**The role of a TLR9 agonist as adjuvant therapy in the treatment of chronic HCV:**

A randomized, double-blind trial of CPG 10101 with concurrent IFN/ribavirin in the treatment of patients chronically infected with HCV

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ICCR Course, IRB Proposal Outline

## Study Purpose and Rationale

The hepatitis C virus (HCV) infects nearly 3% of the world's population with approximately 4 million chronically infected patients in the US (Alter,1999). Nearly 30% of people spontaneously clear infection, an event that correlates with a strong HCV specific CD8+ T cell response (Lauer and Walker, 2002). The majority of those exposed, however, progress to chronic HCV infection. Chronic infection leads to significant morbidity and mortality secondary to cirrhosis, hepatocellular cancer, and end-stage liver disease (Lauer and Walker, 2002). Significant advances have been made leading to the current combination therapy of pegylated IFN (interferon) and ribarvirin (RBV). This combination regimen is highly effective in genotype 2 and 3 with sustained virologic responses (SVR, 6 months after stopping treatment) near 80% (Fried 2002). However, SVR for genotype 1, the most prevalent genotype in North America (Blatt 2000), remains only 42-46% after 48 weeks of combination therapy (Gamban-Galwan and Jacobson, 2008).

CpG 10101 (Actilon; coley Pharmaceutical Group, Inc. Wellesley, MA) is a new class of biologics designed to toll receptor (TLR) 9, expressed on the surface of both B cells and plasmacytoid DCs. Stimulation of plasmacytoid DCs by TLR9 agonists results in the production of numerous cytokines including IFNa and may serve as crucial element in promoting an effective CD8+ T cell response (Krieg, 2007). Recent studies with CpG monotherapy have shown their efficacy for stimulating cytokine production in vivo and concomitant reduction in HCV viral load (McHutchison 2007).

*Hypothesis:* Treatment with CpG 10101 will improve the rate of SVR in patients with chronic HCV treated with pegylated-IFN and ribavirin.

## Study Design

This study will be randomized, double-blind, placebo controlled trial of CpG 10101 performed in the liver clinic at Columbia Presbyterian Medical Center. Randomization will be performed by an independent statistician not involved in patient care or data analysis. Information will be provided to an unblinded pharmacist for dose preparation and assignment. All patients will receive standard pegylated-IFN alfa-2b 1.5 g/kg/week plus daily RBV according to weight based dosing (800 mg for patients weighing <65 kg; 1000 mg for patients weighing 65 to 85 kg; 1200 mg for patients weighing > 85 to 105 kg; and 1400 mg for patients weighing >105 kg but <125 kg) for standard 48 weeks (Jacobson 2008). CpG 10101 or placebo will be given once weekly at 0.75mg/kg SC during week 0-4 and again week 24-28.

**Primary and Secondary Outcomes**

The primary outcome of the study will be SVR as defined as undetectable viral load 24 weeks after treatment. Serum will be collected at 24 weeks, 48 weeks, and 72 weeks (24 weeks post-treatment) for analysis of HCV RNA levels. Quantification of HCV RNA in serum will be performed by the central core laboratory using multicycle RT-PCR.

In addition to SVR, serum cytokine assays will be performed pre- and post- CpG or placebo administration to confirm bioactivity. Assays for IFNa, IFNg-inducible protein 10 will be monitored as surrogates of activity. Previous work has illustrated that the targets of CpG, plasmacytoid dendritic cells (DCs), in patients with chronic HCV remain fully functional, however, the absolute numbers are known to vary. Circulating plasmacytoid DCs will be enumerated using flow cytometry with BDCA-4, a surface marker specific for plasmacytoid DCs.

For analysis of HCV-specific T cell activity, 100cc of blood will be collected at 24, 48, and 72 weeks. CD8+ T cells will be purified using magnetically charged CD8+ antibodies (Miltenyi). Using a peptide library of class I restricted epitopes from the H77 strain of HCV (genotype 1), T cell responses will be tested in IFNg ELISPOT (Kim Blood 2005). Given the specific identification of HLA-A2.1 class I restricted HCV epitopes, PBMCs will be screened by flow cytometry for HLA-A2.1. For monitoring HLA-A2.1-restricted HCV-specific T cell responses, the following peptides will be used: Core 35-44 (YLLPRRGPRL), Core 132-140 (DLMGYIPLV), NS3 1073-1081 (CVNGVCWTV), NS3 1406-1415 (KLTGLGLNAV), NS3 1406-1415A (KLVALGINAV).

**Statistical Procedures**

Anticipating a maximal SVR of 46% in genotype 1 patients with conventional pegylated-IFN and weight based ribavirin, we powered our study to detect a 10% absolute increase in SVR. Chi-square analysis for type I error 0.05 and type II error 0.8 yielded an estimated N = 422. Given the evidence for decreased SVR rates in Latino and African American populations as well as underlying cirrhosis (Rodriguez-Torres, 2009), stratified randomization will be used. The following groups will be independently randomized: Whites with evidence of liver dysfunction, Whites without evidence of liver dysfunction, Non-whites with evidence of liver dysfunction, Non-whites without evidence of liver dysfunction.

**Study Subjects**

Treatment-naïve chronic hepatitis C patients, 18-70 years old with body weight less than 125kg and detectable serum genotype 1 HCV RNA are eligible. Additional inclusion criteria are elevated ALT within 6 months of entry, liver biopsy documented evidence of chronic hepatitis C, and alpha-fetoprotein <100ng/mL within the preceding year. Major exclusion criteria inclue HIV, seropositivity for the hepatitis B surface antigen, and pregnancy. Subjects will be recruited primarily from the liver clinic at CPMC. Fliers will be posted around campus to identify other subjects not currently cared for in the liver clinic. In addition, AIM clinic physicians as well as general GI clinic physicians will be informed of the study and encouraged to refer patients for evaluation.

**Study Drug**
CpG 10101 (Actilion, Coley Pharmaceuticals) is a 22-mer C-class oligodeoxynucleotide designed to stimulate TLR9 in vivo. A phase 1a, placebo-controlled revealed that CpG 10101 was well tolerated up to 20mg SC without serious adverse events or dose limiting toxicities. A subsequent, phase 1b study evaluated the pharmacokinetics, tolerability, and efficacy (McHutchison, 2007). The results revealed a dose-dependent increase in serum cytokines including IFNa and reduction in viral RNA. Side effects were generally limited to site of local injection with >1mg of CpG 10101. One serious adverse effect related to the study drug was urticaria and pruritis without respiratory complications. The symptoms resolved with methylprednisolone and no significant sequlae were noted.

**DMSB and Potential Risks**
A DMSB will be established to monitor adverse effects as defined by Common Toxicity Criteria. Given previous studies with CpG 10101, major adverse effects are not expected. Attention will be given to monitoring local injection reactions. Several adverse effects associated with standard pegylated-IFN and ribavirin therapy exist including anemia and neutropenia. These will be monitored as per standard protocol with laboratory analysis every 2 weeks. If Hb<10mg/dL, the dose of ribavirin will be held until Hb>10mg/dL and then restarted at 200mg/day less.

**Study Questionaire and Confidentiality**
All potential subjects will be required to fill out screening questionnaire with information including age, race, presumed method of HCV exposure, duration of illness, previous treatment, HIV status. Full medical evaluation will be conducted as part of screening. Information will remain confidential as per hospital guidelines. All patients included in the study will be assigned an identifier number to preserve anonymity in analysis and data reporting.

The potential benefits of this study is the identification of immunostimulatory treatment that enhances the rate of SVR in patients with difficult to treat genotype 1 HCV. The alternatives to this study are standard therapy with pegylated-IFN and ribavirin.

References:

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