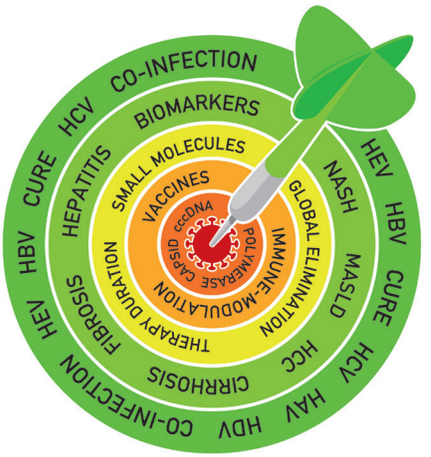
FRONTIERS IN DRUG DEVELOPMENT FOR HEPATOLOGY

HEP-DART 2023

HACIENDA DEL MAR, LOS CABOS • 3-7 DECEMBER 2023



ABSTRACT BOOKLET

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December 3rd, 2023

The US HCV Elimination Plan: What will be Required?

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A national program to eliminate hepatitis C virus (HCV) infection as a public health problem in the U.S. has been proposed by the Biden administration. Legislative language in support of this initiative is expected to be introduced into the Senate soon. Former US National Institutes of Health Director, Francis Collins, has championed this initiative and focusing the elimination plan on three main priorities: (i) increasing HCV diagnosis, linkage to care, and treatment, ii) promoting development and implementation of point-of-care HCV RNA tests, and iii) enhancing access to HCV direct-acting antivirals (DAAs). Indeed, it was the availability of all-oral, well-tolerated, DAAs that cure chronic HCV infection in ≥95% of persons with just 8-12 weeks of therapy, led to call for all countries to achieve HCV elimination targets -- 90% reduction in incidence of new HCV infections, and 65% reduction in HCV-related morbidity and mortality, both from a 2015 baseline – by 2030.

As at least 40% of infected persons are undiagnosed and among those diagnosis, only 23% with HCV infection covered by Medicaid, 28% covered by Medicare, and 35% covered by private insurance were prescribed antiviral therapy within a year of diagnosis. Patient, provider, insurer and system barriers contribute to this low diagnosis and treatment rate. The proposed national program requires a shift in how diagnosis and treatment are done. Decentralization of care and task-shifting to providers on the front-lines are critical -- with the goal of facilitating and supporting a “test-and-treat” approach. This is particularly important, as a high burden of HCV infection lies in those who do not regularly engage with healthcare systems. A “test-and-treat” approach also provides strong impetus to develop point-of-care diagnostics –one of the cornerstones of the national plan. Simplifying treatment and monitoring, removing barriers to access and supporting patients on their treatment journey will require resources and infrastructure. Additionally, the US should seek to develop a comprehensive HCV surveillance system like that used for monitoring HIV infection. The new national plan includes continued efforts in vaccine development. Because injection drug use is the predominant mode of HCV transmission in the US, both HCV harm reduction services and the availability of an HCV vaccine would be critical to reducing incident infections.

Such a program will be essential to get the US back on track for HCV elimination – without such a plan, HCV elimination is projected to go beyond 2050! This highlights the importance of Biden program. There is much hope riding on the delivery of this national plan and continued advocacy with representatives in the US Senate and House of Representatives is needed.

Session 1: Epidemiology and prospects for global elimination of viral hepatitis
December 3rd, 2023

The rocky road to HBV cure

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The global health burden of hepatitis B virus (HBV) infection remains high. Recent estimates suggest ~300 million people are living with chronic infection, which results in 820,000 deaths annually, primarily due to complications of cirrhosis and hepatocellular carcinoma (HCC).

Although currently approved therapies can effectively suppress viral replication, improve hepatic inflammation, and reduce progression to cirrhosis and HCC, they are not curative. Nucleos(t)ide analogues (NAs) are the preferred therapy due to better tolerability and safety profile compare to pegylated interferon alfa-2a. However, only a minority of patients, 1-3%, achieve the desired endpoint of functional cure, defined as the loss of hepatitis B surface antigen (HBsAg) and HBV DNA below the level of quantitation, that permits safe discontinuation of NAs. This is because NAs do not directly target the two sources of HBsAg in serum- covalently closed circular DNA (cccDNA), a non-integrated form of the viral DNA located within the hepatocyte nucleus that serves as the template for viral transcription, and integrated HBV DNA. Consequently, most patients require long-term therapy. Therefore, there is a need for improved, finite duration therapy that that can achieve high rates of functional cure.

Advancements in knowledge of the viral lifecycle, immunopathogenesis of HBV infection and delivery of therapeutics has led to an explosion in drug development for chronic HBV. Proof-of-principle pilot studies have confirmed the mechanism of action of most of these new therapeutics but as with other infectious diseases, a combination of agents with different mechanisms of action will likely be required for achieving functional cure. The most promising combination approaches focus on agents that inhibit viral replication, reduce antigenic burden, and partially enhance/restore immune control against HBV. However, determining the optimal combinations, timing and sequence of use, and duration of treatment remain a challenge. Additionally, different approaches may be required based on factors such as HBeAg status, viral load, HBsAg levels and presence of cirrhosis.

The more complicated the regimen to achieve functional cure, the greater will be the challenge to deliver curative regimens to low-and-middle income countries where the bulk disease burden exists. The ideal regimen would be one that is of finite duration, orally administered, easy to manufacture and scalable. To realize this goal, continued research, investment, and commitment from both the industry and the scientific community will be crucial.

Session 1: Epidemiology and prospects for global elimination of viral hepatitis
December 3rd, 2023

Viral Discovery Programs: Are there still new hepatitis viruses to be found?

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The most recent infectious agents to be discovered causing viral hepatitis were the hepatitis C virus in 1989 and the hepatitis E virus in 1990. Since that time, ongoing efforts to identify hepatotropic viruses that are associated with hepatitis (acute or chronic) have had limited success. There are a number of non-hepatotropic agents known to cause liver injury as a part of a generalized infection and they include, among others, EBV, CMV and most recently SARS-CoV2 has been added to this list. However, a number of cases of acute viral hepatitis remain where no viral agent can be found. This is best illustrated in patients with acute liver failure (ALF), where approximately 5% of cases are still cryptogenic (Ganger 2018). In the context of cirrhosis, there are also a substantial number of cases with a cryptogenic etiology, even after metabolic liver disease has been accounted for. Thus, among patients undergoing liver transplantation in the US, approximately 10% have cirrhosis of unknown etiology (source SRTR). More recently, an outbreak of acute severe hepatitis in children has been described, renewing interest in possible novel viral agents. Some publications have highlighted a possible role for human adenoviruses (adenovirus F type 41) and adeno-associated virus (AAV2), but a clear causative relationship has not yet been confirmed and this outbreak appears to have abated.

Using a metagenomic approach, we have attempted to identify novel viral agents in serum and liver samples from patients with a variety of liver diseases, including:

- Normal, apparently healthy blood donors from China with elevated ALT values but no evidence of known viral hepatitis agents (n=60)
- Patient with cryptogenic ALF (n=9)
- Patients with cryptogenic chronic liver disease, including chronic hepatitis and cirrhosis (n=9)

Our technological approach has evolved over the course of these studies but have largely relied on amplification of RNA and DNA from serum samples, using multiple displacement amplification (MDA). We have developed a variation of this technique that minimizes non-specific amplification, referred to as template-dependent MDA (tMDA) (Wang 2017).

No novel viral agents could be identified among blood donors with elevated ALTs (Li 2019) or among the small number with ALF (Ren 2020). However, a unique sequence was identified in a single subject with cryptogenic chronic liver disease. Serum from this patient included one contig, Seq260, confirmed by PCR in its original sample. Seq260 (387 bp), now in GenBank (MW468091), had no statistically meaningful hit in the NCBI databases, including GenBank, meaning it is not part of any known infectious agent or human DNA. Initial characterization of this contig indicates that:

- It is not a contaminant, as evaluated in the controls and the sample by PCR with DNA/RNA extracted using different methods
- It is DNA in nature, as it can be amplified without the RT step
- It is a virus-like sequence with a score of 0.96 predicted by machine learning
- It is likely from a negative-sense, linear single-strand viral genome as determined by enzyme digestion and gene-walking

We have not yet been able to subsequently find this sequence in serum from 120 other persons so far, including blood donors and patients with chronic hepatitis C virus infection, ALF or HCC.

Summary and Conclusions: We have discovered a novel linear single-stranded DNA sequence from the serum of a patient with cryptogenic cirrhosis using a metagenomic approach. Its association with liver disease is under investigation. It is likely that other viral agents causing hepatitis may exist, but are likely to be be rare or uncommon.

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Award Session
December 4th, 2023

Progress in Hepatitis B: A 4-decade Journey through 3 continents

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The course of chronic HBV infection comprises different phases based on the balance between HBV replication and host immune response. In the early 1980s, chronic HBV infection was viewed as comprising two phases: an early phase characterized by detection of HBeAg and HBV DNA and elevated ALT, and a later phase characterized by undetectable HBeAg and HBV DNA and normal ALT.
Improvement in sensitivity of HBV DNA assays and development of quantitative assays for new HBV markers in the last four decades have changed this simplistic view. Specifically, HBV DNA assays have evolved from semi-quantitative dot-blot hybridization assays with lower limit of detection ~1 million IU/mL to real-time PCR assays with range of quantification 10 – 100 million IU/mL. In addition, quantitative assays for HBsAg, HBV RNA, and hepatitis B core-related antigen (HBcrAg) provide additional insights into immune control of HBV infection and transcriptional activity of cccDNA. We now view the course of chronic HBV infection as comprising five phases: Immune tolerant, HBeAg+ immune active, Inactive carrier, HBeAg- immune active, and Occult HBV. In addition to host and environmental factors, virological factors notably HBV genotype and level of viremia are recognized to be important prognosticators.

In the 1970s, 4 HBV serotypes were recognized. The concept of HBV genotypes emerged in early 2000. Differences in distribution of HBV genotypes in different parts of the world may account for differences in outcomes of HBV infection, e.g., rates of spontaneous HBeAg and HBsAg loss, HCC development, and response to treatment notably interferon. HBV genotypes are also closely associated with precore and core promoter variants.
Integration of HBV DNA into host genome was recognized in the early 1980s as a potential cause of HCC. Integrated HBV DNA sequence is generally incomplete and not replication competent but it was not known to be responsible for HBV protein production until recently when studies showed that integrated HBV DNA is the predominant source of HBsAg in HBeAg- patients. This finding has major implications in achieving HBV “cure”, which is currently defined as sustained undetectable HBsAg and unquantifiable HBV DNA 6 months after stopping treatment.

Standard interferon was the first FDA approved treatment for hepatitis B, in 1992. The first oral antiviral, lamivudine, approved in 1998 was hailed as a savior because it had few side effects and could be safely used in patients with decompensated cirrhosis, but lamivudine is associated with high risk of antiviral drug resistance. Lamivudine has been replaced by entecavir and tenofovir which have negligible risk of antiviral drug resistance allowing these drugs to be used as long-term monotherapy. However, the oral antivirals – nucleos(t)ide analogues (NAs) do not have any direct inhibitory effect on cccDNA or integrated HBV DNA and virological relapse is universal when treatment is stopped, and HBsAg loss rarely occurs. The need for long-term treatment has spurred research into a cure for hepatitis B. Paradoxically, discontinuation of NAs is associated with a higher rate of HBsAg loss than continuing treatment though this strategy is less effective in Asians and can be associated with risk of hepatitis flares and hepatic decompensation.

Major efforts have been invested into a cure for hepatitis B in the past decade. Substantial progress has been made but despite double and triple combinations, few have led to HBsAg loss.
Nearly 60 years after the discovery of Australia antigen, hepatitis B remains an enigmatic disease and a cure elusive. However, safe and effective vaccines have been available for 4 decades and can prevent not only HBV infection but also HBV-related HCC and HBV-related mortality. It has been an amazing journey witnessing the mysteries of HBV and hepatitis B unfold during the last 4 decades. It is sincerely hope that the pace will pick up and a cure for hepatitis B and the goal of HBV elimination will soon be accomplished.

Session 2: Overview of viral hepatitis, pediatrics challenges and NASH
December 4th, 2023

Challenges in accessing pediatric formulations for children with HBV and HCV

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Chronic infections by hepatitis B virus (HBV) and hepatitis C virus (HCV) are major public health threats and causes of advanced liver disease and mortality worldwide. Globally, there is an estimated 3.2 million hepatitis C viremic children below the age of 18 years and 5.6 million children infected with hepatitis B below the age of 5 years. The persistence of HBV and HCV infections through adulthood increase the risk of morbidity and mortality including hepatocellular carcinoma. In addition to the importance of inclusion of this population in elimination strategies before adolescence and age of high risk behavior. The advent of direct acting antivirals for cure of HCV raised expectations for HBV treatment, which is currently far from being curative. The availability and economics of producing pediatric formulations remain key barriers for access to treatment to fulfill the elimination targets in this population. Egypt has exhibited an exemplary model for elimination of hepatitis C in children albeit recommendations for treatment of children with chronic hepatitis B with the currently available medications is limited by lack of data to support treatment in those with high viremia in the absence of evidence of inflammation (immunetolerant). There are various new classes of compounds under investigation with the aim of achieving high rates of HBsAg seroconversion. The candidate drugs are relevant to the pediatric population, as almost all act as immune modifiers to achieve tolerance breakthrough. These medications will undoubtedly alter the burden of HBV in children and adults alike, even if there are still many steps to be taken before they are available to the pediatric community. The likelihood of effective therapies that target tolerance breakthrough will spur more efforts to eradicate HBV at a young age. In conclusion, the WHO viral hepatitis elimination goal for 2030 cannot be achieved without inclusion of adolescents and children. This requires key stakeholders engagement to improve access to early diagnosis and pediatric formulations as well as prevention of mother to child transmission.

Session 2: Overview of viral hepatitis, pediatrics challenges and NASH
December 4th, 2023

Advances in Gene and Cell Therapies for MASLD and MASH

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Introduction: Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) and Metabolic Dysfunction-Associated Steatohepatitis (MASH) pose significant challenges globally, affecting up to a third of the world's population with no approved therapies available. Despite this, recent advancements in understanding molecular drivers of steatohepatitis and fibrosis have opened avenues for innovative approaches, particularly in cell and gene therapy. This lecture provides an overview of these technologies, highlighting specific molecular and cellular targets currently in development for both animals and humans.

Key Concepts:

- **Goals of Gene Therapy**: a) Correcting disease-associated mutations (e.g., PNPLA3 or HSD17B13 in MASH) b) Suppressing gene expression c) Restoring or augmenting gene expression d) Modulating alternative mRNA splicing

- **Types of Gene Therapies** – Carriers and Payloads: Gene therapies aim to modify gene expression transiently or permanently using various carriers and payloads. Carriers include viruses (especially adeno-associated viruses), lipid nanoparticles, and naked DNA. The success of nanoparticle delivery, exemplified by mRNA vaccines for COVID-19, underscores its widespread acceptance. Specific cell targeting is achieved through embedded antibodies or viruses with cell tropism. In the liver, therapies can target receptors such as asialoglycoprotein receptor (ASGPR) for hepatocyte delivery or β-PDGF receptors on activated stellate cells. Regulable gene expression can be achieved using promoters inducible by chemicals, ultrasound, or light (optogenetics). Inhibitory RNAs, particularly siRNA or shRNA, are established and the widely used payloads for liver disease, with emerging RNA editing technologies and CRISPR-based gene editing reaching clinical application.

- **Cell-Based Therapies**: Advances in chimeric antigen receptor (CAR) T cells, initially designed for hematologic malignancies, have extended to solid malignancies and non-malignant diseases, including fibrosis. Ex vivo engineered CAR T cells and in vivo generation, as well as CAR-NK cells and strategies involving other immune cell types, provide new therapeutic avenues. Recent studies demonstrate the potential of CAR T cells in treating hepatic fibrosis by targeting senescent stellate cells and in cardiac fibrosis by targeting fibroblast activating peptide-1 on heart myofibroblasts.

- **Gene Therapies for MASH**: Genome-wide association studies (GWAS) have revealed genetic variants associated with MASH. Inhibitory RNA therapy is being explored in human trials to reduce the expression of PNPLA3 and HSD17B13. For PNPLA3, the goal is to decrease expression of the disease-associated variant (I148M), while for HSD17B13, the objective is to replicate the protective effect of a splice variant (rs72613567:TA) resulting in reduced protein expression. Phase 2 clinical trials of HSD17B13 shRNA show particular promise.

Conclusions/Summary: The rapid expansion of gene delivery and cell therapy technologies presents unprecedented opportunities for treating MASH. Addressing key questions related to the durability, tolerance, off-target effects, and cost of these approaches compared to conventional strategies will be crucial. Additionally, determining whether single-genetic defect treatments are sufficient or if combination approaches are necessary remains an essential consideration in advancing these therapies.

Session 3: Hepatitis B basic science, immunology, and therapeutics (Hepatitis B Foundation sponsored session)
December 4th, 2023

Immunological and antiviral drug approaches to HBV in animal models

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Current treatment strategies for hepatitis B virus (HBV) are life long and rarely curative. Viral evasion and modulation of the immune response is key to the development and maintenance of chronicity. Restoring functional immune responses in chronically infected patients has been the challenging goal of several novel therapeutics. We aimed to decipher the impact of the capsid assembly modulator (CAM) GLP-26 on the viral replication cycle, and ensuing immune responses in the liver, in an HBV infected immunocompetent humanized mouse model.

As HBV only infects humans and chimpanzees, understanding the infectious life cycle and pathological mechanisms has been complicated. We established a xenografted mouse model harboring haplotype-matched humanized immune system and human hepatocytes in the liver (HIS-HUHEP mice). Following infection with HBV, these BRGS A2DR2 uPA-based HIS-HUHEP mice develop persistent viraemia for several months and mount innate and adaptive immune responses characteristic of a chronic infection. Treatment of chronically infected HIS-HUHEP mice with the CAM GLP-26 at 60 mg/kg/day per os for 2 months resulted in clearance of HBV DNA and reduced HBsAg levels (>2 log) in the plasma. This was accompanied by a reversal of some intrahepatic immune phenotypes observed in the chronically infected, notably for activated NK cells and memory $CD8^+$ T cells. Furthermore, CAM treated humanized mice had improved humoral responses with higher titers of anti-HBs IgG in the plasma.

To determine whether antiviral immune responses could be functionally restored, a second cohort of GLP-26 treated mice were followed off treatment for 2 months to investigate potential viral control. Interestingly, viral rebound occurred rapidly in 3/6 mice, whereas 2/6 had a delayed rebound, and 1/6 remained negative throughout. This was accompanied by changes in immune cell phenotypes and development of HBV-specific polyfunctional T cell responses in mice with delayed viral rebound or viral control.

Together, these results show that treatment with the CAM GLP-26 reduced viremia and viral antigens, associated with enhanced immune responses and potentially improved outcomes off therapy.

Session 3: Hepatitis B basic science, immunology, and therapeutics (Hepatitis B Foundation sponsored session)
December 4th, 2023

Do all patients need immunomodulatory therapy to achieve HBV cure?

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Encouraging data demonstrates that new combination therapies are beginning to achieve HBsAg loss in a significant proportion of chronic hepatitis B (CHB) patients. In some patients, HBsAg loss is durable, achieving functional cure, while others relapse, with HBsAg becoming detectable again during follow up. What determines cure vs. relapse remains unclear but, in the absence of a sterilizing cure, the immune system is believed by many to be a critical component to long-term, off-treatment HBV control. Therefore, immunological adjuvants such as IFN-α, therapeutic vaccines, checkpoint inhibitors, and innate immunomodulators are being/will be combined with novel direct acting antivirals (DAAs) to try and increase the durability of cure. Whether immunomodulation will be a requirement for durable cure, or endogenous immunity will be sufficient, is likely to be patient/cohort specific. This presentation will look at immune correlates of viral control, clinical trials where immunomodulation enhances functional cure rates, and immunological questions that need to be addressed in DAA therapies.

Session 3: Hepatitis B basic science, immunology, and therapeutics (Hepatitis B Foundation sponsored session)
December 4th, 2023

Depletion of cccDNA/pgRNA by long-term NUC therapy- HBV Cure

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Hepatitis B virus (HBV) chronically infects nearly 300 million people worldwide, and is the leading cause of liver-related death and hepatocellular carcinoma. HBV is difficult to cure because its genome, the covalently closed circular DNA (cccDNA), persists in every infected cell until it turns over. cccDNA is the template for all viral RNAs, including the pregenomic RNA (pgRNA). Nucleos(t)ide analogues (NAs) inhibit the reverse transcription of pgRNA to relaxed circular DNA (rcDNA), the viral DNA that is encapsidated in infectious virions and that is detectable in blood as HBV DNA. NAs are effective at interrupting reverse transcription sufficiently to reduce blood HBV DNA levels to undetectable levels in most people with chronic HBV (CHB). However, NAs do not directly address cccDNA, meaning that while HBV replication is largely suppressed during NA treatment, cccDNA typically persists indefinitely in CHB. Despite the inactivity of NAs on cccDNA, there is evidence that cccDNA levels decrease during prolonged NA treatment. These declines are likely due to the loss of cccDNA molecules during cell division when viral replication is suppressed. In addition, serum HBV RNA, largely comprising encapsidated pgRNA, appears to decline during NAs. Single-cell analyses with parallel measurements of cccDNA and pgRNA have shown that viral transcription appears to diminish during NAs, although the mechanism(s) underlying this apparent transcriptional silencing are unclear. Clinical investigations of emerging treatments for CHB in combination with NAs should be paired with translational studies to understand whether the novel therapies accelerate loss of cccDNA and whether they affect transcriptional silencing.

Session 3: Hepatitis B basic science, immunology, and therapeutics (Hepatitis B Foundation sponsored session)
December 4th, 2023

Liver Targeting Dihydroquinolizinones against HAV and HBV

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Background
Dihydroquinolizinones (DHQs) are a novel family of small molecules that have been shown to reduce circulating levels of hepatitis B surface antigen (HBsAg) in animals through induction of HBsAg mRNA decay, and block HAV infection via inhibition of TENT4 poly(A/B) polymerases. However, the peripheral neurotoxicity observed for an early lead, RG-7834, raised a safety concern for this series of compounds. To address this problem, we converted a systemically distributed RG-7834 to a liver selective new DHQs with high liver exposure and liver/plasma ratios. We hypothesized that having drugs that are more selective for liver hepatocytes, which are the cells targeted by HBV, is one way to minimize or eliminate unnecessary side effects resulting from the inappropriate distribution of RG7834 to other tissues.

Method
A recognition element for organic anion transporting polypeptide protein 1B1 (OATP1B1) and 1B3 (OATP1B3) which are abundant on liver hepatocytes was installed to the DHQ core. The new DHQ derivatives were designed based on the consideration of distribution coefficient (LogD), absorption, and metabolism. The compounds were evaluated *in vitro* against both HBV and HAV. Selected compounds were also assessed for their absorption, distribution, metabolism, excretion, and toxicity (ADME-Tox) and pharmacokinetic (PK) properties.

Results
Lead optimization through the addition of steric hindrance and modulation of tPSA and LogD of the new molecules resulted in the identification of new leads with low nano molar or sub-nano molar activities against both HAV and HBV. Encouragingly, high liver exposure and liver/plasma ratios were observed from the mouse PK studies. key findings from *in vitro* and *in vivo* studies will be presented.

Conclusions
Through chemical modification, the distribution of a molecule in the body can be changed and directed to the desired site. We demonstrated that incorporation of an acid group into the side chain of RG7834 could help the new derivative recognized by both OATP1B1 and OATP1B3, which facilitated the absorption into the liver. The new leads displayed potent antiviral activities against both HAV and HBV and demonstrated much better hepatoselective distribution in mouse PK studies than RG7834, thus providing an opportunity to improve the safety profiles.

Session 4: New advances in NASH, MASLD, fibrosis, cirrhosis, HCC treatment
December 4th, 2023

Plenary Lecture: Metabolite-induced activation of auto-aggressive T cells as cause of liver damage

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The immune system protects us against infectious pathogens but may also cause tissue damage through immune pathology. While infection of the liver with Hepatitis B Virus (HBV) is controlled by virus-specific immunity, particularly HBV-specific CD8 T cells, a compromised CD8 T cell immunity against HBV underlies chronic viral hepatitis. Also, fatty liver disease, such as NASH, runs a chronic course and causes liver damage that, similar to chronic viral hepatitis, leads to liver fibrosis, cirrhosis and eventually liver cancer. We identified a so far unrecognized effector function of CD8 T cells that is responsible for causing liver damage in NASH patients and preclinical NASH models. We termed this effector function “T cell auto-aggression” because the killing of target cells was not determined by T cell receptor stimulation after recognition of specific peptides presented in the context of MHC molecules. Rather, CD8 T cells killed liver cells indiscriminately and independent from T cell receptor stimulation. These auto-aggressive CD8 T cells are characterized by expression of the marker CXCR6 and display an activated phenotype. Key to the generation of CD8 T cells with auto-aggressive effector function is a single cytokine, i.e., Interleukin 15 (IL-15). Auto-aggressive CD8 T cells are generated in the context of fatty liver disease through the combined activity of short-chain fatty acids, that are released from steatotic hepatocytes, and IL-15, which drives increased expression of effector molecules such as GzmB. Auto-aggressive CD8 T cells require an activation step, which is achieved through signalling via the purinergic receptor P2X7. P2X7 is selectively expressed on CXCR6+ CD8 T cells and recognizes extracellular ATP that is released from steatotic hepatocytes. We found auto-aggressive CD8 T cells to be responsible for tissue damage in preclinical models of NASH and found increased frequencies of auto-aggressive CD8 T cells in NASH patients. Importantly, continuous tissue damage through auto-aggressive CD8 T cells also drives the induction of liver cancer in NASH. We are currently characterizing druggable pathways in auto-aggressive CD8 T cells that could serve as molecular targets for interfering with T cell auto-aggression. This presentation will review and discuss the key mechanisms determining tissue damage in metabolic liver disease and beyond.

Session 4: New advances in NASH, MASLD, fibrosis, cirrhosis, HCC treatment
December 4th, 2023

Unmet Needs for Management of Patients with Cirrhosis

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There are currently an estimated 112 million patients with compensated cirrhosis and 10.6 million with decompensated cirrhosis, representing a significant increase in prevalence over the past several decades. This increased disease burden has resulted in a larger number of patients with cirrhosis potentially needing various types of interventions, other than liver transplantation, to decrease morbidity and improve survival. Cirrhosis is a histological diagnosis of chronic liver disease in an advanced stage, while the term advanced chronic liver disease (ACLD) describes the range of advanced fibrosis and cirrhosis based on liver stiffness measurement (LSM) by transient elastography (TE) and platelet count. The progression from compensated ACLD (cACLD) to decompensated ACLD (dACLD) arises mainly from increased portal pressure, ongoing etiologic injury, systemic inflammation, and hemodynamic changes, and is defined by the development of overt ascites, overt encephalopathy, and variceal hemorrhage. The occurrence of the decompensation phase is a significant event, as the median survival for individuals in the asymptomatic compensated stage is >12 years but can drop to just around 2 years in the decompensated stage. As such there are unmet needs where the goals of treatment are of keeping the patient with cirrhosis alive. Convincing evidence on the use of carvedilol (NSBB) in preventing progression of cirrhosis from cACLD to dACLD in those with clinically significant portal hypertension (CSPH) has led to a recommendation of its use. More recent approval of terlipressin has been of benefit for those with hepatorenal syndrome-acute kidney injury. Major advances in drug development have been in hepatocellular carcinoma (HCC) immunotherapy and other strategies that have revolutionized care of the patient with HCC while they often have underlying cirrhosis. More recent positive Phase 3 studies with elafibranor (PPAR alfa/delta agonist) and seladelpar (PPAR delta agonist) in primary biliary cholangitis (PBC) serve as major advances.

The future holds many challenges for those with various stages of cirrhosis. Until now, the best anti-fibrotic has been an intervention such as directly acting anti-virals (DAAs) for HCV, and HBV suppressive therapy where regression of fibrosis has been noted after effective treatment of these chronic viral infections associated with advanced hepatic fibrosis/cirrhosis. Efforts to specifically regress fibrosis with drugs such as gamma interferon and caspase inhibitors have failed. Current strategies to regress fibrosis in those with metabolic syndrome associated steatohepatitis (MASH) and fibrosis/cirrhosis are again at treating MASH. MASH with fibrosis/cirrhosis represents a huge population that needs effective therapies while several strategies are underway.

Suggested reading:
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Session 5: From bench to bedside: Preclinical and clinical updates on hep B elimination
December 5th, 2023

Assessing the progress towards HBV curative regimens based on gene editing approaches

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Current licensed therapies against HBV efficiently suppress viral replication; however, they do not have significant effects on the intrahepatic covalently closed circular DNA (cccDNA), the viral minichromosome responsible for viral persistence. Thus, life-long treatment is required to avoid viral rebound. Several antiviral approaches are being explored to target different steps of the viral life cycle or to restore antiviral immune responses, but they indirectly target the cccDNA reservoir. Attempts to identify and develop small molecules that would degrade or silence cccDNA have not been successful yet. Novel technologies of gene editing have been developed and were applied to target HBV cccDNA. These approaches include meganucleases, ZNFs, TALENs, CRISPR/Cas9, programmable RNA guided endonucleases, and deaminase base editors. Experiments performed in hepatocyte culture and in mouse models showed that cccDNA can be targeted as well as integrated HBV DNA, resulting in prolonged inhibition of viral replication and HBsAg expression. Novel technologies of RNA guided epigenetic repressors are also evaluated in pre-clinical models. Optimization of delivery systems and careful evaluation of off target effects are being conducted.

Altogether, gene editing may provide proof of concept for direct cccDNA targeting and functional cure of HBV infection.

Session 5: From bench to bedside: Preclinical and clinical updates on hep B elimination
December 5th, 2023

HBV silencing: epigenetic modulation of the hepatitis B virus reservoir

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Infection with the hepatitis B virus (HBV) is a major public health problem of global impact since around 290 million people worldwide are chronically infected and at high risk of developing liver cirrhosis and hepatocellular carcinoma. Licensed polymerase inhibitors target the HBV reverse transcriptase activity, control the disease with long-term therapy but they fail to target the long-lived nuclear reservoir of HBV, the covalently closed circular DNA (cccDNA) minichromosome. Consequently, the production of viral RNAs and proteins, including the Hepatitis B surface antigen (HBsAg) is not abolished. Thus, there is an urgent need for therapeutic approaches able to destabilize or permanently suppress the cccDNA, since silencing of the viral reservoir may promote functional HBV cure. A deeper understanding of the mechanisms involved in cccDNA biogenesis, chromatinization, epigenetic regulation and stability may open new venues to control and eventually cure chronic hepatitis B (CHB). In this presentation, particular focus will be given to therapeutic interventions that may promote silencing of the cccDNA. In this regard, our team has previously employed HBV-infected human liver chimeric mice to investigate the impact of RNA interference strategies targeting all HBV transcripts (siRNA) and pegylated IFN alpha on the viral regulatory protein HBx and on cccDNA transcriptional activity. In particular, we analyzed the fate of the host restriction factor SMC5/6 (structural maintenance of chromosome 5/6 complex), which was shown to be targeted by HBx for degradation. These treatments strongly lowered HBx levels, thus enabling the reappearance of the SMC5/6 complex in human hepatocytes, its recruitment onto the cccDNA and suppression of cccDNA transcription (Allweiss et al. Gut 2022). Remarkably, SMC5/6-mediated silencing of the HBV minichromosome could be maintained for several weeks in vivo after treatment withdrawal by shielding the hepatocytes from new infection events. This was achieved by applying the entry inhibitor Bulevirtide, a compound that has received approval by EMA for the treatment of chronic hepatitis D. Although the impact of SMC5/6-mediated HBV suppression on the epigenetic landscape of the cccDNA minichromosome is still under investigation, independent studies (Peng et al. J. Virol. 2023) also highlighted the key role of new infection events to reactivate SMC5/6-mediated silencing of the cccDNA. Moreover, the association of the cccDNA to the SMC5/6 complex does not appear to hinder cccDNA loss during cell division (Seeger J. Virol. 2023; Allweiss et al. Gut 2018).

Session 7: Industry session: Getting closer to HBV curative approaches
December 5th, 2023

Long-term Dosing with ALG-000184 in HBeAg Positive Subjects Results in Unprecedented Multi-log Reductions in HBV Markers Including HBsAg

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Background: Chronic hepatitis B (CHB) treatment has been challenging due to the persistence of intrahepatic cccDNA. Mechanisms of action which address the establishment and replenishment of cccDNA are needed. In this regard, we are advancing ALG-000184, a prodrug of the potent capsid assembly modulator-empty (CAM-E) ALG-001075 that, in vitro, inhibits both viral replication (1st mode of action (MOA)) and cccDNA establishment (2nd MOA).

Methods: ALG-000184-201 is an on-going multi-part, multi-center, double-blind, randomized, placebo-controlled Phase 1b study (NCT04536337). Here we report emerging safety and antiviral activity data in ongoing cohorts following additional dosing of ≤48 weeks with ALG-000184 ± ETV in CHB subjects.

Results: Safety and antiviral activity data are available in HBeAg+ subjects dosed x ≤48 weeks with 300mg ALG-000184 alone (n=10, ex-China sites) or with ETV (n=11, China sites). Subjects were predominantly HBV genotype C (52%) and B (43%), Asian (95%) and male (57%) with a mean age of 33.8 years. ALG-000184 has been well tolerated with no serious adverse events (AEs) and no discontinuations due to AEs. All treatment emergent AEs (TEAEs) were Grade 1 or 2 in severity, except 4 subjects who experienced Grade 3 TEAEs of transaminase elevations (n=4) and Grade 4 neutropenia (n=1). All transaminase elevation events were associated with HBV DNA, RNA and antigen declines and have resolved or are improving with continued dosing; none were assessed by the study's safety committee as being related to study drug toxicity. Unprecedented reductions in all viral markers were observed during treatment, including mean reductions at Week 48 of 6.7 and 4.6 $\log_{10}$ for HBV DNA and RNA, respectively, as well as 1.2-2.0 $\log_{10}$ mean declines in HBsAg, HBeAg and HBcrAg. No viral breakthrough, measured by HBV DNA, has been observed with ALG-000184 monotherapy.

Conclusions: Dosing with 300 mg ALG-000184 ± ETV up to 48-weeks broadly suppresses production of HBV viral markers, indicating it may potently inhibit cccDNA formation and has the potential to become a cornerstone therapy for treating CHB. Longer dosing in these and additional cohorts is ongoing.

Session 8: Biomarkers, subviral particles, and mitochondrial dysfunction in the context of viral hepatitis and chronic liver disease
December 6th, 2023

Host cell factors associated with HBV virions and subviral particles or involved in their secretion and their role in the viral replication cycle

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Current antiviral therapies against HBV focus on the suppression of viral replication, but rarely lead to functional cure of patients. Host factors exploited by HBV during its lifecycle are potential alternative or additional drug targets to treat chronic hepatitis B patients. One set of such host factors might be proteins involved in the secretion of HBV virions or subviral particles (SVPs) or associated with these particle types. To identify host cell protein involved in HBV particle secretion, we conducted a siRNA-based screen and found that components of the neddylation pathway promote HBsAg production post-transcriptionally. Pharmacological inhibition of neddylation inhibited production of HBsAg encoded by integrants and reduced intracellular HBsAg levels, independent of HBx. To determine cellular proteins associated with HBV virions or SVPs, we established a protocol combining affinity chromatography, size exclusion chromatography, isopycnic centrifugation, and HBsAg-specific immunocapture to enrich HBV particles and SVPs with very high purity as revealed by biochemical analysis of purified fractions. LC-MS/MS proteomics analysis of the purified HBV virions and SVPs from two independent cell lines detected peptides of HBV polymerase, core, and envelope proteins, but no peptides corresponding to HBx or HBeAg. Importantly, our proteomic analysis revealed, in addition to known chaperones such as HSC70 and HSP90, ~160 host factors associated with SVPs and HBV particles and in addition a few host cell proteins present only in the HBV particle preparation. By phenotypic validation using siRNA-mediated knock-down and cells transfected with an HBV 1.1mer plasmid or infected with HBV, we identified 10 factors having various impact on HBV and SVP production. In this way, we confirmed several previously reported HBV host cell dependency factors and identified several novel ones. Their mechanistic role in the HBV replication cycle is under investigation.

Session 8: Biomarkers, subviral particles, and mitochondrial dysfunction in the context of viral hepatitis and chronic liver disease
December 6th, 2023

Serum biomarkers to follow-up chronic hepatitis B

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Currently we are able to treat chronic hepatitis B with nucleoside analogs which suppress viral replication but unfortunately do not lead to functional cure as indicated by HBsAg loss which is sustained off treatment. Traditionally our medical practice is dictated by biomarkers reflecting viral replication such as HBV DNA, HBeAg and quantitative HBsAg, biomarkers reflecting inflammation such as aminotransferases and biomarkers reflecting liver fibrosis using elastography, APRI and FIB 4.

In my talk I will reflect new biomarkers which could help us navigate towards functional cure and which could help us predict complications of liver disease such as liver failure and hepatocellular carcinoma. Biomarkers such as HBV RNA, HBcrAg and HBcAg are reflective of transcriptionally active CCC DNA and will be important not only to assess target engagement of new curate if drugs but they may also help us to predict the sustainability of response after treatment has been discontinued. It is currently unclear whether these biomarkers will have an additive benefit in our daily clinical practice beyond the traditional biomarkers such as HBV DNA and quantitative HBsAg. There is a need for more sensitivity of the new biomarkers as many patients with HBeAg negative disease on nucleoside analogue treatment already have an undetectable level before new curative therapy is even started.

During current investigational treatment strategies, the involvement of immunomodulatory drugs has increased as the combination of antiviral approaches using RNA interference and capsid assembly modulation, have not proved to be efficacious enough to gain functional cure. Therefore, we need an effective immune response to gain sustained functional cure. With that transition there is also an increasing need for immunological biomarkers reflective of immune reinvigoration during new treatments. Immune biomarkers are however difficult to standardize and usually only reflect one aspect of a complex cascade of the innate and adaptive immune response.

Lastly it is important to understand to what extent we can dissect the virological and immunological process is leading to functional cure just with biomarkers measured in blood. As the action mainly takes place in the liver there is an increasing need to assess intrahepatic biomarkers either using full tissue biopsy or by fine needle aspirates. The latter technique has the big advantage of being less invasive and can therefore be longitudinally applied in patients undergoing traditional or new curate if HBV treatment.

Session 8: Biomarkers, subviral particles, and mitochondrial dysfunction in the context of viral hepatitis and chronic liver disease
December 6th, 2023

CD38 is a key mediator of persistent inflammation and diminished antiviral immunity in chronic viral infections.

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Background

Chronic viral infections cause persistent inflammation due to sustained immune activation. Upregulation of CD38 on T cells is a hallmark of immune activation in chronic viral infections. CD38 functions as an ectoenzyme responsible for the degradation of intracellular nicotinamide adenine dinucleotide (NAD+). Prior studies show that CD38 and NAD+ levels are inversely correlated. Since NAD+ is vital for mitochondrial function and influences the immune response, we evaluated the role of CD38 on mitochondrial and immune function in HIV and Hepatitis C Virus (HCV) infections.

Methods

We collected peripheral blood mononuclear cells from patients with HCV infection (before and after SVR), people with HIV (PWH) (virally-suppressed on antiretroviral therapy (ART)), and healthy donors without chronic viral infection. We characterized the immune activation phenotypes of CD8+ T cells by staining with antibodies for CD38 in PWH and patients with HCV before and after SVR. We confirmed the relationship between CD38 and NAD+ by measuring intracellular NAD+ levels in CD38-expressing CD8+ T cells by chemiluminescence (Promega NAD/NADH-Glo™) in the HIV and healthy donor cohorts. We also assessed for mitochondrial function by staining CD38+CD8+ T cells from the healthy donor cohort for mitochondrial-specific dyes to measure mitochondrial superoxide content (Mitosox red) and mitochondrial membrane depolarization (JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethyl-benzimid-azolylcarbocyanine iodide)) and analyzed these cells by flow cytometry. Last, to evaluate changes in viral-specific immunity and T cell function before and after SVR, we stimulated PBMCs from the HCV cohort with HCV peptides and measuring secreted cytokines. We used paired t-tests to determine differences in the above parameters for each of the three cohorts, as appropriate.

Results

T cells from untreated HIV and HCV infections had higher CD38 expression than cells after HIV suppression and HCV SVR. Higher CD38 expression on T cells from HIV and HCV patients exhibited NAD depletion, mitochondrial dysfunction (decreased membrane potential and superoxide production) resulting in a proinflammatory cytokine response. Suppression of HIV and SVR for HCV reduced CD38 levels and inflammatory cytokine response, while enhancing virus-specific T cell responses.

Conclusions

Our findings suggest that chronic viral infections, such as HIV and HCV, induce CD38 expression on T cells that results in reduced intracellular NAD+ levels and impaired mitochondrial function, contributing to diminished antiviral activity and induction of a proinflammatory state. CD38 expression declines after HIV suppression and HCV viral cure, resulting in improved antiviral and reduced proinflammatory responses. These findings reveal that the CD38-NAD+ axis is a novel target to reduce mitochondrial dysfunction and diminished antiviral immunity in patients with chronic viral infections, ultimately reducing end-organ damage and mitigating non-communicable disease morbidity.

Session 8: Biomarkers, subviral particles, and mitochondrial dysfunction in the context of viral hepatitis and chronic liver disease
December 6th, 2023

New HBV biomarkers: how will they change our treatment endpoints

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The goal of antiviral therapy for hepatitis B virus (HBV) infection is to reduce the risk of clinically relevant outcomes, including cirrhosis, end-stage liver disease and liver cancer (HCC). Because outcomes take years or decades to occur, surrogate outcomes are required to determine the effectiveness of novel therapies. Current therapies were approved based on their ability to suppress HBV DNA, normalize liver enzymes, improve liver histology and to a lesser extent, lead to clearance of hepatitis B surface antigen (HBsAg). All of these surrogates have been shown to predict clinical outcomes and thus have been accepted as reasonable endpoints for clinical trial design. However, as we move to a new era in HBV therapy, with the goal of achieving sustained HBsAg loss, also known as functional cure, novel biomarkers have emerged that may provide insights into the mechanism(s) of action of novel therapies as well as offering the potential for redefining therapeutic endpoints.

HBsAg loss is a problematic endpoint. On the one hand, HBsAg loss is associated with an excellent long-term prognosis and thus is meaningful, however it is rarely achieved with current therapies and many who remain HBsAg-positive also have a good prognosis. Understanding who among those that remain HBsAg-positive will not run into problems in the future is currently challenging. In part the challenge derives from the difficulty in determining where HBsAg is coming from. HBsAg may be made from transcription of cccDNA or may come from HBV DNA that has integrated into the host genome. Many theorize that because viral replication only comes from cccDNA, that the goal of antiviral therapy is to clear or transcriptionally silence all cccDNA in the liver. However, even if this elusive goal were achieved, an individual could remain HBsAg-positive due to HBsAg derived from integrated HBV DNA. As a result, it would be helpful to have tools to identify those with and without transcriptionally active cccDNA in the liver. Biomarkers of transcriptionally active cccDNA have been identified including HBV RNA, HBeAg, HBV core-related antigen (HBcrAg), possibly subtypes of HBsAg (S, M or L), and most recently, HBV core antigen (HBcAg). Quantitative surface antigen (qHBsAg) is also used routinely but does not distinguish between cccDNA and integrated HBV DNA-derived HBsAg.

These tools have been studied in different settings ranging from identifying those likely to have inactive disease to identifying patients who can safely stop nucleos(t)ide analogue (NA) therapy. They have also been used to predict HBV reactivation in the setting of immunosuppression and been explored as predictors of HCC development. Although they provide useful information in some settings, all the markers are limited by the current levels of sensitivity, which has been a barrier to their adoption in clinical use. Improved sensitivity assays are being developed that may be able to more reliably predict who has adequate control of cccDNA transcription to stop therapy. Ideally, a very sensitive marker or combination of markers could be used to identify those with no/very limited transcriptionally active cccDNA who have a good prognosis, irrespective of whether they remain HBsAg positive. This could allow for the definition of a partial cure, which would be extremely helpful given the challenges of achieving HBsAg loss with current therapeutic approaches. Ideally any approach to partial cure would be validated against clinical outcomes using well defined cohorts with stored samples.

In addition to their use in potentially defining functional cure, novel biomarkers may also give insights into how novel agents act. Importantly, biomarkers must be considered in the context of the agents being evaluated. For example, reduction of levels of circulating HBV RNA, which generally indicates inhibition of active cccDNA transcription, may also occur with a block to pgRNA encapsidation, as occurs with capsid assembly modulators (CAMs). Thus, reduction in HBV RNA indicates target engagement with CAMs but cannot be used to assess cccDNA transcription. Combining biomarkers may give insights into how specific agents act but there is clearly a need for improved biomarkers, particularly for better defining immune control of HBV replication. There is potential for ex-vivo immune assessments to prove valuable to identify patients best suited for a particular therapy, such as checkpoint blockade. In addition, immune biomarkers can be helpful to understand critical biology, such as the degree to which HBsAg reduction leads to any degree of immune restoration and/or increased capacity for immune stimulation.

The novel HBV biomarkers will be discussed with a focus on their utility with current therapies but more importantly how they may prove useful to define new therapeutic endpoints for clinical trials of HBV cure regimens.

Session 9: Immunotherapy and immune responses towards a functional cure
December 6th, 2023

How to distinguish HBsAg derived from integrated HBV DNA and from episomal HBV DNA

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Hepatitis B viral (HBV) DNA integration is apparently an incidental event in the hepatitis B viral life cycle but is thought to be a key step in HBV induced carcinogenesis. HBsAg is derived from both cccDNA and integrated viral genomes but it is proving difficult to identify a faithful characterisation of HBsAg in serum that provides an accurate picture to track HBsAg protein expression from these two sources.

There is evidence that despite diminishing levels of HBV DNA, there may be a skewing towards HBsAg expression from integrated viral genomes in HBeAg negative patients. Thus despite reductions in HBV replication, the risk of HCC may be increasing in some patients. Natural immunity or antiviral therapy can only remove or suppress replication in hepatocytes that express complete virions and thus the expression of HBsAg from integrated viral genomes may escape host defence mechanisms and thereby maintain a potential for carcinogenesis. HBV integrants can be detected in the majority of HBV related hepatocellular carcinoma.

While whole genome sequencing shows integration occurs at random sites within the human genome, hotspots have been identified in primary liver cancers, which suggest the possibility of enrichment of the HBV integrations and positive selection of hepatitis B virus integrants in the genome of hepatocytes that could progress to hepatocellular carcinoma. Activation of integration hotspot genes such as telomerase reverse transcriptase (TERT) or histone methyl transferase may emulate the insertional mutagenesis of oncogenic retroviruses. HBV integrations in cells can contain unique sequences at the junctions such as virus host chimera DNA which may provide a signature both for HBsAg protein expression and individual HCC, and the prospects of monitoring clonal hyperplasia via circulating junctional fragments as these may gain a growth advantage; as well as perhaps clarifying protein expression from integrated viral genomes.

It has proven difficult to reliably detect the derivation of HBsAg at the protein level although measurement of the relative quantities of the large, middle and small isoforms may provide some indirect evidence of the source of HBsAg. More promising are determinations at the transcriptome level; RNA sequencing and spatial distribution of RNA transcripts, HBsAg and HBcAg may specify the source of HBsAg.

Quantitative HBsAg is a viral biomarker reflecting the transcriptional activity of cccDNA as well as of integrated DNA. However quantitative HBsAg provides only indirect evidence of the derivation of HBsAg in HBeAg-negative patients because of varying expression which may be modulated by viral genotype or other host factors: Transcriptionally active HBV integrations contribute to residual intrahepatic HBsAg expression or may indeed constitute the main source of HBsAg in longstanding HBeAg negative disease. pgRNA and HBcrAg as well as determinations of phosphorylated and non-phosphorylated HBcAg provide indirect evidence of cccDNA transcriptional activity and therefore the relative abundance of these new biomarkers together with HBsAg may infer a dominant source of HBsAg.

There is an urgent unmet need to identify circulating biomarkers to characterised and to identify the source of HBsAg for appropriate monitoring and tracking of the natural history of hepatitis B, and to better understand the mechanism of action and potential limitations of new antiviral therapies including capsid assembly modulators, translational inhibitors and nucleic acid polymers.

Session 9: Immunotherapy and immune responses towards a functional cure
December 6th, 2023

Immune responses mediating HBV functional cure

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Functional cure, i.e. long-term stable control of HBV replication and antigenemia in the absence of therapy, should be a feasible goal of advanced HBV therapies, given that it is the outcome of almost all adult infections. More importantly it can still be observed even after decades of chronic infection, in patients on treatment or without. However, no current treatment protocol achieves substantial rates of functional cure. Despite wide agreement that induction of functional cure will require a combination of antiviral and immunomodulatory drugs with or without the addition of therapeutic vaccines. there is significant uncertainty about the best therapeutic combinations that should go forward into future trials. This is a result of insufficient investments into HBV research after the availability of prophylactic vaccines, leaving us with limited data on the immune correlates of functional cure but also on key immune deficiencies preventing functional cure in the different stages of chronic HBV infection. As a consequence, it is not exactly clear which arms of the immune system require modification and in what CHB populations immune-based therapeutic intervention might be most easily achieved. In addition, a lack of detailed molecular immune analyses in the context of clinical trials with novel HBV agents means that their impact on immune responses also remains unclear.

In this presentation I will discuss the significant challenges posed by HBV as the most complicated of all chronic infections in humans, our current knowledge base of the immune correlates of HBV functional cure, and what I see as the most pressing immunological questions in both HBV natural history and HBV treatment that should be answered to inform novel treatment approaches and the combination of different treatment modalities.

Session 10: Treatment and elimination of HCV in underserved populations
December 6th, 2023

Colocation of HCV and SUD care in patients with substance use disorder: A critical component of HCV elimination

Elana Rosenthal
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Ongoing injection drug use continues to be a major barrier to HCV elimination worldwide. This talk will address strategies for treating and retreating HCV in people who inject drugs. In addition, this talk will elucidate the importance of scaling up substance use disorder treatment and harm reduction alongside HCV treatment, highlighting the additive benefit of concurrent and co-located treatment of HCV and substance use.

Session 11: Non-ABC viral hepatitis: Epidemiology, pathogenesis and treatment
December 7th, 2023

An update on entry inhibition and its synergistic effects on HBV and HDV infection

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The envelope proteins of HBV mediate attachment of HBV and HDV to heparan sulfate proteoglycans on the surface of hepatocytes and the subsequent specific interaction to the receptor NTCP. The myristoylated N-terminal preS1 domain of the large (L-) surface protein is responsible for the specific interaction with NTCP. Bulevirtide (BLV)/ Hepcludex, formerly known as Myrcludex B, mimicks the receptor-binding domain within the L protein and acts as a potent, highly selective entry inhibitor of HDV and HBV into hepatocytes. Monotherapy with BLV is save and results in HDV-RNA decline in most patients, and subsequently BLV became the first approved anti HDV drug and recently achieved standard marketing authorization in Europe. Additionally, small molecules specifically inhibiting the interaction with NTCP are under investigation. The conjugation of bile acids to short preS1 derived peptides have also showed promising inhibitory potency in vitro, offering the possibility of improved entry inhibitors.

As alternative to blocking the receptor on the host cell, antibodies against HBV envelope proteins are used to prevent receptor interaction through targeting viral particles. Hepatitis B immune globulin (HBIG) is used to prevent HBV infection during liver transplantation and vertical transmission from infected mothers to newborns. Currently, new neutralising antibodies targeting the preS1 domain of L protein or the antigenic loop of HBsAg are in preclinical and clinical development.

Achieving functional cure of HBV most likely requires a combination therapy targeting both, viral replication and the viral antigen load, as well as activating the immune response. With BLV treatment of chronic hepatitis D, optimal duration of the treatment, the rates of off-therapy responses, and the occurrence of low and non-response remain to be addressed. Combination therapy with BLV and PEG-IFNα have shown promising synergistic effects on HDV RNA and HBsAg. Similarly, combining entry inhibitors with therapies targeting the viral antigen load should be considered. For HDV, inhibitors targeting viral replication or secretion accelerate the HDV RNA in combination with entry inhibition.

This talk will give an overview of HBV and HDV entry inhibitors that target either the virus or host entry factors. Furthermore, I will discuss the benefits that could arise from entry inhibition regarding prevention and treatment of infection and synergisms that could be expected with other drugs.

Session 11: Non-ABC viral hepatitis: Epidemiology, pathogenesis and treatment
December 7th, 2023

Surprising antiviral activity of dihydroxyquinolones against hepatitis A virus

You Li, Ichiro Misumi, Yanming Du, and Stanley M. Lemon

Despite excellent vaccines, resurgent outbreaks of hepatitis A have caused thousands of hospitalizations and hundreds of deaths within the U.S. in recent years. There is no effective antiviral therapy, and many aspects of the hepatitis A virus (HAV) replication cycle remain to be elucidated. A dihydroquinolizinone (DHQ) molecule RG7834, originally discovered as an HBV antiviral, was surprisingly active against HAV. RG7834 suppressed HAV replication in Huh7.5 hepatoma cell line (IC50 6.11 nM), and orally administered RG7834 potently inhibited HAV replication in mouse models of hepatitis A, sharply reducing serum ALT, hepatocyte apoptosis, and intrahepatic inflammatory cell infiltrates. RG7834 inhibits the noncanonical TENT4A/B poly(A) polymerases which associate with the zinc-finger protein ZCCHC14 to form a TRAMP-like complex. The ZCCHC14/TENT4 complex lengthens and stabilizes the 3' poly(A) tails of HBV viral mRNAs. However, it had no impact on the length of the HAV 3' poly(A) tail, stability of HAV RNA, or cap-independent translation, suggesting a distinct mechanism of action. By contrast, RG7834 blocked incorporation of 5-ethynyl uridine into nascent HAV RNA, indicating that TENT4A/B function in viral RNA synthesis. These results reveal previously unknown requirements for ZCCHC14-TENT4 in hepatovirus RNA synthesis, and suggest TENT4 inhibitors may be useful for preventing or treating hepatitis A in humans. A new generation of hepatoselective DHQ (HS-DHQ) derivatives has been developed that accumulate specifically within the liver, reducing extrahepatic drug exposure. These compounds retain activity against HAV, and likely offer an improved safety profile.

POSTER & ORAL ABSTRACTS

P.01 – HDV RNA and HDAg exhibit long half-lives in HDV-infected hepatocytes

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Background
Chronic hepatitis delta virus (HDV) infection is the most severe form of viral hepatitis, with patients at increased risks of cirrhosis, liver cancer, and mortality. HDV is composed of a 1.7 kb single-stranded circular RNA genome bound by its only viral protein, hepatitis delta antigen (HDAg). Viral replication is mediated by host RNA polymerase(s) copying the circular HDV genomic RNA (gRNA) to the HDV RNA antigenome (agRNA). HDV gRNA serves as the template for a single mRNA that encodes two forms of HDAg regulated through ADAR editing. Small HDAg (S-HDAg) promotes replication in the nucleus, while large HDAg (L-HDAg) promotes viral particle assembly in the cytoplasm. Understanding the viral kinetics, localization, and stability of the HDV RNAs and HDAg in hepatocytes may aid in the development of new treatments for chronic HDV.

Methods
We studied HDV genotype 1 strain (Taylor) in primary human hepatocytes (PHH). Localization and kinetics of the HDV RNAs were determined by confocal microscopy using hybridization chain reaction fluorescent in-situ hybridization. HDAg kinetics and localization were determined by western blotting and immunofluorescence using an antibody that detects both S-HDAg and L-HDAg. Nanopore sequencing was used to measure the percentage of non-edited vs. edited intracellular HDV RNA. HDV RNA half-life and cellular mRNA half-lives were determined following ethynyl uridine pulse labeling and RNA quantification by qRT-PCR. HDAg half-life was determined by confocal IF following cycloheximide treatment to block new protein translation.

Results
In HDV-infected PHH, steady-state levels of gRNA, agRNA, and HDAg were reached by day 4 post-infection. Both gRNA and agRNA localized as distinct clusters within the hepatocyte nuclei, with approximately twice as many gRNA-positive cells as agRNA-positive cells at steady state. HDAg co-localized with both gRNA and agRNA and was predominantly nuclear through day 4 post-infection before transitioning predominantly to the cytoplasm by day 12. Cytoplasmic localization of HDAg corresponded with L-HDAg expression, as evidenced by HDV RNA sequencing and anti-HDAg western blotting. Total HDV RNA exhibited a 5-day half-life in HDV-infected PHH, while cellular mRNAs GAPDH, TBP, and Myc exhibited half-lives of 19, 14, and 5 hours, respectively. HDAg also had a long half-life of 46 hours in HDV-infected PHH compared to the cellular nuclear proteins PML and CDK4, which exhibited half-lives of ~24 hours.

Conclusions
Our data suggest that HDV RNA and HDAg have long half-lives relative to cellular mRNAs and proteins in HDV-infected hepatocytes. The high stability of HDV RNA might contribute, at least in part, to viral persistence in chronically infected patients.

P.02 – Liver concentrations of JNJ-73763989 in patients with chronic hepatitis B (CHB): The INSIGHT Study Panel 3 results

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Background

JNJ-73763989 (JNJ-3989) is a 2:1 combination of two N-acetylgalactosamine-conjugated small interfering RNAs (siRNA) targeting the S and X regions of the HBV genome. In AAV-HBV mice, the half-life of S and X triggers in liver were 12.8 and 15.3 days, respectively. Treatment of CHB with JNJ-3989 and nucleos(t)ide analogs (NA) ± bersacapavir ± pegylated interferon (pegIFN) has shown profound reductions in serum and intrahepatic hepatitis B virus (HBV) markers. Panel 3 of the INSIGHT study (73763989HPB2003) aims to assess liver concentrations of JNJ-3989 in CHB patients.

Methods

INSIGHT is a phase 2 multicenter longitudinal liver biopsy study in CHB patients who are hepatitis B e-antigen (HBeAg) positive and not currently treated (NCT, Panel 1), HBeAg-negative and virologically suppressed by NA (VS, Panel 2), or either (Panel 3). In the single center Panel 3 (the hepatology department of Hôpital Beaujon, Clichy, France), patients received 48 weeks of JNJ-3989 (200 mg per subcutaneous injection Q4W) + NA with optional pegIFN initiated after week 40. Core liver biopsies were collected using standardized procedures at weeks 12 and 40 with an optional biopsy at follow-up week 24. Liver concentrations for JNJ-3989 S and X triggers were measured from homogenized liver tissue using liquid chromatography with fluorescence hybridization. The specificity and sensitivity of the method was established, and baseline separation was achieved for all the target analytes.

Results

4 patients were enrolled: 2 each HBeAg+/NCT and HBeAg-/VS. All 4 had liver biopsy at week 12, and 3 at week 40 and all specimens were received by the central facility in good quality. Biopsy data at follow-up week 24 will be available at time of presentation. Individual liver concentrations (ng of trigger/g of liver) are tabulated below.

	Week 12		Week 40	
Patient	JNJ-3989 S trigger	JNJ-3989 X trigger	JNJ-3989 S trigger	JNJ-3989 X trigger
1	43580	48200	NA	NA
2	77040	103000	168420	263000
3	52960	77000	78830	134000
4	105320	121000	171800	229000

NA = not available

Mean baseline surface antigen was 3.62 $\log_{10}$ IU/mL and decreased ~3 $\log_{10}$ after 48 weeks of treatment. There were no clinically relevant adverse events including ALT flare. The limited sample size precluded any pharmacodynamic assessment with liver concentrations.

Conclusions

To our knowledge, this is the first report of siRNA liver concentrations in humans. Liver concentrations of S and X triggers increased 0.5- to 1.6-fold between weeks 12 and 40, consistent with intrahepatic accumulation due to the estimated long liver half-life.

P.03 – Early virological efficacy of the monoclonal antibody VIR-3434 and siRNA VIR-2218 for the treatment of chronic Hepatitis D Virus: preliminary results from the Phase 2 SOLSTICE trial

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Background

Hepatitis D Virus (HDV) infection is the most severe viral hepatitis with limited treatment options. In the Phase 2 SOLSTICE study (NCT05461170), we are investigating the efficacy and safety of VIR-2218, a small interfering ribonucleic acid (siRNA) targeting the HBx region of the HBV genome and VIR-3434, a Fc-engineered human monoclonal antibody targeting the conserved antigenic loop of HBsAg, in participants with HDV infection.

Methods

To assess the contribution of agents, small cohorts of non-cirrhotic participants received three doses of either subcutaneous (SC) VIR-2218 200 mg (Cohort 1a) or SC VIR-3434 300 mg (Cohort 1b), administered 4 weeks apart. Safety and efficacy with HDV RNA, HBsAg, and PK were assessed. At Week 12, participants who did not achieve ALT normalization *and* virologic response (defined as undetectable *or* ≥ 2 $\log_{10}$ decrease in HDV RNA) when given monotherapy were eligible for combination therapy with VIR-2218 and VIR-3434 given every 4 weeks (Cohort 2c).

Results

Preliminary results from Cohorts 1a, 1b, and 2c at Week 12 are summarized in the Table below. All participants were on nucleoside analogues (tenofovir or entecavir) with undetectable HBV DNA throughout. Twenty percent (1/5) of participants who received VIR-2218 Q4W monotherapy achieved a virologic response at Week 12. Two participants normalized ALT and one other participant (baseline HBsAg >10^4 IU/mL and HDV RNA > 10^5 IU/mL) experienced ALT increase starting at Week 12 (peaked with Grade 4 ALT elevation at Week 15).

Fifty percent (3/6) of participants receiving VIR-3434 Q4W monotherapy achieved a virologic response at Week 12. No ALT elevations have been observed. PK/PD analysis suggests that Q2W VIR-3434 monotherapy could result in greater HBsAg and HDV RNA declines.

Six participants (2 from Cohort 1a, 4 from Cohort 1b) transitioned to combination therapy (Cohort 2c); 5 participants have completed 12 weeks of treatment. Virologic response was observed in 100% (5/5) of participants at Week 12; to date, no ALT elevations have been observed even in participants with HBsAg >10^4 IU/mL. Few treatment-emergent adverse events occurred across all cohorts and were all Grade 1 and 2, including myalgia, headache, and chills.

Conclusions

After only 3 doses of VIR-2218+VIR-3434, all participants achieved HDV virologic response by Week 12. Few treatment-emergent adverse events occurred and were all Grade 1 and 2. Treatment was well tolerated. Evaluation of participants receiving VIR-3434 300mg Q2W monotherapy and *de novo* VIR-3434 and VIR-2218 combination therapy is ongoing.

P.04 – Efficacy and Safety of Bulevirtide in Combination With Pegylated Interferon alfa-2a in Patients With Chronic Hepatitis Delta: Primary Endpoint Results From a Phase 2b Open-Label, Randomized, Multicenter Study MYR204

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Background

Bulevirtide (BLV) is a first-in-class entry inhibitor approved in the EU for the treatment of chronic HDV infection (CHD). This Phase 2 study (MYR204; NCT03852433) evaluated the safety and efficacy of BLV (2 and 10mg) with or without peginterferon alfa-2a (PegIFN) in patients with CHD and compensated liver disease.

Methods

174 patients with CHD were randomized (1:2:2:2) and stratified based on the absence or presence of compensated cirrhosis to receive (A) PegIFN for 48 weeks (w); (B) BLV 2mg + PegIFN or (C) BLV 10mg + PegIFN for 48w, both followed by 48w of monotherapy with BLV 2mg or 10mg, respectively; or

(D) BLV 10mg for 96w. All patients were followed up for 48w after end of treatment (EOT). The primary endpoint was sustained virologic response at W24 after EOT (SVR24) defined as undetectable HDV RNA (<LLOQ, target not detected) with predefined comparison between Arms C and D.

Results

Demographics and baseline characteristics were similar across all arms. The majority were male (71%) and White (87%) with mean age of 41 years (SD, 8.7). Overall, 35% had compensated cirrhosis, mean liver stiffness was 13.1 (7.72) kPa, mean HDV RNA was 5.3 (1.2) log10 IU/mL, mean alanine aminotransferase (ALT) was 114.0 (94.8) U/L, 28% were on nucleos(t)ide analogue therapy, and 48% were interferon experienced.

SVR24 was achieved by 17% of Arm A, 30% of Arm B, 46% of Arm C, and 12% of Arm D (*P*=.0003; Arm C vs D). ALT normalization and composite endpoint at W24 after EOT were superior with BLV 10mg + PegIFN compared to monotherapy. HBsAg loss was only observed with the combination.

The most common adverse events (AE) were leukopenia, neutropenia, thrombocytopenia, influenza-like illness, lymphopenia, and vitamin D deficiency. AEs observed in the BLV + PegIFN combination arms were similar to those with PegIFN monotherapy. BLV dose-dependent bile acid elevations were asymptomatic, and levels returned to baseline after EOT. 6 patients (3%) discontinued treatment; none were assessed as related to BLV.

Conclusions

In patients with compensated CHD, BLV in combination with PegIFN resulted in higher rates of SVR24 and ALT normalization vs BLV or PegIFN monotherapy. Combination therapy was well tolerated with AEs consistent with PegIFN monotherapy. Longer-term off-treatment data at W48 will help define durability of finite therapy with BLV in combination with PegIFN for CHD.

P.05 – Efficacy and safety of siRNA JNJ-73763989, capsid assembly modulator JNJ-56136379, nucleos(t)ide analog (NA), and pegylated interferon alpha-2a (PegIFN-α2a) for treatment of chronic hepatitis B (CHB): final study results from the phase 2 PENGUIN study

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Introduction:
The phase 2, open-label, single-arm, multicenter PENGUIN study (NCT04667104) assessed the efficacy and safety of treatment with JNJ-3989, JNJ-6379, NA, and PegIFN-α2a in virologically suppressed (VS), hepatitis B e antigen positive (HBeAg+) or negative (HBeAg–)patients with chronic hepatitis B (CHB). On-treatment results have been presented previously and here we present the final study results including 48 weeks of follow-up.

Method:
Patients on NA received JNJ-3989 for 24 weeks and JNJ-6379 for up to 24 weeks (some did not start/discontinued JNJ-6379 due to protocol amendment) with PegIFN-α2a added during the final 12 weeks of JNJ-3989 treatment. The primary endpoint was the proportion of patients with ≥2 $\log_{10}$ IU/mL reduction from baseline in HBsAg levels at Week 24. Changes in viral markers from BL and the proportion of patients meeting NA stopping criteria (HBsAg <10 IU/mL, HBeAg–, HBV DNA < LLOQ, and ALT <3× ULN) were also assessed. After 24 weeks of treatment patients were followed with or without NA treatment for 48 weeks.

Results:
A total of 48 HBeAg+ (n=11) and HBeAg– (n=37) VS patients with CHB were enrolled, and 47 reached FUW48. Thirty-one (64.6%) patients met the primary endpoint and one (2.1%) patient achieved HBsAg seroclearance (HBsAg <0.05 IU/mL) at W24, and stopped NA. After a transient increase of HBsAg, HBV DNA, and ALT levels declined again and the patient achieved HBsAg seroclearance at FUW44 and 48. JNJ-3989 ± JNJ-6379+NA resulted in mean (SE) HBsAg changes from BL of –1.43 (0.07) $\log_{10}$ IU/mL at W12, –2.18 (0.08) $\log_{10}$ IU/mL at W24, and –0.71 (0.092) at FUW48 (**Figure**). Forty-four (91.7%) and 23 (47.9%) patients had HBsAg <100 and <10 IU/mL at W24, respectively, and 13 (21.7%) and 2 (4.2%) at FUW48. For HBeAg+ patients, mean (SE) changes from BL in HBeAg were –0.68 (0.09) $\log_{10}$ IU/mL at W12, –0.72 (0.11) $\log_{10}$ IU/mL at W24, and -0.62 (0.176) log10 IU/mL at FUW48. Of 11 HBeAg+ patients, 4 (36.4%), 3 (27.3%), and 3 reached HBeAg seroclearance at W12, W24, and FUW48, respectively. Of 15 (31.3%) patients who met pre-defined NA stopping criteria at W24, 14 stopped NA treatment. Four patients met pre-defined NA re-treatment criteria prior to or at FU week 48 and of the remaining 10, 3 had HBV DNA levels <LLOQ at EOS. All treatments were safe and generally well tolerated; adverse events (AEs) and laboratory abnormalities were in line with the known safety profile of PegIFN-α2a. A total of 8 serious AEs (SAE) were reported, 7 of them during follow-up, none related. One SAE (gastric cancer) led to withdrawal from the study. One AE (grade 4 neutropenia) led to discontinuation of PegIFN-α2a while JNJ-3989 + NA were continued.

Conclusion:
Treatment with JNJ-3989±JNJ-6379, NA, and PegIFN-α2a was generally safe and well tolerated in PENGUIN. While it resulted in a profound HBsAg reduction with 64.6% meeting the primary endpoint and 91.7% of patients achieving HBsAg <100 IU/mL at W24, other studies with JNJ-3989-based regimens without PegIFN-α2a had a comparable pattern of HBsAg decrease. Thus, the addition of PegIFN-α2a did not seem to improve antiviral activity in the studied population. Off-treatment mean HBsAg levels showed a re-increase which appeared to be more rapid than in other JNJ-3989 studies with similar populations.

P. 06 – A Rhesus Macaque Model of HIV/HBV Co-infection

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Background

HIV/HBV co-infection is common due to similar routes of transmission, with an estimated 5-20% of HIV-infected individuals also infected with HBV depending on disease prevalence and transmission rates. HIV/HBV co-infected individuals progress to chronic HBV infection more frequently and exhibit reduced HBV-specific T cell responses, with a higher probability of extensive liver fibrosis and hepatocellular carcinomas. Thus, a greater understanding of the interplay between HIV and HBV infections is urgently needed to design strategies to prevent accelerated liver disease. Rhesus macaques (RM) are a well-established non-human primate model for HIV research, and we discovered recently that antibody-mediated $CD4^+$ T cell depletion in RM leads to long-term, high-titer HBV replication. In this study, we investigated the potential of inducing natural $CD4^+$ T cell depletion via $SHIV_{DH12\ Clone\ 7}$ infection and using it to establish HIV/HBV co-infection in RM.

Methods

Animals were intravenously infected with $SHIV_{DH12\ Clone\ 7}$ ($5x10^3\ TCID_{50}$) followed by challenge with HBV (genotype D, $1x10^9$ virions, i.v) three weeks later. Weekly blood draws were taken to monitor HBV and SHIV infection, and track $CD4^+$ T cells and HBV surface antigens (HBsAg). Liver biopsies were obtained monthly to quantify HBV replication in the liver by quantitative PCR. Alanine transaminase (ALT) levels were also monitored weekly throughout the study timeline.

Results

Preliminary studies showed successful $CD4^+$ T cell depletion in two RM following SHIV infection. However, one RM controlled SHIV infection (Mamu-$B*08^+$) and $CD4^+$ T cells returned concurrent with clearance of HBV. The second RM exhibited SHIV (>10^5 copies/ml) and HBV (>10^4 copies/ml) chronic co-infection (>64 weeks). HBV infection was validated by the presence of HBsAg and HBV DNA in the serum and HBV RNA in the liver. Based on the preliminary results, we repeated the study with nine additional animals and found that four exhibited a similar trend of co-infection.

Conclusions

These results indicate that SHIV-mediated $CD4^+$ T cell depletion helps sustain HBV infection. Thus, we show for the first time an HIV/HBV co-infection model in RM that can be beneficial for studies investigating pathogenesis associated with co-infection; which will be critical for the further development of this model.

P.07 – Novel Diagnostic Assay Testing using Remnant Clinical Samples for Creation and Characterization of SARS-CoV-2 Variant Testing Panels

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Background

Soon after the first appearance of SARS-CoV-2, the need for rapid, accurate, and widely available testing quickly became apparent. As a part of the NIH's Rapid Acceleration of Diagnostics program (RADx), the analytical team at Emory was tasked with verifying SARS-CoV-2 rapid detection tests for accuracy and sensitivity. This need deepened when the virus began mutating and it was unknown how well current tests performed against these rapidly arising new variants. To help evaluate current as well as developing SARS-CoV-2 detection kits, a uniform testing panel is created using the clinical samples that remain after initial testing.

Method

Here, after extensive quality control, remnant clinical samples (RCS) of known lineage are pooled together, serially diluted, blinded (to eliminate bias for visually read tests), and used for analytical testing. As a new variant gains dominance across the country, a new panel is created using RCS of that lineage and used for test verification.

Results

By creating these uniform panels, tests can be directly compared to one another allowing for determination of how well each test was performing against each other and if there was any detection difference between the previous and current variants. Using RCS enables us to prepare panels rapidly, such that tests can be evaluated concurrent with variant appearance.

Conclusions

This data is crucial in ensuring that the SARS-CoV-2 rapid tests on the market, and those in development can detect the newest variant accurately. Three years into the pandemic, it has become clear how essential rapid detection tests are for preventing spread of pathogens, especially now that so many young children are back in school full-time. Knowledge gained from panel-making and testing techniques is also being implemented to evaluate rapid tests that detect other pathogens such as Influenza A, Influenza B, RSV, and Monkeypox.

P.08 – Transgenic rhesus macaques expressing human sodium taurocholate co-transporting polypeptide are susceptible to hepatitis B infection

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Background

Although an effective vaccine against hepatitis B virus (HBV) exists, more than 296 million individuals are living with chronic HBV. With no curative therapies available, nearly one million people die every year as a result of complications arising from chronic HBV infection. The species-specific nature of HBV leads to limited immunocompetent animal models to study HBV treatments. Though rhesus macaques (RMs) are not naturally susceptible, we have shown that HBV infects RMs transduced with an adenoviral (Ad5) vector expressing the HBV entry receptor, human sodium taurocholate co-transporting polypeptide (hNTCP), under the liver-specific transthyretin (TTR) promoter. However, “priming” RMs with Ad5-TTR-hNTCP before HBV infection has drawbacks: 1) the number of HBV-susceptible hepatocytes is limited by Ad5-TTR-hNTCP transduction efficiency, 2) Ad5-TTR-hNTCP transduction results in transient expression due to adenoviral genome loss during hepatocyte division, and 3) Ad5-TTR-hNTCP is immunogenic and could confound HBV replication dynamics.

Methods

To overcome these drawbacks, we created the first transgenic RMs with genomic hNTCP expression. We inserted a TTR-hNTCP piggyBac transposon expression cassette into the genomes of zygotes, then transferred developing embryos into surrogate dams for gestation.

Results

Blood testing revealed integration of TTR-hNTCP in two infant RMs. The presence of hNTCP DNA in the liver, skin, muscle, lymph nodes, and rectum confirmed genomic editing in both RMs. The high specificity of the TTR promoter facilitated mRNA expression exclusively in the liver at levels comparable to humans. Isolated transgenic primary hepatocytes were HBV-susceptible *ex vivo*, with viral antigens detected in the supernatant, and viral DNA and RNA detected within infected cells.

Conclusions

Utilizing gene editing technology, we have generated the first HBV-susceptible transgenic RMs. This innovative approach addresses the lack of physiological relevant animal models in HBV research and provides a unique opportunity for testing HBV therapies.

P.09 – Innate immune response to different HDV genotypes in primary human hepatocytes

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Background

Hepatitis delta virus (HDV) is the most severe form of viral hepatitis affecting 12 to 60 million people worldwide. HDV genotype 1 has been shown to induce a strong innate immune response in hepatocytes via activation of MDA5 and LGP2. While HDV RNA is thought to activate these pattern recognition receptors (PRRs), the specific pathogen-associated molecular pattern (PAMP) has not been determined. We reasoned that the high sequence variability across the eight HDV genotypes (GT) might provide insight into HDV recognition by these PRRs.

Methods

We profiled HDV kinetics and the innate immune response in primary human hepatocytes (PHH) by qRT-PCR and Luminex after infection with the HDV GT1 lab strain (Taylor) or representative clinical HDV GT1-8 strains.

Results

Infection of PHH with an HDV GT1 lab strain resulted in stable HDV replication for >10 days. HDV infection of PHH was accompanied by sustained induction of type III interferon gene expression (IFNL1, IFNL2, IFNL4), interferon-stimulated gene (ISG) expression (RSAD2, IFIT1, MX1), as well as secretion of various chemokines (CXCL10, CXCL11) in a multiplicity of infection-dependent manner. Representative clinical GT1-8 strains all induced an innate immune response, with the degree of induction generally correlating with infection rate. We next evaluated the innate immune response in HDV-infected PHH treated with lonafarnib (LNF), a farnesyl transferase inhibitor which has been shown to increase intracellular viral RNA levels of an HDV GT1 lab strain. Interestingly, LNF significantly increased intracellular HDV RNA levels of some (GT1, 2, 8), but not all (GT3, 5) HDV isolates, and only increased the hepatocyte innate immune response in the former with the strongest response observed in GT1.

Conclusions

The data from this study demonstrates that (i) HDV GT1-8 clinical isolates induce an innate immune response in PHH, and (ii) this response generally correlates with intracellular HDV RNA levels, consistent with MDA5/LGP2 sensing of the HDV (anti)genome. Further molecular characterization of different clinical HDV strains presents an opportunity to advance our understanding of PRR activation by HDV.

P.10 – Transient alanine transaminase increase during bepirovirsen treatment coincides with hepatitis B surface antigen decline in participants with chronic hepatitis B virus infection not currently on nucleos(t)ide analog therapy

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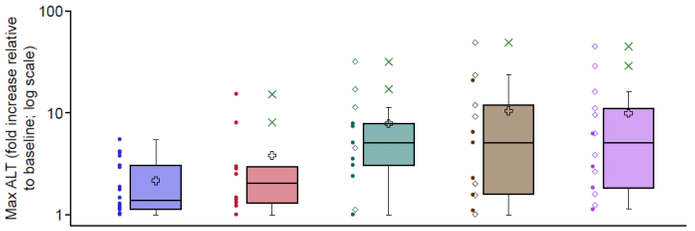
Background: Bepirovirsen is an antisense oligonucleotide that impacts hepatitis B virus (HBV) infection by reducing HBV DNA and viral proteins, including hepatitis B surface antigen (HBsAg), and stimulating the immune system, possibly via toll-like receptor 8 activation. In the B-Clear Phase 2b study, 73% of participants not on nucleos(t)ide analog (NA) therapy (Not-on-NA) who received bepirovirsen 300 mg weekly for 24 weeks achieved a HBsAg decline ≥1 $\log_{10}$ IU/mL at end of treatment. Bepirovirsen treatment was associated with transient increases in alanine transaminase (ALT); most were associated with concurrent HBsAg decline. The aim of this analysis was to characterize the timing and magnitude of on-treatment ALT increases and their relationship with bepirovirsen treatment response.
Methods: B-Clear was a Phase 2b, randomized, parallel-cohort study in participants with chronic HBV infection on stable NA therapy or Not-on-NA therapy. This post hoc analysis assessed on-treatment ALT increases in Not-on-NA participants treated with bepirovirsen 300 mg weekly (with loading dose) for 24 weeks (n=70). Maximum (max) ALT increase from baseline was assessed in relation to max HBsAg decline from baseline (<1.5, 1.5–<3, 3–<4, 4–<4.5, and ≥4.5 $\log_{10}$ IU/mL). Values measured after initiation of new antiviral therapy (rescue medication) were excluded.
Results: Most participants (61/70 [87%]) had a max ALT increase by Week 12. The max increase in ALT ranged from 1.0- to 48.5-fold relative to baseline (**Figure**). Participants with max HBsAg decline ≥3 $\log_{10}$ IU/mL had the greatest median max ALT increase (5.0–5.1-fold from baseline); only two participants with max HBsAg decline ≥3 $\log_{10}$ IU/mL had no ALT increase from baseline. Max HBV DNA decline was greatest in participants with max HBsAg decline ≥3 $\log_{10}$ IU/mL. On-treatment HBsAg loss and HBV DNA <LLOQ was only observed in participants who had a max HBsAg decline of ≥3 $\log_{10}$ IU/mL (n=22). The majority of participants with a ≥3 $\log_{10}$ IU/mL reduction in HBsAg and a max ALT >10x baseline achieved HBsAg loss (10/11); however, HBsAg loss could be achieved with no/minimal ALT increase.

Figure: Max ALT increase and max HBV DNA decline by max HBsAg decline



Max HBsAg decline category, $\log_{10}$ IU/mL*	<1.5 $\log_{10}$	1.5–<3 $\log_{10}$	3–<4 $\log_{10}$	4–<4.5 $\log_{10}$	≥4.5 $\log_{10}$
n	18	10	13	13	14
Max ALT fold increase, mean (SD)	2.1 (1.4)	3.8 (4.5)	7.8 (8.4)	10.4 (13.7)	9.8 (12.6)
Max ALT fold increase, median (range)	1.4 (1.0–5.5)	2.0 (1.0–15.2)	5.0 (1.0–31.6)	5.1 (1.0–48.5)	5.0 (1.1–44.8)
Participants with no ALT increase from baseline, n	3	1	1	1	0
Baseline HBsAg, mean (SD) $\log_{10}$ IU/mL	4.1 (0.8)	4.1 (0.4)	3.4 (1.0)	3.3 (0.6)	3.8 (0.5)
Baseline HBV DNA, mean (SD) $\log_{10}$ IU/mL	5.3 (1.6)	5.2 (1.4)	5.3 (1.7)	4.7 (1.5)	4.7 (1.6)
Max HBV DNA decline, mean (SD) $\log_{10}$ IU/mL	−1.1 (0.7)†	−2.9 (1.4)	−3.7 (1.5)	−3.7 (0.9)	−3.7 (1.0)
Participants with HBsAg and HBV DNA <LLOQ at the same timepoint, n	0	0	5	7	10

HBsAg, HBV DNA and ALT data from Week 1 up to and including Week 24 are included. Max ALT fold increase, max HBV DNA and max HBsAg declines are relative to baseline. The empty diamonds represent participants who reached HBsAg <LLOQ, the filled dots are participants who have not reached HBsAg <LLOQ. The cross and green x are mean and outliers, respectively. *n=2 participants with missing data; †n=15 participants with available data.

Conclusions: Most participants in the B-Clear Not-on-NA cohort with an ALT increase experienced an ALT peak by Week 12. Greater declines in both HBsAg and HBV DNA were observed with higher magnitude ALT increases, suggesting that these transient ALT increases may be a manifestation of immune-mediated clearance of HBV-infected hepatocytes.
Funding: GSK (study 209668)

Disclosures: J Cremer, S You, R Elston, W Jordan, T Lukic, L Maynard, G Quinn, J Singh, S Kendrick, M Paff, and D Theodore are employees of GSK and hold stocks/shares in GSK.

P.11 – Comparative analysis of human, rodent and snake Kolmioviridae replication

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Background
The recent discovery of Hepatitis D (HDV)-like viruses across a wide range of taxa (birds, snakes, bats, rats, deers, marmots, birds, frogs, fishes and insects) led to the establishment of a novel realm, Ribozyviria with a single family, Kolmioviridae, that includes the genus Deltavirus as well as seven other novel genera of kolmiovirids. Recent studies suggest that kolmiovirids can be satellites of viruses other than Hepatitis B virus (HBV), challenging the strict HBV/HDV-association dogma. These discoveries indicated that this new viral family is far more diverse and widespread amongst the animal kingdom than originally thought. Because other kolmiovirids were only recently discovered, most of our knowledge of the biology of these agents stems from research on HDV. Although newly discovered kolmiovirids share similar genome size and organization with HDV, they appear to differ from HDV in many aspects: 1- they are not restricted to the liver of infected animals; 2- they haven't been linked to a Hepadnaviridae co-infection and 3- they do not seem to encode for large delta antigens. The capacity of these satellite viruses to enter virtually many cell types coupled to their exclusive reliance on host factors for replication begs the question: Can kolmiovirids replicate in any cell type they access? This is essential to assess their zoonotic potential.

Methods
Here, we compared replication of three kolmiovirids: human (HDV), rodent (RDeV) and snake deltavirus (SDeV) in vitro and in vivo. Using HDV, RDeV and SDeV infectious clones we characterize their replication in a variety of animal cell lines and in an in vivo mouse model. We use Northern and Western Blots to follow viral RNA and protein accumulation in 9 different animal cell lines. Using human, mouse and snake cells persistently infected with HDV, RDeV and SDeV, we determine the sub-cellular localization of viral RNA and proteins using single molecule RNA FISH (smFISH) and immunofluorescence followed by high resolution microscopy. We also use hydrodynamic tail-vein injection in a mouse model to assess if these viruses are able to replicate in mouse livers.

Results and Conclusions
- Kolmioviridae antigens share both sequence homology and a modular structural organization, suggesting similar biological functions.
- While RDeV appears to be a generalist, able to replicate in most tested cell lines, SDeV seems to have the narrowest host range.
- Viral RNA condensates surrounded by a layer of Delta Antigen proteins, that we refer to as viral hubs, seem to be a defining feature of kolmioviridae accumulation in infected nuclei, as they were observed in human, mouse and snake cells and also in vivo in infected mouse livers.

Unlike SDeV, both HDV and RDeV replicate efficiently in mouse livers and induce an Interferon Stimulated Gene (ISG) signature.

P.12 – In-person interactions impact on HCV, HIV, and Syphilis Rates in At Risk Populations

Ford P[1], Heather House[2] - [1]Omega Specialty Nurses (OSN)/SRx Health Solutions (SRx)[2]

Background:
During Covid-19 restrictions, OSN/SRx worked with two community partners to address Saskatchewan's at-risk population's growing HCV, HIV, and Syphilis rates. This was displayed as a poster at INSHU 2022. Since June 2022, we've partnered with six more community groups. Public Health did not resume STBBI testing and community support when restrictions were eased. Our teams closed this screening gap.

Description of the model of care/intervention:
A nurse, two phlebotomists, a peer support worker, and a community outreach worker make up the OSN/SRx teams. Our teams visit our community partners weekly to test for HCV, HIV, and syphilis and connect clients to additional care, treatment, and support. Needle exchange clinics, drop-in centers, and safe injection sites are included. Addictions and harm-reduction education are also available.

Effectiveness:
200 HCV, 44 HIV, and 13 syphilis positives were found in 368 OSN/SRx tests in 2021. 28 of 80 HCV- positive patients underwent therapy. In 2022, we tested 686 people, the majority were tested between June and December. 274 were HCV positive, 51 were HIV positive, 8 were newly diagnosed with HIV, and 36 were newly diagnosed with syphilis. 44 of 97 HCV-positive individuals underwent therapy.

Conclusion and next steps:
Due to government restrictions on venue capacity during the COVID-19 pandemic, many at-risk communities were unable to access services. HCV, HIV, and Syphilis infections increased in 2022. Anecdotally, the pandemic, lack of services, scarcity of clean needles, and increased isolation that fostered drug use contributed. We can now offer in-person testing and meet clients where they are without restrictions. By adding testing sites, we've tested more people. We expect this knowledge to help conference attendees tackle rising HCV infection rates.

P.13 – Promoting Health Equity for Women with Hepatitis C by Reducing Disparities in Research

Catherine Frenette[1], Stacey Scherbakovsky[1], John Wolf[1], Linda Chen[1] - [1]Gilead Sciences, USA

Background

Significant gender disparity exists in the HCV cascade of care. Researchers must ensure that disparities in data collection and analysis will not inadvertently perpetuate under-diagnosis and under-treatment for women. This is a call for equity in health research to: (1) address the double stigma some women face with injection drug use (IDU) and substance use disorder (SUD), and (2) articulate women's experiences.

Method

The team evaluated published studies and policies focusing on the lived experience of women infected with HCV.

Results

Women and HCV

The WHO (2022) estimated 29 million women worldwide have chronic HCV, with incidence in women increasing. The US Centers for Disease Control (CDC) found a 250% increase in women's HCV infection (2004-2014).

Women with HCV, SUD and IDU

Women face higher HCV risk than men due to overlapping sexual and injection factors, including greater likelihood of IDU sex partners, syringe sharing, injecting after male partners, and being injected by others. Women with HCV and IDU encountered double stigma and were less likely to participate in harm reduction services, receive HCV monitoring and treatment per guidelines, and less adherent when taking chronic medications. Treatment for women must be specifically managed due to potential differences in pharmacokinetic response and adverse events.

Women of Child-Bearing Potential (WOCBP)

The CDC reported an 89% increase in US maternal HCV infection (2009-2014). The Center for Disease Analysis estimated 14.9 million women aged 15-49 worldwide had HCV (2019). The CDC, AASLD, ACOG and IDSA recommend screening or testing for all pregnant women. Studies found significantly higher rates of gestational complications, increased maternal mortality, and those with >6 log10IU/mL had a four-fold risk increase for mother-to-child transmission.

Conclusions

Effective, gender-inclusive research and care models must be consistently implemented to achieve viral hepatitis elimination by 2030.

P.14 – Healthcare seeking behavior and selected gynecological conditions in women with and without hepatitis C: An observational study of women in the United States

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Background:
Women living with chronic hepatitis C (HCV) are believed to have an elevated risk of early menopause (EM, i.e., menopause before the age of 45) or primary ovarian insufficiency (POI, i.e., menopause between the ages of 20-39). In this study we aim to understand rates of selected gynecological conditions including EM/POI in women with and without HCV, as well as how frequently these women receive routine gynecological exams and undergo cervical screening tests (known as Papanicolaou, PAP smears).

Methods:
This is a retrospective, observational cohort study of (IQVIA PharMetrics Plus™ claims data) for women aged 20-44 in the United States from 01 Jan 2006 to 31 Dec 2021. Women with or without HCV were analyzed between 2006 and 2020. HCV infection was defined a ≥2 diagnoses for chronic HCV. The proportion of gynecologic exams and PAP tests yearly was estimated for both groups. Additionally, the proportions of women with polycystic ovarian syndrome (PCOS), endometriosis, and excessive and frequent menstruation (EFM) diagnoses were examined yearly in both cohorts. For EM/POI, age-matched cohorts were utilized, and this analysis was restricted to 2006-2014 (i.e., only during the ICD9 era).

Results:
Between 2006 and 2020, the average size of the yearly cohort with HCV was 542 and the cohort without HCV was 2,088,474. The mean annual frequency of yearly gynecological examinations was 4.3% in women with HCV and 34.1% of women without HCV. The average proportion of women with at least one claim for a PAP smear in a year was also lower in women with HCV (5.3% vs 38.5% respectively). Compared to women without HCV, women with HCV had lower average yearly? rates of PCOS (0.7% vs 2.7%), endometriosis (0.2% vs 0.7%), and/or EFM (1.0% vs 3.5%) but it is unknown if this is related to the significantly lower rates of gynecologic exams resulting in missed diagnoses.
The proportion of women with EM/POI was 14.2% in women living with HCV, and 5.5% in age-matched women without HCV between 2006-2014. Compared to women without HCV, the proportion of women with HCV with POI was higher (9.6% vs 3.7%) and EM was higher (4.6% vs 1.8%). HCV-infected women reported more vasomotor symptoms (13.5% versus 8.9%), osteoporosis (3.9% versus 0.7%) and incontinence (5.6% versus 2.6%) than uninfected women within 365 days of their initial EM/POI diagnosis.

Conclusion:
Women with HCV have claims for gynecologic exams and PAP smears nearly eight times less frequently than women without HCV in this dataset. As women with HCV exhibit substantially lower healthcare seeking behaviors, it is likely that the frequency of PCOS, endometriosis, EFM and EM/POI are underestimated. Despite this, nearly one in seven women living with HCV aged 20-44 had a diagnosis for EM/POI, compared to only one in 18 women without HCV. Better understanding of both disease state extrahepatic risk factors for women and structural barriers to care will help inform solutions that address these disparities and engage women living with HCV in their care.

P.15 – Efficacy and Safety at 96 Weeks of Bulevirtide 2-mg or 10-mg Monotherapy for Chronic Hepatitis Delta: Results From an Interim Analysis of a Phase 3 Randomized Study

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Background:
Bulevirtide (BLV) is a first-in-class entry inhibitor for chronic hepatitis delta (CHD), which was conditionally approved in the Europe in July 2020. Results from the week-48 primary endpoint analysis for MYR301 (NCT03852719), a Phase 3 randomized study, showed monotherapy with BLV at 2 mg/d or 10 mg/d given subcutaneously was superior to no active anti–hepatitis delta virus (HDV) treatment based on the combined virologic and biochemical response. Efficacy was similar at the 2 dose levels, and BLV was generally safe and well tolerated. Here, we present findings from the predefined week-96 interim analysis of this ongoing study.

Methods:
In total, 150 patients with CHD were randomized (1:1:1) and stratified based on the presence/absence of compensated cirrhosis as follows: arm A, no active anti-HDV treatment for 48 weeks followed by BLV 10 mg/d for 96 weeks (n = 51); arms B or C, immediate treatment with BLV at 2 mg/d (n = 49) or 10 mg/d (n = 50), respectively, each for 144 weeks; all groups had 96 weeks of follow-up after end of treatment (ie, up to week 240). The combined response was defined as undetectable HDV RNA or decrease by ≥2 $\log_{10}$ IU/mL from baseline and alanine aminotransferase (ALT) normalization. Other endpoints included virologic response (undetectable HDV RNA or decrease by ≥2 $\log_{10}$ IU/mL from baseline), ALT normalization, $\log_{10}$ change in HDV RNA, and change in liver stiffness (LS) by transient elastography.

Results:
Baseline characteristics were similar between groups and included mean (SD) age 41.8 (8.4) years, 57% male, 83% White, 47% with compensated cirrhosis, mean (SD) HDV RNA of 5.05 (1.34) $\log_{10}$ IU/mL, mean (SD) ALT of 110.9 (69.0) U/L, and mean (SD) LS of 15 (8.9) kPa; 61% were on concomitant nucleos(t)ide analogue therapy. Of 150 patients, 143 (95%) completed 96 weeks of treatment. Week-96 efficacy responses were improved vs week 48. At week 96, similar combined responses were seen in arms B and C. Virologic and biochemical responses were also similar among arms B and C. BLV was well tolerated; there were no drug discontinuations and no serious adverse events or deaths attributed to BLV. Increases in bile acids without a correlation to pruritus or other symptoms were noted with BLV treatment. Injection-site reactions occurred in a higher proportion receiving 10 mg/d dosing.

Conclusion:
BLV continues to be safe and well tolerated as monotherapy for CHD through week 96.

P.16 – N6-Methyladenine Modification of Hepatitis Delta Virus Regulates Its Virion Assembly by Recruiting YTHDF1

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Background

Hepatitis delta virus (HDV) is a defective satellite virus that uses hepatitis B virus (HBV) envelope proteins to form its virions and infect hepatocytes via the HBV receptors. Concomitant HDV/HBV infection continues to be a major health problem, with at least 25 million people chronically infected worldwide. N6-methyladenine (m6A) modification of cellular and viral RNAs is the most prevalent internal modification that occurs cotranscriptionally, and this modification regulates various biological processes. We have previously described a wider range of functional roles of m6A methylation of HBV RNAs, including its imminent regulatory role in the encapsidation of pregenomic RNA.

Methods and Results

In this study, we present evidence that m6A methylation also plays an important role in the HDV life cycle. Using the methylated RNA immunoprecipitation (MeRIP) assay, we identified that the intracellular HDV genome and antigenome are m6A methylated in HDV- and HBV-coinfected primary human hepatocytes and HepG2 cell expressing sodium taurocholate cotransporting polypeptide (NTCP), while the extracellular HDV genome is not m6A methylated. We observed that HDV genome and delta antigen levels are significantly decreased in the absence of METTL3/14, while the extracellular HDV genome levels are increased by depletion of METTL3/14. Importantly, YTHDF1, an m6A reader protein, interacts with the m6A-methylated HDV genome and inhibits the interaction between the HDV genome and antigens. Thus, m6A of the HDV genome negatively regulates virion production by inhibiting the interaction of the HDV genome with delta antigens through the recruitment of YTHDF1. This is the first study that provides insight into the functional roles of m6A in the HDV life cycle.

Conclusions

The functional roles of N6-methyladenine (m6A) modifications in the HBV life cycle have been recently highlighted. Here, we investigated the functional role of m6A modification in the HDV life cycle. HDV is a subviral agent of HBV, as it uses HBV envelope proteins to form its virions. We found that m6A methylation also occurs in the intracellular HDV genome and antigenome but not in the extracellular HDV genome. The m6A modification of the HDV genome recruits m6A reader protein (YTHDF1) onto the viral genome. The association of YTHDF1 with the HDV genome abrogates the interaction of delta antigens with the HDV genome and inhibits virion assembly. This study describes the unique effects of m6A on regulation of the HDV life cycle.

P.17 – Promises and pitfalls of implementing the FIND-C artificial intelligence/machine learning algorithm to improve screening efficiency in a real-world clinical environment

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Background

Universal HCV screening of adults is recommended in all major practice guidelines (AASLD, EASL, CDC, WHO); however HCV prevalence of <1% in the general population yields low case identification rates outside of sub-groups such as PWIDs, prisons, psychiatric populations, unhoused persons and certain geographies. As a result, > 60% of HCV+ patients who could benefit from HCV DAA treatment in North America and Europe remain undiagnosed.
We previously hypothesized that identification of attributes present in HCV+ persons but absent in known HCV- persons from electronic health records related to patients' medical history, geography, and social determinants of health could improve screening efficiency by prioritizing certain patients for HCV testing and diagnosis (Chen et al, EASL 2023).
Gilead initiated the FIND-C (Facilitating INtelligent Diagnosis) program in 2020 to facilitate identification of persons with undiagnosed HCV. In 2023, we piloted a machine learning model in Epividian healthcare clinics in US urban, suburban and rural geographies to determine if the model could identify a higher proportion of HCV+ individuals than universal screening.

Methods

The FIND-C algorithm compared >50,000 HCV+ and 245,000 HCV- patients screened for HCV between 2016-2020 in a de-identified US EHR dataset to train a binary supervised machine learning model to predict the likelihood of HCV infection.
The model utilized 32 features (patient demographics, medical history, prescription medications, and SDoH by zip code data), producing an AUROC of 95%, with 93% precision, and 50% recall. Implementation consisted of an HCP education period, and an alert was embedded into the Epividian clinical decision support system (CDSS). Weekly emails notified practitioners of upcoming patients who had not been tested for HCV according to guidelines, with an individualized description of factors potentially associated with increased probability of HCV positivity.

Results

The HCV risk score was calculated for 173,376 patients without known HCV active infection as of January 2023 at clinics utilizing the CDSS. A pilot study evaluating the effectiveness of the HCV alert is still in progress; the completed visits, HCV antibody tests, and HCV RNA viral load tests for 58,935 patients who are eligible to be screened according to clinical guidelines are being tabulated.
Several factors have arisen as implementation challenges: a) "alert fatigue"; b) generating "risk scores" for all patients (not only high likelihood patients) which reduced the alert value; c) the HCV risk alert was included with other clinical alerts; d) screening tests needed to be added to the patients' primary reason for seeking healthcare; and e) screening was applied only to patients visiting the clinics.

Conclusions

This model appears to be a first for infectious disease; although similar AI/ML algorithms are in use to identify pancreatic cancer (Placido et al., *Nature Medicine*, 2023; Paparrizos, et al., *Journal of Oncology Practice*, 2016) and cardiovascular disease (Avram, et al., *JAMA Cardiology*, 2023).
Algorithm development has been the "easy part", whereas practical implementation requires IT costs, staff education time, and integration into clinical workflows in busy healthcare systems. Practical implementation within specific EHR/CDSS requires substantial resources before the HCP receives the alert. As expected, missing data reduced the sensitivity/specificity of the model. AI-guided medical practice is a novel concept and providers remain wary of blindly following EHR/CDSS recommendations at this time.
A next step will be to proactively apply the algorithm to all patients in a healthcare system and contact "high-risk" patients for screening, diagnostics and treatment. Incorporation of the results of this outreach "re-link" activity is expected to improve model performance, and such models should further improve efficiency, reduce HCV screening costs, and ultimately decrease progression to advanced liver disease, thus accelerating improvement in patient outcomes.

P.18 – Hedgehog signalling and metabolic responses drive CD8 T cell hyperfunction in advanced liver diseases

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Background

Prolonged liver insult in chronic HCV infection (cHCV) and metabolic dysfunction-associated liver disease (MASLD) results in progressive liver damage and increased risk for hepatocellular carcinoma. CD8 T cell function is altered in infectious and non-infectious liver diseases regardless of antigen-specificity. We previously reported long-lasting bulk CD8 T cell hyperfunction in cHCV associating with fibrosis severity, yet specific mechanisms underlying this dysfunction remain elusive. In these studies, we aim to investigate mechanisms of generalized CD8 T cell dysfunction in advanced liver disease.

Methods

We isolated blood CD8 T cells from cHCV or MASLD patients with varying degrees of liver damage. Gene Set Enrichment and Gene Ontology analyses of RNA-seq data were performed on stimulated cells from cHCV, which lead to flow cytometry probing of Hedgehog (Hh) signalling using pathway inhibitors, and cell death assessment. We established mouse models of T cell hyperfunction in liver disease by exposure to hepatotoxin carbon tetrachloride (CCl4), or high-fat, methionine-choline deficient diet (HFMCDD), the latter mirroring human MASLD pathophysiology. T cell function and metabolic activity in these models were assessed by flow cytometry and Seahorse XF Mito Stress test.

Results

RNA-seq identified 362 differential genes in CD8 T cells from cHCV patients with cirrhosis *vs* minimal fibrosis, highlighting genes associated with T cell metabolism and function, including Hh signalling, apoptosis, glycolysis, oxidative phosphorylation, cytoskeletal regulation, inflammatory processes, and cell cycle regulation. RT-qPCR confirmed increased Hh pathway gene expression (*PTCH1*, *GLI1*) in cHCV patients with cirrhosis. Hh signalling inhibition in hyperfunctional CD8 T cells from cHCV patients with cirrhosis restored T cell function to healthy control levels, while functional cells also express higher cell death markers. Hyperfunction was also observed in HCV− MASLD patients with biopsy-proven advanced fibrosis/cirrhosis. In CCl4-treated mice, CD8 T cell hyperfunction was coupled to impaired responses to ectopic tumour growth and immunotherapy. In HFMCDD-treated mice, cells also exhibited hyperfunction, coupled with increased glycolytic activity and mitochondrial respiration. HFMCDD-treated mice also exhibited increased metabolic activity in CD4 T cells, and elevated inflammatory profiles in systemic and hepatic macrophages (see abstract: D. Lawton).

Conclusions

In advanced liver disease, CD8 T cell hyperfunction appears driven by an overall disruption in inflammatory and metabolic processes upon activation, which may involve dysfunctional Hh signalling and a greater propensity for apoptosis. In mice, CD8 T cell hyperfunction is coupled to host and cellular metabolic disruptions, which may contribute to impaired anti-tumour and immunotherapy responses. Understanding mechanisms of chronic immune dysfunction may translate to therapeutic strategies to improve clinical outcomes for individuals living with advanced liver diseases.

P.19 – Impact of IFNα on hepatocyte proteome in chronically-infected primary human hepatocytes

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#equal contribution

Background

Chronic hepatitis B virus (HBV) infections affect around 300 million worldwide with an estimated number of annual deaths of ~800,000. A major role in chronicity is attributed to the nuclear form of the HBV genome, termed covalently closed circular DNA (cccDNA). The cccDNA is the template of transcription of all viral mRNAs and its maintenance and stability in infected hepatocytes is the target of curative therapies. Current approved treatments against chronic HBV infections include nucleoside analogs and pegylated interferon alpha. Nucleoside analogs are very successful in suppressing HBV replication but typically do not eliminate the cccDNA. Interferon alpha (IFNα) has moderate effects on HBV replication with severe side effects.
However, in ~10% of individuals that receive IFNα treatment this can result in a functional cure. While there is a need for IFN-free regimens, understanding the molecular mechanisms of IFNα against chronic HBV may lead to the discovery of novel antiviral strategies with higher efficacy and without the side effects.

Methods

To model chronic HBV *in vitro* we developed a system based on culturing mouse-passaged (mp)PHH isolated from HBV-infected humanized mice. These HBV-mpPHH can be maintained in culture for several weeks. A major advantage is that nearly all hepatocytes are infected and contain high levels of cccDNA. In addition, we established robust CRISPR application methods in mpPHH to interrogate the impact of gene knockouts both in vitro and in humanized mice. These CRISPR applications when combined with highly sensitive proteomics and phosphoproteomics analyses provide a platform to investigate the role of specific host factors and pathways on HBV lifecycle and IFNα activity.

Results

Infected and uninfected mpPHH displayed very distinct protein expression patterns. Forty two proteins induced by IFNa in uninfected mpPHH they were suppressed in HBV-mpPHH. These 42 hits are being validated in CRISPR knockout to determine the impact of them in the context of HBV infection with and without IFNα treatment. Understanding the mechanism of action of these proteins in terms of HBV replication readouts including cccDNA stability and transcriptional silencing is part of these efforts with the goal to identify novel druggable pathways towards eliminating or silencing the cccDNA.

Conclusions

Together, these data are expected to identify host factors that have a crucial role in the context of chronic HBV infection and response to IFNα treatment. Moreover, the in vitro systems we developed together with CRISPR-based applications and systems biology analyses can be extended in other areas of HBV and liver-related diseases.

P.20 – Intrahepatic changes in viral and immune markers following treatment with JNJ-73763989 (JNJ-3989) and nucleos(t)ide analogs (NAs), in patients with chronic hepatitis B (CHB): INSIGHT Week 40 (W40) interim results

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Background:
Treatment of chronic hepatitis B (CHB) with siRNA JNJ-3989 and NA ± JNJ-6379 (capsid assembly modulator Class E) resulted in profound reductions in serum hepatitis B viral (HBV) markers. The aim of the INSIGHT study (NCT04585789) was to assess intrahepatic changes in virological and immunological markers with JNJ-3989 based treatment in CHB patients.

Methods:
INSIGHT is a phase 2 multicenter study in 2 groups of CHB patients: **Group 1** were hepatitis B e-antigen positive (HBeAg+) and not currently treated (NCT) and **Group 2** were HBeAg–negative (HBeAg-) and virologically suppressed (VS) by NA. Patients received 48 weeks of JNJ-3989+NA (±JNJ-6379 discontinued from study following protocol amendment). Paired percutaneous core liver biopsies and fine needle aspiration biopsies (FNABs) were collected using standardized procedures at baseline (BL) and week 40 to investigate changes in intrahepatic viral and immune markers.

Results:
Levels of viral markers in serum and liver were higher for participants in Group 1 versus Group 2 at BL. JNJ-3989 treatment resulted in a mean (SE) serum hepatitis B surface antigen (HBsAg) change from BL of -3.78 (0.481) $\log_{10}$ IU/mL for Group 1 and -2.40 (0.160) for Group 2 at Week 40. One out of nine (11.1%) patients in Group 1 reached HBsAg seroclearance at W40. The estimated mean percentage of HBsAg+ hepatocytes decreased by W40 in both groups while the mean percentage of HBcAg+ cells decreased only in Group 1 (Table). In Group 1, the percentage of HBV RNA+ hepatocytes declined from 90.2-100% at BL to 4.4-28.4% at W40 (n=4 patients in each group with samples profiled at BL and W40) and in Group 2 from 8.6-31.6% at BL to 5.6-15% at W40 (n=4 pairs). The percentage of cccDNA–/HBV RNA– cells increased in both groups, from 0-1.2% at BL in Group 1 and 31.3-64.8% in Group 2; to 48.1-68.9% in Group 1 (n=4 pairs) and 51.1-66.7% in Group 2 (n=4 pairs) at W40. FNAB profiling using scRNA sequencing showed a reduction in CD8+ exhausted T-cells in both groups.

Conclusions:
Treatment of CHB patients with JNJ-3989 resulted in a reduction of HBsAg+ hepatocytes at week 40 with an increased fraction of non-infected hepatocytes. Reductions during treatment were seen in intrahepatic CD8+ exhausted T cells, suggesting that JNJ-3989 + NA may lead to activation of intrahepatic adaptive immunity.

P.21 – Hepatitis B virus genotype H: Epidemiological, molecular, and clinical characteristics

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Background

The hepatitis B virus (HBV) has been a silent threat against humanity, constituting a public health problem worldwide. In 2016, the World Health Organization set forth an initiative for the global elimination of viral hepatitis by 2030. As the target date approaches, Latin America faces challenges in designing and implementing an elimination plan. Mexico launched the "*Plan Nacional para la Eliminación de la Hepatitis C*" in mid-2020. However, HBV elimination strategies are lagging. One step is to fill in the knowledge gaps and consider the host's and virus's genetics and environmental factors that influence the clinical course of infection based on the population's predominant genotype, which in this case is HBV genotype H (HBV/H).

Methods

We assessed literature data covering over 50 years (1970-2022) to portray the state of knowledge about the epidemiological, molecular, and clinical characteristics of HBV/H, endemic to Mexico.

Results

The seroepidemiological studies showed that over the past 50 years, Mexico has experienced intermediate to high rates (2.0%-12.0%) of HBV infection. A shift in risk behaviors has occurred. The most vulnerable are the native groups, hemodialysis patients, liver-diseased patients, and pregnant women. HBsAg prevalence among the general population and blood banks has remained stable, although low and high-risk groups showed increased rates of anti-HBc antibodies. Since the official data primarily focuses on HBsAg prevalence of low-risk groups, the true extent of HBV exposure is not reflected.

This low HBsAg versus high anti-HBc rate profile is suggestive that the diagnostic assays may not be sensitive nor specific for HBV/H. No routine NAT is requested in the clinical setting, only HBsAg, which may lead to misdiagnosis. Therefore, the three-panel test (HBsAg, anti-HBc, and HBV DNA) should be recommended by promoting screening campaigns and research to understand how these markers perform in different risk groups.

HBV/H is endemic to Mexico, as shown by molecular evolutionary studies. HBV/H infections manifest with low viral load and occult hepatitis B. However, minor genotypes may influence the course of infection. The high frequency of antiviral resistance and immune escape mutations could provoke treatment failure and hinder the efficiency of diagnostic kits and vaccine immunization. All these conditions prompt the need to maintain molecular epidemiology surveillance.

HBV/H or G-infected patients with chronic liver disease course with low viral load and low liver function readings; however, HBV/H infection may pass underdiagnosed if international clinical practice guidelines are strictly followed. Mixed infections are common and should be suspected when the viral load rises. Vaccination compliance and coverage are still windows of opportunity to improve in Mexico. Thus, prioritizing diagnostics and prevention/treatment measures are necessary to avoid dissemination and undetected liver damage among risk groups.

Conclusions

Globally, eliminating viral hepatitis is a complex task that requires countries to assess their strengths and weaknesses, develop targeted programs, and implement training, research, and awareness initiatives. Mexico's elimination plan will require collaboration among government, researchers, physicians, specialists, and civil society advocates.

P.22 – Design of potent HBV capsid assembly modulators (CAMs) using free energy perturbation (FEP)

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Background

Chronic hepatitis B virus (HBV) infection remains a major global health burden. Current anti-HBV treatment include a nucleoside analog alone or in combination with pegylated interferon. However, because cure rates are low, most patients will require lifetime treatment. HBV Capsid Assembly Modulators (CAMs) have emerged as a promising new class of compounds as they can affect levels of HBV covalently closed-circular DNA (cccDNA) associated with viral persistence. GLP-26 was shown to alter HBV nucleocapsid assembly preventing transport of the capsid to the nucleus and thus inhibiting viral DNA replication. Despite GLP-26 already high potency, docking of GLP-26 in the reported crystal structure of HBV core protein shows the presence of 1) a small hydrophobic pocket near the pyrrole N-Me group 2) a small space around the propargyl moiety susceptible for optimization. New molecules were designed by exploring these two positions using in-silico methods, leading to the discovery of a new GLP analog (GLP-10a) which is 200 times more potent than reference GLP-26.

Methods

New GLP-26 analogs designed by exploring two key positions were docked into the crystal structure of HBV core protein with CAM, SBA. The docked molecules with docking score better than GLP-26 were taken for molecular dynamics based alchemical relative biding free energy calculations. We implemented FEP method to calculate relative binding free energy change ($\Delta\Delta G$) due to new GLP-26 analogs. FEP has been shown to deliver accurate ligand binding free energies against several protein targets. The molecules with negative $\Delta\Delta G$ values were selected for synthesis. The synthesized compounds were evaluated *in vitro* against HBV in a liver cell-based system and their cytotoxicity determined in several cell systems, including peripheral blood mononuclear (PBM), human T lymphoblast (CEM), African green monkey kidney (Vero), and human hepatocellular carcinoma (HepG2) cells.

Results

Results indicated a clear correlation between FEP calculations and anti-HBV activity, and we GLP-10a which had $\Delta\Delta G$ value -3.44±0.15 kcal/mol and displayed highly potent anti-HBV activity in vitro (EC_{50} = 0.02 nM) and no cytoxicity up to 100 µM. Assessment of cardiac risk through hERG inhibition and reactive metabolite formation through GSH adduct generation confirmed the overall safety of GLP-10a. Detailed CYP inhibition profile of GLP-10a, in a panel of eight relevant CYP enzymes showed no major effects, except for the inhibition of CYP2C8. GLP-10a, alone or in combination with booster ritonavir, displays relatively short half lives in human liver microsomes.

Conclusion

Several GLP-26 derivatives selected and synthesized based on FEP calculations displayed anti-HBV activity in culture superior to GLP-26, including GLP-10a, a sub-nanomolar inhibitor of HBV DNA *in vitro*. Efforts to optimize the overall drug like properties of GLP-10a, while maintaining its high potency are ongoing.

P.23 – Preclinical efficacy, safety and immunogenicity of CLB-3000: a potential treatment for patients with chronic Hepatitis B

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Background

ClearB Therapeutics is developing a therapeutic vaccine candidate, CLB-3000, intended to drive functional cure (FC) in patients with chronic Hepatitis B (CHB). CLB-3000 is comprised of two modified Hepatitis B surface antigens (HBsAg), CLB-405 and CLB-505, purified from *Pichia pastoris* and adjuvanted with Alhydrogel. CLB-405 and CLB-505 were designed to display clearance profile (CP) associated epitopes on HBsAg and were identified from anti-HBs responses of FC patients (Walsh, R et al 2019 Liver Int 39(11) pp2066). Therapeutic vaccination with CLB-3000 was preclinically characterized for efficacy in a murine model of persistent HBV infection, with safety and immunogenicity evaluated in New Zealand white (NZW) rabbits.

Methods

CLB-3000 was delivered intramuscularly (IM) to CBA/CaJ mice with persistent HBV infection (Chou, HH et al 2015 PNAS 112(7) pp2175). Mice were monitored for changes in virological biomarkers by measuring serum HBsAg, anti-HBs and HBV DNA levels. In addition, HBsAg and HBcAg levels via liver immunohistochemistry (IHC) were assessed at end of study. Naïve NZW rabbits (8/sex/group) received 0.9% saline, Alhydrogel (1000 µg) or CLB-3000 [(40, 100 and 250 µg each antigen; 80, 200, or 500 µg total antigen) with Alhydrogel (1000 µg)] via IM injection; administered every 3 weeks for 15 weeks for a total of 6 doses. Evaluated parameters included: viability, clinical observations, local tolerance, ophthalmology, body weights, food consumption, body temperature, hematology, clinical chemistry, coagulation, organ weights, macroscopic and microscopic pathology, and immunogenicity.

Results

Therapeutic vaccination of CHB mice with CLB-3000 resulted in reduction in serum HBsAg to undetectable levels, which was confirmed by liver IHC. Development of CP-associated anti-HBs response was concomitantly detected in 80% of CLB-3000 treated CHB mice that achieved FC. Antibody specificity for CP-associated epitopes was detected, targeting both external loops 1 and 2 of HBsAg, and mimicked that observed in FC patients. CLB-3000 specific antibody responses were also detected. In the GLP toxicity study, all rabbits survived to the scheduled necropsy. CLB-3000-related clinical and microscopic pathology findings were limited to effects at the injection sites; no adverse systemic effects were noted. CLB-3000-related clinical pathology changes at ≥80 µg included increases in fibrinogen, C-reactive protein, and/or creatine kinase, suggestive of an inflammatory response and muscle damage due to injection site reactions. These findings were consistent with expected findings in a vaccine study formulated with adjuvant, did not result in clinical impairment, exhibited some degree of reversibility, and were not considered adverse. CLB-3000 was immunogenic, as assessed by measurement of serum endpoint titers to CLB-405 and CLB-505, thus confirming pharmacologic activity in NZW rabbits.

Conclusions

CLB-3000 was highly efficacious in a murine model of CHB as shown by rapid HBsAg decline and clearance from serum and liver, along with seroconversion to CP-associated anti-HBs which mirrored clinical FC-associated anti-HBs responses. Repeated IM injection of CLB-3000 was well tolerated at doses up to 500 µg total antigen with 1000 µg Alhydrogel/animal, the highest dose tested. Findings in this study were limited to effects at the injection sites, consistent with expected findings in a vaccine study formulated with adjuvant and not considered adverse. The efficacy and safety data further support advancement of CLB-3000 for the treatment of CHB patients in a first in human clinical trial (ANZCTR - Registration) in CHB patients that is currently enrolling.

P.24 – Monkeypox and vaccinia viral inactivation for safe use on molecular, antigen, and drug-discovery assays

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Background

Recent human Monkeypox virus (MPXV) outbreaks have been occurring worldwide, including in the USA, prompting a need for better detection methods and antiviral treatments. The Emory University/RADx/ITAP team conducts studies on the limit of detection, sensitivity, and specificity of newly developed rapid diagnostic tests for several viruses, one of which is MPXV. Our center's work contributed towards Cepheid being granted Emergency Use Authorization for their Point-of-Care Mpox Molecular Test- Xpert Mpox. In addition, drug discovery studies are currently being conducted to find novel therapeutics to help combat recent outbreaks and prepare for future ones. To perform these downstream diagnostics and novel anti-MPXV (and vaccinia-VACV) drug screen assays, studies are needed to prove that available inactivation reagents can successfully inactivate lab-cultured, highly infectious viruses and positive clinical samples. Doing so would allow for subsequent transport of the inactivated virus from the BSL3 to the BSL2*/2, where downstream assays can safely and swiftly be executed for diagnostic testing and drug discovery investigations.

Methods

Several reagents and five commercially available lysis buffers were tested using a new inactivation method and highly concentrated lab-cultured viruses in the BSL2* (VACV) and BSL3 (MPXV) for their potential inactivation of both VACV and MPXV. These lysis buffers from Roche, Qiagen, and Applied Biosystems were selected for study as they are readily available and commonly used in the lab setting. The protocol started with mixing and incubating the undiluted virus with each buffer. Then, to prevent the potential cytotoxic effects of buffers on cells used in assays, the mixture was centrifuged at high speed (+21,000xg) to result in a viral pellet, which was resuspended in fresh media. These resuspended pellets were inoculated into Vero cells for observation of cytopathic effects, and infectivity was measured by ELISpot assays. The cells were monitored over several days until collection and imaging.

Results

All five commercial lysis buffers showed complete inactivation (no cytopathic effect and no cells showing infection by ELISpot) for both viruses.

Conclusion

With safety being of the utmost importance regarding BSL2* and BSL3-level pathogens, it is crucial to safely test for inactivation to transport VACV and MPXV to BSL2. This technique applies to scenarios where viruses are suspended in a potentially toxic matrix. Currently, this protocol is used to resuspend MPXV clinical samples in the medium that would allow for successful infectivity assays to be performed on clinical samples. For drug-discovery assays in our laboratories, lysis buffer (RLT) is used for MPXV inactivation in the supernatant, which is then safely transported from BSL3 to BSL2/2* for DNA extraction and subsequent Real-time PCR to screen for novel anti-MPXV drugs.

P.25 – Antigen-Targeting to cd180 for the treatment of chronic HBV infection

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Background

Anti-viral nucleos(t)ides and immune modulators are the only approved therapies for chronic hepatitis B (CHB) infection, but they do not eliminate HBV from CHB patients. Attempts to induce long term adaptive immunity to achieve functional cures have not been successful, likely due to immune defects, including tolerance, that persist during CHB even under potent antiviral therapy. We have reported that targeting antigens to CD180, a TLR homolog expressed by B cells and dendritic cells (DCs), activates and programs specific antibody and T cell immune responses to the targeted antigens even in immunodeficient settings. Given the important role of B cells in controlling HBV reactivation, we hypothesize that targeting HBV antigens to CD180-expressing B cells and DCs can break tolerance to chronic HBV infection and establish adaptive anti-HBV immunity that includes HBV-specific cytotoxic T cell (CTL) response, resulting in functional cure for CHB patients.

Methods

CD180-stimulated immune responses were examined (i) in mice using a test anti-CD180-OVA conjugate and (ii) in rhesus macaques using recombinant anti-CD180-HBcAg and anti-CD180-PreS1/S2 fusion proteins. The effectiveness of anti-CD180-HBV antigen fusion proteins were further evaluated in SIV+ immune-suppressed rhesus macaques.

Results

Anti-CD180-OVA conjugates induced in vivo expansion of OVA-specific OT1 CD8 T cells, with a substantial percentage of these showing CD44+/CD62L- effector/memory T cell phenotype. Upon boosting, OT1 CD8 cells differentiated into polyfunctional effector cells expressing TNFα and IFNɣ.
Subcutaneous administration of a recombinant anti-human CD180-HBcAg fusion protein, but not soluble HBcAg alone, induced robust HBcAg specific IgG response in rhesus macaques. This antibody response cross-reacted with HBeAg, suggesting that the anti-CD180-HBcAg fusion protein may induce HBeAg seroconversion in CHB patients. Further, ex vivo re-stimulation of PBMCs from anti-CD180-HBcAg immunized rhesus macaques with a HBcAg-derived peptide pool revealed the emergence of HBcAg-reactive CD4 and CD8 T cells. At the peak of response, the frequency of HBcAg-specific T cells was comparable to levels found in HBV-infected individuals who have cleared their infection.
Immunization with anti-CD180-PreS1/S2 fusion protein induced similarly robust PreS1/S2-specific IgG and T cell responses. Importantly, immune sera from a subset of immunized rhesus macaques were able to inhibit HBV infection of HepG2 cells expressing the HBV cellular receptor NTCP.
Moreover, these recombinant proteins could function in immune deficient environments, as they stimulated robust immune responses against HBcAg and PreS1/S2 in SIV-infected macaques.

Conclusions

Overcoming immune defects in chronic HBV infection is likely needed to treat CHB and related hepatocellular carcinoma. Our findings suggest that therapeutic vaccines comprising viral antigens fused to an activating anti-CD180 may provide sufficient potency and selectivity to overcome these barriers and induce strong antiviral immunity in CHB patients as well as antitumor immunity in patients with HBV-driven liver cancer. Research cell banks producing the anti-CD180-HBcAg fusion protein have been generated and this therapeutic vaccine is advancing to IND-enabling studies. (Supported by Abacus Bioscience, Life Sciences Discovery Fund 16721309 to EAC, NIH/NIAID grants R56 AI141494 to DHF and R41 CA257531 to EAC).

P.26 – Concurrent Human Immunodeficiency Virus Infection in Patients with Metabolic Dysfunction Associated Steatotic Liver Disease is Associated with Significantly Greater Risks of Liver Disease Progression, Cardiovascular Disease, and All-Cause Mortality

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Background
Metabolic dysfunction associated steatotic liver disease (MASLD) is highly prevalent among U.S. adults and particularly among U.S. Veterans. While human immunodeficiency virus (HIV) infection increases the risk of hepatic steatosis, it is not clear if concurrent HIV among patients with MASLD is associated with increased risks of liver disease progression, cardiovascular disease, and overall mortality. We aim to evaluate the association between HIV infection status and risks of cirrhosis, hepatocellular carcinoma (HCC), hepatic decompensation, major adverse cardiovascular event (MACE), and overall survival among a national cohort of Veterans with MASLD.

Methods
Adults with MASLD with and without concurrent HIV infection were identified using the national Veterans Affairs database from 1/1/2010 to 12/31/2017 with follow-up through 12/31/2022. Patients with cirrhosis or HCC at baseline or within 6 months of MASLD diagnosis were excluded. We applied propensity score matching (PSM) methods to create comparable cohorts of MASLD patients with vs. without HIV to adjust for potential confounding effects due to differences in baseline characteristics. Variables selected for PSM included age, gender, race/ethnicity, body mass index, diabetes, hypertension, and dyslipidemia. Comprehensive assessment of pharmacy data was performed to determine whether patients were on anti-retroviral therapy (ART). Overall incidence of cirrhosis, HCC, hepatic decompensation, and MACE per 100 person-years was evaluated using competing risks Nelson-Aalen methods. Overall survival was evaluated using Kaplan-Meier methods. Comparisons of incidence rates and survival between groups was compared using log-rank testing and the z-statistic.

Results
The propensity score matched cohort included 1,032 MASLD patients with HIV and 1,032 MASLD patients without HIV. Compared to MASLD patients without HIV, overall incidence of cirrhosis was significantly higher among MASLD with HIV (1.10 vs. 0.79 per 100 person-years, $p<0.05$). However, there was no difference in the incidence of cirrhosis between MASLD-HIV patients on ART vs. MASLD-HIV patients not on ART (1.22 vs. 1.36 per 100 person-years, $p=0.61$). Similarly, when compared to MASLD patients without HIV, overall incidence of cirrhosis-related decompensation was also significantly higher among MASLD with HIV (1.11 vs. 0.72 per 100 person-years, $p<0.01$). No differences in decompensation risk were observed in MASLD-HIV patients on ART vs. not on ART. No significant differences were observed in the incidence of HCC by HIV status or by whether MASLD-HIV patients were on ART. Compared to MASLD patients without HIV, the risk of MACE was significantly higher among MASLD-HIV on ART (5.02 vs. 4.15 per 100 person-years, $p=0.01$) and even higher among MASLD-HIV not on ART (6.00 vs. 4.15 per 100 person-years, $p<0.001$). Compared to MASLD patients without HIV, overall 5-year survival was significantly lower among MASLD-HIV on ART (85.5% vs. 88.6%, $p<0.05$) and even lower among MASLD-HIV not on ART (79.3% vs. 88.6%, $p<0.001$).

Conclusion
Among a national cohort of U.S. Veterans with MASLD, concurrent HIV infection is associated with significantly greater risks of liver disease progression, significantly greater risk of MACE, and lower overall survival. Ensuring timely initiation of ART can mitigate these increased risks associated with concurrent HIV infection.

P.27 – Bepirovirsen has intrinsic innate immune activity, distinct from that of other anti-sense oligonucleotides, and induces innate immune response in the liver

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Background:
Bepirovirsen (BPV) is an anti-sense oligonucleotide (ASO) that is in phase 3 clinical trials for chronic hepatitis B (CHB). Previously, we have reported that BPV induces innate immune activation in human PBMCs and also specific activity in transgenic hTLR8 mice, but not in wild type mice. Here, we investigate whether BPV is unique in its mechanism for innate immune activation compared to other ASOs, and where in the liver the activation originates from.

Methods
WT littermate control and transgenic hTLR8 mice were dosed with BPV, other ASOs (including a scrambled, a minimally inflammatory, and a TLR9 activating ASO), or control treatments. Mice were euthanized four hours after dosing and plasma protein and liver RNA cytokine levels were examined. To investigate the location of cytokine activation, mRNAs of specific genes were stained in fixed liver samples using RNAscope.

Results
BPV had a distinct profile of innate immune activation compared to the other ASOs tested. Levels of plasma TNF-α and IL-6 were significantly higher in hTLR8 mice dosed with BPV than in those dosed with scrambled ASO (TNF-α: $p<0.0001$; IL-6: $p=0.0169$) or the minimally inflammatory ASO 104838 ($p=0.025$, $p=0.0346$). BPV induced TNF-α and IL-6 in hTLR8 mice but not in WT mice, whereas ASO 421856 (activates through TLR9) induced TNF-α and IL-6 in both hTLR8 and WT mice. Upon BPV treatment for 4 hrs, elevated expression of cytokines/chemokines (such as *C*cl2, *Ccl4*, and *Tnf*) was observed in total RNA from the liver. With the RNAscope assay in liver sections (N=2), such inductions were predominantly localized to the Kupffer cells and macrophages rather than hepatocytes.

Conclusions
The innate immune activity of BPV in hTLR8 transgenic mice is distinct from another ASO known to induce TLR9 activity, suggesting that the BPV-mediated innate response is more specific to human TLR8. In addition, BPV mediated cytokine activation is detected in the Kupffer cells and macrophages in the liver.

P.28 – The RNA interference therapeutic imdusiran retains activity against hbsag in chronic hepatitis B subjects with baseline nucleotide polymorphisms within the HBV target site

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Background and Aims

Imdusiran (AB-729) is a *N*-Acetylgalactosamine (GalNac)-conjugated small interfering RNA (siRNA) that targets all HBV RNA transcripts and suppresses viral replication and all viral antigens. Imdusiran is currently being investigated for the treatment of chronic hepatitis B virus (CHB) infection. Imdusiran treatment of CHB subjects resulted in mean HBsAg declines from baseline ranging from 1.8 to 2.6 $\log_{10}$ across all cohorts by end of treatment in clinical study AB-729-001. Here we report the characterization of imdusiran HBV target site variants identified from the HBVdb database as well as from target site sequence profiling in these CHB subjects. The impact of identified target site variants on HBV fitness and imdusiran activity was assessed in a transient transfection HBV cell model.

Methods

CHB subjects were administered single doses (60mg, 90mg, or 180mg) or multiple doses (60mg or 90mg every 4, 8 or 12 weeks) of imdusiran. Prior to imdusiran dosing at baseline, HBV RNA was extracted from plasma, reverse transcribed, and subjected to PCR amplification and ultra deep sequencing (UDS) of the HBx region. Additionally, sequences from the HBVdb database were screened for imdusiran target site variants. To determine the impact of identified imdusiran target site variants on viral fitness and imdusiran activity, point mutations were introduced into a genotype D HBV plasmid by site-directed-mutagenesis and transfected into HepG2 cells. Viral fitness and the impact of target site variants on imdusiran activity were determined by comparisons of variants versus wildtype.

Results

Analysis of HBV sequences from the HBVdb database identified changes in 7 nucleotide positions (T1580A, C1582G, C1587T, G1588A/C/T, C1589A/G, T1590G and T1591C) with ≥0.5% prevalence in any one HBV genotype, or with ≥0.3% prevalence in at least two genotypes. UDS analysis from 44 subjects at baseline showed target site conservation in 35 subjects (79.5%). Single nucleotide polymorphisms (SNPs) were observed at positions 1587, 1590 and 1593 at >15% read count frequency at baseline. *In vitro* analysis of these variants showed their mean viral fitness to range from 37-61% of wildtype. Imdusiran mediated comparable *in vitro* antiviral activity against the identified HBV target site variants in the HBVdb database and against the clinically identified target site variants.

Conclusion

SNPs in the imdusiran HBV target site were identified in sequences obtained from the HBVdb database and were observed at baseline in some CHB subjects in AB-729-001. *In vitro* testing confirmed retention of imdusiran activity against tested variants, suggesting that these SNPs have no obvious influence on individual or mean HBsAg declines observed in subjects treated with imdusiran.

P.29 – VIR-2218 and VIR-3434 therapy is efficacious in preclinical models of Hepatitis Delta Virus infection

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Background

Chronic Hepatitis Delta Virus (HDV) infection represents the most severe form of viral hepatitis with limited treatment options. HDV is a satellite virus of Hepatitis B Virus (HBV) that depends on HBV-derived HBsAg for envelopment and viral dissemination. VIR-2218 is an investigational RNAi therapeutic that targets a highly conserved region within the HBV X open reading frame and demonstrates potent knockdown of all HBV transcripts including of HBsAg. VIR-3434 is an investigational monoclonal antibody targeting the antigenic loop of HBsAg, inhibiting viral entry, and reducing circulating HBsAg in preclinical models and in early-stage clinical trials. This study aims to investigate the antiviral effect of VIR-2218 and VIR-3434 on HDV infection in preclinical models.

Methods

In vitro antiviral efficacy of VIR-2218 was determined in an HBV/HDV co-infection model of primary human hepatocytes (PHH). Secreted HBsAg was quantified using ELISA and secreted infectious HDV virions by re-infection of naïve Huh7-NTCP cells. *In vitro* neutralization potency of VIR-3434 was determined against HDV enveloped with HBsAg of eight different HBV genotypes. *In vivo,* efficacy of VIR-2218/VIR-3434 mono- and combination treatments were evaluated in HBV/HDV co-infected liver-chimeric mice.

Results

VIR-2218 treatment *in vitro* reduced HBsAg and secreted infectious HDV with picomolar efficacy. VIR-3434 neutralized HDV infection *in vitro* with >10,000-fold higher potency than Hepatitis B Immunoglobulins. Neutralization activity was pan-genotypic as tested with HDV enveloped with HBsAg of HBV genotypes A-H. Combination treatment of co-infected PHH with VIR-2218 and VIR-3434 reduced levels of infectious HDV virions. *In vivo*, mono-treatment with VIR-2218 or VIR-3434 decreased HBsAg and HDV RNA serum levels by >0.5 log and >1 log, respectively. Combination treatment reduced HBsAg and HDV RNA serum levels by >2 log.

Conclusions

VIR-2218 and VIR-3434 have previously shown antiviral efficacy against HBV infection in multiple *in vitro* and *in vivo* models. Due to the shared use of HBsAg by HBV and HDV, targeting HBsAg also reduces concurrent HDV infection. VIR-2218 and VIR-3434 exert antiviral efficacy against HDV as single agents and in combination by reducing HBsAg secretion, circulating HBsAg and HDV virions, as well as by blocking entry into hepatocytes. These data support the clinical development of VIR-2218 and VIR-3434 for treatment of patients with chronic HDV infection.

P.30 – A candidate for NASH therapy, TB-840 attenuates ER-stress and Inflammation in RAW 264.7 cells by the regulations of RORα and FGF21

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Background

Retinoic acid receptor-related orphan receptor α (RORα) is known as one of transcriptional factors of fibroblast growth factor (FGF21). FGF21 has been studied as one of the important hormones that functions to maintain intracellular homeostasis related to lipid metabolism, ER stress and inflammatory response inhibition in liver and fat tissues. Many studies have indicated that ER stress-related proteins (e.g., PERK, IRE1, ATF6, CHOP, xbp1) modulate inflammatory responses through NFκB-mediated signaling, which is known as a critical regulator of inflammatory responses. Furthermore, it has been reported that cytokines such as TNFα, IL-1β, and MCP1 induce ER stress. These findings provide supporting results on the correlation between ER stress and inflammation. However, the functions of RORα and FGF21 in regulating inflammation responses and ER stress pathway in macrophages have yet to be defined. In this study, we aimed to investigate the correlation of RORα and FGF21 with our compound TB-840, which is a RORα agonist that has been studied with healthy subjects in Phase 1 clinical trial in Korea (NCT05045534), related to the inflammatory response and ER stress in macrophage RAW 264.7 cells.

Methods

Cell culture: RAW 264.7 cells were cultured in DMEM (Gibco, 11965-092) containing 10% FBS (Gibco, 16140-089) under 37 °C, 5% CO2 conditions with antibiotics.
Immunoblot: Cells were exposed to LPS (L4391; sigma), 4-Phenylbutyric acid (4-PBA, P21005; sigma) or Thapsigargin (TG, T9033; sigma) at the indicated times. The proteins were detected by appropriate antibodies. The antibodies against anti-CHOP (2895S), BiP (C50B12) and β-Actin (3700) were obtained from Cell Signaling Technology. Antibody against anti-FGF21 (ab171941) was obtained from abcam.
Real-Time PCR: Total RNA was prepared by RNAiso Plus (TaKaRa) and, cDNA was synthesized using a reverse transcription kit (Invitrogen). cDNA was amplified in a CFX96 Real-Time PCR Cycler (Bio- Rad) using appropriate primers. Table 1 lists the primers used.
Data are expressed as means ± standard deviation (SD). Differences among mean was one-way analysis of variance (ANOVA) for multiple groups followed by Tukey's honest significant difference (HSD) test using the SPSS software (IBM). Significance was evaluated at a level of $p < 0.05$.

Results

LPS was used as an inflammatory stimulating factor and it was found that the protein expression of ER- stress marker, Bip and CHOP, was increased in RAW 264.7 cells after LPS treatment in different concentrations of 5, 10, 20, 40 ng/ml. The same increase of Bip and CHOP proteins was observed at different time points of 2, 4, 8 hrs after LPS treatment in 10 ng/ml concentration. We confirmed that the treatment with chemical chaperone 4-PBA suppressed the increased expression of Bip and CHOP by LPS and TG.
In addition, when analyzing Bip and FGF21 expression at each time point after treating RAW 264.7

P.31 – Hepatitis C virus RNA is 5' capped with flavin adenine dinucleotide

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Background and Aims

RNA viruses have evolved elaborate strategies for protection of their genomes, including 5' capping. However, so far no RNA 5' cap was identified for hepatitis C virus (HCV). Based on previous observations that flavin adenine dinucleotide (FAD) is required for HCV replication, the conservation across HCV isolates of 5' A on the negative strand, and partially on the positive strand, we hypothesized that HCV RNA is 5' capped with FAD.

Methods

To probe RNA FAD capping, we modified the CapZyme-seq methodology using the FAD specific decapping enzyme AtNUDX23, developed an RT-qPCR reduction assay for analysis of *in vivo* samples, and optimized mass-spectrometry methodology for FAD detection. HCV replicons were used to assess FAD dependency of replication, and *in vitro* replication initiation assays were used to study NS5B RNA-dependent RNA polymerase *de novo* initiation.

Results

We demonstrate that FAD is used as noncanonical initiating nucleotide by the viral NS5B polymerase resulting in a 5' FAD cap on the HCV RNA. The HCV FAD capping frequency is ~75%, which is the highest observed for any RNA metabolite cap across all kingdoms of life. FAD capping is conserved among HCV isolates for the negative strand and partially for the positive strand. It is also observed *in vivo* on HCV RNA isolated from patient sera and the liver and serum of the uPA-SCID human liver chimeric mouse model. HCV replication is abrogated in the absence of the FAD precursor, riboflavin, for highly FAD-capped isolates.
Furthermore, we show that 5' FAD capping protects RNA from RIG-I mediated innate immune recognition but has no effect on HCV RNA stability.

Conclusions

These results establish capping with cellular metabolites as a novel viral RNA capping and innate immune evasion strategy, which could be used by other viruses and contribute to viral persistence. Furthermore, the virus specific FAD cap could be a novel target for antiviral therapeutics.

P.32 – Safety and efficacy of REP 2139-Mg against chronic HBV / HDV co-infection with advanced liver disease

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Background

REP 2139 inhibits HDV replication in the nucleus via direct interaction with HDAg (Fonte et al., HBV meeting 2023). In the secretory pathway, it interferes with SVP assembly and HDV RNP envelopment via a post- ER interaction with the host chaperone DNAJB12, driving high rates of HBsAg loss in HBV infection.
In fibrotic patients, REP 2139-based combination therapy achieves high rates of functional cure of HBV and cure of HDV stable through 7 years of treatment free follow-up. Compassionate use of subcutaneously administered REP 2139-Mg (Replicor Compassionate Access Program, RCAP [NCT05683548]) is underway in cirrhotic HBV / HDV co-infection with HDV non-response or viral rebound during previous therapy with pegIFN and or bulevirtide (BLV).

Methods

Data is available for 31 patients (France, Austria, Italy, Turkey, Germany and Canada). Two patients also have HIV co-infection. Existing NUC or HIV combination therapy was supplemented with 250mg REP 2139-Mg qW SC scheduled for 48 weeks. In patients with compensated cirrhosis, 90ug pegIFN qW SC was also added. Safety and biochemical response were monitored weekly and virologic response every 4 weeks using standard assays for quantitative HBsAg and anti-HBs, HBV DNA, HDV RNA, HIV RNA and HBcrAg.

Results

As of submission, 20/31 patients have completed > 24 weeks of therapy. Two patients have had therapy extended beyond 48 weeks. REP 2139-Mg is generally well tolerated with transient grade 1 injection site reactivity in a majority of patients. Four ALT flares > 5 X ULN have occurred with pegIFN present, 3 self- resolving without dose alteration and one resolving after removal of pegIFN therapy. ALT has normalized in 11/31 patients. Reversal of decompensation and or ascites has occurred in 3 of 5 patients with decompensated cirrhosis. One cirrhotic patient has reverted to fibrosis. Hepatic venous pressure gradient reductions of 18% and 19% were observed in two additional patients 12 weeks after the start of therapy.
Compensated cirrhosis was present in 26/31 patients, 19 with failure/rebound during BLV and 7 with failure to pegIFN. HDV RNA decline > 2 log10 from baseline has occurred in 18/26 and HDV RNA loss in 10/26 patients. HBsAg decline > 2 log10 has occurred in 10/26 and HBsAg loss in 5/26 with anti-HBs seroconversion in 4/26. In the two HIV+ patients, HIV viral load has remained undetectable. Two patients have completed REP 2139-Mg + pegIFN therapy; one has achieved functional cure of HBV with HDV cure, the second remains HBsAg and HDV RNA undetectable with anti-HBs seroconversion 6 months on TDF monotherapy.
Decompensated cirrhosis was present in 5/31 patients. HDV RNA decline > 2 log10 is present in 4/5 with HDV RNA loss in 2/5. HBsAg < 10 IU/mL has occurred in 3 patients with HBsAg loss in 2 and anti-HBs seroconversion in 1. Liver transplant in two patients occurred before the end of therapy and explant analysis in the first of these patients has showed no detectable HDV RNA in the liver after 10 weeks of REP 2139- Mg therapy. One patient has completed therapy and is currently HBsAg and HDV RNA undetectable with anti-HBs seroconversion for 2 months on TDF monotherapy.

Conclusions

REP 2139-Mg is safe and effective against HBV/HDV and HBV/HDV/HIV infection in the presence of compensated and decompensated cirrhosis. REP 2139-Mg can clear HDV RNA from the blood and liver and can establish HBV functional cure and HDV cure in advanced liver disease.

P.33 – mTORC1 activation promotes systematic M1 macrophage polarity in virus and diet-induced liver fibrosis

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Background

Monocyte-derived macrophages (MoMϕs) infiltrate in large quantities to augment the resident liver Kupffer cells (KC) and play a central role in maintaining the homeostasis of the liver and mediating the development of fibrosis and liver healing. Diversity and plasticity are hallmarks of macrophages which permit responses to the local microenvironment, distinguished by cytokine production (IL-1, TNF-a, IL-6, IL-10, TGF-) and phenotype. The frequency and localization of infiltrating inflammatory (M1) or anti- inflammatory (M2) phenotypes promote or attenuate liver disease pathologies and influence surrounding immune cell activity. We have previously identified M1 macrophages as key producers of IFN-γ, and this was amplified in HCV infection with cirrhosis. Activation of the mammalian target of rapamycin complex 1 (mTORC1) has previously been identified to induce the release of M1 macrophage-associated cytokines in several inflammatory conditions including chronic liver disease. It remains unclear how macrophage polarity is impacted in chronic liver disease with advanced fibrosis and if mTORC1 activity is promoting M1 macrophage dysfunction.

Methods

Macrophage subsets were differentiated *in vitro* with M-CSF, polarized, and stimulated with lipopolysaccharide (LPS) from blood monocytes of healthy and treatment-naïve HCV^+ individuals with minimal or advanced fibrosis (< 9 KPa or > 12.5KPa). Male C57BL/6 mice were fed a high-fat methionine and choline-deficient diet (HFMCDD) *ad libitum* for 16 weeks to induce advanced liver fibrosis. Tibia and femurs were collected to generate bone marrow-derived macrophages (BMDMs) and livers homogenized for Kupffer cell analysis. To impair M1 macrophage development, mTORC1 inhibitor (rapamycin) was added in conjunction with polarizing cytokines. Cell phenotype and function were assessed by flow cytometry.

Results

Inhibition of mTORC1 with FDA-approved rapamycin during polarization of MDMs impairs M1 differentiation, including IFN-γ secretion. Inhibition of mTORC1 via rapamycin treatments was confirmed by a significant reduction of phosphorylated S6 protein. Untreated, HCV-derived advanced fibrosis- derived macrophages appeared to prefer an M2-like phenotype ($CD80^-CD163^+$) while producing high amounts of IFN-γ compared to healthy controls. M1 polarization and LPS activation induced increased CD80 expression with advanced fibrosis than in minimal fibrosis. HFMCD mice exhibited significant F4 fibrosis post-16 weeks of diet when compared to regular chow controls. Both male and female mice showed elevated inflammatory markers including IFN-γ in polarized macrophages in both BMDMs and KCs, which may be reversed with mTORC1 inhibition.

Conclusions

We have identified significant systemic dysfunction of monocyte-derived macrophages in cHCV infection with advanced fibrosis and this was replicated in BMDMs and KC in an animal model of diet-induced liver fibrosis. Enhanced inflammatory macrophage activity, characterized by elevated IFN-γ, predominates systemically and in the liver in advanced liver fibrosis. These findings suggest rapamycin may be a therapeutic target to restore systemic and hepatic macrophage polarity.

P.34 – Low prevalence of HDV testing and worse disease outcomes among HDV patients in NYC – results from the INSIGHT database.

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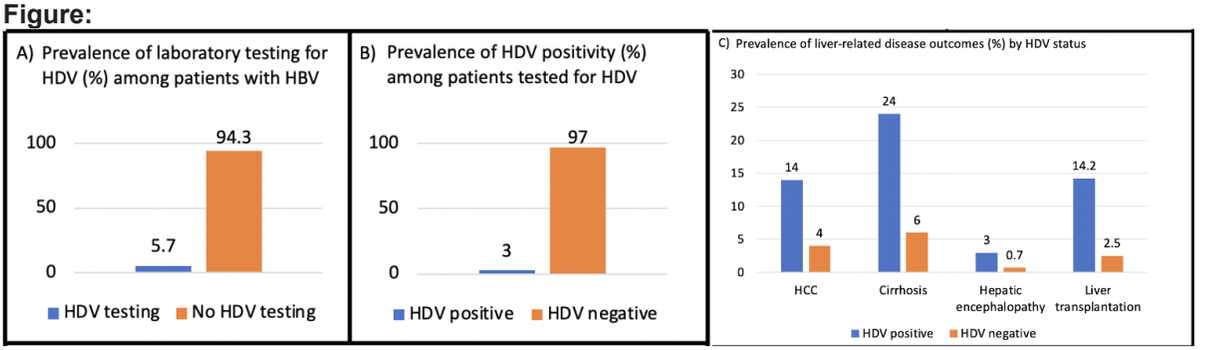
Background:
Hepatitis delta virus (HDV) is the most severe form of viral hepatitis and is associated with rapid progression to cirrhosis and hepatocellular carcinoma (HCC). Although New York City (NYC) has been called a "hot spot" for HDV, in part due to its large immigrant population, there are limited data on HDV testing and disease outcomes among HDV patients in NYC.

Methods:
We utilized the INSIGHT Clinical Research Network (CRN), a robust database that includes comprehensive longitudinal clinical, laboratory, procedural, and social determinants of health data from ~19 million patients from 5 different NYC institutions. Among HBV patients, we determined the prevalence of HDV positive laboratory status and/or diagnosis code evidence of HDV. We compared the prevalence of cirrhosis, HCC, liver transplantation, and other relevant outcomes between patients with and without evidence of HDV. To solicit feedback and input at all stages of the research process, we developed an HDV Community Advisory Board comprised of NYC residents living with HDV and other stakeholders.

Results:
We identified 36,765 individuals with HBV based on diagnosis or laboratory codes. Among these, we identified 2,102 individuals (5.7%) who had laboratory testing for HDV (see **Figure**). Of those with HDV testing, 65 (3%) were found to be HDV positive. Those who had HDV testing were more likely to be male (58% vs. 53%), Asian (50% vs. 40%), and less likely to be Hispanic (5% vs. 15%), all p-values<0.001. Patients positive for HDV were also more likely to be male (71% vs. 58%, p=0.04). Race and ethnicity were similar among those with and without HDV positive status. In regards to clinical outcomes evaluated, HDV positive individuals were more likely to have a diagnosis of HCC (14% vs. 4%), cirrhosis (24% vs. 6%), and hepatic encephalopathy (3% vs. 0.7%) and were more likely to undergo liver transplantation (14.2% vs. 2.5%) than patients without HDV. On multivariable logistic regression analysis adjusted for age, sex, race, and liver disease comorbidities (i.e., HCV and metabolic associated liver disease), patients with HDV were more likely to develop cirrhosis (OR=5.90, 95% CI: 5.00-6.97) and HCC (OR=5.52, 95% CI: 4.49-6.82) and were more likely to undergo liver transplantation (OR=7.47, 95% CI: 6.10-9.15).

Conclusions:
Prevalence of testing for HDV among HBV patients in NYC was extremely low (~6%). Among those tested for HDV, around 3% were found to be positive. There were significant demographic differences among those tested, suggesting potential disparities in HDV surveillance and access to care. Liver disease outcomes were uniformly worse among patients with HDV compared to those without HDV.

Figure:



P.35 – Design of a Small-Molecule Bispecific tether (miniBiT) for the Treatment of Influenza Infections and expansion of the platform to treat chronic hepatitis B

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Background

Centers for Disease Control and Prevention (CDC) estimates that during the 2022-2023 flu season, there have been 27-54 million flu-related illnesses, 12-26 million medical visits, 300,000-650,000 hospitalizations and 19,000-58,000 deaths in the USA. While flu vaccines are widely available, they have been only 19- 60% effective over the past decade. While approved drugs such as oseltamivir and baloxavir are effective when taken in very early stages of infection where they reduce recovery times by a day or two, they become ineffective in later stages of infection, arguing that a major unmet need exists for developing novel flu therapeutics, especially for treating more severe and later stages of infection.

Methods

We developed a bispecific small-molecule adapter (EV21) with a dual mechanism of action that harnesses the power of the host's innate immune system to eradicate viral infection. It's a platform technology comprising of a targeting ligand and a payload conjugated via a suitable linker. Because neuraminidase is expressed on both the influenza virus particles and the infected host cell surface, we repurposed the neuraminidase inhibitor zanamivir for use as a targeting ligand. We linked zanamivir to two distinct haptens that bind to two different naturally occurring antibodies in humans. Once recruited, these antibodies engage the innate immune effectors to simultaneously kill the infectious virus particles and virus-infected cells. Preclinical in vitro and in vivo experiments were performed to evaluate the efficacy, safety, and pharmacokinetics of EV21.

Results

When tested in BALB/c mice supplemented with physiological levels of human IgG and infected with 10xLD50 of influenza virus (H1N1, A/Puerto Rico/8/1934), EV21 elicited excellent antiviral activity. A single intranasal dose of EV21 administered at a later stage of infection (96hpi) resulted in 100% survival of the infected mice and significantly higher reduction of lung viral titer compared to the standard of care (SOC) drugs. Although EV21 exerts its antiviral effect by engaging the innate immune system, it did not give rise to any local or systemic cytokine storm. Moreover, EV21 demonstrated superior in vitro and in vivo activity against an oseltamivir resistant influenza strain (A/H1N1/HK/2369/2009-H275Y), making it a promising alternative to treat drug-resistant influenza infections. Furthermore, EV21 showed excellent safety and pharmacokinetics profile in mice making it a promising pre-clinical candidate

Conclusions

Our zanamivir-targeted dual-hapten immunotherapy (EV21) has the potential to treat both early and late- stage influenza infections including drug-resistant infections more effectively and rapidly than the SOC drugs. We are currently expanding the platform to fight against other enveloped viruses with significant public health concerns. We hypothesize that targeting HBsAg with a similar ligand-dual-hapten conjugate will selectively destroy the HBV virions, subviral particles (SVP) and virus-infected hepatocytes - leading to a sustained functional cure for chronic hepatitis B by achieving rapid HBsAg clearance and restoration of a fully functional immune response. However, it may not be applicable as a first-line therapy due to the excessively high titer of SVP and virions in chronic HBV patients, but it might be very effective in getting rid of the residual particles and infected cells when followed by an antigen-suppression therapy i.e., siRNA, ASO etc. and thus help in achieving a sustained functional cure.

P.36 – Safety, pharmacokinetics, and pharmacodynamics of multiple ascending oral doses of ALG-055009, a thyroid hormone receptor beta agonist, in hyperlipidemic subjects and relative bioavailability/food effect of a solid formulation in healthy volunteers

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Background:
Thyroid Hormone Receptor Beta (THR-β) agonists reduce atherogenic lipids, decrease hepatic fat, and improve liver histology in MASH. ALG-055009 is a novel next generation THR-β agonist with β selectivity and potency which exceeds that of first generation THR-β drugs.

Methods:
ALG-055009-301 was a multi-part Phase 1 study (NCT05090111). In Part 2 (double-blinded), the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of multiple ascending doses of ALG-055009/ placebo was evaluated in subjects with mild hyperlipidemia. For each Part 2 cohort, 8 subjects were randomized 4:1 to receive ALG-055009 or placebo solution formulation once daily for 14 days. Part 3 (open label) assessed in healthy volunteers (HV) the relative bioavailability of a softgel capsule vs. solution formulation of ALG-055009 and food effect potential (softgel capsule). In Part 3, eight HVs received single 0.6 mg ALG-055009 doses in a fixed sequence: solution (fasted), softgel capsule (fasted) and softgel capsule (fed); a washout period of ≥10 days occurred between doses. Safety assessments (adverse events (AEs), vital signs (VS), physical examination (PE), ECG, and laboratories), and plasma PK samples were collected throughout study conduct. Reported here are complete Part 2 (unblinded) and Part 3 safety, PK and PD data; placebo data in Part 2 were pooled.

Results:
In Part 2, a total of 50 subjects (75 – 100% male, mean age 33.4–49.4 years, mean BMI 24.7– 28.4 kg/m^2) with mild hyperlipidemia received oral solution doses of ALG-055009 (0.3, 0.5, 0.6, 0.75 or

1.0 mg) or placebo for 14 days. In Part 3, 8 HVs (75% male, mean age 39.1 years, mean BMI 24.7 kg/m^2) were dosed. Across Parts 2 and 3, there were no serious AEs or treatment-emergent AEs (TEAE) leading to study drug discontinuation. All TEAEs were Grade 1 or 2 in severity. No clinically concerning laboratory (including thyroid hormones), PE, VS, or ECG findings were observed. In Part 2, plasma

ALG-055009 concentrations increased in a dose proportional manner with low variability (geometric coefficient of variability <30%) and a terminal t1/2 of ~20 hours. Dose responsive increases in sex hormone binding globulin and reductions in LDL-C, apolipoprotein B, and triglycerides were observed. In Part 3, geometric mean ratios (90% confidence interval [CI]) of area under curve from time zero to infinity and maximum plasma concentrations of softgel capsule versus solution formulations were 0.86 [0.81- 0.91] and 0.86 [0.82–0.91], respectively, and between fed and fasted for the softgel capsule formulation were 1.06 [1.0-1.13] and 0.97 [0.91-1.03], respectively, suggesting the 2 formulations were bioequivalent and there was no evidence of a food effect for the softgel capsule formulation.

tConclusion:
Favorable safety, PK and PD effects were observed with multiple (14 days) dosing of up to

1.0 mg ALG-055009 (solution) in subjects with mild hyperlipidemia, supporting further evaluation in longer term studies. Single doses of an ALG-055009 softgel capsule formulation were well-tolerated in HVs, with exposures similar to (~86%) the solution formulation and no food effect.

P.37 – REP 2139 targets the Hepatitis Delta Virus (HDV) ribonucleoprotein (RNP) and exerts a direct antiviral effect on HDV replication

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Introduction

The nucleic acid polymer (NAP) REP 2139 has multiple molecular mechanisms: 1) targeting the HSP40 chaperone DNAJB12 to block HBV subviral particles assembly and secretion (Boulon et al, Hepatology (S1) 2020; Angelo et al, HBV Meeting 2023), an effect which also blocks HDV ribonuclear protein (RNP) envelopment and HDV secretion from infected cell; 2) direct binding to S-HDAg and L-HDAg (Shamur et al, Hepatology (S1) 2017). A phase II study (Bazinet et al, Lancet GastroHep 2017) and current compassionate use of REP 2139 in HBV/ HDV infection have shown a more robust and early response toward HDV RNA as compared to HBsAg, suggesting a second direct acting antiviral mechanism. Here we have investigated the direct REP 2139 antiviral activity in relevant HDV cell infection models *in vitro*.

Methods

REP 2139 endosomal release *in vitro* was restored by UNC 7938 as previously described (Blanchet et al, Antiviral Res 2019). Clinical supply of REP 2139-Mg (lot FAB-22-0001) was used for dosing in HDV infected (10 ge/cell) HepG2-NTCP cells and primary human hepatocytes (PHH).
Intracellular HDV viral genome levels were assessed by qRT-PCR. HDV RNA and Hepatitis Delta Antigen (HDAg) association to form the HDV ribonucleoprotein (HDV RNP) was monitored by anti-HDAg RNA immunoprecipitation (RIP) followed by HDV qRT-PCR (Abeywickrama-Samarakoon N, Nat Comms 2020).

Results

A single dose of REP 2139-Mg reduced intracellular HDV viral genome levels by ~1 log10 in HepG2-NTCP and PHH cells at 400nM and 600nM respectively. Loss of antiviral activity at higher doses could be recovered by increasing UNC 7938 concentration, indicating that REP 2139 endosomal release is influenced by both REP 2139 endosomal concentration and UNC 7938 dosing. REP 2139-Mg was active in reducing both genomic (~1Log) and antigenomic (~1.5Log) HDV RNA. A single REP 2139-Mg dose also reduced the intranuclear association of HDV RNA with HDAg by ~60% (@ 600nM) in HepG2- NTCP and by 65% (@ 400nM), without changing the HDAg protein levels. Finally, REP 2139-Mg inhibits HDV replication in HBV-HDV coinfected PHHs. In HBV-HDV coinfected PHHs, REP 2139 inhibits HBsAg secretion without affecting intracellular pgRNA levels or HBeAg secretion.

Conclusions

REP 2139 has a direct acting antiviral effect against HDV RNA replication which appears to involve blocking HDV RNA interaction with HDAg during the morphogenesis of the HDV RNP. These antiviral effects bear further investigation and may explain the more rapid decline of HDV RNA versus HBsAg in human studies.

P.38 – First-in-human application of a novel HBsAg-specific TCR T cell therapy (SCG101) shows antiviral and anti-tumor effects in patients with HBV-related hepatocellular carcinoma

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Background

Hepatitis B virus (HBV) infection accounts for 75-80% of virus-associated hepatocellular carcinoma (HCC). HBV DNA integration into the host cell genome may trigger carcinogenesis and result in the expression of HBV antigens, mainly HBsAg. HBsAg-positive cells can be effectively targeted by T cells grafted with HBsAg-specific T-cell receptors. SCG101, a first-in-class autologous HBsAg-specific TCR-T cell therapy, uses a high avidity TCR that showed profound anti-viral and anti-tumor activity in preclinical studies. Here, we present the evaluation of SCG101 in subjects with HBV-related HCC in an investigator-initiated trial.

Methods

he trial enrolled six HLA-A*02:01(+), HBsAg(+), HBeAg(-) subjects with advanced HBV-related HCC, BCLC B/C stage, who had received one to three prior systemic therapies and at least 12 months of nucleoside analog treatment. All subjects intravenously received a single dose of 5x10e7 or 1x10e8 cells/kg of SCG101 TCR-T cells after lymphodepletion with cyclophosphamide-fludarabine. Safety, pharmacokinetics, antiviral, and antitumor activities were evaluated.

Results

Following infusion, SCG101 TCR-T cells showed significant dose-dependent proliferation and persisted during the study period. Antiviral and/or antitumor activities were observed in all six subjects treated with SCG101. Serum HBsAg dropped in all six subjects, with 4/6 >1 $\log_{10,}$ and maintained suppressed at 20IU/mL throughout the follow-up period for up to 90 weeks. Transient ALT elevation correlating with HBsAg reduction was observed in all subjects, indicating on-target activity of SCG101. Tumor reduction was observed in all 4 patients with >1 $\log_{10}$ serum HBsAg reduction, with two patients showing partial response and two stable diseases as per mRECIST criteria. Patients with less than 1 $\log_{10}$ reduction in serum HBsAg showed no tumor response. The medium overall survival was not reached until the data cut-off date (end of Aug 2023). The treatment was well-tolerated, and no dose-limiting toxicity or neurotoxicity was observed.

Conclusion

SCG101, as a single agent, demonstrated significant antiviral and antitumor activity in subjects with HBV-related HCC. The persistence of TCR T cells, reduction of serum HBsAg, and tumor response proved the on-target activity of SCG101. A phase I/II clinical trial has been initiated to systematically evaluate the safety and efficacy of SCG101.

P.39 – HCV reinfection among people who use drugs (PWUD) treated for HCV infection: A long-term view

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Background

In order to eliminate HCV infection as a public health concern by 2030, there is a need to develop comprehensive programs for its treatment among priority populations such as PWUD. A key concern associated with such initiatives has been the need for strategies to mitigate the risk of reinfection after successful therapy. Metanalyses suggest the rate may be 5/100 person-years (py), but some real-world cohorts report much higher rates. We hypothesize that continued engagement in care after successful therapy would serve to reduce reinfection rates and increase the benefits of HCV therapy among PWUD.

Methods

In our program, we provide HCV therapy to PWUD within the context of a comprehensive, multidisciplinary addressing all medical, social, psychologic, and addiction-related needs with antiviral medications administered daily, or weekly as most appropriate, to maximize adherence and achievement of cure. Subsequently, patients continue to be integrated in care to minimize behaviors associated with reinfection risk, with HCV RNA testing repeated annually, or more frequently if clinically indicated. We conducted an evaluation of the last 282 successful courses of HCV therapy administered to PWUD in our center to determine the rate of HCV reinfection and its correlates.

Results

Among the 282 individuals treated between March 2019 and August 2023, we observed a median age of 47 years (range: 22-83), with 28.2% being female, 19.3% identifying as indigenous, 53.87% being unstably housed, and 92.18% continuing active drug use even after successful HCV cure. At the 3-year mark (with a median follow-up of 3 months), we documented 8 cases of reinfection, resulting in a rate of 0.88 cases per 100 person-years. Of these 8 reinfected patients, 2 were female, 2 identified as indigenous, and all were experiencing unstable housing and ongoing drug use.

Conclusions

To our knowledge, this is the largest single center longitudinal evaluation of HCV reinfection rates among active PWUD in a cohort enriched for factors of instability (high rates of fentanyl use, unstable housing). We demonstrate that a comprehensive, multidisciplinary approach to HCV therapy with maintenance in follow up after cure is achieved is associated with rates of reinfection below 1/100 py. Implementation of programs such as ours will contribute significantly to the goal of eliminating HCV infection as a public health concern by 2030, particularly in this vulnerable population.

P.40 – cccDNA clearance and elimination in HBV infected chimeric mice with humanized liver

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Background

Hepatitis B virus (HBV) infection requires the establishment of covalently closed circular DNA (cccDNA), which is considered stable in the nuclei of infected cells. A strategy to directly target cccDNA molecules, though it is difficult, is nonetheless recommended to eliminate cccDNA and cure chronic HBV infection. Clinical evidence shows a dynamic evolution of the cccDNA population. For instance, wild type (WT) viral population in serum or WT cccDNA in the liver can be cleared and replaced with mutant populations in chronic hepatitis B (CHB) patients with treatment that does not directly act on cccDNA, or without the treatment, which raises the possibility that cccDNA can be spontaneously cleared from infected cells. We hypothesized that the in vivo HBV infected cells can spontaneously clear cccDNA and we tested this hypothesis in uPA/SCID chimeric mice with humanized liver.

Methods

cccDNA level and kinetics were investigated in 43 HBV infected chimeric mice from 3 infection experiments under the conditions of no treatment or blocking of two cccDNA replenishment pathways, which combines entecavir to reduce the de novo rcDNA synthesis mediated cccDNA replenishment, with a new HBV therapy candidate HBVZ10 that expresses sustained high level of anti-HBs antibody after a single injection to block de novo infection mediated cccDNA replenishment. Each of the 43 livers was randomly sampled 20 times (9 of them were sampled 40 times), resulting in a total of 1040 cccDNA samples analyzed. cccDNA and rcDNA copies per cell were also simultaneously analyzed at a single individual nucleus level by Absolute Q (ABQ) Digital duplexing PCR in addition to qPCR assay of cccDNA copies isolated from bulk cells.

Results

HBVZ10 expresses high levels of anti-HBs antibody ranging between 100,000 and 1,000,000 mIU/ml and stays steady for at least 200 days. Most of the individual nuclei in the untreated group just contained a single copy of cccDNA but it still drives a robust replication, which features high serum HBsAg and HBV
DNA levels as well as progressive accumulation of HBV envelope proteins (up to 100,000 copies/cell) and virions (up to 400 copies/cell) in infected cells. Both bulk cells and single nucleus-based assays detect spontaneous cccDNA loss during the spread of infection as well as persistent infection phases. cccDNA replenishment is required to compensate for the spontaneous cccDNA loss. Blocking of cccDNA replenishment pathways reduces cccDNA levels by an average of >100-fold (ranging from 1 to 4 log or to undetectable level) in a few months, which is corroborated by a progressive reduction of serum HBeAg and HBsAg to undetectable level among all 17 mice with complete blocking of de novo infection.
Intrahepatic HBsAg level is also reduced to undetectable level or becomes barely detectable as analyzed by ELISA, western blot, and immunostaining of liver sections.

Conclusions

In vivo infected cells can spontaneously clear cccDNA to stay noncytopathic but continuous cccDNA replenishment is allowed to maintain a steady cccDNA level in the humanized livers. Our results devise an unconventional cccDNA elimination strategy that does not directly target cccDNA but aims to transform spontaneous cccDNA loss into progressive cccDNA elimination through blocking of cccDNA replenishment, which works effectively upon combining this new HBV therapy candidate HBVZ10 with entecavir.

P.41 – A long-term 3D cell culture model for hepatitis B virus studies

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Background:
In vitro investigation of HBV infection typically relies upon 2D culture of primary human hepatocytes (PHH) or cell lines. However, 2D cultures are limited as they do not necessarily accurately represent the 3D environment of hepatocytes, and alternate 3D cell culture models have therefore been developed and have become increasingly widely used. Various successful applications of 3D culture models in the study of HBV infection have been reported, including drug toxicity, screening and metabolism studies. Existing 3D models however are themselves limited as they are composed of hepatic cell lines, with limited physiological relevance, or of PHH, which have a tendency to de-differentiate and have limited longevity in culture. More rigorous and malleable 3D culture models are therefore desirable for the study of HBV infection, and in particular the study of chronic HBV infection.

Method:
Mouse-passaged primary human hepatocytes (mpPHH) were infected with HBV (HBV-infected) or were treated with mock infection conditions (mock) and subsequently formed into 3D spheroids utilizing ultra-low adhesion (ULA) plates and a centrifugation protocol that we have developed. Various outputs were subsequently measured - including viral antigen and human albumin (hAlb) - to monitor infection and hepatocyte health. Additionally, the efficacy of an antiviral capsid-assembly modulator (CpAM) (GLP-26) in the context of the model was investigated.

Results:
3D mpPHH spheroids of various sizes were reliably produced and validated. Spheroids maintained measurable HBV infection for more than two months. Spheroid health was maintained for most of this duration, as determined by hAlb. Infection was controllable using an antiviral compound with known efficacy against HBV infection *in vitro*, and infection was observed to rebound after drug removal.

Conclusions:
We here report a 3D mpPHH model as a novel and advantageous alternative to current systems, that shows promise for use in a range of studies including anti-HBV drug screens. The model overcomes many limitations of widely used 3D and 2D alternatives due principally to its physiological relevance and longevity, and therefore is predicted to enable the study of chronic HBV infection.

P.42 – Preclinical efficacy and safety of ARCUS-POL nucleases for chronic hepatitis B: a potentially curative strategy

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Background
Gene editing with ARCUS nucleases is a potentially curative approach for chronic hepatitis B (CHB) capable of eliminating or inactivating hepatitis B virus (HBV) covalently closed circular DNA (cccDNA) and integrated HBV DNA. We previously demonstrated that ARCUS-POL nucleases engineered to recognize a conserved target sequence in the HBV polymerase gene durably reduce HBV surface antigen (HBsAg) in vitro and in vivo. The previously described ARCUS-POL nuclease, construct, and formulation were each optimized for increased activity and safety (ARCUS-POL v.2), then further engineered to enhance specificity for the HBV target site (ARCUS-POL v.3). The efficacy and safety of ARCUS-POL v.2 and v.3 were tested in preclinical models.

Methods
To evaluate efficacy of ARCUS-POL v.2 in non-human primates (NHP), animals were dosed with a cccDNA-surrogate AAV containing the HBV target sequence then two administrations of 0.5, 1.0 or 2.0 mg/kg ARCUS-POL v.2 mRNA encapsulated in lipid nanoparticles (LNPs). The activity and specificity of ARCUS-POL v.2 and v.3 were evaluated side by side in a liver cell line with integrated HBV DNA and an AAV-surrogate mouse model similar to the AAV-surrogate NHP study. ARCUS-POL v.3 was further tested in HBV-infected primary human hepatocytes (PHH) and a transgenic HBV mouse model.

Results
In NHPs, the optimized ARCUS-POL v.2 was well tolerated and resulted in cccDNA-surrogate elimination and editing, achieving 99% total inactivation at the highest dose and >90% at all dose levels. Compared to ARCUS-POL v.2, the v.3 variant showed enhanced specificity, eliminating editing at off-target sites, with similar HBsAg reductions in a HepG2 cell line with integrated HBV DNA. In a cccDNA-surrogate mouse model, treatment with ARCUS-POL v.2 or v.3 resulted in high levels of viral inactivation and durable reduction of serum HBsAg. In HBV-infected PHH, ARCUS-POL v.3 reduced HBsAg, HBeAg, HBV DNA, and HBV RNA and eliminated or inactivated the majority of cccDNA. Finally, ARCUS-POL v.3 edited integrated HBV DNA and durably reduced HBsAg, HBeAg, HBV DNA, and HBV RNA in a transgenic mouse model.

Conclusions
The fully optimized ARCUS-POL v.3 eliminates or inactivates both cccDNA and integrated HBV DNA resulting in durable reductions in HBsAg with high levels of specificity for the target HBV DNA sequence, and this approach represents a potentially curative therapy for CHB.