson - Clinical Investigator: Coley Pharmaceutical Group  
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Eric Lawitz - Clinical Investigator: Coley Pharmaceutical Group  
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**THE ROLE OF KRÜPPEL-LIKE FACTOR 6 (KLF6) IN DIFFERENTIATION OF EMBRYONIC STEM CELLS INTO HEPATOCYTES** *Xiao Zhao<sup>1</sup>, Nobuyuki Matsumoto<sup>3</sup>, Valerie Gouon-Evans<sup>2</sup>, Steven Yea<sup>1</sup>, Johnny Loke<sup>1</sup>, Gordon Keller<sup>2</sup>, Scott L. Friedman<sup>1</sup>*; <sup>1</sup>Division of Liver Diseases, Mount Sinai School of Medicine, New York, NY; <sup>2</sup>Department of Gene and Cell Medicine, Mount Sinai School of Medicine, New York, NY; <sup>3</sup>Division of Gastroenterology and Hepatology, St. Marianna University School of Medicine, Kanagawa, Japan

Background: Krüppel-like factor 6 (KLF6), a zinc finger transcription factor and tumor suppressor gene, plays an important role in regulating proliferation and development. Our previous analysis of *Klf6*<sup>-/-</sup> mice indicates that they are embryonic lethal by embryonic day (E) 12.5, and exhibit markedly reduced hematopoiesis and angiogenesis; they also lack a definable liver (Blood 2006, 107:1357). Consistent with this phenotype, *Klf6*<sup>-/-</sup> embryonic stem (ES) cells generated by homologous recombination also display significant hematopoietic defects following differentiation into embryoid bodies (EBs). In order to further characterize the role of KLF6 in liver development, our aim was to assess the capacity of *Klf6*<sup>-/-</sup> ES cells to differentiate into a hepatic lineage, compared to *Klf6*<sup>+/+</sup> and *Klf6*<sup>+/-</sup> ES cells. Methods/Results: *Klf6*<sup>+/+</sup> and *Klf6*<sup>+/-</sup> mouse ES cells were first differentiated into an endodermal progenitor population at day 4 in serum-free conditions using high doses of activin A, as confirmed by analysis of *Hnf3β*/*Foxa2* and *brachyury* mRNA expression. Next, in addition to activin A, FGF and BMP4 were added to induce hepatic specification, and expression of hepatic differentiation markers was quantified by real time PCR (qRT-PCR) until day 12. The differentiated *Klf6*<sup>+/+</sup> and *Klf6*<sup>+/-</sup> ES cell-derived cultures both expressed similar levels of endoderm markers, as well as early (e.g., alpha fetoprotein, transthyretin), and late (albumin) hepatic mRNAs. Cytoplasmic albumin expression was also apparent by immunocytochemistry. Differentiation was accompanied by a biphasic increase in *Klf6* mRNA on day two and again after day four. In contrast, *Klf6*<sup>-/-</sup> ES cells displayed significantly reduced expression of endoderm markers, including *Hnf3β*, *Gata4*, *Sox17*, and *Cxcr4*, a marker for definitive endoderm, and did not express hepatocyte markers (alpha fetoprotein, transthyretin, albumin). Furthermore, forced

expression of KLF6 at specific time points using a tet-inducible system increased endoderm and hepatic gene expression. Conclusions: KLF6 regulates endoderm formation and hepatic differentiation in a temporally specific manner in ES cells. It is unclear whether these defects are restricted to liver or affect all endoderm-derived tissues (e.g. pancreas). These results reveal a novel role of the KLF6 transcription factor in the highly regulated process of liver differentiation.

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The following people have nothing to disclose: Xiao Zhao, Nobuyuki Matsumoto, Valerie Gouon-Evans, Steven Yea, Johnny Loke, Gordon Keller, Scott L. Friedman

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**THE HEPATITIS C VIRUS E2 PROTEIN IS A NOVEL KINASE THAT CONTROLS ENDOCYTOSIS** *Martina Buck; University of California San Diego, San Diego, CA*

Background: The HCV entry and trafficking remain poorly understood. The intracellular roles of E2 are unknown. Auxilin 2/Cyclin G-associated kinase (GAK) is a master regulator of clathrin-mediated trafficking (CMT), cell signaling and function. GAK regulates CMT by phosphorylating the clathrin adaptor protein AP50. Phosphorylated AP50 binds to membrane receptors and the clathrin coated vesicles, thereby controlling endocytosis. Hypothesis: In this study, we found that the HCV E2 protein has homology to the kinase region of GAK. Therefore, we postulate that E2 is able to perform many of the cellular functions of GAK. Methods: E2 was expressed and purified as a recombinant protein. Mutagenesis was performed using PCR to generate single amino acid changes in 10 separate motifs, homologous to the kinase region of GAK. These E2 proteins were used to phosphorylate heat-inactivated, immuno-purified AP50. The internalization of both transferrin and EGF was measured with <sup>125</sup>Iodine- ligands. E2 was transfected both as a DNA, and as a protein using a cell-permeable HIV-tat peptide. Primary hepatocytes were isolated by perfusion with collagenase and cultured on collagen coated plates. Activities of kinases were measured with phosphorylation-specific antibodies. Hepatocyte proliferation was assayed by [<sup>3</sup>H]-thymidine incorporation. Results: We found that E2 phosphorylates AP50 and is auto-phosphorylated in vitro. E2 associates with AP50 in normal hepatocytes, stimulating CMT of endocytic vesicles. In primary hepatocytes transfected with the E2 protein, the internalization of transferrin is markedly enhanced compared to control hepatocytes. Single amino acid mutations of the E2 regions, homologous to GAK, disrupt endocytosis of transferrin. Like GAK, E2 decreases the internalization of EGF, inhibiting the cellular response to EGF. Unexpectedly, E2 also activates the PI-3K pathway in the absence of extracellular stimuli. HCV E2 induction of PI-3K includes the activation of ERK 1/2 and Akt which leads to the phosphorylation/inhibition of BAD, resulting in a blockade of the apoptotic pathway and an increase in hepatocyte proliferation. Several of the E2 mutants are unable to induce this pathway, indicating mechanistically important motifs. Conclusion: HCV E2 is a novel kinase that by phosphorylating AP-50 stimulates endocytosis and regulates the internalization of both transferrin and EGF in normal hepatocytes. Through this pathway, E2 increases the activities of powerful growth kinases, ERK1/2 and Akt. Thus, E2 pushes normally quiescent hepatocytes toward proliferation and away from apoptosis.

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The following people have nothing to disclose: Martina Buck

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**TLR4 SIGNALING MEDIATES HEPATIC FIBROGENESIS BY ENHANCING TGF-BETA SIGNALING WITH DOWNREGULATING BAMBI IN HEPATIC STELLATE CELLS** *Ekihiro Seki, Samuele De Minicis, Yosuke Osawa, Christoph Oesterreicher, David A. Brenner, Robert F. Schwabe; Medicine, Columbia University College of Physicians and Surgeons, New York, NY*

Background and Aims: The pattern recognition receptor Toll-like receptor (TLR) 4 is a key regulator of innate immune responses. During hepatic fibrogenesis, systemic and portal levels of the