INTRODUCTION

Lack of a robust small animal model of hepatitis C virus (HCV) infection has impeded the development of effective antiviral therapies and understanding of HCV biology. By transplanting normal human hepatocytes into the liver of immunodeficient mice (SCID/Beige) carrying the urokinase type plasminogen activator transgene linked to an albumin promoter (Alb-uPA), we generated mice with human liver tissue (1, 2, 3). Mice engrafted successfully with transplanted human hepatocytes screened with ELISA for human alpha-1 antitrypsin (hAAT) are capable of supporting long-term stable infection with HCV. We evaluated therapeutic agents against HCV and HBV infection that have been clinically validated. These included interferon alpha 2B (4) and a protease inhibitor previously reported to decrease HCV titers effectively (BILN 2061) (5). In addition, we studied the impact of Lamivudine on hepatitis B virus (HBV) infection in this model (6,7).

EXPERIMENTAL STUDIES: MODEL VALIDATION

Since this mouse model was published in Nature Medicine in August 2001, we have validated this system in the context of development of antivirals and HCV therapeutic strategies. The following studies have been carried out with this animal model system:

- Interferon alpha 2B treatment of HCV infection- 4 independent experiments (Study 1)
- Assessment of anti-HCV activity of a serine protease inhibitor (BILN 2061) reported to result in decreased HCV viral titers in clinical trials (Study 2)
- Verification of anti-HBV effect of the nucleoside analogue lamivudine on established HBV infection in chimeric mice (Study 3)
- Evaluation of gene therapy approach against HCV infection using modified BID (oral presentation O-61, abstract N. 281, 10th HCV meeting, Kyoto)

**Study 1: Interferon alpha 2B treatment of HCV**

**Study Design**

- **Patients (n=8)**
- **Study**: Interferon alpha 2B treatment of HCV
- **Administration**: Twice daily, oral gavage
- **Courses**: 4 weeks treatment and 1 week follow-up post-treatment

**Assays**

- hAAT for monitoring human hepatocyte grafts
- Roche Amplicor for HCV RNA quantitation
- Light Cycler PCR for HBV DNA quantitation

**Results**

- Significant difference in the net decrease of HCV viral RNA exists between BILN 2061 and Vehicle (p<0.0001), INF and vehicle (p< 0.005) and INF and BILN 2061 and INF (p<0.03), respectively (ANOVA and LSD test)
- HCV viral RNA decreased 1.2 log(6.7±0.32 to 4.5±0.6) in INF group after 4 weeks treatment
- HCV viral RNA rebound after stopping INF

**REFERENCES**


**CONCLUSIONS**

- This small animal model has proven predictive of clinical treatment impact for antiviral compounds for HCV as demonstrated by positive outcomes in the mouse model with Interferon alpha 2B and a protease inhibitor (BILN 2061), the only two therapies to date demonstrated to be effective in clinical application
- Results with lamivudine therapy for HBV infection in the model also mirror clinical outcomes. The mice can be infected with both HCV and HBV
- Other therapeutic strategies can also be evaluated with this system, including passive immunotherapy and gene therapy. We have previously reported the ability of Hepatitis B immune globulin to prevent infection with HBV in this model (B)

**ACKNOWLEDGEMENTS**

Aukerman L, Hashash A, Kovelovsky K, MacKichon M, Ni ZJ, Platter J, Shoenaker K, and Weiner A from Chiron Corporation are acknowledged for their contributions to the study, and Lewis J, Bouzidi J and Yakunin A for technical support in these studies.

**www.kmthepatech.com**

**Study 2: Effect of protease inhibitor BILN 2061 on HCV**

**Study Design**

- **Patients (n=6)**
- **Study**: Anti-HBV effect of Lamivudine in chimeric mice
- **Administration**: Twice a day, oral gavage
- **Courses**: 4 weeks treatment and 1 week follow-up post-treatment

**Assays**

- hAAT for monitoring human hepatocyte grafts
- Roche Amplicor for HCV RNA quantitative and qualitative testing

**Results**

- HBV viral load reduced consistently in lamivudine treated patients, 20 mg/kg b.i.d. by oral gavage
- HBV viral load decreased steadily and resulted in a significant (p<0.001) decrease of 1.0 log from baseline to day 14
- A significant difference in the net decrease of HCV viral RNA exists between BILN 2061 and Vehicle (p<0.005), INF and vehicle (p<0.05) and INF and BILN 2061 and INF (p<0.03), respectively (ANOVA and LSD test)
- Results parallel reported impact of BILN 2061 in HCV-infected patients (Fig 3)

**Study 3: Anti-HBV effect of Lamivudine in chimeric mice**

**Study Design**

- **Patients (n=20)**
- **Study**: Anti-HBV effect of Lamivudine in chimeric mice
- **Administration**: Twice daily, oral gavage
- **Courses**: 4 weeks treatment and 1 week follow-up post-treatment

**Assays**

- hAAT for monitoring human hepatocyte grafts
- Light Cycler PCR for HBV DNA quantitation

**Results**

- HBV viral load reduced consistently in lamivudine treated patients, 20 mg/kg b.i.d. by oral gavage
- HBV viral load decreased steadily and resulted in a significant (p<0.001) decrease of 1.0 log from baseline to day 14
- A significant difference in the net decrease of HCV viral RNA exists between BILN 2061 and Vehicle (p<0.005), INF and vehicle (p<0.05) and INF and BILN 2061 and INF (p<0.03), respectively (ANOVA and LSD test)

**SUMMARY**

- We evaluated and validated our SCID/bg/lupgraft chimeric mouse model (KMT mouse) for testing anti-HCV and anti-HBV agents (INF alpha 2B, protease inhibitor BILN 2061, Lamivudine) and therapeutic strategies (gene therapy for HCV and passive immunotherapy for HBV). HCV viral load was reduced 0.7, 1.0 and 2.0 log (means) after 1, 2 and 4 week treatment with INF alpha 2B (1350 IU/kg/d), respectively. A small molecule protease inhibitor (BILN2061, 10mg/kg/bid) resulted in 2.0 and 1.4 log reduction of HCV viral load after 4 and 7 day treatment courses. Differences of reduction of HCV viral load between INF and BILN2061 treatment groups and corresponding controls were statistically significant (at level of p<0.05). Chimeric mice infected with HBV also demonstrated a positive response to lamivudine treatment (nucleoside analogue, 20 mg/kg/d). HCV viral load decreased steadily and resulted in 2.1 log decrease after 4 weeks treatment. In our repeated tests with INF alpha 2B and BILN2061, outcomes of HCV viral load were consistent and reproducible. This data provides validation of the KMT mouse model for testing anti-viral therapies for HCV and HBV.