FRONTIERS IN DRUG DEVELOPMENT FOR HEPATOLOGY

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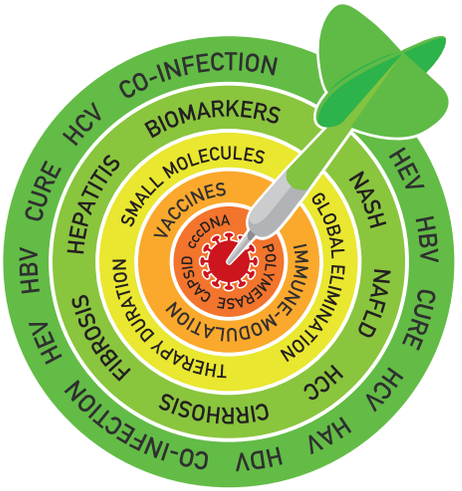

ABSTRACT BOOKLET

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Session 1: Epidemiology and prospects for global elimination: Viral hepatitis and chronic liver disease
December 5th, 2021

Global viral hepatitis epidemiology and prospects for global elimination

Homie Razavi
CDA Foundation

The global prevalence of chronic hepatitis C has been declining as result of increased treatment due to national hepatitis programs. However, much of the global HCV treated patients has been concentrated in one country – Egypt. As the Egyptian program reaches it targets, the global number of HCV treated patients is expected to decrease. This is exacerbated by the COVID-19 pandemic that resulted in a 40% decline in treated patients in high income countries and 70% decline in low- and middle-income countries in 2020. New efforts are needed to maintain the global treatment rate and reach the elimination targets by 2030.

HBV prevalence has been declining as result of infant vaccination, but the liver related deaths are expected to continue to increase among the population who was infected prior to the start of the HBV vaccination program. Current treatment eligibility criteria will ensure that few, if any, country will achieve the HBV mortality reduction targets by 2030. A growing body of evidence suggest that a 60% reduction in HCC cases can be achieved by treating all HBV patients as compared to the current guidelines. A new approach to HBV treatment (test and treat all HBsAg positive patients) will be required to achieve the 2030 elimination targets.

Session 1: Epidemiology and prospects for global elimination: Viral hepatitis and chronic liver disease
December 5th, 2021

HBV and Chronic Liver Disease in Mexico

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Hepatitis B virus (HBV) has ten genotypes (A-J) and over 40 subgenotypes with distinct worldwide distribution. The clinical outcome of HBV infection depends upon virus genotype/subgenotype and host interactions. These facts lead to the need for regional population-based analyzes to register such features. Basically, in the Latin American region, HBV genotypes H and G are endemic to Mexico, and genotype F (subgenotypes F1-F4) predominate in Central and South America. The Asian genotypes B and C and the European A and African D genotypes have reached the Americas primarily by human migration.

The most frequent genotypes among the Mexican population are H, G, and A, followed by D and F and a minor proportion B and C. This distribution is highly concordant with the genetic history and social class of the population. HBV genotype H was initially hosted by the ancestral multiethnic Amerindian populations (First Nations Mesoamerican people). Currently, the descendants of these first settlers comprise at least 25.7 million Native Mexicans living mainly in rural areas. Modern-day Mexicans are an admixture of the Amerindian, Caucasian, and African lineages that vary from region to region. These features may originate deviations in the canonical clinical outcomes of HBV infection reported worldwide.

Chronic liver disease is the 4th cause of mortality in Mexico, in which the three leading etiologies are alcoholic liver disease, viral hepatitis, and, recently, NASH. Despite the high number of patients with cirrhosis, the prevalence rate of liver cancer is low. Concerning HBV infection, Mexico is considered a region of low HBsAg endemicity; however, we have identified high prevalence rates among the Amerindian population (7.99% HBsAg, 45.69% anti-HBc). Moreover, HBV infection is also underdiagnosed. Among the general population, the prevalence of HBV is 0.37%, rising to 1.66% when the anti-HBc marker is tested, increasing even more in adults above 25 years of age. Therefore, considering that Mexico´s 130 million inhabitants are genetically heterogeneous and predominately living in poor economic status, estimates are that at least 16 million people have evidence of HBV infection, of which more than 2.5 million may be active carriers.

HBV infection is acquired mainly by males through risky behaviors involving multiple sexual partners, intravenous drug use, and men who have sex with men. Nearly 70% of HBV infections acquired within the risk groups are genotype H: Amerindians or among the admixed populations such as patients with HIV or hepatitis C, deferred blood donors, and children from low-income families.

We have characterized the kinetics of an acute HBV infection, which features a fast resolution of the infection where occult hepatitis B occurs in a short period (HBsAg negative, HBV/DNA positive). These data agree with the clinical manifestations of HBV infection seen among the Mexicans: low viral load related to OBI; however, when the viral load is high, we observe advanced liver damage and mixtures of HBV genotypes. In this case, mixed infections are with genotypes H and G or A2 and D4 genotypes, the latter of European and African origin, respectively. Both are common among patients living in urban areas.

Genotypes H and F are phylogenetically related and have circulated endemically among the native populations inducing a selective pressure on the host´s immune system, adapting to the virus. This trait may explain the high endemicity of HBV infection, low detection of HBsAg but responsiveness to anti-HBc testing, occult infection, moderate clinical manifestations, and almost null complications such as liver cancer. Likewise, a plausible protective factor against liver cancer is the high consumption of aflatoxin-depleted maize products treated with a well-established pre-Hispanic lime treatment. Thus, it seems that both the host population and the endemic viruses have adapted to each other, reaching a steady interaction within a specific regional environment.
Finally, given the reported high endemicity, occult infection, and underdiagnosis, we recommend that patients at risk be routinely tested for HBsAg, anti-HBc, and HBV/DNA to detect liver damage timely. This action can benefit risk groups, immunocompromised patients, and those diagnosed with NAFL/NASH, a metabolic abnormality rising due to the ongoing obesity epidemic, to discard underlaying HBV-related liver disease.

Session 1: Epidemiology and prospects for global elimination: Viral hepatitis and chronic liver disease
December 5th, 2021

HBV and chronic liver diseases in South America

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South America is a heterogeneous region composed of 14 countries. Brazil is the 1st largest country by population (6th in the world in 2021)(1), followed by Colombia, Argentina, Peru, Venezuela amid the 14 countries. According to the Global Burden of Disease Project(2), cirrhosis and other chronic liver diseases as causes of burden account for an average of 1.94% (from 0.83% in Colombia to 3.08% in Chile). However, when we analyze as a percentage of total deaths, the numbers increase to 3.09% (from 1.39% in Uruguay to 4.56% in Chile).
Hepatitis B and C are major causes of liver-related deaths, and the World Health Organization (WHO) set the year 2030 as the target for Hepatitis B and C elimination program(3).
The Polaris Observatory estimates that HBV infection in South America accounts for approximately 2.4 million cases (0.5%)(4, 5). Only 18% of them had the diagnosis, and only 1% of them is treated. According to this database, in absolute numbers, Brazil has the highest number estimated (1.1 million), 30% of them already with the diagnosis, 27% of them treated. Analyzing the Vaccine birth dose, Colombia has the highest coverage with 84%. The coverage of three or more doses of the HBV vaccine is 89% in South America (range from 80 to 97%).
The same database estimates that HCV infection in South America accounts for approximately 2,1 million cases (0.5%). Only 14% of them were treated. Brazil has the highest estimated number of 1,8 million cases (0.9%), with 8% of them with the diagnosis and 2% of them treated. Venezuela has an estimate of 118,000 cases, with 25% of them diagnosed, but no data on treatment. However, the whole scenario of HCV in South America is still upcoming.

Table1. Summary of hepatitis B and C situation in South America and comparison with Global and other regions situation.

	South America	Global	North America	Europe	Asia	Africa	Oceania
Hepatitis B							
HBsAg+ Infections	2,404,000 (0.5%)	284,781,000 (3.8%)	2,125,000 (0.4%)	8,133,000 (1.1%)	195,719,000 (4.4%)	75,266,000 (6.2%)	802,000 (7%)
Total Diagnosed	18%	11%	23%	21%	13%	4%	5%
Total on-Treatment Rate	1%	2%	5%	2%	3%	0%	0%
≥3 Dose Vaccination	89%	86%	88%	77%	89%	81%	65%
Progress toward WHO 2030 elimination targets							
Diagnosed (90% of total infections)	21%	13.14%	25.76%	23.02%	16.10%	4.04%	5.12%
Treated (80% of eligible are treated)	1.96%	2.35%	5.36%	2.51%	3.18%	0.19%	0%
Birth Dose Vaccination (90%)	51.63%	41.81%	68.58%	43.64%	57.08%	7.50%	42.92%
Three Dose Vaccination (90%)	79.73%	87.89%	76.24%	89.72%	91.78%	84.58%	48.33%
Year of achieving elimination targets							
Prevalence among 5 y.o. (<0.1%)	2015%	No data	2018%	2017%	No data	No data	No data
HCV							
Viremic Infections	2,160,000 (0.5%)	64,416,000 (0.9%)	3,417,000 (0.6%)	9,283,000 (1.2%)	35,493,000 (0.8%)	13,699,000 (1.1%)	126,000 (1.1%)
Viremic Diagnosed	14%	21%	47%	34%	19%	12%	9%
Annual Initiated Treatment Rate	2%	2%	7%	2%	2%	3%	0%
Progress toward WHO 2030 elimination targets							
Diagnosed (90% of Total Infections)	21%	32%	62%	40%	27%	33%	12%
Treated (80% of eligible are treated)	8%	16%	35%	11%	11%	27%	0%
Year of achieving elimination targets							
All Goals	2051	2051	2051	2051	2051	2051	2051
Diagnosed (90%)	2051	2051	2029	2051	2048	2051	2051
Treated (80% of eligible)	2051	2051	2029	2051	2051	2051	2051
Mortality (65% reduction)	2051	2051	2051	2051	2051	2051	2051
Incidence (90% reduction)	2051	2051	2049	2051	2051	2051	2051

Data from the Polaris Observatory (https://cdafound.org/polaris/)(5).
Comparison of hepatitis B and C situation in South America in comparison with Global and other regions is presented in Table 1. The total diagnosed HBV and HCV cases in South America is behind North America and Europe. The HBV vaccine program in South America (89%) is one of the highest compared with other regions, however treatment of eligible patients with chronic hepatitis C is far behind.
Other chronic liver diseases includes hepatitis delta with a geographic distribution predominating in the Amazon area(6). Metabolic associated fatty liver disease (MAFLD) is highly prevalent in all continents, but with one of the highest rates in South America (32%)(7). To finish, although alcohol-related liver disease data on prevalence is limited, it is a major cause of advanced liver disease in South America, being a main cause of cirrhosis in Brazil, Chile and Peru(8).

Session 1: Epidemiology and prospects for global elimination: Viral hepatitis and chronic liver disease
December 5th, 2021

Elimination of HBV and HCV on the African continent

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Over 60 million individuals in Africa have chronic hepatitis B and ten million have chronic hepatitis C. This includes 4.8 million children under the age of five with HBV and one million children with HCV. Still though, only about one out of every 10 people has access to viral hepatitis testing and treatment. According to the WHO, viral hepatitis is a greater threat to Africa than HIV/AIDS, malaria, or tuberculosis, causing about 1.34 million deaths per year. HBV is primarily spread from mother-to-child, whereas the majority of hepatitis C infections are caused by unsafe injection practices in both health care facilities and the community. Intravenous drug use is also becoming a major source of HCV transmission. If no intervention is implemented, it is estimated that over 2 million Africans with chronic HBV and HCV will have progressive liver disease in the next few decades. Modelling studies suggest that investing in the global elimination of viral hepatitis is feasible and cost-effective. Universal access to viral hepatitis screening and linkage to care and treatment is achievable due to the availability of effective generic antiviral therapy for hepatitis B that costs $30 per year and affordable curative HCV direct acting antiviral agents. A few nations though are executing a government-led public health approach and making considerable progress towards the 2030 elimination targets. Cape Verde, Uganda and Rwanda have committed more resources to ensure a 99% birth dose vaccination rate, free national hepatitis B treatment and free treatment for hepatitis B and C. Egypt uniquely verified the feasibility of universal HCV screening and treatment in an exemplary model program, reaching 50 million adults and 9 million adolescents and children. Nevertheless, expansion in implementation of the hepatitis B birth-dose vaccine as a critical intervention for elimination of HBV and HDV still has low coverage in sub-Saharan Africa. Poor community and political awareness, inadequate data for decision-making, limited access to affordable testing and treatments, insufficient funding, and a short of experienced health workers are all significant barriers to building an effective African response to viral hepatitis. It will take a well-coordinated public health response, government leadership and community participation to build an enabling climate for collaboration and partnership. In addition to mobilising finances, training, and the successful implementation of a country-specific hepatitis response.

Session 2: Overview of viral hepatitis, co-infections and NASH
December 6th, 2021

Clinical update on drugs and trials for hepatitis B elimination.

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First- and second- generation new treatments are being evaluated singly and in combination clinical trials to provide a cure for hepatitis B. The life cycle of HBV includes several well- categorized druggable targets for new treatments. A considerable pipeline, and multiple strategies are in development. The notion of a functional cure of hepatitis B (sustained loss of HBsAg) has been accepted as the major endpoint of current clinical trials. Progress has been observed including improved on treatment decrease of hepatitis B DNA, reductions in HBV RNA and a decline in HBsAg with different strategies. The difficulty of depleting or inactivating cccDNA, targeting integrated viral genomes and overcoming antigen specific immune dysfunction means that a cure remains a major challenge. A partial functional cure defined as a decline in HBsAg concentrations to lower levels after finite treatment may need consideration. This overview of major trials will focus on RNAi strategies and HBsAg assembly inhibition and capsid inhibitors. Entry inhibitors and monoclonal or vaccinial antibodies are being assessed.

More profound suppression of hepatitis B replication through the addition of capsid inhibitors with nucleoside analogues can be achieved. Several combination strategies have been evaluated; results of combination studies in HBeAg positive and negative, and nucleoside analogue (NUC) naïve versus NUC suppressed patients have been published. Capsid inhibitor induced reductions of pgRNA (HBV RNA) are most significant in HBeAg-positive NUC-naïve patients. Reductions of HBsAg occur in HBeAg positive NUC-naïve patients, but lower reductions occur in HBeAg-positive NUC- suppressed or HBeAg negative patients. The toxicity versus overall efficacy of new capsid inhibitors require evaluation. The efficacy of combination studies requires re-evaluation. Important progress including on - treatment reductions of HBsAg in phase 2 studies, to a degree that is not achieved with chain terminators have been published with RNAi and antisense oligonucleotides. Nucleic acid polymers induce profound reductions in HBsAg and probable sequential ALT increases. Progress in immunomodulatory therapy has lagged that of antiviral therapy. Newer biomarkers including HBV RNA and HBcrAg may refine phenotyping of chronic hepatitis B. Although unproven, restoring the multilayered impaired and dysfunctional immune response in hepatitis B is perhaps more likely and feasible after a reduction in host antigen burden. Other potential experimental strategies include CRISPR- Cas9 genome- editing nucleases to specifically target cccDNAs. A perspective will be required, given the delay in diagnosing and treating the worldwide burden of hepatitis B, and the efficacy and low cost of current nucleoside analogues.

Session 2: Overview of viral hepatitis, co-infections and NASH
December 6th, 2021

Molecular Analysis and Personalized Medicine in NASH Fibrosis and HCC

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Background
Advances in technologies to sequence individual cells have revolutionized our understanding of complex diseases. This is nowhere more relevant than in studies of non alcoholic steatohepatitis (NASH) and hepatocellular carcinoma (HCC). These technologies have not only clarified cellular contributions to disease, but also uncover cell-cell interactions and identify novel therapeutic targets. Concurrently, rapid sequencing of either whole exomes or genomes, has yielded major insights into genetic variants and mutations that may either create or predispose to disease.

Methods and Results
This lecture reviews both published and unpublished data that greatly clarify the role and heterogeneity of hepatic stellate cells in pathogenesis of fibrosis, both in experimental and human NASH. Specifically, ongoing studies define subgroups of hepatic stellate cells and related mesenchymal cells, thereby unearthing novel therapeutic targets. The theme of heterogeneity has been further expanded through analysis of different fibroblast and fibrogenic populations across tissues. Software platforms including Cellphone DB leverage these large datasets to define candidate autocrine and paracrine interactions that were previously unappreciated using conventional cell biologic approaches. Highly refined sequencing methodologies also offer the opportunity to better understand the cellular and molecular basis of fibrosis regression, which is still poorly understood. This is vital unmet clinical need, since fibrosis regression has now been clearly linked to improved clinical outcomes enlarge cohorts of patients with NASH, but no targets to accelerate fibrosis regression have been identified. Molecular analyses have also been used to identify specific cell surface molecules that mark senescent stellate cells, providing a target for clearance of these cells using CAR T cells.

Sequencing technologies have also transformed our ability to define genetic determinants of disease, disease risk and progression in NASH. Whole exome sequencing can be used to identify specific mutations that explain disease in patients who present who present with atypical features of the disease. Additionally, key single nucleotide polymorphisms, for example in the genes encoding PNPLA3, and HSD17B13, not only represent novel therapeutic targets, but can be combined to define genetic risk scores that will influence management. Big data approaches have also allowed us to identify existing drugs that can be repurposed for therapy of NASH without a pre-existing biologic rationale. For example, we recently identified a GABA B receptor agonist, AZD 3355, through computational methods that link gene expression analysis in cultured cells screened with a range of drugs already tested and safe in humans. Finally, high throughput sequencing methods combined with single cell analyses have defined unique features of the stromal microenvironment in NASH livers with HCC, suggesting a unique milieu that will require different therapies than in patients with HCC due to other etiologies. They can also be used to diagnose disease features using liquid biopsy.

Conclusions / Summary;

1. Fibrosis is marked by heterogeneity. Functional implications of this heterogeneity not fully clarified. Epigenetics (ATACseq) and secretome also require analysis.
2. Studies of NASH HCC suggest a unique immunologic milieu but unclear if this extends to mechanisms of fibrosis.
3. NASH will benefit from sequencing methods and personalized medicine through:
    - Drug repurposing for novel therapies
    - Whole exome sequencing in patients with atypical disease
    - Risk assessment based on SNPs that impact disease progression
    - Defining subgroups of disease with unique drivers
    - Assessment of immunologic drivers of HCCs in NASH

Session 3: Hepatitis B basic science (Hepatitis B Foundation sponsored session)
December 6th, 2021

HBV transcriptional regulation : New insights in the role of HBx

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Background
N6-methyladenosine (m^6A) modification, the most prevalent internal modification, occurs in cellular RNAs and viral transcripts and regulates the fate of cellular and viral RNAs. Hepatitis B virus (HBV) transcripts are m^6A methylated in the consensus DRACH motif localized within the epsilon stem-loop structural element. This RNA modification differentially regulates the viral life cycle depending on the m^6A position in the 5′- or 3′-epsilon stem-loop. We have investigated the emerging myriad functional roles of m6A modification and found that it plays key roles in the viral life cycle and liver disease pathogenesis associated with infection. These include from RNA stability, translatability, Interferon sensitivity, encapsidation of pregenomic RNA, cotransrciptional methylation of viral and host RNAs and tumor suppressor function of a host protein with possible role in hepatocracionogenesis (HCC). In this presentation, we present evidence for the pivotal role of HBV-encoded protein X (HBx) protein in recruiting cellular m^6A machinery onto HBV minichromosome and host PTEN chromosomal locus to add m^6A modification co-transcriptionally. Induced m^6A modification of HBV RNAs and PTEN mRNA by HBx decreases their stability.

Methods
HBV genomes defective in HBx failed to induce m^6A modifications of HBV RNAs during infection/transfection, while ectopic expression of HBx restores m^6A modifications of the viral RNAs but not the mutant HBx carrying the nuclear export signal. Using chromatin immunoprecipitation (ChIP) assays, we provide evidence that HBx and m^6A methyl-transferases complexes are localized on the HBV minichromosome to achieve co-transcriptional m^6A modification of viral RNAs. HBx interacts with METTL 3/14 to carry out methylation activity and also modestly stimulates their nuclear import.

Conclusions
N6 metheyladenosine modification of RNA is speculated to occur cotranscriptionally. Our work provides insight into how HBx recruits RNA methylation machinery to m^6A modify RNAs on cccDNA. This role of HBx in mediating m^6A modification also extends to host PTEN mRNA with consequences in the development of HCC.

Session 3: Hepatitis B basic science (Hepatitis B Foundation sponsored session)
December 6th, 2021

Mechanistic insights into hepatitis B virus (HBV) cccDNA biogenesis

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Background
HBV is a liver-tropic virus that infects human and chimpanzees. An estimated 2 billion people have been exposed to HBV, of whom 250-400 million are chronically infected. Approximately 887,000 people die each year from HBV-related liver diseases. Currently, there is no cure for chronic HBV infection, and patients need to be on lifelong antiviral regimens. The root cause of HBV chronicity is the formation of the covalently closed circular DNA (cccDNA), which is formed by the repair of lesion-bearing HBV relaxed circular DNA (rcDNA) delivered by the virions to hepatocytes. However, the molecular mechanisms of cccDNA formation are poorly understood.

Methods
Here, we have developed novel biochemical systems where HBV rcDNA can be repaired to form cccDNA in vitro. Using this biochemical system, we examined the molecular mechanisms by which each individual lesion of rcDNA is repaired.

Results
We have previously identified five components involved in DNA lagging strand synthesis to be essential in cccDNA formation in vitro. We have now identified that the repair of the plus and minus stands of rcDNA require different set of factors. The repair of the plus strand requires all five factors and its repair resembles the maturation of Okazaki fragment, while the repair of the minus strand only requires two of these five factors. We found that inhibitors targeting these factors could restrain cccDNA formation in vitro and/or in cell culture.

Conclusions
We have established a biochemical system to fully reconstitute cccDNA formation with purified human proteins. We have provided mechanistic insight in rcDNA repair in vitro and potential therapeutic approaches to block cccDNA formation.

Session 3: Hepatitis B basic science (Hepatitis B Foundation sponsored session)
December 6th, 2021

Hepatitis B virus (HBV) DNA integration and functional cure

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Hepatitis B virus (HBV) exists as 2 persistent forms: the replication-competent covalently closed circular (ccc)DNA; or as the replication-deficient integrated HBV DNA. The latter has been strongly associated with liver cancer, but the specific mechanisms that drive this pathogenesis is unknown. More importantly, as a highly stable source of HBV surface antigen, integrated HBV DNA is a likely barrier to HBV functional cure (defined as the loss of circulating HBV surface antigen). Thus, the design of therapeutic strategies must take into account the underlying biology, role in pathogenesis, and influence on viral persistence driven by integrated HBV DNA.

Using highly sensitive and quantitative molecular methods, our group has aimed to understand these contributions. We and others have shown that HBV DNA integration into host cell genome occurs early in infection and independent of viral replication. We have also identified specific clinical characteristics associated with increased frequency of integration. Finally we have investigated the role of integrations in functional cure, with evidence that some clearance of cells containing integrated HBV DNA is necessary.

Together, our data emphasises the need to understand the dynamics of HBV DNA integration and how it may limit upcoming therapeutic approaches against chronic HBV infection.

Session 4: From bench to bedside: Clinical updates on hep B elimination
December 6th, 2021

What will it take to achieve HBV cure?

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It is generally accepted that sterilizing cure with eradication of both cccDNA and integrated HBV DNA and elimination of risk of HBV reactivation is not feasible, at least not in the foreseeable future. Thus, experts have agreed that the goal is to achieve functional cure defined as sustained clearance of HBsAg and HBV DNA after completing a finite course of treatment. In this scenario, cccDNA and integrated HBV DNA will still be present but are not transcriptionally active and risk of HBV reactivation and HCC will be reduced but not eliminated.
Combination therapy will be necessary to achieve functional HBV cure. This will require not only development of novel classes of direct-acting antiviral drugs but also immune modulatory therapies and existing drugs: nucleos(t)ide analogues (NA) and interferon (IFN). It is envisioned that three targets must be met: suppression of HBV replication, decrease in HBsAg production, and restoration of HBV-specific immune response. Suppression of HBV replication may involve combinations of NA, capsid assembly modulators (CAM), and entry inhibitors. Decrease in HBsAg production may involve combinations of entry inhibitors, siRNA/antisense oligonucleotides, and drugs that block release of HBV or viral proteins. It is currently unclear whether immune modulatory therapy is needed for all patients or only those in whom immune response is not restored despite suppression of HBV replication and HBsAg production.
In addition to the development of new drugs, development of standardized assays to confirm target engagement and to confirm “cure” need to occur in parallel. Success of HBV cure programs will require multiple weapons and multiple collaborators: scientists, medicinal chemists, pharmaceutical partners, clinicians, regulatory authorities AND patients.

Session 4: From bench to bedside: Clinical updates on hep B elimination
December 6th, 2021

Stopping Nucleos(t)ide analogs: Is the glass half full or half empty?

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Discontinuing NA therapy has been the subject of debate in several recent trials. NA therapy cessation aims to sustain virologic suppression while potentially clearing HBsAg, which may or may not be related to ALT flares. Evidence to support stopping NAs in HBeAg negative patients is limited and highlights the need for long-term consolidation therapy and close follow-up monitoring. Patients with advanced liver fibrosis should continue NA therapy indefinitely.

The results published thus far suggest that stopping NAs is difficult because many patients experience virological relapse (up to 55%), sometimes with potentially dangerous flares Although an off-treatment hepatitis flare might signal or induce HB sAg loss, it has been challenging to distinguish these ‘good’ flares from the ‘bad’ ones . Limitations of these studies were the small sample size or retrospective study design.

The 6-year cumulative HBsAg loss rate ranged widely from 13-55% in HBeAg negative patients. However, prediction of HBsAg loss, virological relapse or sustained virological suppression remains challenging. Low levels of serum HBsAg at treatment withdrawal were associated with HBsAg loss. Further questions to be answered concern the optimal duration of NA consolidation therapy, the association between HBsAg loss and ALT flares, patient selection, usage of new biomarkers and when to retreat.

Session 4: From bench to bedside: Clinical updates on hep B elimination
December 6th, 2021

Clinical Trials Conducted by the Hepatitis B Research Network (HBRN)

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Background
The HBRN is a consortium of 11 centers of excellence, funded by the NIH. It has assembled a cohort of more than 2,500 adults and children to study the natural history of hepatitis B viral (HBV) infection in North America. In addition, the consortium has conducted three clinical trials of treatments for chronic HBV infection.

Methods
The first two of these trials focused on individuals with immune tolerant HBV infection, one trial being done in adults and the other in children while a third study was confined to adults with immune active chronic hepatitis B.

Results
The immune tolerant studies assessed the effect of a combination of nucleoside analogue (entecavir) antiviral therapy with peginterferon alfa-2a. In the adult study, only 1 of 28 participants cleared HBeAg and none met the primary endpoint of both HBeAg loss and HBV DNA ≤ 1,000 IU/mL 48 weeks after treatment. ALT elevations > 5 times the ULN (i.e. flares) occurred in eight (29%) participants but none were associated with jaundice. Forty-eight weeks posttreatment, HBV DNA rebounded to baseline levels in all participants, including the participant who lost HBeAg, and ALT values returned to near baseline levels in all but four participants. In a similar study conducted in 60 children, the combination of entecavir and peginterferon for up to 48 weeks rarely led to loss of HBeAg with sustained suppression of HBV DNA levels in children in the immune-tolerant phase of HBV infection, and treatment was associated with frequent adverse events.
A randomized controlled trial was conducted in adults with immune active chronic hepatitis B. The study design compared tenofovir alone for 4 years vs. peginterferon alfa-2a for 1 year with tenofovir for 4 years. Briefly there was no significance difference in rates of loss of HBsAg between groups, but HBeAg loss occurred more frequently and earlier with tenofovir plus peginterferon alfa-2a. HBV subgenotype A2 was a strong predictor of HBsAg loss, independent of treatment but HBsAg loss was rare (~1%) in immune active chronic hepatitis B.
The effect of treatment withdrawal was also studied in this clinical trial. Of the 201 subjects enrolled in this study, 43 participants in the TDF arm and 49 in the TDF+PEGIFN arm were eligible for treatment discontinuation. It was found that ALT flares after TDF withdrawal were not associated with subsequent HBsAg decline or loss. Those experiencing HBeAg seroconversion on therapy had better outcomes post-withdrawal than participants who started treatment with HBeAg-negative IA disease.

Conclusions
These clinical trials conducted by the HBRN offer insights into treatment of patients with chronic HBV infection in North America.

Session 5: New advances in NASH, NAFLD, fibrosis, cirrhosis, HCC treatment
December 7th, 2021

Does HCC occur in Nash without hepatitis B core antibody?

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Background
HCC is the most rapidly growing cancer in the US today. NASH and hepatitis B are certainly involved separately and possibly together in the etiology of HCC. The looming question is whether infection with hepatitis B and clearance leaving only hepatitis B core antibody will increase the likelihood of HCC in NASH patients with or without cirrhosis.

Methods
There is at least one reference that suggests that HCC does not occur in NASH without a core antibody and there are many studies showing that core antibody increases the risk of HCC in alcoholic liver disease and hepatitis C.

Results
Some new work by Patrick Kennedy in London has shown that events associated with hepatocarcinogenesis can be present at any phase of chronic hepatitis B infection and that HBVDNA integration into the host genome is an early event and thought to drive carcinogenesis via several mechanisms.
Other studies reveal that in patients with core antibody there may be core antigen that's being expressed, implying that there is an occult active hepatitis B infection without measurable HBVDNA in the blood. That of course, would be driving carcinogenesis.

Conclusions
There is much yet to learn about what happens when hepatitis B s antigen is cleared but leaves core antibody, integrated HBVDNA and other markers like hepatitis b core antigen, markers perhaps of a low-grade or occult HBV infection which can lead to HCC in any patient.

Session 6: Academic/ industry session: Advances in therapeutics for NASH, NAFLD, and liver disease
December 7th, 2021

Phase 3 development of resmetirom for the treatment of patients with NASH and significant liver fibrosis

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Background
MAESTRO-NASH NCT03900429 and MAESTRO-NAFLD-1 NCT04197479 are 52 week Phase 3 registrational double blind placebo controlled clinical trials to study the effect of resmetirom, a selective thyroid receptor beta agonist in more than 2000 NASH patients. A goal of MAESTRO-NAFLD-1, a 1200 patient “real life” NASH study is to identify non-invasive markers that correlate with patient response to resmetirom treatment. The 169 patient 100 mg open label (OL) arm completed the 52 week study in July 2021.

Methods
Eligibility required at least 3 metabolic risk factors (Metabolic syndrome), fibroscan kilopascals (kPa) consistent with ≥F1 fibrosis stage, and MRI-PDFF≥8%. The primary and key secondary endpoints of MAESTRO-NAFLD-1 including safety, relative percent reduction of MRI-PDFF (week 16), LDL cholesterol (LDL-C) (week 24), Apolipoprotein B and triglycerides, fibroscan and 52 week endpoints were analyzed in the OL arm.

Results
Mean age was 55.6 (11.5 (SD)), female 68%, BMI 36.1 (6.0), diabetes 48%, hypertension 68%, dyslipidemia >70%, ASCVD score 11.6%; fibroscan (kPa 7.7 (3.3)), and MRI-PDFF 17.8% (7%). Statistically significant ($p<0.0001$) reduction of MRI-PDFF -53% (3.3% (SE)) overall, and in several subgroups were observed at week 52 (figure). Liver volume (LV) was elevated at baseline (2202 cm (535)) by ~50% relative to normal controls and ~15% after correction for BMI (Euro J of Radiol 106, 2018, 32–37). Resmetirom reduced LV -21%(1.0%), -23%(1.0%) respectively, at weeks 16 and 52 ($p<0.0001$), in all demographic groups. LV reduction was greater than predicted by % reduction in MRI-PDFF, a measure of liver fat content (Clin Gastroenterol Hepatol. 2015 13: 561–568); LV-corrected mean MRI-PDFF reduction was -61% (2.4%). Weight loss ≥5% occurred in ~21% and was linked to resmetirom exposure (SHBG). At week 52, MRE (-0.4, $p=0.014$); fibroscan CAP (-53(4.6)) and VCTE (-1.9; -20%) ($p<0.0001$) were reduced relative to baseline. LDL-C (-21% (1.9%), apolipoprotein-B (-22% (1.6%)), triglycerides (-22%(2.6) were statistically significantly reduced ($p<0.0001$). Decreases from baseline in liver enzymes were ALT -20 IU, AST -11 IU, GGT -25 IU ($p<0.0001$). Significant reductions in inflammatory and fibrosis biomarkers, reverse T3, ELF, and M30 and an increase in adiponectin were observed. No safety flags were identified; BP (systolic, diastolic) was reduced by ~2mm Hg, ($p=0.02$); bone mineral density (DEXA) was unchanged at 52 weeks.

Conclusion
In this 52 week Phase 3 OL study, noninvasively identified NASH patients treated with 100 mg per day of resmetirom for up to 52 weeks demonstrated rapid and sustained reduction in in 1-hepatic fat and liver volume 2-fibrosis as assessed by biomarkers, MRE and fibroscan; 3- LDL and atherogenic lipids, 4-liver enzymes and inflammatory biomarkers, providing support for the use of non-invasive tests to monitor individual NASH patient response to resmetirom treatment.

Session 6: Academic/ industry session: Advances in therapeutics for NASH, NAFLD, and liver disease
December 7th, 2021

IBAT inhibitors and liver disease targets

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The causes of cholestasis may involve genetic, inflammatory, obstructive, or toxic initiators. However, the root cause of disease from cholestasis is, by definition, the excess intrahepatic retention of bile acids. Over the past 25 years we know in great detail the genes, enzymes and transporters involved bile acid formation (from cholesterol precursors) and the enterohepatic circulation, However, there have been very few means to address the inherent accumulation of bile acids in cholestatic conditions. Utilizing the knowledge of key therapeutic target points in the enterohepatic circulation, it is clear that interruption of the return of bile acids from the lumen of the intestine back into the portal circulation can be achieved with potent luminally-focused inhibitors of the Ileal Bile Acid Transporter—IBAT (aka ASBT or SLC10A2). Very recently, two IBAT inhibitors have been studied in 2 cholestatic diseases of childhood—Alagille Syndrome and PFIC, and shown to reduce disease progression and debilitating pruritus. These two agents, maralixibat for Alagille Syndrome and odevixibat for PFIC, were recently approved in 2021 for these indications—the first proven therapies for cholestasis in > 20 years. Preclinical and clinical analyses have revealed that IBAT inhibitors reduce hepatic retention of bile acids in mouse models as well as serum bile acids in pediatric cholestatic disorders. Based on these findings, IBAT inhibitors are now being studied in a variety of disorders including NASH, PBC, and biliary atresia. The hope and promise of IBAT inhibitors are the substantial safety profile of luminally-restricted agents, limited side effect profiles, and the opportunity to begin to address the root cause of cholestatic pathophysiology—therapeutic reductions of the intrahepatic retention of bile acids.

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Session 6: Academic/ industry session: Advances in therapeutics for NASH, NAFLD, and liver disease
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Long-term efficacy, safety and natural history comparisons of maralixibat treatment in patients with Alagille syndrome and cholestatic pruritus

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Background
Alagille syndrome (ALGS) is a rare genetic disease that often presents with severe cholestasis and pruritus. Pruritus is a leading indication of liver transplantation. Until recently, there were no approved drugs for management. Maralixibat, an apical, sodium-dependent, bile acid transport inhibitor, prevents enterohepatic bile acid recirculation and was approved by the US FDA for treatment of cholestatic pruritus in patients with ALGS 1 year of age and older on Sep 29, 2021 (LIVMARLI). Long-term safety and efficacy of maralixibat was evaluated in the pivotal ICONIC study. In addition, an analysis of 6-year event free survival (EFS) was conducted to compare maralixibat treated ALGS patients to a natural history external control.

Methods
ICONIC was a placebo-controlled, randomized withdrawal period (RWD), phase 2b study with open-label extension in children (aged 1–18 years) with Alagille syndrome (NCT02160782). Eligible participants had more than three times the normal serum bile acid (sBA) levels and intractable pruritus. After 18 weeks of maralixibat 380 µg/kg once per day, participants were randomly assigned (1:1) to continue maralixibat or receive placebo for 4 weeks. Subsequently, all participants received open-label maralixibat until Week 48 and could continue through a long-term extension study. Cholestastic pruritus, serum bile acids, growth, xanthomas, quality of life and safety were assessed over time (204 weeks reported). In addition, a separate independent 6-year EFS analysis was conducted by the GALA Study Group, which compared maralixibat-treated patients across the clinical program (n=84) versus a natural history external control cohort from the GALA clinical database (n=469).

Results
Between Oct 28, 2014, and Aug 14, 2015, 31 participants (mean age 5·4 years [SD 4·25]) were enrolled in ICONIC and 28 analyzed at Week 48. Of the 29 participants who entered the RWD, 10 (34%) were female and 19 (66%) were male. In the RWD, participants switched to placebo had significant increases in sBA (94 µmol/L, 95% CI 23 to 164) and pruritus (1·7 points, 95% CI 1·2 to 2·2), whereas participants who continued maralixibat maintained treatment effect. From baseline to Week 48, sBA (–96 µmol/L, –162 to –31) and pruritus (–1·6 pts, –2·1 to –1·1) improved. In participants who continued to Week 204 (n=15), all improvements were maintained. Improvements in xanthomas and height z-score were also observed. The most frequent adverse events were gastrointestinal related. Most adverse events were self-limiting in nature and mild-to-moderate in severity. In the natural history comparison conducted by GALA, 6-year EFS was significantly improved in the maralixibat cohort, HR=0.305 (95% CI: 0.189–0.491), p<0.0001, demonstrating a 70% reduction for clinical outcomes (defined as liver transplant, death, surgical biliary diversion and hepatic decompensation), with maralixibat treatment vs natural history in patients with ALGS.

Conclusion
Maralixibat is the first approved agent for the treatment of cholestatic pruritus in patients with ALGS age 1 year and older. It is the first agent to show durable and clinically meaningful improvements in pruritus and other clinical parameters. A 70% reduction for clinical outcomes with maralixibat when compared to a natural history control group further suggests that it may provide a potential. alternative therapy to liver transplant.

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Efficacy of a higher daily aramchol dose on fibrosis improvement in the phase 3 open label part ARMOR study

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Background
Aramchol is a partial inhibitor of hepatic stearoyl-CoA desaturase (SCD1), a mechanism that directly affects fibrogenesis, i.e; collagen production by HSC›s. In a 52-week phase 2b study, improvement in fibrosis by ≥1 stage without worsening of NASH was observed in 17.5%, 21.3% and 29.5%, in the placebo, Aramchol 400 mg and 600 mg daily arms, respectively. A 53% higher exposure is achieved when dividing 600mg QD Aramchol to 300mg twice daily (BID). Since this higher exposure was expected to improve efficacy, and potentially over a shorter treatment duration, Aramchol 300mg BID was selected for a phase 3 study in patients with NASH and fibrosis. An Open-Label Part is ongoing that is designed to explore the kinetics of histological outcome measures and non-invasive tests as a function of treatment duration.

Methods
150 patients with histologically confirmed NASH and fibrosis are being enrolled to receive Aramchol 300mg BID in the Open-Label Part of the study. Patients are randomized 1:1:1 to perform a post-baseline liver biopsy at weeks 24, 48 or 72. The primary efficacy endpoints are the kinetics of fibrosis Improvement without worsening of NASH and NASH Resolution without worsening of fibrosis for the different treatment durations. Biopsies are read by 3 independent pathologists individually, followed by a consensus reading.

Results
Herein we report the results from the first 20, F1-3 patients that received Aramchol in whom the scheduled post-baseline biopsy was performed. At baseline, mean age ±SD was 58.3±8.9 years; 69% were females; 81% White; mean BMI 34.0±2.8 kg/m2; 94% had type 2 diabetes; 10 patients had stage 3 fibrosis; 4 stage 2, and 2 stage 1; Mean NAS was 4.9±1.3. Post-baseline biopsies were performed for 9 patients at 24 weeks, 9 at 48 weeks and 2 at 72 weeks. Altogether 12 of 20 patients (60%) showed fibrosis improvement by ≥1 stage (5 of 9 after 24 weeks, 6 of 9 after 48 weeks and 1 of 2 after 72 weeks). In 5 patients, fibrosis was reduced by 2 points. In 9 of 20 (45%) patients there was fibrosis improvement without worsening of NASH. Aramchol continues to show good safety and tolerability.

Conclusion
Treatment with Aramchol(tm) 300mg BID reduced fibrosis progression measured by histology in 19 out of the 20 patients completed as of data cutoff. 12 of the 20 patients (60%) showed fibrosis improvement. The data presented here, albeit preliminary, is aligned with the hypothesis that higher Aramchol exposure results in an improved efficacy profile and that a direct anti-fibrotic effect may be manifested as early as 24 weeks.

Session 7: Academic/ industry session on advances in therapeutics for HBV (non-CME accredited)
December 7th, 2021

Janssen's evolving strategy for development of novel combination therapies towards achieving functional cure (FC) for chronic hepatitis B (CHB)

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Background

In CHB, sterilizing cure is thought unlikely due to presence of transcriptionally silent cccDNA, thus the current treatment goal is functional cure (FC; HBsAg and HBV DNA negative for ≥ 6 months off treatment) that depends on effective HBV-specific T cell control (mostly CD8+), as well as B cell support. High levels of HBsAg are immunosuppressive, leading to upregulation of PD-1 on T (and B) cells, rendering them exhausted and non-functional. Achieving FC might require the combination of various mechanisms of action including immunomodulating approaches.

Janssen is developing combination regimens in three generations that are defined by increasing immune modulation.

Generation 1 regimens involve only direct acting antivirals (DAA) with a requirement for significant HBsAg lowering. Removal of immune suppressive effects through lowering of HBsAg to very low or undetectable levels is expected to allow recovery of T cell function and achievement of FC in some patients. Janssen Generation 1 regimens include two novel agents that are combined with NA standard-of-care: (i) JNJ-3989, siRNA comprising both X and S triggers and previously shown to significantly reduce HBsAg in CHB patients with 3 monthly doses (*ARO-HBV1001*), and (ii) JNJ-6379, a capsid assembly modulator of the class that forms normal shaped empty capsids (CAM-N) previously shown to block HBV replication in CHB patients with daily dosing over 28 days (***6379HPB1001)***.

Generation 2 regimens build upon Generation 1 by combining DAA and immune modulator(s) (other than a vaccine), and **Generation 3** regimens also include a therapeutic vaccine.

Methods

REEF-1 Ph2b study: HBeAg positive or negative, NA-experienced or -naïve CHB pts were enrolled and treated for 48 weeks with JNJ-3989 (SC injection q4wks) ± JNJ-6379 (by daily oral dosing) as follows, all on top of daily oral NA therapy.

- siRNA (3 arms, N=90 each): JNJ-3989 at 40, 100 or 200 mg
- siRNA + CAM-N (1 arm, N=90): JNJ-3989 at 100 mg + JNJ-6379 at 250 mg.
- CAM-N (1 arm, N=45): JNJ-6379 at 250 mg
- Placebo (SC) + placebo (oral) (1 arm, N=45).

Results

Interim REEF-1 end-of-treatment results will be presented at AASLD in November 2021. At HepDART, key findings will be summarized and then discussed in the context of important learnings and open questions towards developing a strategy to achieve FC in CHB patients.

Conclusion

This large Ph2b study is expected to provide results that are informative to the field and that will shape Janssen's strategy to develop a finite treatment regimen for FC in CHB.

Session 7: Academic/ industry session on advances in therapeutics for HBV (non-CME accredited)
December 7th, 2021

Combination Approaches Towards a Functional Cure for Chronic Hepatitis B

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Background
Chronic Hepatitis B (CHB) is a worldwide epidemic, affecting >300 million people. Current standard of care can effectively block viral DNA replication but must be given for life and rarely leads to sustained loss of HBsAg or functional cure.

Methods
Aligos is developing multiple drug candidates that target distinct, clinically validated stages of the HBV life cycle in order to achieve marked reductions in HBsAg and further suppression of viral replication. Our goal is to identify the most safe and effective combinations of these investigational agents that can achieve high rates of functional cure. Our approach includes the development of novel oligonucleotides that target HBsAg production, including S-antigen Transport-inhibiting Oligonucleotide Polymers (STOPS™), antisense oligonucleotides (ASO) and small interfering RNAs (siRNA) as well as small molecule replication inhibitors, such as capsid assembly modulators (CAMs).

Results
We have advanced three compounds, ALG-010133 (STOPS), ALG-000184 (CAM) and ALG-020572 (ASO), with best-in-class non-clinical properties into Phase 1 clinical trials. To date, all three compounds have demonstrated acceptable safety and PK profiles supportive of their continued evaluation in CHB subjects (ALG-010133, ALG-000184) and HVs (ALG-020572). Additionally, 50-100 mg doses of ALG-000184 given for 28 days have been shown to potently reduce HBV DNA and RNA levels in CHB subjects to below the lower limit of quantitation (<LLOQ) in ≥75% and 100% of subjects, respectively.
Our siRNA agent, ALG-125755, has demonstrated picomolar activity in in vitro studies and excellent activity and durability in HBsAg reduction in the AAV-HBV mouse model. ALG-125755 has advanced into GLP toxicology studies and is on track to enter Phase 1 clinical trials in H1 2022.

Conclusion
We have discovered and selected four potentially best-in-class compounds across distinct, clinically validated mechanisms of action for the treatment of CHB. Three of these agents, our CAM, STOPS and ASO molecules, have entered clinical development and continue to be evaluated in Phase 1 studies. Our siRNA is on-track to begin dosing in humans in H1 2022. Our goal is to initiate Phase 2 studies to identify the most promising combinations of these drug candidates in CHB patients in H2 2022.

Session 7: Academic/ industry session on advances in therapeutics for HBV (non-CME accredited)
December 7th, 2021

Development of HBV core inhibitors for the treatment of chronic hepatitis B infection

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Core inhibitors are a new class of antivirals with the potential to deepen viral suppression and improve cure rates in patients with chronic hepatitis B (cHBV) infection when administered in combination regimens. Core inhibitors have multiple mechanisms of action (MOA) including (1) inhibition of pgRNA encapsidation, which prevents assembly and release of new viral particles, and (2) disruption of incoming capsids, which prevents de novo cccDNA formation. We believe activity against both MOAs is likely to be important for maximal clinical benefit. Assembly Biosciences has a portfolio three core inhibitors in development, with each core inhibitor having increasing potency against both MOAs. Vebicorvir (VBR) is the most advanced molecule and is currently in phase 2 combination studies. ABI-H3733 is in phase 1 and ABI-H4334 was recently nominated for development and has entered IND-enabling studies.

VBR belongs to the dibenzothiazepin structural class and has an in vitro EC_{50} of 0.15 mM against the formation of new viral particles and 2 mM against de novo cccDNA formation. Phase 1a evaluation in healthy subjects indicated increasing exposure with doses of 100 mg up to 1000 mg and a mean half-life of approximately 24 hours. During 28-day phase 1b studies in cHBV patients, VBR demonstrated dose dependent antiviral suppression of both serum HBV DNA and serum RNA when given once daily at doses ranging from 100 to 300 mg, with maximal suppression achieved at 300 mg (mean maximum reduction in HBV DNA of 2.8 $\log_{10}$ IU/mL as compared to baseline).

During phase 2 development, treatment-naïve (TN) HBeAg positive and virologically suppressed (VS) HBeAg positive or HBeAg negative cHBV patients were randomized to either VBR or placebo plus nucleoside reverse transcriptase inhibitor (NrtI) for 24 wks. The combination of VBR + NrtI resulted in deeper antiviral suppression compared to nucleoside alone in both the TN and VS populations. Eligible patients subsequently received VBR+NrtI in an extension study. Patients in the extension study meeting treatment discontinuation criteria (total HBV nucleic acid [DNA + pgRNA] <20 U/mL and HBeAg ≤5 IU/mL for ≥6 months at week 52 or later) stopped combination therapy to assess for sustained virologic response (SVR). Following treatment discontinuation in 41 patients that met stopping criteria, universal relapse was observed between weeks 4 and 16, despite the deeper level of viral suppression for an extended period of time. Stopping criteria will be refined for future studies. VBR was well-tolerated with a mean duration of treatment of 76 weeks. Viral sequence analysis found no enrichment of core inhibitor substitutions during treatment and no association with core inhibitor substitutions with time to relapse post treatment discontinuation. Based on its antiviral activity and safety profile, VBR is being evaluated in two ongoing phase 2 studies in combination with NrtI and third agents. In the first study, the combination of VBR+NrtI is being tested with Arbutus Biopharma's siRNA AB-729. In the second study, the combination of VBR+NrtI is being evaluated with Peg-IFNα.

Overall, through completed phase 2 studies VBR has a favorable safety profile, is well-tolerated, and when added to NrtIs, results in deeper HBV DNA and pgRNA suppression than NrtIs alone. Current phase 2 triple combination studies are actively investigating the potential for third mechanisms to build on the antiviral activity of VBR + NrtI as a key component of finite and curative regimens.

Session 7: Academic/ industry session on advances in therapeutics for HBV (non-CME accredited)
December 7th, 2021

Progress toward an HBV cure combination therapy

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Background
The combination of therapeutic agents with different mechanisms of action is proven effective in treating HIV and in curing HCV. It is widely viewed that a combination strategy will be necessary to cure chronic hepatitis B. In considering a combination strategy to deliver an HBV cure, we envisioned the need to achieve several key outcomes. These include the need to suppress viral replication and the ability to stop replenishment of the cccDNA pool, reduce HBsAg levels and boost the host immune response to mediate the clearance of infected hepatocytes.

Results
We are internally developing several agents that in combination with nucleos(t)ide standard of care provides the possibility of delivering an HBV cure, in addition to evaluating other approved and investigational agents. These internal agents include AB-729, our phase 2a GalNAc conjugated liver-targeted RNAi agent, that suppresses the production of HBsAg and other viral proteins, our next-generation capsid inhibitor (CI) AB-836 that blocks viral replication and cccDNA replenishment and our small molecule PD-L1 inhibitor that functions to reawaken the host immune response.
AB-729, when administered to chronic HBV infected patients, has been shown to be safe and well tolerated after multi-dosing over 48 weeks. In HBV patients AB-729 shows an approximate mean 1.8 $\log_{10}$ reduction of HBsAg as well as a reduction of other HBV biomarkers after extended dosing.
AB-836 our next-generation HBV CI is characterized by high intrinsic potency: EC_{50} values of ≤10 nM in cell culture models, the ability to engage CI's second mechanism of action (MOA) at a therapeutically relevant dose and the ability to address certain key clinically relevant CI resistant viral variants.
Our PD-L1 small molecule is positioned to reawaken the immune system by breaking immune tolerance in a targeted manner. Our PD-L1 inhibitor disrupts the PD-1/PD-L1 interaction (EC50 < 20nM) via a novel MOA, activates primary human T cells, restores T cell activity from CHB patient blood and liver samples ex vivo and demonstrates in vivo animal model efficacy.
The profile of each of these internal agents will be presented.

Conclusion
We believe that combination therapy is the key to achieving an HBV cure with a finite duration of therapy. Our strategy of providing a regimen to suppress viral replication, reduce antigen load and boost the immune system is the most comprehensive approach to delivering an HBV cure.

Session 7: Academic/ industry session on advances in therapeutics for HBV (non-CME accredited)
December 7th, 2021

Phase 1 results for ATI-2173, a novel active site polymerase inhibitor nucleotide, in HBV-infected patients

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Background

ATI-2173 is a novel phosphoramidate liver-targeted prodrug of clevudine-5'-triphosphate that functions as an active site polymerase inhibitor nucleoside (ASPIN). In preclinical studies, ATI-2173 demonstrated enhanced anti-hepatitis B virus (HBV) activity and significantly reduced systemic clevudine exposure. ANTT101 included a phase 1b 28-day study of ATI-2173 in treatment-naive patients with chronic HBV infection eligible for treatment under EASL guidelines.

Methods

The phase 1b portion of the ANTT101 study was a randomized, double-blind, placebo-controlled trial of ATI-2173 in chronic HBV-infected patients conducted at sites in Moldova and Ukraine. Predominantly hepatitis B e antigen-negative, hepatitis B surface antigen (HBsAg)-positive patients (n=25) were randomized 6:2 to doses of ATI-2173 10, 25, or 50 mg or placebo (PBO) orally once daily for 28 days. Safety laboratory assessments and pharmacokinetics were evaluated on days 1, 2, 3, 7, 10, 14, 21, and 28 on treatment and days 2, 3, 4, 7, 10, 14, and 28 off treatment. Antiviral activity was measured at baseline and on days 7, 14, 21, and 28 on treatment and days 4, 10, and weeks 4, 12, and 24 off treatment. HBV DNA and RNA were measured using a Roche Cobas 6800 (LLOQ = 10 IU/mL and 10 copies/mL, respectively). HBsAg was measured using a Roche Elecsys (LLOQ = 0.05 IU/mL).

Results

ATI-2173 was generally safe and well tolerated. There were no apparent dose-related adverse events (AEs) or serious adverse events. The most common AE was headache. One patient withdrew on day 3 for personal reasons and was replaced. Mean values for clevudine exposure following repeated administration of ATI-2173 10, 25, and 50 mg were 5%, 13%, and 34%, respectively, of the plasma clevudine exposure previously reported with the 30-mg clevudine dose. Viral load responses on day 28 for 10 mg (N=6), 25 mg (N=5), 50 mg (N=6) or PBO (N=7) doses of ATI-2173 were −2.8, −2.7, −2.8 and +0.2 $\log_{10}$ IU/mL, respectively. There was no change in HBsAg on day 28. After treatment, 3/6 patients in the 10-mg cohort, 4/5 in the 25-mg cohort, 5/6 in the 50-mg cohort, and 0/7 in the PBO cohort had HBV DNA below the limit of quantification (BLQ; <10 IU/mL). Of patients who were BLQ, 9/9 remained BLQ at 4 weeks, 4/9 remained BLQ at 12 weeks, and 1/9 remained BLQ at 24 weeks off treatment. All patients receiving ATI-2173 25 or 50 mg with detectable baseline HBV RNA (N=8) had on-treatment decreases in HBV RNA, with continued declines for 4 weeks off treatment; each patient had HBV DNA that was BLQ at 4 weeks off treatment.

Conclusions

ATI-2173 is a novel ASPIN with potent anti-HBV activity (−2.7 $\log_{10}$ IU/mL), and substantially reduced systemic clevudine exposure after 28 days of treatment. 65% of ATI-2173-treated patients were BLQ at end of treatment. Sustained off-treatment responses were noted 4 to 24 weeks after ATI-2173 discontinuation. The 25- and 50-mg doses of ATI-2173 have been advanced into a phase 2a study in combination with tenofovir disoproxil fumarate in both HBV mono-infected and HBV/hepatitis Delta virus co-infected populations.

Data included in this abstract have been presented in full at The International Liver Congress™; June 23-26, 2021; Virtual; Posters PO-1240 and PO-1251.

Session 8: Immunity, vaccines and transplantation in the context of viral hepatitis and chronic liver disease
December 8th, 2021

Organ transplantation from HCV-positive donors to HCV-negative recipients: How short can we go?

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Background

Development of direct-acting antivirals (DAAs) has revolutionized treatment for hepatitis C virus (HCV) infection with cure rates consistently above 98% in people without cirrhosis in both clinical trials and routine clinical practice. During the same period, the devastating overdose crisis has led to an increasing number of potential organ donors with HCV infection. Poor outcomes restricted use of HCV nucleic acid test (NAT) + donors, however DAAs have opened the possibility of performing organ transplantation from people with HCV infection to uninfected recipients with prompt treatment of HCV following transplantation. After initial pilot studies showing the feasibility of this approach, strategies to simplify and shorten HCV therapy in transplant recipients have been progressing.

Early Results

The first studies done Univ of Pennsylvania and Johns Hopkins University evaluated immediate treatment with grazoprevir/elbasvir after kidney transplantation, both showing universal cure in recipients of kidneys from HCV-infected donors. However, a key distinction between the trials was the timing of treatment initiation. In the U Penn THINKER-1 trial, treatment was started 3 days post-transplant and although everyone was ultimately cured with a standard course of 12 weeks of treatment, all 10 individuals developed high level viremia prior to treatment. In contrast, in the EXPANDER-1 trial at JHU, the first dose was given on call to the OR, pre-transplantation. While everyone still received a full 12-week course of therapy (and all were cured), only 3 recipients developed quantifiable HCV RNA levels after transplant. This key result suggested that prophylactic/pre-emptive treatment might allow for prevention of infection and possible shortened duration of treatment.

Further Studies

After the initial success, various approaches have been taken to D+/R- transplantation. Some institutions have adopted this approach as standard of care and offered transplants from HCV NAT+ donors to HCV-negative recipients with a plan to treat HCV post-transplant as standard of care, often weeks or months after hospital discharge. Other sites have worked to initiate treatment early, including pre-transplantation and have explored other approaches to shorten the duration of treatment. Our group has evaluated strategies to reduce transmission, including using ultraviolet C radiation during ex-vivo organ perfusion prior to transplantation in an effort to sterilize the organ and reduce transmission. Others have tried very short courses of therapy, with as little as a single dose of prophylactic sofosbuvir/velpatasvir (SOF/VEL). While 1 and 3-dose regimens were sub-optimal, a number of studies have now evaluated shorter courses than standard DAA durations. Woolley and colleagues showed that 4 weeks of SOF/VEL was adequate to achieve 100% SVR in heart and lung transplant recipients from NAT+ donors. We added ezetimibe to glecaprevir/pibrentasvir (GLE/PIB) based on its effects at preventing HCV entry into hepatocytes. When ezetimibe was given with GLE/PIB as a single dose before transplantation and then daily for 7 days post-transplant, it prevented chronic infection in recipients of heart, lung or kidney transplants. This regimen was recently reported to be universally effective in a multi-centre study presented at AASLD 2021. Whether ezetimibe is truly required is not clear.

Future Directions

Results from various strategies have proven remarkably effective and it is unclear if a particular approach is better than another. Some concerns with delayed treatment have been rare reports of HCV-related complications such as glomerulonephritis or severe HCV infection with fibrosing cholestatic HCV. However, challenges to short therapy have been raised including issues with reimbursement and concerns about treating people while still in the immediate post-operative setting. The pros and cons of early vs late treatment, as well as different strategies to both approaches will be discussed and contrasted.

Session 8: Immunity, vaccines and transplantation in the context of viral hepatitis and chronic liver disease
December 8th, 2021

Serum HBsAg clearance has minimal impact on CD8+ T cell responses in mouse models of HBV infection

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Background

Antibody-mediated clearance of hepatitis B surface antigen (HBsAg) from the circulation of chronically infected patients (i.e., seroconversion) is usually associated with increased HBV-specific T cell responsiveness. However, a causative link between serum HBsAg levels and impairment of intrahepatic CD8+ T cells has not been established.

Methods

Here we addressed this issue by using HBV replication-competent transgenic mice that are depleted of circulating HBsAg, via either spontaneous seroconversion or therapeutic monoclonal antibodies, as recipients of HBV-specific CD8+ T cells.

Results

Surprisingly, we found that serum HBsAg clearance has only a minimal effect on the expansion of HBV-specific naive CD8+ T cells undergoing intrahepatic priming. It does not alter their propensity to become dysfunctional, nor does it enhance the capacity of IL-2-based immunotherapeutic strategies to increase their antiviral function.

Conclusions

In summary, our results reveal that circulating HBsAg clearance does not improve HBV-specific CD8+ T cell responses in vivo and may have important implications for the treatment of chronic HBV infection.

Session 8: Immunity, vaccines and transplantation in the context of viral hepatitis and chronic liver disease
December 8th, 2021

Do we need direct immune modifying drugs to reach HBsAg loss in HBV?

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HBsAg loss and functional cure for chronic hepatitis B (CHB) are two separate endpoints, both of which are optimistically within reach. HBsAg loss is self-explanatory and does not directly indicate that a patient has achieved functional cure. It is loss of HBsAg in the serum. Functional cure is maintenance of HBsAg loss after a finite course of treatment, no longer requiring antiviral therapy. We have witnessed an incredible expansion of direct acting antiviral (DAA) therapies for chronic hepatitis B (CHB) aimed at new viral targets with novel mechanisms of action. Of these, RNA interference (siRNA, antisense oligos), HBsAg secretion inhibitors (S-antigen Transport-inhibiting Oligonucleotide Polymer, STOPs; nucleic acid polymers NAPs), and anti-HBsAg monoclonal antibodies have demonstrated the capacity to eliminate HBsAg from the serum. However, their ability to achieve functional cure remains questionable because none of these agents target the covalently closed circular DNA (cccDNA) or integrated DNA expressing HBsAg. Therefore, at this stage, an immunological response is believed necessary to eliminate hepatocytes containing cccDNA or expressing HBsAg from integrated HBV DNA. This talk will discuss the mechanism of action for each new class of DAA, where it falls short of functional cure and how combination with immunotherapeutic strategies might overcome the threshold to achieve functional cure in a significant number of CHB patients.

Session 9: Diagnostics, liver disease and COVID-19
December 8th, 2021

History and biology of coronaviruses and what they can teach us about SARS-CoV-2

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There will be a description of the history and biology of coronaviruses and how this is informs 1) the origin of SARS-CoV-2; 2) how variants emerge and why some are “of concern”; 3) antiviral and vaccine approaches; and 4) what to expect in the future.

Human respiratory coronaviruses that cause the common cold have been studied since the 1970s, followed by a large amount of basic coronavirus biology research performed on murine coronavirus, as well as avian, bovine and porcine coronaviruses. This work enabled the rapid identification and characterization of SARS-CoV in 2002-2003. The SARS epidemic was followed by the emergence of MERS-CoV in 2012 and then SARS-CoV-2 in 2019. While both SARS-CoV and MERS-CoV have their origins in bats and are transmitted through intermediate species, the origin of SARS-CoV-2 is not yet completely known and will be discussed,

The coronavirus viral life cycle will be described with detail on entry pathways and conserved replicase proteins, both of which inform antiviral strategies. Coronaviruses enter cells by two pathways, the direct plasma membrane route and via the endosome. The pathway used is determined by the proteases present in the host cells and the sequence of the viral spike protein and may be cell type dependent. The pathways used determines the efficacy of drugs directed at entry. Coronavirus genomes encode sixteen conserved proteins in the replicase locus, many of which are enzymes. These include an RNA dependent RNA polymerase (RdRp), helicase and primase, two or three proteases depending on the virus, as well as several enzymes mediating mRNA capping, two ribonucleases and several proteins involved in host antagonist activities. Many of these are enzymes and serve as potential targets for antiviral drugs. Since coronavirus genomes are highly conserved, any drug inhibiting SARS-CoV-2 is likely to be effective against many if not all known and future zoontic coronaviruses.

The talk will describe the emergence of SARS-CoV-2 variants which occur as a natural part of coronavirus genome replication and are selected for enhanced spread among humans. Variants may be of concern due to enhanced interactions with the ACE2 receptor, increased cleavage of the spike protein in the producer cell and the consequent increased spread and/or mutations in epitopes recognized by antibody responses.

Finally, the talk will discuss the lessons learned about coronavirus organ tropism from the murine coronavirus, mouse hepatitis virus, model system. There are many strains of MHV displaying different tissue tropisms while using the same CEACAM1a receptor. Use of recombinant chimeric viruses derived from hepatotropic and non-hepatotropic strains showed that post entry events such as antagonism of host innate immune responses are strong determinants of hepatitis.

The talk will end with speculation about what we might expect for SARS-CoV-2 going forward and how to prepare for future emergent zoonotic viruses

Session 9: Diagnostics, liver disease and COVID-19
December 8th, 2021

An intersection of a pandemic and a vital organ: COVID-19 and the Liver

Robert G Gish, MD
Medical Director, Hepatitis B Foundation

COVID-19 has resulted in the largest number of severe infections and deaths since the influenza pandemic of 1918. The liver is involved in many coronavirus infections due to the presence of ACE-2 receptors on many hepatic cell types. The pathophysiology of hepatic involvement by SARS-Cov2 has been dissected by continued research that overlaps liver physiology and pathology with many other organs involved in this infection, although the primary involvement is the lung and airway system that results in the greatest number of complications including the risk of death. There has also been a major impact of the pandemic on the management of many other forms of liver disease. The decrease in diagnosis and treatment of hepatitis C and hepatitis B are examples of this global impact, especially a delay in the elimination efforts as the health care system redirected resources and patients avoided or declined health care.
In addition, the background of chronic liver disease sets the scene for more complications of COVID-19. The crucial need to diagnose underlying liver disease accurately to provide better care for each patient is clear from multiple publications. Refining the care of immune-suppressed patients over the course of pandemic took place due to the rapid response of academic and clinical providers to publish their experiences in the peer reviewed literature. Vaccination of liver patients was the next major step in the evolution of the pandemic. Both safety and efficacy data emerged as new vaccines reached clinics showing that the benefit risk ratio as substantial and the need for booster vaccines became clear quickly including liver transplant patients. Long-term consequences of SARS-Cov2 are also being detailed in general and special populations. This includes, in liver patients, a syndrome of vanishing bile ducts that can result in a form of chronic liver disease.

Session 11: Non-ABC viral hepatitis: Epidemiology, pathogenesis and treatment
December 9th, 2021

Discovery and development of Bulevirtide

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Background

Hepatitis B (HBV) and Hepatitis D Virus (HDV) infection requires the interaction of the shared envelope proteins with the hepatocyte specific receptor sodium taurocholate co-transporting polypeptide (NTCP). This species-specific interaction is mediated by an evolutionary adaptation of an essential sequence (the receptor binding domain (RBD)) within the myristoylated preS1-domain of the HBV large surface protein (L-protein) to NTCP. Following the concept that chemically synthesized lipopeptides representing this RBD are potent inhibitors of hepadnaviral infection, both the identity of the long-sought HBV receptor was uncovered and the first approved entry inhibitor for HBV/HDV infection (bulevirtide/Hepcludex) was developed.

Results

In this lecture I will provide a historical survey on the development of bulevirtide (formerly called Myrcludex B). I will highlight the initial limitations that HBV research faced at a time when only the duck hepatitis B virus (DHBV) infection system was available to study hepadnaviral entry processes. I will elaborate on how we successfully transferred the concepts established in the DHBV model to HBV using primary human hepatocytes (PHH) and HepaRG cell lines. Although some of these concepts could not be verified in both infection systems one finally resulted in the identification of a peptidic lead substance that entered into clinical trials (bulevirtide). I will further highlight some very surprising findings that were associated with the pharmacological behaviour of HBVpreS-1-derived peptides *in vivo* (e.g. liver targeting, stability) that fundamentally differed from other therapeutically applied peptides. I will finally summarize arguments that strongly support the use of an entry inhibitor to counteract HDV (and probably HBV) persistence and therefore may lead to elimination of the respective viral template (nuclear circHDV RNA and HBV cccDNA).

Conclusion

The pharmaceutical development of bulevirtide was an untypical enterprise, not only regarding its identification as an optimized “fragment” of an “endogenous” viral structure but also regarding its unusual properties as a stable and highly efficient peptidic drug. Its recent approval in the European Union and its successful application to HDV/HBV coinfected patients raises justified hope to become part of a curative regimen in the future.

Session 11: Non-ABC viral hepatitis: Epidemiology, pathogenesis and treatment
December 9th, 2021

The future cure for hepatitis delta virus

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Hepatitis delta virus (HDV) infection is a defective virus requiring hepatitis B virus (HBV) for its complete replication cycle. HDV is a small hepatotropic RNA virus and around 15 to 25 million people worldwide are living with chronic hepatitis delta (CHD) infection. However, the prevalence of HDV may be underestimated, and screening is frequently insufficient. HDV infection remains endemic in several regions including Central and West Africa, the Mediterranean basin, the Middle East, Eastern Europe, Northern Asia, certain areas of Southeast Asia and the Amazon basin of South America. The best preventive strategy to decrease HDV infection is to improve coverage of the prophylactic HBV vaccine. HDV infection may occur by HBV-HDV co-infection or superinfection, and the latter is usually more severe. CHD is associated with a higher risk of cirrhosis and hepatocellular carcinoma (HCC) compared to HBV mono-infection. Pegylated interferon alpha (PEG-IFNα) therapy is limited by moderate effectiveness (around 20%) and its adverse effects. The entry inhibitor, bulevirtide (BLV, Hepcludex®), which was recently approved in Europe at a dose of 2 mg in sub-cutaneous injection per day, is indicated for the treatment of CHD in adult patients with compensated liver disease and positive HDV viremia. BLV can be administrated in monotherapy or in combination with PEG-IFNα. Nucleos(t)ide analogues can be used in combination for underlying HBV infection. The optimal treatment duration has not yet been determined and treatment should be continued if a clinical benefit is observed. There are other promising therapies such as IFN lambda (IFNλ) (immunomodulator), lonafarnib (prenylation inhibitor) and nucleic acid polymers (Inhibitors of HBsAg release). In this presentation, we will present an update on CHD and future promising treatments.

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Session 11: Non-ABC viral hepatitis: Epidemiology, pathogenesis and treatment
December 9th, 2021

Evolution of non-ABC viral hepatitis

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Hepatitis Delta Virus (HDV) and HDV-like agents in human and animal hosts

The emergence and rapid transmission of viruses poses increasing risks and challenges to modern societies, threatening public health and economic stability. A thorough understanding of basic virology is therefore critical for an informed development of preventive and control strategies. Although for over 40 years the only known member of deltaviruses was the human Hepatitis Delta Virus (HDV), it was recently discovered that HDV-like viruses are present in a variety of animal vectors and reservoirs including bats, rats, snakes, birds, and insects. In this talk, I will give an overview of the research performed on HDV during the last decades, focusing on fundamental aspects of HDV infection and replication in human hepatocytes. I will then describe our current knowledge of newly identified HDV-like viruses, that have been reported over the past few years in several animal species. In fact, metagenomic data indicate that these satellite viruses possess an unrecognized host shifting capacity enabling them to cross species barrier. I will reflect on the implications and open questions emerging from these recent discoveries. Finally, I will outline strategies and technologies used in our laboratory to functionally characterize the interaction of HDV and HDV-like viruses with cellular host factors in both human and animal host cells. Such approaches will guide the development of novel antiviral strategies and will have profound implications for understanding of the ecology and evolution of these newly discovered, yet mysterious, viral elements.

ORAL ABSTRACTS

Oral Abstract O1

Active site polymerase inhibitor nucleotides (ASPINs) are novel nucleos(t)ide analogues with a unique mechanism of action

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Background
Current hepatitis B virus (HBV) therapies require long-term administration and rarely lead to functional cure. Nucleos(t)ide reverse transcriptase inhibitors (NRTIs) compete with native bases for incorporation into HBV DNA, causing chain termination. Active site polymerase inhibitor nucleotides (ASPINs) have the favorable pharmacokinetics (PK) of NRTIs but broader inactivation of HBV polymerase, resulting in unique clinical properties.

Methods
A search of PubMed and Embase identified publications describing the antiviral mechanism of action (MOA) of ASPINs compared with HBV NRTIs, as well as the preclinical and clinical development of clevudine (CLV) and ATI-2173. Search results for 6 manuscripts and 2 abstracts published from 2001 to 2021 are summarized below.

Results
NRTIs, such as tenofovir (TFV) and entecavir (ETV), are incorporated by HBV polymerase into lengthening HBV DNA, resulting in chain termination. ETV-triphosphate (TP) and TFV-diphosphate cause chain termination during DNA synthesis and at distinct early stages of replication priming: initiation of protein priming and primer polymerization, respectively, while all NRTIs block HBV DNA elongation. CLV, an L-nucleotide thymidine analogue, noncompetitively inhibits HBV polymerase. CLV-TP binding to HBV polymerase distorts the active site of the enzyme and inhibits priming driven by any nucleotide. CLV-TP inhibits all stages of early HBV DNA synthesis. CLV showed a potent antiviral effect in a chronic HBV woodchuck model with significant reductions in covalently closed circular DNA and a delayed off-treatment return of viremia for up to 12 weeks. CLV clinical trials confirmed the potent anti-HBV activity on-treatment and continued suppression up to 24 weeks off-treatment. ATI-2173 is a liver-targeting prodrug of CLV. Similar anti-HBV activity along with a 3- to 8-fold reduction in CLV exposure compared with the 30-mg CLV dose was confirmed in the phase 1 ATI-2173 program. In vitro studies of CLV and ATI-2173 combined with NRTIs have demonstrated additive to synergistic activity.

Conclusions
ASPINs have a unique MOA compared with other NRTIs and function like non-nucleotide polymerase inhibitors while retaining the favorable PK properties of nucleotides. ATI-2173 is a second-generation ASPIN that offers improved PK compared with CLV, delivering more CLV-monophosphate to the liver and decreasing systemic exposure. Combining an ASPIN with an NRTI has the potential to completely suppress HBV polymerase activity, leading to a higher rate of functional cure.

Oral Abstract O2

Novel dimeric HBV Capsid Assembly Modulators

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Background
Current approved therapeutic approaches to eliminate HBV fail to target the HBV covalently closed-circular DNA (cccDNA; associated with viral persistence) or the virus capsid which is essential for virus persistence. Our innovative approach has led to highly potent low nanomolar monomer capsid assembly modulators (CAM) which is essential for replication, as DNA synthesis from cccDNA occurs exclusively within the capsid encoded particle. We recently designed novel dimeric structures by linking two CAM moieties (or "Warheads") with the hypothesis that such structure could increase the potency of simple monomers by interacting with two distinct sites of one capsid, or eventually connect two capsids together. We also hypothesized that these compounds could have a different effect on HBV capsid assembly than known class I or II CAMs.

Methods
Using a standard "click" linking protocol, we were able to synthesize several dimers of our lead CAM monomer GLP-26 connected with various alkyl or PEG linkers. The compounds were evaluated *in vitro* against HBV in a liver cell-based system and their cytotoxicity determined in several cell lines, including peripheral blood mononuclear (PBM), human T lymphoblast (CEM), African green monkey kidney (Vero), and human hepatocellular carcinoma (HepG2) cell lines.

Results
Results indicated a clear correlation between length of the linker and anti-HBV activity and we identified a novel GLP-26 dimer (14 carbons linker) 30 times more potent than GLP-26 itself (EC_{50} = 3 nM for monomer vs ≤ 0.01 nM for dimer).

Conclusion
Several CAM homodimer derivatives displaying anti-HBV activity in culture in the picomolar range. Optimization of the linker moiety (length, nature) in order to improve potency and overall drug like properties of these compounds is providing new insights on the potential of these potent novel compounds. Initial mechanism of action studies suggest that these new dimer derivatives function as Class II CAMs as determined by electron microscopy studies.

Oral Abstract O3

Targeting hepatitis B cccDNA with a sequence-specific ARCUS nuclease to eliminate hepatitis B virus *in vivo*

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Background
Hepatitis B virus is a major, worldwide health concern with more than 250 million people chronically infected. Following acute HBV infection, 5% to 10% of adults and up to 90% of young children fail to produce an immune response adequate to clear the infection and subsequently develop the chronic infection of the virus, chronic hepatitis B (CHB). Current HBV therapies can slow progression of disease but fail to eliminate covalently closed circular DNA (cccDNA), therefore requiring patients to receive lifelong treatment. We hypothesized that a nuclease-mediated double-stranded break could lead to degradation of the cccDNA or generate mutated, replication-incompetent cccDNA, with both outcomes potentially leading to reductions of the immunosuppressing HBV protein, surface antigen (HBsAg). Here we describe a potentially curative therapeutic approach using an engineered ARCUS nuclease (termed HBV-POL) targeting the HBV polymerase gene in cccDNA.

Methods
Nuclease characterization was performed in vitro using assays for both activity and specificity, as determined by on-target editing percentage and a genome-wide, unbiased oligo-capture assay, respectively. Using an HBV-infected primary human hepatocyte (PHH) model, we were able to determine nuclease potency by evaluating cccDNA levels by southern blot, editing of cccDNA by NGS, extracellular HBV DNA by qPCR and HBV sAg levels by immunoassay (CLIA). Editing of an integrated target sequence was performed by analysis of indels and HBsAg reduction from a HepG2 derived cell line that contains 1 integration of a partial HBV genome and produces HBsAg. To test our gene editing approach in vivo, we utilized an AAV model in which episomal AAV served as a surrogate for cccDNA and delivered our nuclease systemically via lipid nanoparticles (LNP) containing HBV-POL nuclease mRNA. We have evaluated this model in mice and adapted it to a novel non-human primate (NHP) model.

Results
HBV-POL nucleases were engineered through five rounds of optimization yielding a highly specific and active nuclease targeting HBV. In HBV-infected primary human hepatocytes transfected to express the HBV-POL nuclease, we achieved substantial reductions in cccDNA, secreted HBV DNA, and HBsAg. In mice treated with an LNP-containing HBV-POL nuclease mRNA, we detected a 60% decrease in AAV copies, up to 86% indels in the remaining AAV, and a 96% sustained reduction in serum HBsAg after a single LNP administration. In NHPs, we administered LNP twice ~6 weeks apart and observed progressive editing after each LNP dose, ultimately achieving a 70% reduction in AAV copies and an average of 34% indels. However, despite utilizing an immunosuppression regimen, HBsAg was cleared from circulation, preventing its use as a biomarker for HBV-POL activity in NHPs.

Conclusions
We have developed a gene editing approach using an ARCUS nuclease, HBV-POL, with high on-target activity and specificity against the HBV polymerase gene in cccDNA and have shown the ability to edit and degrade cccDNA in vitro in HBV-infected PHH. Furthermore, HBV-POL nucleases delivered by therapeutically relevant LNP in mice and NHPs were effective at cutting and degrading episomal AAV, used as an HBV cccDNA surrogate. Together, these data support a gene editing approach for elimination of cccDNA and a potential cure for HBV infection.

Oral Abstract O4

Improved response rate of immune checkpoint inhibitors when used in combination with locoregional treatment in patients with advanced HCC

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Background: The prevalence of hepatocellular carcinoma (HCC) continues to increase and its health and economic burden are significant. Current therapeutic options include surgical resection, liver transplantation, locoregional therapies and systemic agents. Although liver transplantation is a curative therapy, the majority of cases are identified beyond current criteria for transplantation. Immune checkpoint inhibitors (ICI) are emergent systemic treatments that target the PD-1/PD-L1 pathway. They work by enhancing the anti-tumor immune responses and reduce the risk of recurrence in 20-30% of patients. We report improving efficacy of ICI when combined with locoregional treatments (LRT).

Methods: Between May 2017 and May 2021, 459 patients (pts) who were diagnosed with HCC were referred to the liver cancer clinic. This is an analysis of 96 consecutive pts who were referred to the ICI and LRT treatment program. Pts were prescribed ICI as adjunctive therapy to LRT (primarily TACE, and Y-90). ICI was infused per the package insert and pts were followed per standard of care. Labs, imaging, locoregional interventions, disease progression, transplantation candidacy, and survival were collected. Of the 96 pts, 7 were denied ICI treatment by the insurance provider, 15 did not start ICI treatment. The subjects of this analysis are 74 pts who received at least 2 doses of ICI.

Results: 74.3% were males. 77.0% Hispanic, 94.6% white. Mean age was 69.1±15.1 years. Main underlying liver disease etiology was HCV. All were Child-Pugh A or B. Lesions: Prior to starting ICI, the mean number of lesions was 1.9±1.5 and the median size of lesions was 2.9cm (0.4-13). The median treatment duration was 6 months (1-28). Safety and Tolerability: Liver enzymes and MELD score were stable throughout treatment period. 9 pts discontinued due to rash and 3 pts due to severe immune reaction. Efficacy: 78.3% are still alive and 35.1% were not eligible initially for liver transplant due to tumor burden. Recurrence was seen in 4 pts while on ICI and 8 pts after treatment discontinuation. Survival: Median survival from HCC diagnosis was 28 months in pts who did not receive ICI versus 46 months in those who did receive ICI. Pts who received ICI were further divided into three groups by LRT and lesion viability prior to receiving ICI (see table).

Conclusions: Our analysis shows that ICI is: 1) safe and well-tolerated, 2) more effective than LRT alone, and 3) once ICI was initiated, patients required less LRT and stayed disease-free. In addition, treatment with ICI maintained MILAN criteria for patients listed for liver transplant and decreased the dropout rate from the list compared to published data. In summary, combining Immune Checkpoint Inhibitors with locoregional treatments prolonged disease-free survival of patients with advanced HCC. This data could impact the selection of advanced HCC pts for liver transplant beyond the MILAN criteria. Further analysis is in progress comparing the various ICI treatments.

Status of 96 patients	No ICI (Group 1)	Received ICI (Group 2)			
		No LRT Pre-ICI	Pre-ICI LRT and No Viable Lesion Pre-ICI	Pre-ICI LRT and Viable Lesion Pre-ICI	Total Received ICI (Group 2)
Number of patients	**22**	13	32	29	**74**
LRT Pre-starting ICI	**21 (95.4%)**	Not Applicable	32 (100%)	29 (100%)	**61 (82.4%)**
LRT Post-starting ICI	**N/A**	9 (69.2%)	8 (25.0%)	11 (37.9%)	**28 (37.8%)**
Outcome Alive	**9 (40.9%)**	6 (46.1%)	17 (53.1%)	11 (37.9%)	**34 (45.9%)**
Average survival since HCC diagnosis (months)	**28**				**46**

Oral Abstract O5

The liquid liver biopsy: External validation of serum protein models of liver steatosis, inflammation, hepatocyte ballooning, fibrosis, and at-risk NASH

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Background
The definitive diagnostic test for Nonalcoholic steatohepatitis (NASH) is liver biopsy, which carries risks and cannot be used for frequent monitoring. There is no single noninvasive method that can accurately and simultaneously capture steatosis, inflammation, hepatocyte ballooning and fibrosis, the four major pathologic components assessed by biopsy. We show that large scale proteomics has promise as an alternative to liver biopsies in clinical trials of NASH. Furthermore, we show that the outcomes of four independent liver biopsy liver component models can be successfully combined to predict a diagnosis of "at-risk NASH' (i.e., fibrotic NASH) in an external validation cohort.

Methods
Predictive (e.g., machine learning-based) models for each liver biopsy component were previously developed using 636 participants from the NASH CRN natural history and longitudinal clinical trial samples[1]. An additional outcome, at-risk NASH, which is defined as having a NAFLD activity score (NAS) greater than 4 and at least stage 2 fibrosis, was created by multiplying the outputs of each individual model (i.e., the predicted probabilities). Each of the models for the 4 liver biopsy components and the derived output for at-risk NASH were assessed on an independent validation dataset (n = 322) from the Liver Investigation: Testing Marker Utility in Steatohepatitis (LITMUS) consortium. The LITMUS Metacohort is a retrospective collection of samples from participants diagnosed with Non-Alcoholic Fatty Liver Disease (NAFLD) in the United Kingdom and European Union (13 countries and 25 clinical sites), with the goal of identifying non-invasive biomarkers for the diagnosis, risk stratification, and monitoring of patients with NAFLD. The Metacohort was assembled from European biobanks with samples collected over the last 10 years[2]. Model predictions were generated for each of the five outcomes (4 liver biopsy components and at-risk NASH) and performance was assessed using AUC, sensitivity, specificity, and accuracy.

Results
The four individual liver biopsy models and the combined output for at-risk NASH performed well in the independent LITMUS Metacohort validation data set, where performance for each outcome was evaluated compared to expert pathologist read liver biopsy results. Table 1 displays the performance metrics with 95% confidence intervals for each outcome.,.

Test Name	AUC	Sensitivity	Specificity	Accuracy
Steatosis	NA*	0.847*	NA*	NA*
Ballooning	0.754 (0.679, 0.828)	0.727 (0.658, 0.796)	0.667 (0.510, 0.824)	0.717 (0.653, 0.780)
Inflammation	0.681 (0.612, 0.750)	0.647 (0.531, 0.763)	0.692 (0.613, 0.772)	0.677 (0.612, 0.743)
Fibrosis	0.869 (0.824, 0.914)	0.609 (0.523, 0.695)	0.915 (0.851, 0.979)	0.722 (0.659, 0.784)
At-risk NASH	0.801 (0.747, 0.856)	0.744 (0.657, 0.831)	0.731 (0.644, 0.818)	0.737 (0.676, 0.799)

*All LITMUS study participants had biopsy-confirmed steatosis, therefore AUC, specificity, accuracy could not be calculated

In addition to the NASH-specific models, proteomic models previously validated for predictions of cardiometabolic health, including 4-year risk of a cardiovascular event, glucose tolerance, liver fat, and adiposity measures and cardiorespiratory fitness were evaluated in the LITMUS cohort, that included 50% at-risk NASH (NASH with significant fibrosis)/50% NAFLD patients. The at-risk NASH population had significantly higher predicted cardiovascular risk, impaired glucose tolerance, body fat, and lower cardiorespiratory fitness. These results demonstrate that at-risk NASH is associated with poor cardiometabolic fitness and increased risk of a cardiovascular event.

Conclusions:
- Four proteomic models developed using machine learning techniques were able to simultaneously and noninvasively measure each component of liver biopsy from a single blood sample.
- An additional outcome, calculated by combining the output of the four individual models was further able to characterize a diagnosis of at-risk NASH, identifying the patient population most likely to progress to poor outcomes.
- Each of the five outcomes were successful in predicting NASH components and at-risk NASH in an independent validation cohort, performing at or above performance in the original model development.
- The at-risk NASH population was further predicted to have poorer cardiometabolic risk and increased risk of a cardiovascular event.
- These tests are a non-invasive alternative to liver biopsy and provide the ability to identify and monitor changes in each biopsy component throughout drug development and treatment.

1 Ostroff, Alexander, & Williams, 2020
2 Hardy T, Wonders K, Younes R, et al., 2020

Oral Abstract O6

SARS-CoV-2 infection in patients with concurrent chronic liver disease is associated with more severe disease presentation and significantly greater risk of severe pneumonia and respiratory failure

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Background
The novel coronavirus, SARS-CoV-2, and the resulting COVID-19 pandemic have contributed to significant morbidity and mortality worldwide. Patients with concurrent chronic liver disease (CLD) are immunocompromised due to underlying liver disease and are hypothesized to have more severe clinical disease when infected with SARS-CoV-2 infection. Existing studies evaluating SARS-CoV-2 in patients with CLD have been limited to small cohort studies. We aim to provide a comprehensive analysis of COVID-19 outcomes among a large U.S. population with CLD to determine whether underlying CLD is associated with more severe presentation of SARS-CoV-2 infection outcomes.

Methods
Using data from Office Ally, a large U.S. claims database provided by the COVID-19 Research Database consortium (https://covid19researchdatabase.org) containing over 100 million patients with both inpatient and outpatient data representing >3,000 different payers, we focused on patients with the four leading etiologies of CLD (hepatitis C virus (HCV), hepatitis B virus (HBV), alcoholic liver disease (ALD), and nonalcoholic fatty liver disease/steatohepatitis (NAFLD/NASH)), which were identified using previously validated ICD-9/10 algorithms. SARS-CoV-2 infection was identified using ICD-10 code: U07.1. Our study period spanned from April 1, 2020 to August 31, 2021 (with a 12-month look-back to April 1, 2019 for identification of CLD and baseline characteristics). Our outcomes included incident pneumonia, severe pneumonia (pneumonia + severe sepsis or septic shock), and respiratory failure. Outcomes were evaluated using Cox proportional hazards models and compared between patients with COVID-19+CLD vs. COVID-19 without CLD.

Results
Among 1,082,396 adult patients with COVID-19 (55.1% women, 44.9% men, 33.7% age 18-44y, 53.1% age 45-65y, and 13.2% age ≥65y), 3.61% had COVID-19+CLD and 96.39% had COVID-19 without CLD. Over a median follow up of 245-259 days, 51,183 (5.78%) patients developed pneumonia, 11,651 (1.08%) developed severe pneumonia, and 42,472 (4.36%) developed respiratory failure. Compared to COVID-19 patients without CLD, COVID-19+CLD had significantly greater risk of incident pneumonia (11.09% vs. 5.58%; HR 1.85, 95% 1.79-1.19, p<0.0001), severe pneumonia (3.00% vs. 1.00%; HR 2.71, 95% CI 2.55-2.86, p<0.0001), and respiratory failure (8.48% vs. 4.20%; HR 1.85%, 95% 1.78-1.92, p<0.0001). Compared to men, women with COVID-19 had significantly lower risk of pneumonia (4.90% vs. 6.95%; HR 0.73, 95% CI 0.72-0.74, p<0.0001), severe pneumonia (0.78% vs. 1.44%; HR 0.59, 95% CI 0.57-0.61, p<0.0001), and respiratory failure (3.46% vs. 5.52%; HR 0.66, 95% CI 0.64-0.67, p<0.0001). Compared to patients age 18-44y, COVID-19 patients age ≥65y had significantly greater risk of pneumonia (10.04% vs. 2.24%; HR 4.68, 95% CI 4.54-4.82), severe pneumonia (1.96% vs. 0.23%; HR 8.44, 95% CI 7.82-9.12, p<0.0001), and respiratory failure (8.37% vs. 1.31%; HR 6.56, 95% CI 6.33-6.79).

Conclusions
Among one of the largest cohorts evaluating COVID-19 outcomes in patients with underlying CLD, we observed that patients with CLD had significantly greater risk of developing severe disease presentation and respiratory complications when infected with SARS-CoV-2. These data emphasize the importance of SARS-CoV-2 vaccination in patients with CLD and close monitoring of CLD patients with COVID-19.

Oral Abstract O7

A fully-automated artificial intelligence algorithm in differentiating contrast-enhanced phases in liver computer tomography

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Background
Hepatocellular carcinoma (HCC) has become the 4th leading cause of cancer-related deaths, with high social, economical and health implications. Imaging techniques such as multiphase computed tomography (CT) have been successfully used for diagnosis of liver tumors such as HCC. However, in health facilities where there is a high load of exams and patients who need to be diagnosed as fast as possible, automatizing HCC diagnosis is fundamental. Over the past few years, this has been addressed by the development of tools based on artificial intelligence algorithms, especially by convolutional neural networks, which are the most suitable algorithm for imaging analysis. Nonetheless, liver segmentation contour algorithms face many challenges related to the input images, which need to be provided not only in a specific format and orientation, but also with each phase already identified. In addition, due to the double liver vascularization (from both portal vein and hepatic artery), phase characterization is particularly important in the evaluation of focal lesions. Considering the importance of organizing the images of CT scans and identifying the corresponding phases in liver segmentation and HCC identification pipelines, we successfully implemented an algorithm to automatically identify the CT scan phases in the Hospital das Clínicas (HCFMUSP) database in the city of Sao Paulo, Brazil.

Methods
A total of 208 scans, from unique patients in the HCFMUSP in São Paulo, Brazil, were collected in DICOM format. For each CT scan, we had radiologists annotating the 4 main contrast phases and, for each phase, we selected 150 slices approximately evenly spaced. The scans were randomly split into training (60%), validation (15%) and testing (25%) sets. The model was a Convolutional Neural Network (CNN) with a dense final classification layer, which was implemented using Python 3.8 with Machine Learning packages Keras and Tensorflow. Furthermore, we used the Hyperband approach to select the best hyper parameters, including number and size of convolutional layers, and learning rate. The algorithm's predictions of the correct phase were analyzed in three evaluation levels: individual slices, volumes and exams, and its performance was assessed by F1 score, Area under the ROC curve (AUC), accuracy, precision and recall metrics.

Results
We evaluated this algorithm by comparing its predictions with the radiologists' annotation, achieving an accuracy of 85% and 92%, and AUC of 97% and 99% in the testing data set for the slice and volume evaluation, respectively. Among the other metrics, the highest average was the precision one in the Unenhanced, Arterial and Portal phases. Thirty-eight out of fifty-two test exams (73%) had all four phases correctly classified and no exam had more than two phases incorrectly classified.

Conclusions
Our study is the first investigation to specifically identify the four phases of contrast-enhanced abdominal CT scans using a Convolutional Neural Network (CNN) and will aid in the proper and fundamental standardization and quality control of input data for automatic liver segmentation and identification of HCC lesions.

Oral Abstract O8

Establishing a protocol for management and DAA treatment of HCV during pregnancy: adherence to a co-located care protocol

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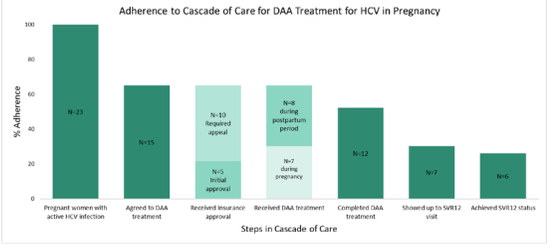
Background
Current AASLD, USPSTF, CDC, and ACOG guidelines have recommended universal screening for HCV during pregnancy. However, there are currently no recommendations for directly acting antiviral (DAA) therapy during pregnancy. Pregnancy provides an opportunity to cure maternal HCV and reduce adverse pregnancy outcomes but also presents unique challenges. Emerging data suggests that DAAs are safe and effective during pregnancy. We evaluated our center experience in implementing DAA treatment during pregnancy.

Methods
After establishment of a sub-specialty Women's Liver Clinic (WLC) co-located in the ambulatory obstetrics practice and implementation of universal antepartum HCV screening across our health system, we offered DAA treatment during pregnancy to women with active HCV. We evaluated adherence to each step in the HCV treatment cascade of care: 1. Patient uptake of DAA treatment when offered, 2. Insurance approval for DAA +/- whether appeal needed, 3. Receipt of DAA treatment prior to delivery, 4. DAA treatment completion, 5. SVR12 visit adherence and achievement of SVR. We also evaluated rates of perinatal outcomes.

Results
Twenty-three pregnant women with active HCV infection were referred to WLC for consideration for HCV treatment; they were of median age 31 (IQR 24, 36); predominantly white (39%) non-Hispanic (52%), and medicaid-insured (78%); 7 (30%) with injection drug use as a risk factor; 2 (9%) who acquired HCV through perinatal transmission; 6 (26%) with no known risk factors. Fifteen (65%) reported that HCV was initially diagnosed during pregnancy. Among those offered DAA during pregnancy, 15 (68%) agreed to DAA treatment (**Figure**). Initial DAA ordered was ledipasvir/ sofosbuvir for 7 (42%), ledipasvir/ velpatasvir for 7 (42%) and glecaprevir/ pibrentasvir for 2 (12%). Five (21%) had initial approval by insurance; 10 (63%) required appeal for coverage; six (60%) had successful appeal. Among those with DAA approval, nine (45%) filled their DAA prescription while pregnant and seven (35%) initiated DAA treatment during pregnancy; eight (40%) initiated postpartum. Twelve (70%) had documented completion of DAA treatment; only 7 (35%) showed up to their SVR12 check; 6/7 (86%) had documented SVR12; one patient required re-treatment. In regards to pregnancy outcomes, 35% had intrahepatic cholestasis of pregnancy; two (9%) had preterm birth; one patient had pregnancy loss prior to DAA treatment initiation. There was one documented mother-to-child transmission in a patient treated with DAA postpartum.

Conclusions
Pregnancy offers an important opportunity to link women with HCV to treatment – the majority were diagnosed initially during routine screening during pregnancy. Cholestasis of pregnancy was a prevalent complication and further studies should evaluate whether HCV eradication can decrease incidence of ICP in women with HCV. HCV treatment during pregnancy is feasible and will likely offer benefits to maternal and child health, however significant challenges remain to ensure adherence through the vulnerable postpartum period. Similar to the management of other chronic conditions in the postpartum period, linkage to care and case management programs designed specifically for postpartum women should be evaluated to improve DAA therapy completion and documentation of SVR12.



Oral Abstract O9

Humanized NSG-PiZ mice support the study of hepatitis B virus antiviral therapies

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Background
We previously investigated a gene editing approach to hepatitis B virus (HBV) treatment that used adenoassociated virus (AAV) vector-mediated delivery of *Sa*Cas9 to target HBV cccDNA in liver humanized FRG mice. We demonstrated HBV DNA gene editing, but the cost of humanized FRG mice limited our ability to study this approach further.

Methods
We established the liver humanized NSG-PiZ mouse model in our lab and evaluated its permissiveness to HBV replication. NSG-PiZ mice are easy to humanize, requiring only pre-conditioning via injection of a hepatotoxic agent prior to intra-splenic human hepatocyte transplantation, and need no extensivehusbandry. Notably, NSG-PiZ mice can be humanized for less than 1/10 the cost of commercially available humanized uPA-SCID and FRG mice, making them affordable for academic researchers.

Results
We transplanted NSG-PiZ mice with human hepatocytes and achieved engraftment levels comparable to uPA-SCID and FRG mice, routinely achieving hAlbumin levels of 1-10mg/mL within ten weeks. Importantly, humanized NSG-PiZ mice are fully permissive to HBV infection. NSG-PiZ mice challenged with a genotype D clinical HBV isolate have been followed for 24 weeks and infectious virus can be passaged sequentially through mice. Levels of viremia correlated with hAlbumin levels and peaked above 109 IU/mL. HBV+ mice secrete HBsAg, with peak levels seen by 10 weeks, and express HBsAg in hCK18+ human hepatocytes, indicating that species-specific replication occurs in humanized liver. At necropsy, liver chimerism was quantified by ddPCR detection of hRPP30 and mRRP30, as well as liverassociated total HBV DNA and cccDNA. We then performed a set of experiments to demonstrate that the NSG-PiZ model is suitable for the study of HBV therapeutics. We first investigated the effect of the RTi entecavir (ETV) on HBV replication +/- the capsid assembly inhibitor Ciclopirox (CPX) to establish efficacy and to determine whether combination therapy provides an additive antiviral effect. Viral loads were followed in viremic NSG-PiZ mice for six weeks, encompassing four weeks of ETV or ETV/CPX therapy followed by two weeks of no drug therapy to determine the effects of single or combination drug therapy. Mice treated with ETV showed an approximately 3-log reduction in viral loads over four weeks, which rebounded upon ETV withdrawal. This level of inhibition is comparable to that seen in HBV+ uPA-SCID or FRG mice. Combination ETV/CPX therapy showed no additional effect on mice treated with ETV alone. Finally, we determined whether AAV vectors could efficiently transduce HBV+ human hepatocytes in NSG-PiZ mice. HBV+ NSG-PiZ mice were administered hepatotropic AAV3B or AAV.LK03 vectors expressing GFP, and gene transfer was analyzed after 28 days in chimeric livers. Both vectors transduced human and mouse hepatocytes, as well as HBV+ human hepatocytes.

Conclusions
Our results show that liver humanized NSG-PiZ mice support HBV replication and can be used to monitor the efficacy of antiviral small molecule therapies. They also appear well suited for the study of AAVmediated antiviral therapies that target HBV cccDNA.

Oral Abstract O10

Multi-pathway innate immunity antagonism by the hepatitis E virus ORF1 protein

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Background
Hepatitis E virus (HEV) is a (+) ssRNA virus in the *Hepeviridae* family that is responsible for over 3.3 million infections and approximately 66,000 deaths per annum, with pregnant women representing a particularly vulnerable population. The HEV genome is divided into three open reading frames (ORFs) that work in concert to accomplish its replication cycle: ORF1 encodes the viral replicase, ORF2 the capsid protein, and ORF3 a multi-functional phosphoprotein necessary for viral egress. Virally encoded protease mediated innate immunity antagonism is well characterized across other well known (+) ssRNA viruses, such as hepatitis A virus (HAV), hepatitis C virus (HCV), and many of the flaviviruses such as dengue virus (DENV), West Nile virus (WNV), and Zika virus (ZIKV), though the existence of such a domain within HEV has yet to be fully characterized. Prior bioinformatic analyses suggest the presence of a papain-like cysteine virally encoded protease (PCP) domain within ORF1, with the highly conserved residue C483 as part of the putative catalytic dyad, though functional characterization of this domain is still debated in the field. Further, the ability of the HEV ORF1 protein to antagonize and blunt the host innate immune response is not completely understood. Using comparative innate immunity profiling, our work highlights HEV ORF1 protein's ability to antagonize host innate immunity across multiple signaling pathways via a virally encoded protease *independent* mechanism, and investigates other potential mechanisms as to how HEV ORF1 protein accomplishes this.

Methods
Human hepatoma cells individually stably expressing either the catalytically active or catalytically inactivated protease domain from one of nine different viruses were independently subjected to innate immunity stimulation via agonists to the RIG-I like helicase/MAVS, Toll-like receptor 3/TRIF, or cGAS/STING signaling pathways. For HEV, we introduced a cysteine to alanine substitution in position 483 within the PCP, which renders ORF1 non-functional. These protease domain bearing cells demonstrated the ability to respond to type-I interferon (IFN) stimulation downstream of IFN induction using a live cell reporter, were subsequently harvested for cellular RNA and total protein, and were analyzed both by RTqPCR -to measure transcript levels of four antiviral interferon stimulated genes (ISGs), as well as IFN-β and IFN-λ - and immunoblotting for the quantification of host innate immunity signaling proteins. Further, HEV ORF1 mutant bearing cells exposed to proteasome inhibition were altered in their ability to blunt innate immune signaling.

Results
Cells stably expressing the catalytically active or inactive domains of eight viruses, or HEV ORF1 protein were demonstrated to be signaling competent via type-I interfering signaling. The presence of wild type HEV ORF1 protein robustly blunts innate immune signaling across the RIG-I like helicase, Toll-like receptor 3, or cGAS/STING signaling pathways. ORF1 protein reduces expression of the critical innate immune signaling adapter proteins MAVS and STING, and does so via a virally encoded protease *independent* mechanism. Comparative innate immunity profiling likens HEV ORF1's innate immunity antagonism profile to that of HCV, though HCV accomplishes its own antagonism via its virally encoded NS3/4A protease. Further, HEV ORF1's reduction of adapter protein expression is not accomplished via global transcription/translation repression, and the presence of a proteasome inhibitor altered the innate immune signaling profile for the ORF1 mutant bearing cells.

Conclusions
Our data demonstrate that the presence of wild type HEV ORF1 creates a cellular environment conducive to viral replication by blunting the cell's ability to sense and respond to infection, though the mechanism by which it accomplishes this remains yet to be fully determined. Our data further support the notion that HEV blunts innate immunity via the action of the PCP domain and a mechanism that does not suppressing globally host transcription/translation. Notably, inhibition of the host proteasome alters the innate immunity antagonism profile exhibited by HEV ORF1, opening up a new and exciting avenue of investigation.

Oral Abstract O11

Heterologous prime-boost immunotherapy circumvents tolerance and protects against hepatitis B and D co-infection and hepatitis D super-infection.

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Background
Chronic hepatitis B and D virus (HBV/HDV) infections are a major cause of severe liver disease and cancer that generally require lifelong therapy. A hallmark of chronic HBV infection is the profound T cell dysfunction/tolerance to viral proteins, and strategies to break this tolerance may therefore contribute to achieving functional cures. The HDV antigen acts as a heterologous antigen and supports HBV-specific immunactivation, leading to enhanced anti-HBV antibody and T cell responses. Its incorporation into an immunotherapeutic approach to enhance neutralizing antibody and T-cell responses to HBV preS1 is therefore being investigated.

Methods
Plasmid DNA and fusion proteins containing HBV preS1 and HDAg sequences were expressed in E. coli. Antibody and T-cell responses directed against preS1 and HDAg after DNA-prime protein boost immunization were assessed in rabbits and in wild type and HBsAg-transgenic mice. Protection against HBV/HDV co-infection, and HDV super-infection was assessed in uPA-Scid mice repopulated with human hepatocytes against in vivo.

Results
A DNA prime protein boost immunization resulted in superior anti-preS1 and anti-HDAg antibody and T-cell responses in mice and rabbits compared to repeated immunization with DNA or protein alone. Antibody titres resulting from immunization were similar across HBV genotypes A-H. Immunization of wild-type and HBsAg transgenic C57BL/6 mice resulted in equivalent antibody titres, supporting the role of HDAg in circumventing T cell tolerance to HBV. Passive transfer of antiserum from immunized wild-type mice resulted in protection against HBV/HDV co-infection in 3/3 mice with humanized livers compared to 0/4 control animals that received serum from un-immunized mice. Passive transfer of antiserum from immunized wild-type mice resulted in protection against HDV superinfection in 3/3 mice with humanized livers that had been infected with HBV 8 weeks previously, compared to 0/4 control animals that received serum from un-immunized mice

Conclusions
This novel prime-boost immunotherapy circumvents immune tolerance and induces endogenous production of high levels of neutralizing preS1 antibodies and T cells against preS1 and HDAg, supporting its further investigation as a curative strategy for HBV monoinfection and HBV/HDV co-infection.

Oral Abstract O12

Leveraging machine learning to identify clinical attributes and social determinants of health associated with Hepatitis C virus to improve screening efficiency

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Background

Hepatitis C virus (HCV) poses a significant public health challenge with an estimated 58 million infected patients globally as of 2021. HCV is substantially underdiagnosed, with approximately 45% of patients unaware of their HCV infection status in the United States (US). In alignment with the World Health Organization's goal of eliminating viral hepatitis by 2030, the US Department of Health and Human Services aims to increase the proportion of people with cleared hepatitis C infection up to 80% by 2030. To support this effort, Gilead initiated the HCV FIND-C (Facilitating INtelligent Diagnosis) program to help identify patients with undiagnosed HCV. Practical limitations of implementing universal screening guidelines as stated from Centers for Disease Control and Prevention, and US Preventive Services Task Force, currently yield low positive screening rates. This study used a predictive machine learning (ML) algorithm to define attributes related to patients' medical history, geography, and social determinants of health (SDoH) to identify patients with potentially undiagnosed HCV.

Methods

This study analyzed a large-scale US dataset consisting of deidentified electronic health records (EHR) of patients who underwent screening for HCV between January 2016 and June 2020. Patients >11 years of age with at least 2 years of available medical history were included. Patients were stratified into two classes: 1) HCV-positive (at least one positive result for HCV screening or at least one HCV medication code in the EHR), and 2) HCV-negative (at least one negative result for HCV screening (RNA or antibody), no HCV medication codes, and absence of positive result for HCV screening (RNA or antibody)). Cross-sectional data of medical history-related attributes, including demographics, diagnosis, labs, treatments, and procedures, were recorded. To account for geographic-level variance in patient populations, SDoH data at the zip-3 code level from publicly-available nationwide datasets were merged with the EHR data. A supervised ML algorithm was used to train a binary classification model to predict the likelihood of HCV infection.

Results

A total of 295,512 patients (HCV-positive: 50,726; HCV-negative: 245,236) were identified. The model produced an area under the receiver operating characteristic (AUROC) of 96% with 98% precision.

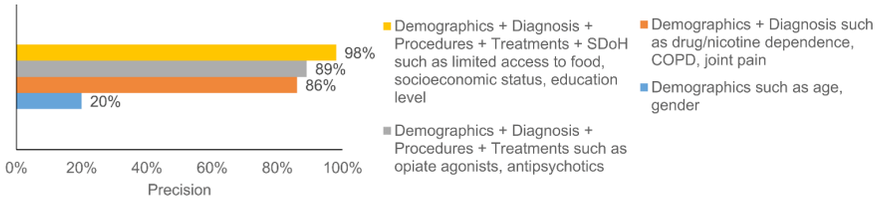

Figure 1: Improvement in model precision with step-wise addition of features

The model highlighted several features important for HCV prediction, such as drug/nicotine dependence, opioid usage, joint pain, chronic obstructive pulmonary disease, treatment with antipsychotics, elevated levels of aspartate aminotransferase, elevated levels of alanine aminotransferase, and SDoH including socioeconomic status and limited access to food. The model involving only clinical attributes exhibited 89% precision, while further incorporation of SDoH attributes improved precision to 98% (Figure 1).

Conclusions

This study described the development of a novel ML algorithm that can identify patients with undiagnosed HCV with high precision using clinical markers, geographic and SDoH data to predict the likelihood of HCV infection while reinforcing known risk factors for HCV. Implementing this model across healthcare networks may help flag patients with undiagnosed HCV, considerably improving screening and subsequent diagnosis. This may further help mitigate the risk of progression to advanced liver disease, improving patient outcomes.

POSTER ABSTRACTS

Poster Abstract P1

Ph-dependent interaction of NAPs with the HSP40 chaperone DnaJB12

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Background
Nucleic acid polymers (NAPs) inhibit assembly and secretion of HBV subviral particles (SVP) but not HBeAg or Dane particles. This oligonucleotide effect is driven by length- and phosphorothioate (PS)-dependent hydrophobic interactions independent of sequence or base / sugar modification. NAPs act in the acidified lumen of post-ER, pre-Golgi vesicles (ERGIC) where SVP morphogenesis occurs. A differential NAP interactome screen in HepG2.2.15 cell lysates at pH 7.4 identified the HSP40 chaperone DnaJB12 as a putative host target for NAPs inhibiting SVP assembly. DnaJB12's involvement in SVP assembly was confirmed by DnaJB12-knockdown in HepG2.2.15 cells inhibiting HBsAg but not HBeAg secretion. Continued analysis of NAP interactions at acidic pH was performed to examine physiologically relevant binding activity within the ERGIC.

Methods
MS/MS interactome analysis with biotinylated NAPs in HepG2.2.15 lysates was conducted at pH 7.4 and 6.5. Protein interactions were validated by interaction ratios between the active REP 2139 (40mer PS, hydrophobic) and REP 2179 (20mer PS), REP 2147 (40mer phosphodiester, non-hydrophobic) and REP 2031 (40mer PS poly C – hydrophobic but inactivated at acidic pH by homo tetramerization). shRNA mediated DNAJB12 knockdown was confirmed by western blotting. Effects on secretion of HBsAg (GS EIA 3.0, Biorad) and HBeAg (ETI-EBK PLUS N0140, Diasorin) were monitored by ELSIA and normalized to total cellular protein (as determined by BCA assay).

Results
shRNA knockdown of the previously identified casein kinase 1D (CSNK1D) inhibited secretion of HBsAg and HBeAg. A 53-fold reduction in the ratio of REP 2139 : REP 2147 interaction with CSNK1D was observed pH 6.5 vs 7.4, indicating a loss of hydrophobic interaction. Moreover, the CSNK1D interaction of REP 2139 at pH 6.5 was comparable to the inactive REP 2031. In contrast, a 40-fold increase in the ratio of REP 2139 : REP 2147 interaction of REP 2139 to DNAJB12 occurred at pH 6.4 vs 7.4, indicating an increased hydrophobic interaction. At acidic pH, the interaction of REP 2139 with DNAJB12 was 10-fold greater than REP 2031. REP 2139 : REP 2179 ratios declined ~2 fold at pH 6.5 vs 7.4 with both targets, suggesting similar binding affinities.
Previous 48h shRNA knockdown of DNAJB12 resulted in 40-50% decline in secreted HBsAg. Extending this knockdown to 6 days increased inhibition of secreted HBsAg to 90%, consistent with the inhibition of HBsAg secretion with REP 2139.

Conclusions
Hydrophobic (antiviral) interactions of REP 2139 with DNAJB12 are strongly enhanced at acidic pH. This is consistent with the location of DNAJB12 within the acidified ERGIC and the assembly of SVP and its inhibition by NAPs within the ERGIC. The loss of hydrophobic interactions of NAPs with the cytoplasmic CSNK1D at pH 6.5 is consistent with a non-physiological interaction and the knockdown of CSNK1D driving the non-physiological inhibition of HBeAg.

Poster Abstract P2

Long-acting entecavir prodrugs for HBV treatment

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Background
Hepatitis B virus (HBV) is a hepatotropic virus that can establish persistent, chronic infection through immune anergy, affecting >250 million people globally. Current first-line therapies require lifelong, daily administration, making patients susceptible to missed treatments and disease relapse. As adherence to therapy is imperative to achieve and maintain viral suppression, a long-acting HBV therapy with minimal dosing requirements would significantly improve treatment outcomes.

Methods
We applied our LAI platform to develop a LAI based on prodrugs of an approved NARTI i.e. Entecavir (ETV) to improve adherence and limit viral breakthrough and disease progression in patients with HBV. More than 30 prodrugs were synthesized and subjected to formulation development and subsequent rodent and non-rodent PK studies.

Results
ETV prodrug mCEC540 identified as early lead for solution-based depot strategy, with lower Cmax and favorable IM release in rats relative to parent entecavir. On the aqueous suspension front, lead prodrug mCMQ657 provided differentiated PK profile in dogs with extended duration of therapeutic exposure relative to parent entecavir for more than 3 months after a single intramuscular injection dose.

Conclusions
Lead Prodrugs of ETV have been determined to provide plasma levels higher than therapeutic levels for more than 3 months, thereby providing exciting opportunities for improving adherence via LAI approach.

Poster Abstract P3

Investigation of the anti-HBV activity of TL020 and its effects on host protein expression

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Background
Current drug treatments for hepatitis B virus are not sufficient to achieve desired endpoints and it is expected that a combination therapy will be important in improving patient outcomes in years to come. As such antivirals with novel mechanisms of action are promising drug leads. Cyclotriazadisulfonamide (CADA) and its analogues have shown activity against a broad range of viruses. In an *in-vitro* screen a CADA analogue, TL020, was shown to potently inhibit HBV replication with minimal effects on cellular viability. CADA has demonstrated its ability to reduce levels of specific proteins by interfering with their co-translational translocation in a signal peptide dependent manner. This interference results in mis-localization and degradation of the protein. TL020 and its effect on protein synthesis were investigated to elucidate the mechanism of its anti-HBV activity.

Methods
HepG2.2.15 cells expressing HBV virions were treated with varying concentrations of TL020 for 4 days followed by Southern blot to quantify viral DNA levels. Neutral red uptake was used to quantify toxicity. To determine the effects on cellular protein levels HepG2 cells were treated with 20 μM of TL020 for 48 hours. The cells were lysed in RIPA buffer and cellular debris separated by centrifugation. Proteins were trypsin digested and analyzed by LC-MS/MS. Alternatively after lysis, RNA was isolated using a spin column and reverse transcribed into cDNA. Transcript levels were determined by qPCR using SYBR Green dye.

Results
In the initial screen, TL020 was highly effective at reducing HBV DNA levels. The concentration required to reduce HBV DNA levels by 50% (EC_{50}) or by 90% (EC_{90}) was determined for both intracellular and excreted virions. For the excreted virions the EC_{50} was between 0.49 to 0.63 μM and the EC_{90} was between 1.2 to 1.6 μM. For intracellular HBV DNA the EC_{50} was 1.2 μM and the EC_{90} was 5.9 μM. These values were much lower than the concentration required to reduce cellular viability by 50% (CC_{50}) which was between 38 to 53 μM. Looking at the effect of TL020 on HepG2 cells using LC-MS/MS revealed significant variation in protein levels for 385 genes out of the 4,680 proteins detected. Pathway analysis revealed ECM-receptor interaction, protein processing in the ER, bile secretion, pyruvate metabolism, and metabolic pathways were the most significantly affected. In considering the mechanism for interfering with co-translational translocation, a subset of 30 proteins were down-regulated and contained signal peptides necessary for co-translation translocation. These were selected for analysis via qRT-PCR which revealed 18 proteins whose mRNA levels were either steady or increased, indicating a post-transcriptional mechanism.

Conclusions
Investigation of TL020's effect on host protein expression revealed that this analogue is likely less specific than CADA. The list of most down-regulated proteins was enriched for signal peptide-containing proteins. Follow up studies are necessary to determine which proteins are being directly targeted by the proposed mechanism, and to determine which proteins are affecting HBV replication. The work presented here is useful for prioritizing proteins for these further studies.

Poster Abstract P4

Treatment Intensification with Vebicorvir in Patients with Chronic Hepatitis B Infection and Partial Virologic Suppression on Nucleos(t)ide Reverse Transcriptase Inhibitors

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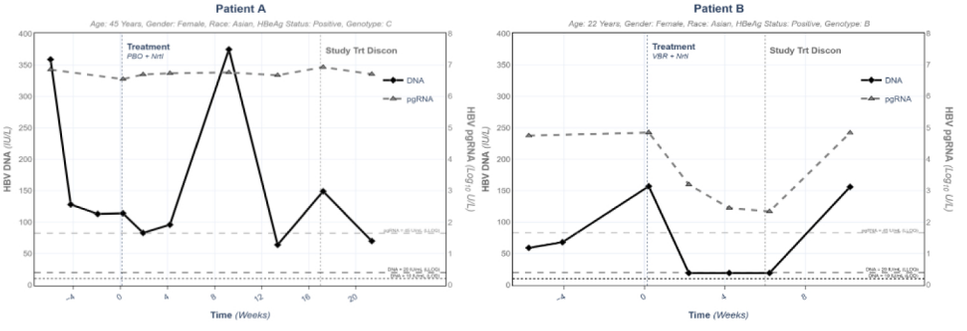
Background
Nucleos(t)ide reverse transcriptase inhibitors (NrtIs) are standard-of-care therapy for chronic HBV infection (cHBV). The goal of NrtI treatment is complete virologic suppression (VS) as measured by HBV DNA less than lower limit of quantification (<LLOQ). Failure to achieve complete VS is a risk factor for HCC and hepatic events. While NrtIs suppress HBV DNA in most patients, ~30% of HBeAg positive, and up to 10% of HBeAg negative, patients are not able to achieve HBV DNA <LLOQ after 1 year of treatment; many do not achieve complete VS even after many years of NrtI therapy. Treatment-intensification approaches are needed for such patients with partial VS. Vebicorvir (VBR) is an investigational first-generation core inhibitor being developed for cHBV. The Phase 2 Study 205 (NCT04454567) evaluated treatment intensification with VBR in patients with partial VS on NrtI therapy. This case report describes data from the 2 patients enrolled in this study, both of whom discontinued treatment prematurely.

Methods
Eligible patients had partial VS following a stable NrtI regimen administered for more than 12 months, with HBV DNA >LLOQ on 2 occasions during screening. At the time of study termination, 2 patients were randomized. Patient A received placebo (PBO)+NrtI for 12 weeks with 4 weeks of follow-up. Patient B received VBR+NrtI for 4 weeks with 4 weeks of follow-up. HBV DNA was measured by COBAS TaqMan 2.0 (LLOQ=20 IU/mL), and HBV pgRNA was measured by an Assembly Biosciences assay (LLOQ=45 U/mL). Safety was assessed through reporting of adverse events (AEs) and laboratory abnormalities.

Results
Patient A was a 45-year-old Asian female with genotype C, HBeAg positive cHBV who started with tenofovir alafenamide 1.5 years before entering the study. Patient B was a 22-year-old Asian female with genotype B, HBeAg positive cHBV who started with entecavir 3.6 years before entering the study. VBR was well tolerated by the two patients. Two AEs were reported, both by Patient A and unrelated to study drug: COVID-19 infection and Grade 1 flu-like symptoms secondary to COVID vaccine. There were no clinically significant abnormalities in laboratory evaluations, vital signs, or ECGs reported in either patient. Individual HBV DNA and HBV pgRNA profiles are presented in Figure 1. In Patient A (PBO+NrtI), HBV pgRNA remained unchanged and HBV DNA fluctuated around LLOQ and up to 400 IU/mL during treatment. In Patient B (VBR+NrtI), treatment intensification with the addition of VBR led to a reduction of HBV pgRNA and HBV DNA was <LLOQ throughout the treatment interval. Rebound of HBV DNA and HBV pgRNA to baseline occurred after cessation of VBR.

Figure 1: Patient A (PBO+NrtI) and Patient B (VBR+NrtI): HBV DNA (log_{10} IU/mL) and HBV pgRNA (log_{10} U/mL)



Conclusions
Patients with partial VS receiving standard-of-care NrtI therapy may benefit from treatment intensification to achieve the goal of undetectable HBV DNA. In Phase 2 clinical trials, VBR demonstrated a favorable safety profile following long-term administration and addition of VBR to NrtIs may allow patients to achieve complete VS, a status that correlates with improved long-term clinical outcomes.

Poster Abstract P5

Novel dihydroquinolizinones for HBV surface antigen (HBsAg) reduction with liver targeting properties

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Background
Chronic hepatitis B (CHB) is characterized by high levels of hepatitis B virus (HBV) surface antigen (HBsAg) in blood circulation. A major goal of CHB interventions is to reduce or eliminate this antigenemia; however, the current standard of care medications with either pegylated interferon alpha or nucleos(t)ide analogues (NUCs) have failed to do this. A novel family of dihydroquinolizinone (DHQ) has been shown to reduce circulating levels of HBsAg in animals, representing a first small molecule with reliable and promising potential. Reductions of HBsAg were a result of the compound's effect on HBsAg mRNA levels. Since the first report of dihydroquinolizinone (DHQ) compound RG-7834 as an effective hepatitis B virus expression inhibitor in 2018, dozens of structurally diversified DHQs have been disclosed in both scientific journals and patent. However, the CNS liability observed for RG-7834 raised a safety concern for this compound. Commercial development by Roche of RG-7834 was stopped due to undisclosed toxicity issues.

Methods
Our rationale, contrary to the regular medicinal practice of pursuing a highly systemically bioavailable lead compound, is to convert a systemic RG-7834 to a liver selective new DHQs that have low to moderate bioavailabilities but high liver exposure and liver/plasma ratios. We believe 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. Therefore, hepatoselective DHQ compounds should have great potential to improve the safety of this novel family of anti-HBV compounds. Here, we report our approach to develop liver targeting DHQ derivatives through the installation of a recognition element for organic anion transporting polypeptide protein 1B1 (OATP1B1) and 1B3 (OATP1B3) which are abundant on liver hepatocytes.

Results
we successfully developed RG-7834 derivatives as substrates of OATP1B1 and OATP1B3, resulting in a lead compound that is potent in biochemical and cellular assays, has low risk for penetrating blood-brain-barrier (BBB), and demonstrates high hepatoselectivity in liver versus plasma in a mouse pharmacokinetic (PK) study .

Conclusions
Based on the structure of RG7834 and the analysis of its ADME and PK profiles, we have incorporated an additional acid group into the side chain of RG7834. Through the increase in tPSA and the modulation of the cLogP/LogD of the new molecules, we have identified a new lead to be potent in both the PAPD 5 and 7 enzyme assays and HBV mRNA degradation cellular assay. Further evaluation showed that unlike RG7834, the new lead is a substrate of both OATP1B1 and OATP1B3, which may facilitate the absorption into the liver. This in vitro result was translated into an in vivo setting: the new lead demonstrated much better hepatoselective distribution in a mouse PK study than RG7834, with an average liver/plasma ratio of 37.8 over 8 h. More importantly, the new lead demonstrated a low risk for crossing the BBB in comparison to the moderate risk of RG7834.

Poster Abstract P6

Therapeutic vaccination with the pre-S based vaccine VVX001 - interim analysis

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Background
VVX001 is an HBV vaccine candidate based on a preS polypeptide fused to grass pollen allergen peptides. It is studied if vaccination with VVX001 can induce an immune reaction in vaccine-naïve adults, non- or low responders, in chronic hepatitis B patients and in patients infected with chronic hepatitis B virus (HBV).

Methods
Four cohorts are studied in a prospective double-blind, placebo-controlled clinical trial (NCT03625934). Cohort 1: vaccine naive subjects; cohort 2: subjects having failed to seroconvert upon vaccination with a licensed HBV vaccine (non- or low responders); cohort 3: chronic hepatitis B patients classified as inactive carriers; cohort 4: patients with chronic HBeAg negative hepatitis B who are on long-term treatment with nucleo(t)side analogues (NUCs). Participants received 5 injections (s.c.) of either VVX001 or placebo in 4-week intervals (randomized 3:1) at visits 2, 3, 4, 5, 6, respectively. Inclusion criteria for cohort 3 and cohort 4a, respectively, are: HBV DNA <2000 IU/ml (cohort 3), not detectable (cohort 4a); HBsAg <3000 IU/ml (cohort 3), <2000 IU/ml (cohort 4a). In the still ongoing cohort 4a, NUCs were stopped after three vaccinations (visit 4). After treatment phase completion, patients were followed until week 52 (visit 9). IgG and CD4+/CD8+ responses specific for recombinant preS were studied by ELISA and FACS-based CFSE dilution assay (cohort 3). Respective results for cohort 4a are pending. 17 of the 20 included cohort 4a patients completed treatment and follow-up at this point.

Results
All 9 actively treated subjects of cohort 3 and all 6 actively treated subjects of cohort 1, who completed vaccination, developed robust preS-specific antibody responses peaking one month after the last injection and declining at month 12. A modest CD4+ and CD8+ preS-specific T cell response was observed in naïve subjects.
Except local injection site-reactions, VVX001 was well tolerated in all participants and no SAEs were reported.
In cohort 4a, HBV DNA became again quantifiable in 5 patients during follow up, in 2 of them associated with a marked increase in alanine aminotransferase (ALT). In these 2 patients, NUC therapy was restarted. In the remaining 3 patients HBV-DNA decreased again below or to <20 IU/ml. 12 patients (70.6%) remained HBV-DNA negative with normal ALT, and a total of 15 required no NUC treatment (88%).

Conclusions
VVX001 is safe and well tolerated and induces a preS-specific immune response in vaccine naïve subjects and in patients with chronic hepatitis B infection. Whether VVX001 induces this preS-specific immune response also in patients with chronic HBeAg-neg hepatitis B (cohorts 4a and 4b) who discontinue NUC therapy is currently studied. In 88% of patients in cohort 4a of this ongoing study, after stopping NUCs, no repeated treatment with NUCs was necessary. The precise mechanism how this was achieved is still under investigation.

Poster Abstract P7

Subcutaneous Administration of REP 2139-Mg in the Compassionate Treatment of Cirrhotic HBV / HDV Co-infection

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Background
REP 2139-Mg based combination therapy achieves high rates of HBsAg loss, therapeutic transaminase flares and functional cure of HBV and HDV when administered by weekly IV infusion. Like all phosphorothioate oligonucleotides (antisense oligonucleotides, ASOs), subcutaneous (SC) injection site reactions are common with REP 2139 but, like all NAPs, are significantly stronger because of their increased length. Chelate complex formulation of NAPs (REP 2139-Mg) neutralizes administration reactivity. The safety and efficacy of SC injection of REP 2139-Mg in combination therapy was assessed in a cirrhotic patient with chronic HBV / HDV co-infection.

Methods
The patient (male, Senegalese, 51 years old) had confirmed cirrhosis and chronic HBV / HDV co-infection since 2005 (HDV GT3) and had failed previous therapies with TDF (300mg) + pegIFN (180ug) and later with TDF + pegIFN (180ug) + bulvertide (2mg) and was currently receiving only TDF. Eight months following discontinuation of pegIFN + bulvertide, TDF therapy was supplemented with 90ug pegIFN and 250mg REP 2139-Mg given as two subcutaneous injections of 125mg once each week. Safety assessments included liver, kidney and hematological function. Virologic assessments included HDV RNA (Robogene MK II), HBV DNA (Abbott), HBsAg and anti-HBs (Abbott Architect quantitative).

Results
No evidence of pain or inflammation at the injection sites for REP 2139-Mg was observed for the first 9 weeks. Thereafter, mild to moderate discomfort post injection was transient and not accompanied by inflammation. Mild pruritis after week 6 responded well to supportive therapy. Two mild and superficial indurations were not accompanied by pain or inflammation.
Virologic response was rapid, with HDV RNA becoming undetectable at week 4 and HBsAg becoming < 0.05 IU/mL at week 15 and HBsAg seroconversion evident at week 12 of therapy. A strong host-mediated transaminase flare (ALT, AST and GGT) developed after week 6, with its nadir (ALT 373 U/L) at week 9 and rapid normalization (current ALT is 54 U/L at week 16). Liver and kidney functions have remained normal throughout therapy with stable hematological parameters (RBC, WBC, platelets).

Conclusions
SC REP 2139-Mg was safe, well tolerated and highly effective against HBV and HDV infection in combination with TDF and low dose (90ug) pegIFN in this cirrhotic patient. The therapeutic transaminase flare was not associated with any adverse effects and was correlated with HBsAg loss.

Poster Abstract P8

Mitochondrial stress in patients with chronic heptatis B and advanced fibrosis

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[&] Equal contribution

Background
Hepatitis B virus (HBV) infection causes oxidative stress and alters hepatic mitochondria. The aim of the study is to look for the role of mitochondrial stress in the progression of fibrosis in chronic hepatitis B (CHB).

Methods
One hundred thirty-two (n=132) treatment-naïve CHB mono-infected patients and available liver biopsies were included in the study. Liver mitochondrial DNA (mtDNA) damage was screened by long PCR and sequencing. The expression of cytochrome c oxidase subunits 1 (COX1) and 2 (COX2), Parkinson juvenile disease protein 2 (PARKIN), Phosphatase and Tensin-Induced Putative Kinase-1(PINK1), Lon Peptidase 1(LonP1), HSP60, HSP70, TNFα and IL6 was investigated by RT-qPCR and Western blotting. Patients with advanced fibrosis (F3-F4; n=41) were compared to mild-moderate fibrosis (F0-F1-F2; n=86). Patients with advanced fibrosis-cirrhosis (Metavir score, F3-F4) were compared to patients with no-mild-moderate fibrosis (F0-F1-F2).

Results
We identified various mtDNA damages including strand breaks and multiple mtDNA deletions in patients with F3-F4 as compared to patients with F0-F2. mtDNA damage was associated with alterations in mitochondrial function, mitochondrial unfolded protein response, mitophagy, and liver inflammation in patients with CHB and advanced fibrosis-cirrhosis. In vitro, significant increases of the mitochondrial formation of superoxide and peroxynitrite as well as mtDNA damage and nitration of the mitochondrial respiratory chain complexes occurred in HepG2 hepatocytes transiently expressing either HBV or Hepatitis B virus X protein (HBx).

Conclusions
Our results emphasized the importance of mitochondrial oxidative stress, mtDNA damage, and associated alterations in mitochondrial function and dynamics in the progression of fibrosis in patients with CHB.

Poster Abstract P9

Hepatitis B virus infection and extra-hepatic manifestations: a systemic disease

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Background
People living with hepatitis B virus (HBV) chronic infection are exposed to high rates of liver complications including end-stage liver disease and hepato-cellular carcinoma. Extrahepatic manifestations of HBV infection have long been under-estimated.

Methods
“Search strategy and selection criteria”. For this review, we used the search terms « HBV, chronic hepatitis B, extra-hepatic manifestations, PAN, vasculitis, cryoglobulinemia, arthritis, kidney, GN, cardiac manifestations, cutaneous manifestations, lymphoma, asthenia, quality of life, ischemic heart disease, stroke», to search PubMed, Ovid MEDLINE, the Cochrane Central Register of Controlled Trials, and Web of Science for articles published in English from January 1, 2009, to January 1, 2021. Conference proceedings, abstract books, and references from relevant studies were also examined.

Results
Several of these extrahepatic syndromes have been well described, including systemic vasculitides, glomerulonephritis, and cutaneous manifestations. Other manifestations have been more recently described such as hematological malignancies and neurological diseases. These extra-hepatic manifestations are associated with significant morbidity and mortality. Although not completely understood, underlying mechanisms include HBV-induced local and systemic inflammation. The crucial pathogenic mechanism in most extra-hepatic manifestations is believed to be driven by immune responses against HBV, with the deposition of immune complexes (IC) in targeted tissues and a generalized inflammation. Several guidelines have been published for the management of patients with HBV infection. There is hope for a HBV cure increasing the number of candidates for therapy, beyond the present guidelines. An awareness and recognition of these manifestations are important to allow early diagnosis and treatment. Suppression of HBV replication usually improves extra-hepatic manifestations. This review will discuss how HBV induces inflammation and the associated systemic extra-hepatic manifestations of HBV infection to guide clinical management.

Conclusions
Clinicians should be aware that HBV-infected patients may present many extra-hepatic manifestations. Patients with HBV infection require checking for extra-hepatic manifestations and conversely patients with a rare systemic disease should be tested for markers of HBV infection. Suppression of HBV replication usually improves extra-hepatic manifestations. Most HBV infected patients with symptomatic extrahepatic manifestations and/or organ damage should probably receive antivirals regardless of viral and/or liver indications. We need more information, whether we should consider fatigue or decreased quality of life as an indication for treatment.

Poster Abstract P10

Electronic health engagement improves heptatis C care cascade in PWID

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Background
There are multiple challenges in the hepatitis C [HCV] care cascade. Electronic health engagement could emerge as a tool in linking patients to the HCV care cascade. There is an emerging epidemic of HCV among people who inject drugs (PWID). Our aim is to link PWID that are HCV RNA positive to care and utilize our online patient database management system (PDMS) with Substance Abuse Treatment Centers to fulfill the linkage portion.

Methods
Longitudinal prospective cohort study with HCV screening and linkage to care through utilization of a HIPPA compliant PDMS (www.linkagetocare.com). A centrally located Linkage to Care Specialist (LTCS) is notified immediately when an individual's information is entered in the system by the treatment center or self-referred. The LTCS educates the individual and proceeds to link them to care.

Results
January 2017 – January 2020, 1867 patients were referred to LTC; 52% self-referred and 48% referred from 40 facilities in 29 states; 51% were uninsured; 60% were between the ages of 21-40; 57% males. 1012 (54%) of patients were from sober living homes, 470 (25%) from treatment facilities, 133 (7%) from medical clinics and 252 (14%) were from charitable facilities. 1034 HCV RNA positive patients; 888 (86%) patients were contacted by LTCS; 470 (53%) referred to a medical provider; 16% patients awaiting lab results; 4.5% lost contact. 210 (37%) patients made it to their first appointment; 122 initiated therapy; 49% were completing evaluation; 51% finished therapy or achieved SVR12; 3 patients relapsed to drug use; 91% were seen in office vs. 9% through telemedicine.

Conclusions
Targeting PWID is an effective way of reducing the prevalence of HCV infection and ultimately eliminating HCV in our communities. Our study demonstrates a promising role for patient engagement in electronic health portals as a tool in linking patients to the hepatitis C care cascade. With the LTC program, there is an increase in patient compliance with linkage to care as compared to current care models.

Poster Abstract P11

Response guided long-term treatment of chronic hepatitis D patients with bulevirtide - results of a "real world" study

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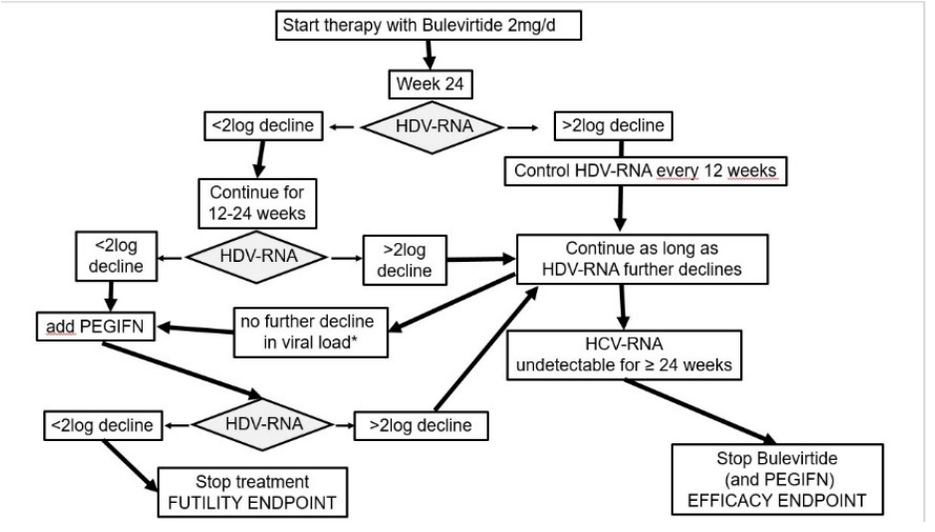
Background
Bulevirtide (BLV) blocks the uptake of the hepatitis D Virus (HDV) into hepatocytes via the sodium/bile acid cotransporter NTCP. BLV was conditionally approved by EMA but real-life data on BLV efficacy are limited.

Methods
BLV was provided by MyrPharma (Leipzig/Germany) in a compassionate use program until 8/2020, and thereafter was prescribed. HDV-RNA was determined by PCR (according le Gal et.al,J.Clin Microbiol 2005; LLQ:100 copies/mL). BLV dosing and treatment duration was at the discretion of the investigator. Patient were classified as responder (≥2log drop of HDV-RNA within 24 weeks followed by further decline), partial responder (≥2log drop within 24 weeks and no further decline), non-responder (<2log drop of HDV-RNA within 24 weeks).

Results
17 patients (m/f:6/11; mean age: 50.2 years (range; 29-68; 11 with cirrhosis; ALT:98±20 IU/mL; median HDV-RNA: 950000 [range:100-59000000] copies/mL) received BLV (2mg/d in n=15; 10 mg/day in n=2). In 15 patients (84.6%), BLV was combined with NUCs (n=3 ETV, n=10 TDF, n=2 TAF), n=13 patients had a previous nonresponse to PEGIFN.Four patients were classified as responders: BLV treatment was terminated in 2 patients with undetectable HDV-RNA for >6 months after a total of 63 and 130 weeks, respectively, with one patients remaining HDV-RNA negative also 20 weeks after cessation of BLV therapy, and with the other patient (compensated cirrhosis) becoming HDV-RNA positive again 4 weeks after cessation of BLV therapy (BLV treatment was resumed in the latter patient). 2 responders are still on BLV treatment.
In 4 non-responders treatment with peginterferon-alfa2a (PEGIFN) was added – leading to a rapid decline of HDV-RNA in all (log drop within 12 weeks: 0,97; 1.19; 1,23; 1.54). 6 partial responders are still on BLV monotherapy. Two patients dropped out due to noncompliance after 8 and 24 weeks, and one patient underwent liver transplantation at week 25 of BLV, respectively.
ALT levels normalized in 11 (84.6%) patients. During BLV therapy HBsAg changes levels did not change and bile acid levels increased without pruritus.

Conclusions
Long-term BLV is safe and effectively decreaseg HDV-RNA and ALT. Optimal treatment duration to achieve sustained HDV suppression has not been established - but likely requires >1 year. Our data indicate the need for an individualized approach when using BLV for HDV treatment (see proposed BLV treatment algorithm, Figure-1).



Poster Abstract P12

The *LIMT-2* study: a phase 3 study of 48-week treatment with peginterferon lambda in patients with chronic hepatitis delta virus (HDV) infection

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Background
Hepatitis Delta Virus (HDV) infection leads to the most aggressive form of chronic viral hepatitis, for which there is no FDA-approved therapy. Worldwide prevalence of HDV infection is 15-20 million. Peginterferon Lambda (Lambda) has previously demonstrated a good tolerability profile in >3000 HBV HCV, and HDV patients, with fewer and less severe cases of cytopenia, flu-like and psychiatric symptoms compared to peginterferon alfa. In a prior Phase 2 study, 33 patients with chronic HDV infection were treated with once-weekly subcutaneous injections of Peginterferon Lambda for 48 weeks. 36% of patients achieved a durable virologic response (DVR) defined as HDV RNA level below the limit of quantitation (BLQ) at 24-weeks post-treatment. The goal of the LIMT-2 study (NCT05070364) is to evaluate the safety and efficacy of Peginterferon Lambda monotherapy in a registrational study of 150 patients with chronic HDV

Methods
This is a randomized, open-label, parallel-arm study that will allocate patients with HDV to one of two treatment groups (2:1) -- Peginterferon Lambda 180 mcg QW for 48 weeks with 24 weeks follow-up (Arm 1, n=100), or no treatment for 12 weeks followed by Peginterferon Lambda treatment for 48 weeks with 24 weeks of follow-up (Arm 2, n=50). All patients will receive concomitant therapy with a potent 2nd generation anti-HBV nucleos(t)ide analogue (NUC) throughout the study duration.

Results
We describe key eligibility criteria and approximately 50 sites across 13 countries. Key inclusion criteria include: chronic HDV infection, quantifiable HDV RNA by RT-PCR, suppression of HBV DNA (< 100 IU/mL) following at least 12 weeks of anti-HBV NUC treatment, serum ALT > upper limit of normal (ULN) and < 10 × ULN, Child-Turcotte-Pugh score of ≤ 5 with well compensated liver disease. Key exclusion criteria include: history or current evidence of decompensated liver disease (episodes of variceal bleeding, ascites or encephalopathy), treatment with interferons (IFNs) or immunomodulators within 12 months of randomization or refractory response to prior IFN treatment.

Conclusions
Screening has initiated with the first patient expected to be randomized in 2021. The primary analysis will compare the proportion of patients with a DVR, or HDV RNA BLQ at 24-weeks post-treatment in the Peginterferon Lambda treatment group (Arm 1) to the proportion of patients with HDV RNA BLQ after 12 weeks of no treatment in the comparator group (Arm 2). Approximately 150 patients will be enrolled in 13 countries across 50 investigator sites. Enrollment to this study will be competitive.

Poster Abstract P13

Increasing the performance of a fully automated, quantitative, assay for the detection of circulating HBV pregenomic RNA

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Background: Hepatitis B virus pregenomic RNA (HBV pgRNA) has been proposed as a potential circulating biomarker for the activity of covalently closed circular DNA (cccDNA) that is present in infected hepatocytes of HBV patients. There are an increasing number of studies showing the utility of HBV RNA quantitation in monitoring the effectiveness of both experimental and standard of care therapies and it is being investigated as an endpoint for clinical trial effectiveness and therapy removal. We have previously reported on the development of a fully automated dual-target, quantitative assay for the measurement of HBV pgRNA with a lower limit of quantitation (LLOQ) of 1.65 log U/mL (~152 copies/mL). Here we report on a modified assay v2.0 with increased overall sensitivity by 7-15-fold.

Methods: A Research Use Only (RUO) fully automated real-time PCR assay was developed for the Abbott *m*2000 (Abbott Molecular Diagnostics, Des Plaines, IL, USA) platform and previously described (v1.0). Briefly, targets in conserved regions of the HBV x and core genes are used to ensure robust detection in the presence of mutations, and the assay is standardized against a WHO secondary DNA standard. An internal control is included to detect PCR interference. Assay LLOQ was measured by Probit analysis to be 1.65 log U/mL (~152 copies/mL) using a 0.2 mL sample volume input and 95% detection threshold. Modifications were made to the reagent formulation, cycling parameters, and sample input volumes which improve analytical performance. Performance (linearity, sensitivity, and standard deviations) of the new assay (v2.0) was compared with v1.0 using serial dilution panels from selected HBV clinical samples. A patient sample with high levels of HBV RNA was selected from which a panel of 11 serial dilutions into negative human plasma was made. Target HBV RNA concentrations ranged from 1.00E6 log U/mL (~3.41E6 copies/mL) down to 3.13 U/mL (~10-11 copies/mL) and either 3 or 20 replicates at each dilution were tested with the v1.0 and v2.0 assays.

Results: Overall sensitivity of the v1.0, defined as 95% detection was determined to be 25 U/mL (~86 copies/mL). The v2.0 assay with a 0.2 mL input volume showed enhanced sensitivity down to 6.3 U/ml (~22 copies/mL). Increasing the sample input volume to 0.6 mL further increased the overall sensitivity to 3.1 U/mL (~10-11 copies/mL). Linear regression analysis showed a highly linear relationship between expected and quantitated HBV RNA levels (R^2 >0.998) using the v2.0 assays. Standard deviations in quantitation between replicates were also lower with the v2.0 assays, suggesting further enhancements were made with regards to run-to-run variability. Lastly, quantitated values of samples above the v1.0 assay LLOQ were indistinguishable from quantitated values from the v2.0 assays, confirming the ability to compare results between the different assay versions.

Conclusions: Here we have shown increased sensitivity with the HBV RNA v2.0 assay resulting in a 7-15-fold increase in sensitivity over the first-generation assay and reducing the limit of detection to ~10-11 copies/mL. Importantly, this increased sensitivity does not impact quantitation at higher HBV RNA levels compared to the v1.0 assay, allowing for future studies to compare results with those that were run on the v1.0. Future studies will be conducted to determine if improved HBV RNA sensitivity yields additional clinical insights regarding therapy effectiveness and/or off therapy outcomes.