Resumen del CROI 2024 sobre hepatitis víricas

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Intrahepatic HDV Activity Is Fueled by Integrated HBV DNA-Derived HBs Independently From cccDNA Size

Oral Abstract #162 (Hepatitis and Tuberculosis)

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This work explores Hepatitis B virus (HBV) and Hepatitis Delta virus (HDV) replicative activity and their interplay using liver biopsies. HDV exploits the surface proteins (HBsAg) for entry into hepatocytes and for the release of viral progeny. Various mechanisms underpinning HDV persistence have been proposed, including HBV integration as a potential source of HBsAg for sustaining HDV infection, a facet explored within this research.

The study cohort comprised 68 individuals who were HBeAg negative, categorized into two groups: those with HDV chronic co-infection (n=32) and those with HBV chronic mono-infection (n=36). The intrahepatic levels of total HBV-DNA, covalently closed circular DNA (cccDNA), pregenomic RNA (marker of, and HDV RNA were quantified using highly-sensitive droplet digital PCR (ddPCR). To discriminate the quantity of HBsAg derived from cccDNA versus integrated HBV-DNA, two distinct ddPCR assays were employed. One assay quantified total HBs transcripts, while the other specifically targeted HBs transcripts derived solely from cccDNA. The difference between these measurements were considered as integrated HBV-DNA to HBs transcripts.

Both groups of the study were comparable in terms of age and NUC treatment. HBV mono-infection group had higher levels of serum HBV-DNA compared to HDV co-infected [IQ]:3.6 [2.4-4.9] vs 1.3 [0-1.5]; p<0.0001). HDV co-infection group had higher levels of fibrosis (indicated by Ishak score≥5 (%); 53.3% vs 19.4%; p=0.005) and higher ALT levels (U/L [IQR]: 68 [45-89] vs 28[21-49]; p=0.001) than HBV mono- infected group, which indicates higher liver inflammation. When they compared peripheral markers with intrahepatic markers, they observed that HDV co-infected group had high levels of HDV viraemia (median [IQR]: 6.0 [3.8-6.7] which correlated positively (Rho=0.62 p=0.006) with intrahepatic levels of HDV-RNA (median [IQR] copies/1000cells: 784 [1-4266] indicating that serum HDV-RNA is a reliable marker of intrahepatic HDV activity.

Comparing intrahepatic HBV markers, they observed significant differences in **HBV reservoir size**, especially in cccDNA, between HDV co-infected and HBV mono-infected groups (p<0.01), with higher levels in HBV mono-infected group.

When they analyze **HBs transcripts** in the overall population, a positive correlation between the total amount of HBs transcripts and serum HBsAs was detected (Rho=0.5 p=0.001). Analyzing the source of HBs transcripts, more than 99% derived from integrated HBV-DNA with a very limited contribution of cccDNA transcriptional activity.

Total and integrated HBV DNA-derived HBs transcripts were comparable between both groups. However, the production of cccDNA-derived HBs transcripts tended to be lower in the setting of HDV co-infection, although this trend did not reach statistical significance (p=0.07).

When they focus on HDV co-infected group and divided them according cccDNA levels (<1 cccDNA, n=17 or >1 cccDNA copies/1000 cells, n=15), they observed lower levels of **intrahepatic HBV markers** total HBV-DNA (p=0.001), HBV pgRNA (p=0.01) and cccDNA (p<0.0001), in the group with <1 cccDNA copy/1000 cells. However, **HDV-RNA levels** were comparable independently of the amount of cccDNA which suggest the existence of pathways underlying HDV activity independent from HBV reservoir. This data was confirm analyzing 7 individuals HDV chronic co-infected with no cccDNA, no cccDNA-derived Hbs transcripts and undetectable HBV viraemia. In these participants, they observe an intensive HDV activity at both serum (median [IR]: 6.0 [5.9-7.3]) and intrahepatic levels (intrahepatic HDV-RNA, median [IQR]: 1,659 [660-12,261]; integrated HBV DNA-derived HBs transcripts, median [IQR] copies/1000 cells: 3 [2-690], suggesting that HDV persistence can be sustained by HBsAg derived from integrated HBV-DNA. In summary, chronic HDV co-infection is marked by elevated levels of intrahepatic HDV replication despite the presence of a restricted HBV reservoir. The pathways supporting HDV activity are independent from the size of HBV reservoir and are sustained by an abundant production of HBs transcripts, mainly derived from integrated HBV-DNA.

This study is important for two main reasons. First, it establishes a strong correlation between serum and intrahepatic data, which simplifies sampling procedures. Secondly, the results confirm the existence of unconventional mechanisms promoting HDV persistence, even independently from HBV, which warrant further investigation as they may represent significant barriers to HDV eradication.

Novel Biomarkers as Determinants of HBsAgLoss in Persons with HIV/HBV on Tenofovir

Poster #733; Poster Session-I4 (HBV and HDV Treatment and Cure)

Authors: **Lorin Begré**, Anders Boyd, Marie-Laure Plissonnier, Barbara Testoni, Charles Béguelin, Franziska Suter-Riniker, Caroline Scholtes, Juergen K. Rockstroh, Karine Lacombe, Lars Peters, Massimo Levrero, Andri Rauch, Fabien Zoulim, Gilles Wandeler

HBsAg loss is associated with improved clinical outcomes in individuals with hepatitis B virus (HBV) infection. However, the relationship between novel biomarkers, such as circulating HBV RNA and hepatitis B core-related antigen (HBcrAg), and this outcome in HIV/HBV co-infected individuals remains unexplored.

In this study, Begré et al. examine the rates of HBsAg loss and associated risk factors in Euro-B, a multi-cohort collaboration including participants from the Swiss HIV Cohort Study and EuroSIDA.

The study included participants with a positive HBsAg (qHBsAg≥0.05 IU/ml) at tenofovir start and at least one qHBsAg measurements after >180 days of follow-up on tenofovir-containing antiretroviral therapy (ART). Quantitative measurements of HBsAg (qHBsAg), HBV DNA, HBcrAg, and HBV RNA were conducted at the initiation of tenofovir treatment. The investigation assessed rates of HBsAg loss, defined as qHBsAg <0.05 IU/mL, and evaluated its predictors using multivariable logistic regression. Risk factors were evaluated in two separate multivariable logistic regression models due to collinearity between HBcrAg and HBV RNA.

Participants included in the study were classified by ART exposure status at start of tenofovir therapy, as ART naïve (N=259) or ART-experienced (N=340). Median age was 40 (IQR 34-46) vs 41 (36-47), 55/259 (21.2%) vs 55/340 (16.2%) were female at birth with a median CD4 count of 308 (IQR 181-438) vs 410 (271-587) cells/mm^3 and of 79/177 (44.6%) vs 131/222 (59%) were HBeAg positive. At tenofovir start, 143/599 (23.9%) participants had a qHBsAg <1000 IU/mL, 177/596 (29.7%) had HBV DNA <20 IU/mL, 126/587 (21.5%) had HBcrAg ≤3 log_{10} U/mL and 247/566 (43.6%) had HBV RNA <10 copies/mL.

After 2 years of tenofovir therapy 66/510 (12.9%) showed qHBsAg<0.05 IU/ml and 109/599 (18.2%) experienced HBsAg loss during a median follow-up of 8.2 years (IQR 3.6-13.1).

In both multivariable logistic regression models, qHBsAg <1000 IU/mL at baseline, low HBV RNA levels and being born in a country with high HBV prevalence were associated with HBsAg loss. In HBeAg-positive participants, lower qHBsAg levels, lower HBV RNA levels and higher HBV DNA levels, but not HBcrAg were associated with HBsAg loss. In HBeAg-negative participants, qHBsAg <1000 IU/mL and higher HBcrAg but not HBV RNA were associated with HBsAg loss.

In summary, the most robust predictor of HBsAg loss is the low baseline qHBsAg level at the initiation of tenofovir therapy. High HBcrAg levels in HBe Ag-negative individuals and low HBV RNA levels in HBe Ag-positive individuals were associated with HBsAg loss.

This study focuses on finding other biomarkers to predict HBsAg loss. The study of non-invasive biomarkers of HBV infection is of great interest in order to choose the most appropriate therapeutic strategy.

Hepatitis B Reactivation in PLWH With Anti-Core Antibody After Switch to an Anti-HBV Sparing Regimen

Poster #737; Poster Session-I4 (HBV and HDV Treatment and Cure)

Authors: Giulia Morsica, Riccardo Lolatto, Sara Diotallevi, Valentina Svicher, Costanza Bertoni, Alessia Siribelli, Hamid Hasson, Sabrina Bagaglio, **Tommaso Clemente**, Arianna Forniti, Romina Salpini, Caterina Uberti-Foppa, Antonella Castagna, Nicola Gianotti

In this study, the authors investigated HBV reactivation in PLWH with antiHBc who switched from combination therapy containing nucleos(t)ide analogues [(NAs) tenofovir disoproxil fumarate (TDF) or tenofovir alafenamide (TAF)], drugs active against HIV and HBV, to antiHBV sparing regimens (antiHBVsr). This issue is important to address because some individuals with isolated anti-HBc and in cases of severe immunosuppression with antiHBc and HBs antibodies (antiHBsAb) showed HBV reactivation.

Cohort study involved 41 PLWH with antiHBc (n=7) or antiHBc with antiHBsAb (n=34), switched from NA-containing regimens to antiHBVsr dolutegravir/rilpivirine (DTG/RPV) or cabotegravir/rilpivirine long-acting (CAB/RPVLA). They were followed-up from the date of switching (baseline, BL) to the date of antiHBVsr discontinuation. HBV reactivation was assessed by ALT above the upper limit of normal (normal <60 IU/L). For PLWH with ALT above the normal range, HBV DNA quantification was performed for further assessment of reactivation. Highly sensitive digital droplet PCR (ddPCR) was used to quantify HBV-DNA and HBV-RNA (as a surrogate marker for cccDNA persisting in hepatocytes) from plasma samples when HBV reactivation was detected.

Both groups were formed by male individuals (100%) and were comparable in age and years of HIV infection as well as HIV-RNA (<50cp/ml, except one participant of antiHBc-HBsAb positive had 50-200 cp/ml before switching to DTG/RPV), CD4+ cells count and HBV-DNA <10 IU/ml. Median follow-up after switch to antiHBVsr was of 8.91 months [(IQR 6.78 - 24.14) in overall population; in PLWH with HBsAb was 8.96 months (IQR 6.78 - 24.14) and in PLWH with isolated antiHBc 6.97 months (IQR 5.56 - 43.22); p=0.742].

HBV reactivation was detected in one individual from antiHBc group (1/7, 14.3%) 3 months after switching to CAB/PRVLA. However, this individual progressively decrease HBV-DNA and normalize transamisases levels after receiving FTC/TAF.

The authors concluded that HBV reactivation after switching to antiHBVsr is unlikely in PLWH with antiHBc and HBsAb however, close monitoring of ALT and possibly HBV-DNA is mandatory in PLWH with isolated antiHBc switching to antiHBVsr.

This study reinforces that those co-infected with isolated anti-HB core antibodies should remain on anti-hepatitis B-containing ART. Switching to regimens without FTC/3TC should be monitored for hepatitis B reactivation.

Preclinical Pharmacokinetic Assessment of a Hepatitis C Virus Long-Acting Injectable Formulation

Oral Abstract #161 (Hepatitis and Tuberculosis)

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Presenting author: **Usman Arshad**; University of Liverpool

This study explores an alternative approach for the treatment of Hepatitis C through the formulation of a long-acting injectable (LAI) using glecaprevir (GLE) and pibrentasvir (PIB). Long-acting therapies are useful alternatives to reduce loss of patients to follow-up and to improve medication adherence. Based on their physicochemical and pharmacokinetic properties (water solubility, oral pharmacokinetic half-lives and target plasma concentrations) GLE an PIB are potential candidates for parenteral long-acting formulations.

For preclinical characterization, male Sprague Dawley rats were injected intramuscularly into both thighs, with LAI suspensions of GLE and PIB alone, and both drugs in a fixed dose combination (FDC) of 1:1 ratio. GLE and PIB were co-formulated as a fixed dose combination LAI (250mg/ml of each drug) with dosing volumes of 0.3, 0.15 and 0.075 ml (representing active doses of 75, 37.5 and 18.75 mg). Plasma samples were collected from the tail vein and terminal liver samples collected. Plasma GLE and PIB concentrations were quantified using LC/MS-MS.

PIB concentration in rats over the study period of 3 months showed PK stability in all formulation although the higher exposure was obtained with the FDC. For GLE, FDC formulation was the one with a good terminal half-life as seen by the protection of the PK. Their results evidence that combination of both drugs in a FDC maintains a longer terminal half-life for GLE and improves exposure for PIB, maintaining plasma concentrations above the human oral C_{trough} during 13 weeks (exceeding the 8 weeks of current oral treatment in humans). Analysis of dose combination showed an increase exposure for increase doses. To interpreted the PK data semi-mechanistic compartmental pharmacokinetic models were applied in order to understand what ratio of the drugs would be required in FDC. For GLE, there was an initial period of rapid release followed by an intermediate release and at the end a slower release. However, for PIB, they observed some differences in the drug absorption kinetics with a slow release for PIB consistent with its low water solubility and higher lipophilicity. Application of these models allowed to predict doses and drug ratio of 3:1 (GLE: PIB) to achieve exposures above median human C_{trough} up to 13 weeks in rats. When they compared the liver:plasma concentration ratios of this LAI formulation with those of oral administration, similar levels of the drug were observed. Finally, no signs of toxicity or tolerability were described but GLP toxicity assessments are in development to support clinical translation.

To sum up, this GLE/PIB LAI formulation achieves sustained therapeutic concentrations in rats above the oral C_{trough} beyond the 56-day treatment duration with similar hepatic drug penetration compared to previously published data for oral dosing in humans.

This LAI formulation is a promising approach and it could be a great alternative for the treatment of hepatitis C in patients with poor adherence to medication or difficult to follow up because the treatment would be administered in a single dose.

HepB-CpG Vaccine Is Superior to HepB-alum in People with HIV and Prior Vaccine Nonresponse: A5379

Oral Abstract #209 (Special Session: Clinical Late-Breaking Oral Abstracts)

Authors: **Kristen Marks**, Minhee Kang, Triin Umbleja, Andrea Cox, Karen J. Vigil, Ngan T. Ta, Ayotunde Omoz-Oarhe, Jennifer C. Price, Josphat Kosgei, Leolin Katsidzira, Hugo Perazzo, Kevin Knowles, Beverly L. Alston-Smith, Kenneth E. Sherman, for ACTG 5379 (BEe-HIVe) Study Team

Presenting author: **Kristen Marks**, Weill Cornell Medicine

This work presents the results of the **group A of the ACTG A5379 study**. A5379, is a phase III, prospective, open-labeled, interventional, two group study (group a and B) conducted at US and non-US sites looking at hepatitis B vaccination in adults living with HIV. Group A includes people with HIV with non-response to conventional Hepatitis B vaccine (HepB-alum, Engerix®).

Participants of the study were randomized 1:1:1 to receive i) HepB-CpG (HEPLISAV-B®) 2 doses (at entry and week, n=187) ii) HepB-CpG (HEPLISAV-B®) 3 doses (at entry and at weeks 4 and 24, n=188) and iii) HepB-alum (Engerix®) 3 doses (at entry and at weeks 4 and 24, n=186).

They were stratified by sex at birth and diabetes and participants of the study for 72 weeks. Both vaccines used in this study contained 20 mcg of HBsAg.

The primary objectives of this study were i) to compare the seroprotection response (SPR) of 2-dose HepB-CpG to 3 dose HepB-alum (non-inferiority) ii) to compare SPR of 3-dose HepB-CpG to 3-dose HepB-alum (superiority) and iii) to describe safety.

Data presented were for the period up to 28 weeks, which was the primary analysis. Primary SPR was defined at week 12 for 2-CpG and week 28 for 3-CpG and 3-alum.

516 participants were recruited from 41 sites in 10 countries and their baseline characteristics were comparable between three arms: 64% were male, 42% Black, 35% White, 17% Asian, 22% Hispanic. Median age was 46 years (range 18-70), 56% enrolled in the US, 21% Africa, 17% Asia, 6% S. America. Median CD4 was 638 cells/mm^3, 94% had HIV-1 RNA <40 copies/mL, 26% BMI >30, and 13% diabetes. Primary results obtained from 508 participants showed a 93.1% SPR in 2-dose HepB-CpG, 99.4% SPR in 3-dose HepB-CpG and 80.6% in 3-dose HepB-alum. The sensitivity analysis that included participants with imputed results showed similar values: 92.3% (n=182), 99.4% (n=181) and 77.8% (n=180) respectively. When they compared HepB-CpG SPR to HepB-alum they observed that SPR difference between 2-dose HepB-CpG and 3-dose HepB-alum was 12.5% (97.5% CI: 4%, 21%), achieving non-inferiority and indicating superiority. SPR of 3-dose HepB-CpG was superior to 3-dose HepB-alum with a difference of 18.4% (97.5% repeated CI: 10%, 26%). The proportion of participants achieving seroprotection was more rapid and higher in both arms of Hep-CpG. When they analyzed the distribution of anti-HBs titers they observed that 3-dose HepB-CpG arm achieved a 78% of participants with Anti-HBs titers >1000 mIU/ml compared to 26 % and 35% in 2-dose HepB-CpG and 3-doses HepB-alum respectively and 96% with Anti-HBs titers >100 mIU/ml.

An interesting thing observed in the HepB-CpG arms was that these participants achieved high levels of seroprotection even in those individuals with risk factors (older age, diabetes, higher BMI, low CD4 and HIV viremia) for not responding to

conventional vaccination. In terms of safety, post-vaccination AEs (grade 2 or higher within 4 weeks of vaccination) were experienced by 33%, 45% and 43% of 2-CpG, 3-CpG and 3-alum participants, respectively. The most frequent AEs were injection site pain, fatigue, headache, malaise and myalgia.

The authors highlighted the low representation in the study of some predictors of non-response to conventional vaccination such as low CD4 an low viral load.

To summarise, in this study PWH non-responder to conventional HBV vaccine achieved superior SPR after 2 and 3 dose of HepB-CpG compared to 3-dose of HepB-alum. Moreover, 3-dose HepB-CpG achieved a higher proportion of Anti-HBs titers above 1000mIU/ml compared to 2-dose HepB-CpG and 3-dose of HepB-alum. Finally, no safety or deaths were reporter.

These results are encouraging and we await the results at 72 weeks to know the durability of response and safety.