HBV FORUM 7– VIRTUAL WEBINAR SERIES
SAFETY PANEL WEBINAR

July 22, 2020

12:00-2:00pm ET

Presenters:

- Jessica Weber, *Forum for Collaborative Research*
- Kosh Agarwal, *King's College Hospital*
- Gaston Picchio, *Arbutus Biopharma*
- Ohad Etzion, *Soroka University Medical Center*
- Anuj Gaggar, *Gilead Sciences*
- Eric Hughes, *Novartis*

Panelists:

- Robert Fontana, *University of Michigan*
- Aimee Hodowanec, *U.S. Food and Drug Administration*
- Gabriel Westman, *Swedish Medical Products Agency*

Moderator:

- Norah Terrault, *University of Southern California*

Presentations

Presenter: Jessica Weber, *Forum for Collaborative Research*
Title: Welcome and Introductions
Slides:
https://forumresearch.org/storage/documents/HBV_Forum_7/HBV_Forum_Safety_Panel_Webinar_IntroSlides_072020v2_Final.pdf

Overview of the webinar:

- This webinar takes advantage of the broad membership and expertise of the HBV Forum to discuss recent safety events in clinical trials
- This webinar is the first in a series of three designed to replace the in-person HBV Forum 7 meeting, which was scheduled to coincide with EASL
- Other HBV Forum webinars will include a follow-up from HBV Forum 6 and an Intrahepatic Panel, which will include presentations from various stakeholders on biopsies and FNAs.

Participation:

- The Forum restricts industry participation to experts with the necessary scientific knowledge with a clear commitment to advancing the therapeutic field related to HBV.
- Presentations, discussions, comments, and questions are not for attribution. Participants speak as individuals and express views that may not represent those of their organizations.

Presenter: Kosh Agarwal, *King's College Hospital*
Title: A General Discussion of the Springbank Catalyst Study
Slides: https://forumresearch.org/storage/documents/HBV_Forum_7/Agarwal_HBV_Forum.pdf

Overview:

- This presentation will focus on general terms since Springbank is still reviewing the data.
- Kosh Agarwal is the lead author of the study and the data will be presented during the ILC 2020 meeting as a late breaker abstract.

MOA:

- Dr. Agarwal showed a summary slide describing the MOA of SB9200.
- SB9200 is known to have direct antiviral and immunomodulatory effects. SB9200 was also used as a drug in Hepatitis C.
- MF Yeun et al presented a dose escalation study at EASL in 2019 focused on Inarigivir monotherapy for 12 weeks followed by a switch to Tenofovir 300mg for 12 weeks. There were 5 Inarigivir cohorts: 25mg, 50mg, 100mg, 200mg and placebo. The study achieved its primary endpoints of safety and HBV DNA reduction; changes in all of the targeted biomarkers were observed.

Catalyst study:

- The first cohort of the study focused on *stop and shock* in 20 patients. The patients were all HBeAg negative, immunosuppressed, non-cirrhotic, and on 3 years of NUC suppression. NUCs were stopped for 4 weeks and Inarigivir was started for 12 weeks. The goal was to determine if immune engagement would take place and evaluate the potential for flares with the goal of clearance of HBsAg.
- The second cohort focused on *stop and suppress* in two arms in a total of 40 patients. Arm B was based on treatment response. If patients were stable, they were monitored on a monthly basis; patients negative for HBV RNA 3 times during their monthly follow up, could potentially discontinue Inarigivir. Patients were monitored for flares and the study involved a flare management program with the potential to restart NUCs if needed.
- Dr. Agarwal explained the study's protocol of Inarigivir dose reduction based on a patient's on-treatment ALT elevation.

<u>Catalyst Adverse Events:</u>

- A gradual increase in ALT was observed. Approximatively 40% of patients had abnormal ALT levels at week 8, which did not require a dose reduction, and 88% of patients had abnormal ALT levels at week 16. The patients in Cohort 2 had more significant symptoms.
- Between December 15-19 adverse events started to accrue.
    - A patient in London developed abdominal pain, mildly elevated ALT, lactic acidosis, pancreatitis, and liver failure. He was transferred to another hospital and died. The trial halted immediately after these adverse events.
    - Following this incident, 7 other patients were admitted. There was significant heterogeneity in liver malfunction, heterogenous LFTs, abdominal pain and vomiting, which continued to evolve post cessation of dosing – up to three weeks after the last dose.
- As noted above, the trial ceased.
- Two patients had serious cholestasis and coagulopathy. This could not have been predicted in preclinical studies.
- Biopsies in several patients reflected foamy cytoplasmic change, micro-vesicular steatosis and large lipid droplets with a variable amount of inflammatory infiltrate, which was established up to two weeks after cessation of dosing.
- The abnormalities took significant time to resolve.
- These heterogenic, adverse events are likely due to DILI and dose regulation. A low-level ALT could have been indicative of future events, but patients taking part in the study were monitored for immune engagement or flare; adverse events were not indicated in previous studies.
- A poster related to this study was presented during the ILC 2020 and a manuscript is underway.

Presenter: Gaston Picchio, *Arbutus Biopharma*
Title: *Key Finding Leading to the Discontinuation of a Capsid Inhibitor (CI), AB-506, in Healthy Subjects and Chronic Hepatitis B Subjects by Gaston Picchio*
Slides:
https://forumresearch.org/storage/documents/HBV_Forum_7/Picchio_HBVForum_July_22_2020new.pdf

Introduction:

- This study was previously presented as part of AASLD in 2019 and HEP DART 2019. It summarizes the discontinuation of AB-506.
- Distinguishing between drug induced and host induced ALT flares is challenging considering the natural history of CHB infection. Multiple dose studies in healthy subjects are rarely conducted longer than 7-14 days to assess the potential for drug toxicity before dosing the target population.
- AB-506 is a class 2 selective HBV capsid inhibitor with activity against HBV genotypes A-H and nucleoside resistant variants in vitro.

Study Background and Details:

- No transaminase elevations were noted in the 28-day or 90-day AB-506 toxicology studies.
- The primary objective was to study the safety and tolerability of single and multiple doses of AB-506 in healthy subjects for 10 days and DNA+ CHB subjects for 28 days.
- The inclusion and exclusion criteria along with the baseline characteristics of patients were presented. An imbalance was noted in the baseline HBV DNA levels between the 400mg and 160mg cohorts.
- AB-506 decreased HBV DNA and RNA in patients.
- One subject in the 160mg cohort did not experience a change in HBV DNA. This patient had a pre-existing I105T variant at baseline, which correlated to the absence of change. The prevalence of I105T in the HBV database is low at 0.6%, but among the subjects screened for the AB-506 study the prevalence was 7.7%.

Safety Findings:

- The safety findings in healthy volunteers were unremarkable; there were no elevations in transaminases or changes in any other laboratory parameters.
- In the 400mg cohort, 2 patients were observed with Grade 4 ALT elevations, which occurred around day 21 while HBV DNA was declining. AB-506 was discontinued immediately, after which ALT levels began to normalize. One investigator started one of these subjects on TAF following discontinuation of AB-506, and the patient showed sustained antiviral response including HBsAg and HBeAg decline after ALT normalization.
- In the 160mg cohort, 2 subjects had Grade 2 ALT elevations and 2 subjects had Grade 4 ALT elevations. These elevations began around day 20, while HBV DNA was declining. The subjects had normal bilirubin, INR, LFTs, and the frequency and severity of ALT elevation did not correlate with the AB-506 dose, CMAX or AUC at Day 1.
- All Grade 4 and Grade 2 ALT elevations occurred in subjects of Asian descent.
- The patient who was switched to TAF after AB-506 discontinuation, sustained a significant decline in all antigens and HBV DNA which persisted until day 302 (end of the follow up period).
- Cytokine profiling in serum was conducted in Grade 4 ALT elevation subjects. Among the CHB subjects there was significant elevation in IP-10 coinciding with the ALT elevation peak in all Grade-4 cases. Patients who experienced a profound HBsAg decline also experienced an increase in IFN-γ and IL-17α preceding ALT elevation, suggesting a potential beneficial immune component to the ALT flares.

Study of AB-506-003 in Healthy Subjects:

- After noting the ALT elevations in CHB patients, a 28-day follow-on study in healthy subjects was conducted to explore longer dosing durations and evaluate the safety observations. There were two cohorts: Cohort A with Caucasian patients and Cohort B with Asian patients. Study participants received 400mgs of AB-506 for a 28-day period, extended from the initial study of 10 days.
- In Cohort B 2 patients had Grade 4 ALT elevations beginning at day 18. The subjects were hospitalized for a short time and recovered; ALT elevations rapidly resolved after discontinuation of AB-506. The serum IP-10 of the 2 patients increased concomitantly with ALT elevations.

Conclusions:

- AB-506 demonstrated inhibition of HBV replication with mean declines in HBV DNA of 2.8 $\log_{10}$ and RNA of 2.4 $\log_{10}$.
- A 28-day study in two cohorts of Caucasian and Asian health subjects indicated that the transaminase elevations observed in a subset of Asian CHB subjects were drug-related.
- Following these results, the development of AB-506 was halted.

Presenter: Ohad Etzion, *Soroka University Medical Center*
Title: Tolerability and Safety of Peginterferon Lambda in Chronic HDV Infection
Slides: https://forumresearch.org/storage/documents/HBV_Forum_7/Etzion_HBV_Forum.pdf

Overview of HDV and Treatments:

- Always associated with HBV
- Causes most severe form of chronic viral hepatitis
- Leads to rapid progression to liver cirrhosis and cancer
- No FDA approved Rx which represents an unmet medical need.
- Off-label use of PEG-IFN α for 48-96 weeks leads to HDV RNA negativity in around 25-40% of patients, but relapse rates are high. Treatment is limited by its tolerability.
- Peginterferon Lambda is a first in class Type III interferon and better tolerated than Peginterferon alpha. Its use is expected to be associated with fewer of the typical systemic side effects associated with alpha.
- It was developed by BMS for of the treatment of HCV and HBV and was evaluated in more than 3000 patients in 17 separate clinical trials with similar efficacy as PEG-IFN α but with fewer side effects.

LIMT HDV “Mono”: Phase 2 Study

- The *Study to Evaluate Pegylated Interferon Lambda Monotherapy in Patients With Chronic Hepatitis Delta Virus Infection* (LIMT) was a randomized open label study with the goal of evaluating the safety, tolerability and efficacy of Lambda monotherapy for 48 weeks. The primary endpoint was undetectable HDV RNA at 12 and 24 weeks after the end of treatment. It was conducted at 4 clinical sites.
- Patients were either given 120 µg or 180 µg administered as a subcutaneous injection once weekly for 48 weeks. Dose reduction was permitted. Major inclusion criteria included HDV RNA+ at baseline, ULN< ALT< 10x ULN, and patients with compensated liver disese. Tenofovir or Entecavir were started at baseline.

- Baseline characteristics were presented. Of note, 9 patients (27%) were cirrhotic, and 21 patients (64%) had prior use of interferon alpha during their disease course.

Results:

- Treatment with Interferon Lambda at both doses was associated with a meaningful decline in viral load at the end of treatment but was more pronounced at the 180 µg. More than 1/3 of patient treated with the 180 µg reached ALT normalization and a durable virological response at 24 weeks after stopping therapy.
- The vast majority of patients reported side effects at some time point during the treatment phase of the study. The vast majority of the side effects (>90%) were either Grade 1 or 2 and none of the study participants terminated therapy due to systemic side effects. There was no incidence of depression and irritability unlike patients treated with Interferon Alpha. Only one patient developed neutropenia, which was managed with dose reduction.
- A total of 17 events required dose reduction, interruption or drug discontinuation- 88% of those were hepatobiliary events, which included jaundice and ALT flare. Drug discontinuation occurred in 8 patients, 62% of those were in the Pakistan cohort.

Related Studies and Findings:

- The results of the LIMT study are consistent with the *Peginterferon lambda for the treatment of HBeAg-positive chronic hepatitis B: A randomized phase 2b study (LIRA-B)*.and overall, there were fewer systemic side effects in the Lambda versus the Alpha.
- In the LIRA-B Study, ALT and bilirubin elevations were more common in the Lambda arm; the overall treatment discontinuation rate was similar between the groups but attributed to different causes. In the Alpha arm it was attributed to neutropenia, and in the Lambda arm it was due to ALT flares > 5xULN or bilirubin elevation.
- In the LIMT study, drug discontinuation was mostly associated with ALT flares and hyperbilirubinemia. The course of these events was mostly benign, with the majority of patients showing no symptoms or signs of decompensation. Labs normalized within several weeks following treatment discontinuation.
- The nature of the hepatobiliary abnormalities observed in patients treated with Lambda, was further explored using DILIsym, which is a computational model for investigation of DILI mechanisms during the process of drug development. It is based on the evaluation of ALT/AST and Tbili excrusions in Lambda treated patients.
- The predicted hepatocyte loss based on this model in LIMT patients was less than 25%. The bilirubin elevations were not consistent with significant liver necrosis. The most plausible cause for bilirubin elevation were alterations in transport and metabolism rather than hepatotoxicity.

Conclusion:

- Lambda shows a favorable tolerability profile in HDV infected patients.
- There were incidences of bilirubin and ALT elevations in a subset of patients. Clinical status was not compromised in affected patients and modeling suggest changes observed are not associated with substantial hepatocyte loss.
- Liver enzymes and bilirubin levels returned to baseline values following dose reduction, interruption or drug discontinuation.
- Lambda is promising as an efficacious drug for chronic HDV and should be developed further. It is currently being evaluated in combination with Lonafarnib.

Patients treated with Lambda should be monitored closely for alterations in liver enzymes and bilirubin with treatment adjusted or stopped as needed.

Presenter: Anuj Gaggar, *Gilead Sciences*
Title: Liver Safety Learnings
Slides:
https://forumresearch.org/storage/documents/HBV_Forum_7/Gaggar_HBV_Forum_Safety_Panel_2020_Final.pdf

Overview:

- Dr. Gaggar described part of the liver safety program at Gilead Sciences. Based on the MOA (direct acting antivirals, host-interacting antivirals, or immune agonists), safety considerations involve monitoring the on-target and/ or off-target toxicity. There can be potential on-target activity that leads to liver safety findings; for example, direct acting antivirals can cause an accumulation of viral proteins. Previous presentations have discussed the possible off-target toxicity of direct acting antivirals or host-interacting antivirals. In immune agonists, there is the potential for on-target toxicity due to an overwhelming response against the viral antigens or as part of the natural pathway for cytokines.
- This presentation focuses on a host-interacting antiviral, and mechanisms of toxicity that were assessed.

GS-5801 Description:

- GS-5801 is a liver targeted prodrug that inhibits the lysine demethylase 5 enzyme (KDM5). Inhibiting KDM5 results in the accumulation of methylation at k4 on the tail of H3 histone, which is important for the viral transcription of cccDNA.
- GS-5801 in vitro shows activity in primary human hepatocyte system. With increasing concentrations of GS-5801, there was decrease in HBV RNA, HBV DNA, HBeAg and HBsAg with minimal toxicity at high doses.
- For the in-vivo study, the GS-5801 molecule was altered to be more liver directed, to ensure modulation of histone methylation only at the site of the virus. This molecule was designed to minimize exposure of such a broad agent in the body. The liver was not identified as the target organ in preclinical studies, which suggested that doses used in the study would not result in any toxicity.

GS-5801 Clinical Trials:

- Phase 1a and Phase 1b were concurrently run studies. Phase 1a evaluated 2mg and 6mg doses of GS-5801 in healthy volunteers. Results indicated that 6mg dosing increased the pharmacodynamics response and after dosing for 7 days GS-5801 led to ALT increases. As a result, the Phase 1b trial used a lower dose of 4mgs for 7 days. Phase 1b showed no change in viral parameters with 7-day dosing. ALT levels were increased in some patients with CHB, which peaked at the end of dosing period or shortly thereafter. There were no significant changes in HBsAg, HBVcrAg and HBV RNA.
- No adverse events were associated with ALT elevations in the patients. There were no changes in any other liver parameters, including ALP, total bilirubin, albumin or HBV DNA. They did not conduct routine liver imaging, autoimmune panels or viral serology. There was limited evaluation overall on liver safety.

Safety Considerations and Conclusions:

- Preclinical studies did not clearly indicate a mechanism for the ALT elevation.
- ALT elevations were seen in both healthy volunteers and patients with CHB for the same dose of GS-5801 and for the same duration. While this does not indicate the mechanism of the ALT elevation, it demonstrates that it is not viral specific.
- Gilead Sciences determined that there was not sufficient antiviral activity to justify the risk-benefit of further developing GS-5801 for CHB. Clinical development for this molecule was discontinued.
- One conclusion from this study is that including healthy volunteers with CHB patients minimized the number of participants put at risk through the clinical trial.

Presenter: Eric Hughes, *Novartis*
Title: *Alpharetta (ALP-189) for the treatment of chronic hepatitis C infection*
Slides: https://forumresearch.org/storage/documents/HBV_Forum_7/Hughes_Alpharetta_ALP-189_Safety_Example_HBV_Forum_Eric_Hughes.pdf

Overview:

- Names and designations have been changed for this presentation.
- Alpharetta (ALP-189) is an HCV NS5b nucleoside inhibitor with pan-genotypic antiviral activity in vitro.
- It appeared promising and had a high genetic barrier to resistance (S282T).

ALP-189 Clinical Trials:

- There was no hERG signal in the HEK cells.
- Effects of high exposures for a 6-month duration at non-tolerated doses indicated multiple target organ toxicities. Effects at tolerated doses were limited to skeletal muscle degeneration at low multiples of the projected clinical exposure. Since skeletal muscle degeneration correlated with increases in standard serum chemistry analytes (transaminases and creatinine kinase) was deemed safe with appropriate monitoring up to 200mg per day.
- Various doses of Alpharetta were given for 14 days with a 200mg dose showing the maximum effect with the largest decrease in HCV RNA.
- Phase 2 studies took place over 12 weeks with 4 cohorts of 25mg, 50mg and 100mg of ALP-189 + pegIFN/RBV as well as placebo. The safety data appeared comparable with pegIFN/RBV.
- The efficacy was consistent with what was expected; 66% of patients achieved a sustained viral response (SVR) after 12-week triple therapy followed by 12 weeks of PEG/RBV. It was suspected that by increasing the dosage of Alpharetta, 90-100% of patient could achieve SVR.
- The study was amended to reflect new knowledge in the field and pegIFN was replaced with daclatasvir (DCV).

Case Study:

- A 25 year-old white male with history of chronic HCV infection, opioid dependence, cocaine abuse and mild depression who received 200mg of ALP-189 and DCV for 40 days was admitted to the hospital in cardiogenic shock.
- Up until then, there were no significant clinical events during the initial three visits.

- On day 39, the patient reported nausea and vomiting and was prescribed prochlorperazine. The following day, he was hospitalized for shortness of breath and had pulmonary edema, acute renal failure, and shock liver. A TTE demonstrated an LVED of less than 10%.
- This patient was the longest case at the highest dose (200mg).
- The main change was a significant, small T wave amplitude depression seen on the ECG.
- The study was discontinued within 3 days of this case.

Response and Lessons Learned:

- Advantages of Study Model Related to Termination:
  - The sites touched base with all patients twice a week.
  - They had a low threshold for immediate referral to tertiary care.
  - The company obtained permission from patients to directly contact them.
  - Company letter provided patients information and emergency contact.
- Established a "data sharing room" for other companies working on this MOA.
- A long-term outcome study was established with the Duke Clinical Research Institute (DCRI) to provide expertise and patient management. DCRI established CHF Centers of Excellence Referral Network for patients exposed to ALP-189.

- Preclinical data can be exposure and species specific.
- Nothing replaces informed and attentive medical monitoring of the patients.
- Wellbeing of subjects must guide decision-making.
- Communication both internally and externally is critical.
- Collaboration and sharing of data should become standard.

Panel and Audience Discussion

The panel was composed of Kosh Agarwal, *King's College Hospital*, Ohad Etzion, *Soroka University Medical Center*, Anuj Gaggar, *Gilead Sciences*, Eric Hughes, *Novartis*, Gaston Picchio, *Arbutus Biopharma*, Robert Fontana, *University of Michigan*, Aimee Hodowanec, *U.S. Food and Drug Administration*, and Gabriel Westman, *Swedish Medical Products Agency*.

Panelists Questions and Comments:

1) In the first presentation, "*A General Discussion of the Springbank Catalyst Study*" the histological picture was suggestive of mitochondrial damage. Was this evident in preclinical data? What is the role of NUCs?

- There were heterogenous manifestations of the drug related toxicity. Patients that presented with moderate ALT elevations deteriorated rapidly, which was thought to be due to lactic acidosis. Some patients evolved to various liver disfunctions. One group developed significant cholestasis and coagulopathy. In conclusion, not all patients had the mitochondrial pathology. In addition, the histology of all patients is not yet available. Some biopsies were conducted 2.5 weeks after drug cessation with variable results, which cannot be linked to data from preclinical toxicology studies. Currently, the clinical trial results do not fit into any known paradigm. Finally, additional review will need to be conducted on the role of NUCs in the study results.

2) Panelists were asked to discuss drug toxicity related to longer duration therapies in clinical trials.

- DILI cannot be narrowly defined, complicating recognizing, predicting and preventing it. Any hepatic manifestation can be a mimicker of DILI, such as transaminase elevations, ALT elevation, AST elevation, mitochondrial toxin, inflammation, etc. Each presentation highlighted various phenotypes of the laboratory results, which occurred relatively early on in the studies. Despite several presentations discussing rapid and severe drug toxicity, it's important to recognize that there is a spectrum of toxicity. Dr. Agarwal's presentation on Springbank's Catalyst study included a biopsy with evidence of mitochondrial toxicity, which leads to unanswered questions about whether the adverse events are related to a primary toxicity from the drug or an intracellular drug-drug interaction.

- A panelist questioned whether the Catalyst study participants were on tenofovir concomitantly or if some patients were solely prescribed the investigational agent.
    - In Cohort 1, patients were only taking the investigational agent. In Cohort 2, all patients were concomitantly taking a NUC. Thus, it is feasible that the toxicity came from an interaction of Inarigivir with the NUC but it is difficult to investigate.
    - A panelist indicated that some earlier HBV agents and early HIV drugs of this class have been shown to cause a depletion of mitochondrial proteins when taken chronically. So even if they were stopped before beginning the investigational drug, there could still be a low mitochondrial reserve in these patients.
    - Even with a single agent in a prospective study, there will be a spectrum of liver injury patterns.
    - Regulators indicated that drugs with different MOAs cause different liver injuries. A better understanding of the MOA, in trials such as the Springbank study, is important. Potentially confirming that the adverse events were related to a drug-drug interaction instead of a primary toxicity, could allow investigations to continue with a change of protocol. As HBV combination treatment studies progress, its important to understand the effects on the mitochondria when mixing drugs together. This leads to the question about how companies can simulate or model drug-drug interactions preclinically to better understand and prevent DILI in a clinical trial.

3) Does data contradict the idea that NUCs are always the benign therapy? Does this influence the types of populations included in these drug studies? Should we rethink how we see NUCs in combination?

- NUCs are thought of as a good treatment background and are considered safe because there is less liver inflammation. Drug-drug interactions should be evaluated in the preclinical profile. It is important to have a good, healthy volunteer profile to understand the effects of the drug in the absence of a NUC. What assessments would have to be conducted preclinically to determine a drug-drug interaction?

4) Should we rethink using NUCs in combination? Would looking at a drug profile without the inclusion of NUCs in the study background be preferred?

- One lesson learned from previous drug trials is that the inclusion of additional study time for healthy volunteers can be critical to identifying the signal. Duration might be essential even in healthy volunteers, but that may not be true for other types of capsid inhibitors or medications.

5) <u>There are now liver chips available. What is the level of enthusiasm and/ or interest in using human in vitro test systems?</u>

- Industry representatives indicated that the use of liver chips in clinical trials would depend on how predictive they are and whether signals demonstrated in the clinic are reproducible in this preclinical model. Are there predictive systems that will better reflect what is being seen in the clinic?
- There is a consortium of pharmaceutical companies looking at ways of predicting DILI in non-clinical phases of drug development. A survey they conducted indicated there is wide variability in the approach and use of these types of technologies, which ranges from companies that will abandon drug development to companies that will not utilize these types of instruments. There is still too much to learn about them to use them confidently.

- One regulator questioned if there is a preclinical test system for mitochondrial toxicity that's practical. Gary Peltz published the paper "*Can 'humanized' mice improve drug development in the 21st century?*" which indicated that specific drug toxicity could be reproduced using chimeric human cells in mice, which is a mitochondrial toxicity phenotype. This leads to the question about whether there is a system that could be used in advance of testing combination therapy in preclinical trials.
- Another regulator indicated that NUCs and mitochondrial toxicity vary significantly between drug classes and analogs. Adverse events may not be due to drug-drug interactions, and novel compounds will have to be reviewed in detail. It's important to note that these clinical trials are not treating patients nearing death, rather they are treating patients on NUCs with a good long-term prognosis. Development programs and end-products should have a good safety profile. The field should move forward as swiftly as possible, while also minimizing risk. Clinical trials are seeing various types of DILIs and enhanced preclinical models could be useful to reduce the likelihood of DILIs developing in patients.

6) <u>Is it worthwhile to conduct more studies in healthy patients using NUCs in combination with a new agent as opposed to only studying the new agent alone?</u>

- Regulators indicated agreed that type of study would be informative and worth considering. Engaging in longer trials with combination therapies would depend on the product and what is already known about the safety concerns. There should not be undue risks to healthy volunteers, especially if there is concern about carcinogenicity or genotoxicity. However, in certain situations, these types of trials could be informative.

7) Are there differences or other factors (genetics, duration of disease, etc.) that influence the risk of toxicity between Asian and non-Asian populations? Is this relevant in other drug classes? How should this influence testing in the future? There are HBV patients from all over the world and studies are often divided into Asian and Caucasian populations.

- There is a huge proportion of HBV patients from sub-Saharan Africa. In the Springbank late breaking abstract, there was no difference in age, gender, ethnicity or NUC therapy between those who developed liver injury. In Cohort 2, there was an overrepresentation of patients with a Pan-Pacific Asian background, but there were also Caucasian and African patients. The patient who succumbed and the patient with the cholestatic and coagulopathy issues were older than other patients in the cohort.

8) Should the Springbank clinical trial have lowered the threshold of concern and further investigated patient's adverse events with various methodologies (i.e. liver biopsy, fna, etc.)?

- A patient advocate provided perspective on HBV clinical trial participants. She indicated that the Hepatitis B Foundation surveyed 2000 patients living with Hepatitis B around the world and conducted in-depth interviews. People living with Hepatitis B noted their interest in participating in clinical trials, if the medication could contribute to a cure. Patients are often not thinking about safety concerns. They need to be adequately educated about questions to ask and symptoms of concern. It is difficult to share this type of information, so it's not overwhelming but also accurate.
- Maximizing safety measures around participation is the most important thing that can be done to help patients understand how they will be monitored during the process.

- One commenter noted there are assays from the 1980s, which are well documented in the literature, that investigate mitochondrial toxicity. Clinical trials should be conducting mitochondrial testing in human cells for at least 14 days in order to be valid. DDC can be used as a positive control side by side with 3TC as a negative control. This model works and there is no need to develop a new model.
    - The Springbank company conducted standard development. A prolonged exposure in preclinical trials is noteworthy and the use of other models could be useful.
    - This webinar is designed to promote this type of discussion and consider lessons learned.

9) Do we need extra caution for some drugs, such as immunomodulatory agents? Do we need different monitoring?

- The adverse events related to GS-5801, are likely dose related. It is unknown whether the Inarigivir adverse events are due to an immunomodulatory agent or specific molecule issue. Gilead Sciences is cautious about using immunomodulatory drugs in clinic, especially related to augmenting the CD8 T cell response. The article, "*Liver safety assessment in clinical trials of new agents for chronic hepatitis B*" by Robert Fontana, et. al. and created in collaboration by the Liver Safety Monitoring sub-Working Group provides key guidelines to monitor toxicities and react.

- A participant indicated that in Hepatitis B and Hepatitis Delta, some of the ALT flares may be beneficial. Current tools for assessing liver toxicity are limited, and better tools should be developed to assess liver functionality. Clinical trials should be able to detect patients who develop ALT flares or bilirubin elevations that may precipitate viral clearance versus patients who may develop severe liver toxicity. Currently, when ALT flares and/ or bilirubin exceeds a certain threshold, patients are mandated to stop a trial. There should be better tools for continuing medications if liver function is maintained.
    - There are emerging companies looking at uptake cholic acid and the trajectory of certain interventions, as tools to assess liver function abnormalities on treatment.
    - Tools should be more sensitive.

10) Is the current recommendation of ALT 10x ULN to stop a drug and ALT 5xULN for investigating a drug the right threshold? Should clinical trials further investigate any ALT elevation? Should there be lower ALT thresholds for different classes of drugs?

- Regulators from the FDA supported asking these types of questions, but the answers are unknown. The "*Liver safety assessment in clinical trials of new agents for chronic hepatitis B*" article currently contains the most reasonable thresholds. Additionally, various factors will affect these thresholds, such as the patient population, MOA, and where the drug is in the development process. Looking at symptoms or other lab values suggesting decompensation may also provide valuable information. Sponsors are encouraged to collect this type of exploratory, biomarker data. Sponsors can also involve a DILI adjudication committee, with a high level of expertise, to review the data for patients with elevated ALT under investigation.
- Other regulators from the EU noted that temporal aspects are also important. Perhaps longer studies should be conducted on smaller populations, especially in relation to immunomodulatory agents, since it's a novel field. Radiology and biopsy could be used on a more regular basis, if needed. There should be further consideration about early clinical trial issues and, if possible, learnings from preclinical trials.

11) Are there any recommendations for updates to the "*Liver safety assessment in clinical trials of new agents for chronic hepatitis B*" article?

- The ALT thresholds should not be changed to make them more conservative. Values should be considered as part of a larger picture, since some HBV patients will begin clinical trials with an abnormal ALT. Now is the time to test the newer tools, such as human tissue chips or spheroids. We're closer to human physiology than we have been before, and drugs were not previously tested with these more recent technologies. When lamivudine first came out, some patients had an elevated ALT; regardless, it is a safe and beneficial drug. Ultimately, data should be considered in totality.