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Major open questions in the Hepatitis B and D field – proceedings of the inaugural International Emerging Hepatitis B and Hepatitis D Researchers Workshop

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Abstract

The early and mid-career researchers (EMCRs) of scientific communities represent the forefront of research and the future direction in which a field takes. The opinions of this key demographic are not commonly aggregated to audit fields and precisely demonstrate where challenges lie for the future. To address this, we initiated the inaugural International Emerging Researchers Workshop for the global Hepatitis B and Hepatitis D scientific community (75 individuals). The cohort was split into small discussion groups and the significant problems, challenges, and future directions were assessed. Here, we summarise the outcome of these discussions and outline the future directions suggested by the EMCR community. We show an effective approach to gauging and accumulating the ideas of EMCRs and provide a succinct summary of the significant gaps remaining in the Hepatitis B and Hepatitis D field.

Keywords

Hepatitis B; Hepatitis D; hepatocellular carcinoma; professional development; emerging researchers; early and mid-career researchers; challenges

Introduction

On the 19th of September 2023, 75 early and mid-career researchers (EMCRs) working in the Hepatitis B and D virus (HBV and HDV) research fields gathered in Kobe, Japan for the inaugural International Emerging Researchers Workshop, held directly before the 2023 International HBV Meeting.

The concept of this meeting was developed by Hepatitis B Foundation Emerging Scientific and Medical Advisory Board for 3 complementary purposes: 1) to ensure that EMCRs (particularly those just beginning in the field with few personal connections) could network with each other prior to the meeting, enhancing global collaboration; 2) to provide a platform for EMCRs to exercise their professional development (e.g., workshop organisation, chairing discussion panels, networking across groups); and 3) to brainstorm new research approaches by those closest to the benchwork, in a peer-led context outside the potentially intimidating environment during the meeting proper.

After a short “ice-breaker” session to stimulate interaction and informal discussion amongst the group, the cohort was randomly broken up into 10 small discussion panels of approximately 8 people. Each discussion panel was devoted to a specific topic (Table 1). After a brief round of introductions, an expert in the field (generally an EMCR) led semi-structured discussions to survey the thoughts of the EMCR community. Questions used as prompts are listed in Table 2 and discussion points were recorded by panel leads. Discussions were held for 10 minutes before each participant was randomly assigned to

a new panel. In total, each participant took part in 3 rounds of discussions. Here, we summarise the outcome of these discussions and outline the future directions suggested by the EMCR community.

Experimental Models

HBV and HDV research rely on the use of suitable models (Allweiss and Dandri, 2016b). Therefore, the discussion was divided into 3 main parts: in-vitro, in-vivo, and novel models.

In vitro *models*

A main point of discussion was the high variability of the different *in vitro* infection models in the field. Since the discovery of the HBV entry receptor NTCP (Yan et al., 2012), many labs have generated their own NTCP-over-expressing cell lines, usually based on HepG2 and Huh7 cells (Iwamoto et al., 2014; Ko et al., 2015; Michailidis et al., 2017; Ni et al., 2014). While most of these clones were selected on a single-cell level for the highest permissiveness and infectivity, the expression pattern of these clonal cell lines may vary considerably. This offers the opportunity to achieve independent research findings but limits the reproducibility of published results. Therefore, the EMCRs stressed the need for more standardised in-vitro cell-culture models. To achieve this, a better understanding of the biology of the different cell lines and optimised generalisable protocols for HBV and HDV infection would be necessary.

The discussion also focussed on the use of primary human hepatocytes as a leading *in vitro* infection model (Lucifora et al., 2020; Schulze-Bergkamen et al., 2003). Here, the researchers recognised the need for a better supply and longitudinal primary cell samples. Thus, the field would benefit from a method to proliferate primary hepatocytes in-vitro and to freeze/thaw the cells at a high viability. Further, optimising the current protocols could also help increase infection rates and extend the lifespan of the cultured cells.

In vivo *models*

Development and evaluation of novel treatment options and the validation of *in vitro* results are often performed in *in vivo* animal models (Wettengel and Burwitz, 2020). Little focus was placed on surrogate models in related hepadnaviruses (e.g., WHV in woodchucks or DHBV in ducks), but instead discussion centred on human HBV models. While HBV naturally only infects humans and chimpanzees, several other infection animal models in tupaias (Walter et al., 1996), mice (Dandri and Lütgehetmann, 2014; Du et al., 2021), or macaques (Biswas et al., 2022; Burwitz et al., 2017) have been established with different advantages and disadvantages. EMCRs addressed the point, that there is a critical need for immunocompetent animal models for HBV. The most suitable models would be mice and/or non-human primates.

Novel models

The discussion also included perspectives on novel models to overcome the limitations of the current *in vitro* and *in vivo* models (Allweiss and Dandri, 2016a). Bridging the gap between 2-dimensional cell culture-based research and *in vivo* or *ex vivo* research in the

liver, the field would benefit from more available technologies such as the liver on a chip (Ortega-Prieto et al., 2018) or *in vitro* cultivated 3D liver organoids (Nie et al., 2018; Rao et al., 2021). While costs and extensive methodologies currently restrict the broad use, advances in these technologies could help to improve *in vitro* results and reduce the use of animal models.

Viral entry and cccDNA biology

Understanding the viral entry and cccDNA biology of HBV is crucial for comprehending the host tropism of HBV, unveiling the viral life cycle and developing effective antiviral strategies. The discussion focused on several topics:

Mechanism of Receptor Binding and Internalization

The exact mechanism of how HBV attaches to host cell receptors and is internalized is not fully understood.

What host factors are involved in HBV entry?—Identification and characterization of additional host factors that play a role in HBV entry are ongoing challenges. A more comprehensive understanding of the host cell machinery involved in viral entry could reveal potential targets for therapeutic interventions.

Why is HBV entry not pH dependent?—The entry of HBV is not strongly dependent on pH (Hagelstein et al., 1997), unlike some other viruses that rely on an acidic environment for fusion with host cell membranes (Grove and Marsh, 2011). The pH dependence of viral entry is typically associated with viruses that enter cells through endocytosis, where the acidic conditions in endosomes trigger conformational changes in viral proteins, leading to membrane fusion and release of the viral genome into the cytoplasm. The process of HBV entry may include receptor-mediated endocytosis, but unlike some other viruses, the subsequent steps leading to the release of the viral genome are not critically dependent on acidic conditions. Instead, HBV may undergo uncoating and release its genetic material in a manner that is less influenced by the pH of the cellular environment. The exact details are still not fully understood.

What is the difference between HBV and HDV regarding entry?—While HBV can independently infect hepatocytes, HDV is a satellite virus that relies on HBV for its entry into host cells. HDV utilizes the envelope proteins of HBV to facilitate its own entry process (Negro and Lok, 2023). Whether there are differences regarding receptor-mediated endocytosis between these two viruses need further investigation.

Endosomal Trafficking and Uncoating

The process of endosomal trafficking and uncoating of HBV after internalization is not well characterized. Understanding the fate of the viral capsid and the events leading to the release of the viral genome in the cytoplasm remains an active research area.

How does rcDNA go into the nucleus?—The pathway through which rcDNA enters the nucleus is not fully understood. During the early stages of HBV entry into the host cell,

the viral capsid undergoes uncoating, leading to the release of rcDNA into the cytoplasm (Zhao et al., 2023). It is plausible that the rcDNA, in association with viral or host proteins, could be directed toward the nucleus. The exact mechanisms involved in this process are not well-defined.

cccDNA Formation

How cccDNA formed?—While it is known that the viral genome enters the nucleus and forms cccDNA, the precise mechanisms and host factors involved in these processes are still not fully elucidated. Understanding the regulation of cccDNA formation is crucial for developing strategies to eliminate or control chronic HBV infection.

Regulation of cccDNA

The cccDNA is a key component in the life cycle of HBV. It serves as a stable template for the transcription of viral RNAs. The regulation of cccDNA is crucial in understanding the persistence of chronic HBV infection. Current antiviral treatments primarily target viral replication but have limited impact on cccDNA. Further research needs to identify new targets and develop approaches to modulate cccDNA levels, which is essential for achieving a functional cure for chronic hepatitis B.

What is the epigenetic profile of cccDNA?—DNA methylation and histone modifications play a role in the epigenetic regulation of cccDNA. Methylation of CpG motifs in the cccDNA minichromosome has been observed in some studies, and it is associated with transcriptional repression. Histone modifications, such as histone acetylation and methylation, also influence the accessibility of cccDNA to transcriptional machinery (Xia and Guo, 2020).

Why is cccDNA copy number low in infected hepatocytes?—HBV has evolved to efficiently replicate and transcribe its genome within hepatocytes. The cccDNA serves as a template for transcription, leading to the production of viral RNAs. However, the efficiency of transcription and replication may not always result in a high copy number of cccDNA(Xia and Guo, 2020). The viral life cycle is finely tuned to balance productive infection with avoiding host immune responses.

What is the half-life of cccDNA?—The half-life of cccDNA is a topic of active research and may vary among individuals. Several studies have suggested a long half-life for cccDNA, contributing to the chronic nature of HBV infection (Boyd et al., 2016; Lythgoe et al., 2021), whereas genetic studies of resistance mutation accumulation and revision indicate a much shorter half-life (Huang et al., 2021). Precise measurements of cccDNA half-life can be challenging due to technical and ethical considerations. The technical gap in measuring cccDNA lies in the complexity and specificity required for accurate quantification. Traditional methods often lack the precision needed to differentiate cccDNA from other forms of HBV DNA, leading to challenges in understanding its dynamics and persistence during infection and treatment.

What makes cccDNA difference from host DNA (ex. Smc 5/6 recognition)?

—cccDNA is different from host chromosomal DNA in several ways. One of the distinguishing features is the episomal nature of cccDNA, which sets it apart from the integrated host DNA. Additionally, cccDNA is recognized and restricted by specific host cellular factors, such as SMC5/6 (Decorsiere et al., 2016). However, there are too many unanswered questions. The recognition of cccDNA by cellular factors like SMC5/6 underscores the host cell's efforts to regulate and control viral infection. The interaction between cccDNA and host cellular components contributes to the dynamic interplay between the virus and the host. Understanding these interactions is crucial for developing therapeutic strategies aimed at targeting cccDNA.

HBV expression, replication, and egress

The discussion focused mostly on HBV expression and replication, highlighting major gaps in knowledge in the steps leading to cccDNA formation and early transcription and on the biology of HBV RNAs. One crucial question addressed the timeframe of the beginning of cccDNA transcription respect to cccDNA appearance in the nucleus and the kinetics of Smc5/6 potential silencing vs HBx transcription. Increasing evidence has shown that cccDNA formation is an early event after HBV entry and that HBV RNAs are detectable at around 24h post-infection (Ko et al., 2018; Locatelli et al., 2022; Luo et al., 2017). However, the possibility that certain HBV transcripts might be produced before or concomitantly to DNA repair/histone deposition during the conversion of rcDNA to cccDNA has never been explored. HBx RNA, which has been demonstrated in virions that enter cells during infection, could prevent Smc5/6-dependent cccDNA silencing (Niu et al., 2017; Stadelmayer et al., 2020) upon entry, though this remains to be confirmed. If that would not be the case, what is preventing Smc5/6 complex from silencing cccDNA before the expression of HBx protein?

This leads to additional questions: i) what additional interactors of HBx or regulators of Smc5/6 might be involved in cccDNA transcriptional regulation; ii) if entry signaling pathways might be involved in the subsequent cccDNA formation and transcription; iii) whether host factors regulating HBV expression differ across CHB phases; iv) whether HBc would play a role in cccDNA formation and transcription. In this respect, it has been demonstrated that *de novo* synthesis of HBc is not required for cccDNA transcription (Tu et al., 2021; Zhong et al., 2022), but what about HBc incoming with the virions? Indeed, these proteins have been observed to have a long half-life and to associate to cccDNA as soon as it is detectable in the nucleus, as well as at later time points (Locatelli et al., 2022; Lucifora et al., 2021).

Regarding HBV RNAs, the discussion highlighted the struggle in understanding and studying the complexity of HBV transcripts species (overlap of sequences, presence of spliced variants). In addition, there is no clear “best practices” in the use of PCR-derived and sequencing techniques allowing to appreciate the pros and cons of the techniques and, thus, to help in correctly interpreting the results. Part of the discussion touched on miRNAs and their role in regulating HBV expression. A huge amount of literature is present on the subject, but in diverse models, thus rendering difficult a clear picture of the crucial miRNA

involved in HBV biology. Interestingly, the question was raised if HBV itself was able to produce miRNA and, more broadly, non-coding RNAs, as it has been observed for other viruses (Li et al., 2022).

When the discussion verted on potential therapeutic targets, cccDNA and pre-genomic RNA were elected as the central molecules to tackle. Questions were raised about the pertinence of using a CRISPR/Cas9 approach and the advent of Cas9-derived technologies not necessitating DNA double strand breaks (reviewed in (Martinez et al., 2022; Zhang and Tu, 2023)) and how it would be possible to accelerate cccDNA degradation and if it would be better to block cccDNA formation or accelerate its degradation. Regarding pre-genomic RNA, much interest is gathered around the host factors that could interact with the epsilon loop (which might include epitranscriptome modifiers (Kim et al., 2022)), and the fundamental amino acids of HBV polymerase involved in retro-transcription.

Genotypes, variants, and evolution.

There are a multitude of new therapies being studied for chronic HBV infection. An important question for the field is “Will there be genotype specific responses to these therapies for patients with different genotypes?”. This question extends to basic research work in the field. There has been a bias towards work done with genotype D. The molecular tools used by many have been developed using this genotype, including the stable cell lines currently used for virion production for *in vitro* infection studies (Ladner et al., 1997; Sells et al., 1987). It will be important to determine if there may be genotype differences across all aspects of the viral ‘life cycle’. Are there also differences in the host-virus interactions for HBV of different genotypes, or differences in the immune response? To aid in answering these questions, the field needs cheaper and simpler diagnostics for genotyping and to also assess disease progression.

Despite the available effective vaccine, there remain concerns about vaccine escape variants. Is the efficacy of the current vaccine equivalent across all genotypes? Are boosters required for different genotypes? Are different epitopes targeted by neutralizing antibodies in different genotypes? Do we need new vaccines, which will need testing and implementation, using standardised correlates of protection?

Accumulation of mutations or variants occurs during chronic infection and may be involved in disease progression and pathogenesis (Kao et al., 2003; Liang et al., 2021). The mechanisms by which these variants are selected within the diverse viral population within an individual needs to be determined. How common is dual infection, does this occur by superinfection or co-infection? The selection and identification of potential antiviral resistance variants to new therapies may also be an emerging challenge for the field.

Research into the evolution of HBV has undergone a revolution in the past few years, with the discovery of ancient HBV (Krause-Kyora et al., 2018; Muhlemann et al., 2018). This has led to many more questions than answers. What and where was the origin of HBV? Has it co-evolved with each host? What are the origins of the different genotypes and what is the evolutionary rate of this virus? Are there potentially new genotypes still

undiscovered? Could additional ancient samples be detected from around the globe? What are the differences between ancient and modern viruses? Can we learn from the ancient viruses that have become 'extinct'? In addition, there are now many more members of the *hepadnaviridae* family identified (Lauber et al., 2017; Revill et al., 2020). There needs to be more work done to examine the potential for cross-species transmission creating a zoonotic event, given some of these members also use NTCP as an entry receptor (de Carvalho Dominguez Souza et al., 2018; Drexler et al., 2013; Shofa et al., 2023).

Viral pathogenesis

Discussions of viral pathogenesis centred around 4 major themes: viral molecular factors, cellular molecular factors; clinical challenges; and current technical shortcomings.

Viral molecular factors

A major highlighted unknown was how HBV infection affects cellular pathways and the exact mechanisms as to how it does so. The effects of HBV proteins and integrated HBV DNA have not been well characterised and may play some roles in disease progression.

Potential roles for HBV pathogenesis were not limited only to HBV as represented by a single clone, but also how these pathways are affected by HBV genomic diversity (e.g., different mutations in viral proteins). Moreover, viral pathogenesis does not occur with HBV alone: in many cases, co-infection with other viruses (including HDV, HIV, and HCV) exacerbates pathogenesis (Cheruvu et al., 2007; Mathurin et al., 2000; Singh et al., 2017). Greater exploration and characterisation of the underlying molecular interactions between HBV and these other viruses is needed to understand this enhanced disease and develop ways to prevent it.

Host molecular factors

There was a recognition that the complex host system may contain several factors that affect how HBV is regulated (e.g., diurnal fluctuations impacting antiviral immunity, ALT level, or viral replication (Zhuang et al., 2021)). Not only the level, but also quality of the antiviral immune response is likely to be important in pathogenesis with participants highlighting the need to determine the specific factors and dynamics in this response that lead to pathogenic vs. antiviral responses (e.g., "bad" flares vs. "good" flares (Ghany et al., 2020)). This quality of antiviral response is likely to vary from time of infection acquisition (e.g., neonatally vs. adult exposure) and is reflected in the outcome of infection (acute vs. chronic) (McMahon et al., 1985), but little is known about the underlying mechanisms.

The contributions of non-hepatocyte factors (including liver extracellular matrix, stellate cells, immune cells, and intrahepatic gradients) to pathogenesis also remain unclear. Some of these may even be targets of viral entry and expression through non-NTCP entry or transfer of HBV into non-hepatocyte cells (such as reported replication in kidney cells, PBMCs, nail-bed tissue, and hair (Komatsu et al., 2022; Torii et al., 2003; Wang et al., 2024)) to unknown effect.

Clinical aspects

A clearly recognised shortcoming in developing HBV curative therapies is understanding to what extent the various endpoints for cure reduce risk of liver cancer and whether this differs between different treatment modalities (e.g., interferon-containing vs -free treatments) or outcomes (e.g., HBsAg-negative vs. anti-HBs positive). This requires an understanding of the underlying mechanisms driving cancer and liver injury.

Gross histological changes can occur in patients who have previously been categorised as “immune tolerant” and with normal ALTs (Lin et al., 2022). However, it is unclear if molecular changes associated with this pathogenesis occur earlier (and if so, how much earlier). Understanding this could enable development of biomarkers necessary for earlier diagnosis and measuring the effect of potential therapeutics.

There is also a growing concern amongst the patient population regarding the side-effects of nucleoside therapy. It is unclear what drives the broad range of symptoms associated with long-term antiviral use (Liu et al., 2023), but this should be an additional field of research as more patients are put on reverse transcriptase inhibitor therapies.

Technical needs

To answer these scientific questions, accurate and representative models are required, but are still lacking. The current cell culture and animal models are unlikely to reflect most of the aspects occurring in patients (both in quality and quantity). This is the major barrier to link results from basic science experiments to the implementation of potential diagnostics and treatments. Timely and accurate models of liver cancer and inflammation remain the biggest issues in the viral pathogenesis field.

Immunology

While the focus of the immunology discussions was on the protective role of immune responses in viral control, participants also discussed how the immune response might contribute to the pathogenesis of progressive liver disease, including specific circumstances such as flares post-pregnancies. All groups of EMCRs who joined the discussions agreed that future investigations will focus on three significant scientific challenges: 1) studies focusing on liver-specific immunity in the context of HBV infection; 2) mechanistic understanding of HBV-specific immune dysfunctions and how therapeutic interventions can overcome them; 3) the establishment of immune correlates of viral control and/or clearance and the importance of biomarkers to access HBV-specific immune responses in chronic HBV patients.

Liver-resident HBV-specific immunity

As HBV exclusively infects human hepatocytes, immune responses occurring directly at the site of infection are seen as crucial for viral control. Although studies of intrahepatic HBV-specific immunity have been performed in patients for over 30 years (Barnaba et al., 1989; Ferrari et al., 1987; Maini et al., 2000), recent technological advancements have significantly expanded our understanding of the intrahepatic immune landscape (Pallett and

Maini, 2022). The EMCRs discussed the ongoing progress in this area, noting that novel techniques such as single-cell RNA sequencing and spatial transcriptomics, combined with tools to visualize HBV-specific T and B cells, will better define liver-resident immune cell populations and their topological relationships. While recently established less invasive liver sampling via fine-needle aspiration facilitates even longitudinal studies in tissue (Genshaft et al., 2023; Gill et al., 2018; Gill et al., 2019; Nkongolo et al., 2023; Pembroke et al., 2015; Sprengers et al., 2005), both pre- and post-therapies, or as liver disease progresses, EMCRs highlighted the need for comparative studies in blood to identify correlates that can be studied in larger patient cohorts.

One group highlighted the challenge of modeling chronic HBV infection and mechanisms of HBV-specific immune dysfunction in mice (Ploss et al., 2021). They suggested using woodchucks and WHBV for immunological studies instead of relying solely on dually engrafted humanized mice. Woodchucks offer a better model for studying the natural history of chronic infection, but we lack immunologic tools.

Mechanisms of HBV-specific immune dysfunction

Immunology studies in the context of chronic HBV infection mostly investigate the mechanisms leading to HBV-specific B and T cell dysfunction (Alfei et al., 2019; Boni et al., 2007; Das et al., 2008; Fisicaro et al., 2017; Kurktschiev et al., 2014; Lopes et al., 2008; Pallett et al., 2017; Pallett et al., 2015; Peppa et al., 2013; Sandalova et al., 2012; Schmidt et al., 2021). We now know that they differ from classical hierarchical T cell exhaustion observed in response to persistent viral or tumor antigen stimulation in LCMV or cancer models (Virgin et al., 2009; Wherry, 2011). HBV-specific T cells in chronic patients, however, show a vast heterogeneity in terms of phenotype, functionality, and metabolic profiles (Cheng et al., 2019; Heim et al., 2020; Hoogeveen et al., 2019; Schuch et al., 2019; Winkler et al., 2023), even within the same patient. Hence, the underlying mechanisms for HBV-specific T cell dysfunction are probably heterogeneous, and likely due to the variable degrees of HBV antigen presentation and the particularities of hepatic T cell priming (Benechet et al., 2019) within the tolerogenic liver environment (Thomson and Knolle, 2010). The EMCRs discussed that archiving a detailed understanding of these mechanisms will be crucial for the development of novel immunotherapies that could lead to functional cures.

Biomarkers to access HBV-specific immune response:

The EMCRs discussed the significance of immune reconstitution in restoring dysfunctional virus-specific T and B cells as a likely requirement for a functional cure of chronic HBV infections (Fanning et al., 2019). However, they emphasized that one of the major challenges in the field is the absence of immunological biomarkers capable of identifying correlates for viral clearance and/or long-term control. This view is shared among established researchers in the field, and efforts are taken to standardize immunological assays, to allow their implementation in clinical studies of novel therapeutic approaches (Gehring et al., 2022). Presently, only the detection of HBV-specific T cells following *in vitro* expansion was shown to predict viral control upon therapy discontinuation (Garcia-Lopez et al., 2021; Rivino et al., 2018). However, there is a consensus that simpler and more scalable assays,

requiring minimal *ex vivo* manipulation, ideally performable with small blood volumes, are needed for their implementation into routine patient management (Bertoletti, 2022; Bertoletti and Le Bert, 2023; Chua et al., 2023; Li et al., 2023; Rossi et al., 2023). Whether these assays will effectively identify patients more likely to respond to particular therapies remains to be seen.

Antivirals and immunotherapies

Direct antivirals

The EMCRs acknowledged the importance of recent innovations and the progress made in further developing advanced direct antiviral therapies, including entry inhibitors, capsid assembly modulators, RNA interference approaches, as well as applications targeting cccDNA and their overall benefits in promoting a functional cure (Fung et al., 2022). It was highlighted that new antivirals are required to effectively target and equally affect all HBV genotypes, alongside the aforementioned necessity to avoid a research bias towards specific genotypes. The financial and timely benefits of investigations into drug repurposing as novel antiviral therapies for CHB in the future were also discussed. Having the patient's direct benefits and needs during treatment regimens in mind, the group supported the notion that novel therapies are developed ideally for oral application.

Immunotherapies

While direct- and indirect-acting antivirals are key aspects of therapeutic research and essential part of developing future curative CHB therapies, the EMCRs also centered their discussion around adoptive immune cell applications, including CAR-T cell, T-cell, and NK cell therapies that are at the forefront of immunotherapeutic developments and complement the application of checkpoint inhibitors and the development of therapeutic vaccines to promote the elimination of infection (Bertoletti and Le Bert, 2018; Hoogeveen and Boonstra, 2020). The group underlined the necessity of tailored HLA-specific or, alternatively, HLA-independent approaches. On new and upcoming technologies, participants discussed the idea and the need for more individual and personalized medicine approaches during immunotherapy and to look ahead into new and upcoming technologies, including the incorporation of artificial intelligence in our overall research endeavors towards HBV cure (Dost et al., 2023).

Key questions and future challenges in therapy development

Key issues in therapy development were highlighted, including target specificity and off- / on-target toxicity, but also the overall feasibility of multi-layered and time-consuming therapy approaches to tackle the complex disease of CHB (Lim et al., 2023). There is consensus that further focus needs to be applied to accessibility and involved costs of these future therapies for patients with limited access to quality health care, particularly in developing countries. Concerns about the broad applicability of novel antivirals and immunotherapies remain, including the role of individual patient clinical features such as age, underlying coinfections, long-term infection, and advanced progressive liver disease. The EMCRs also shared their thoughts on the major challenges and tasks lying ahead of our research community. The primary focus in therapy development remains the effective

prevention or risk reduction of hepatocellular carcinoma in chronic HBV patients. Overall, the greatest importance was given to the need to effectively target episomal cccDNA and HBV DNA integrations within hepatocytes to eliminate the infection and cure CHB (Zoulim and Testoni, 2023).

Epidemiology and Hepatitis B Elimination

Discussion on the epidemiology and global elimination of hepatitis B included challenges and questions for how to address hepatitis B/D across the entire care continuum: from improving awareness, to prevention, to getting people tested, and into care and treatment. The group questioned why hepatitis B and liver cancer continue to be neglected diseases, and discussed the great need to create urgency, particularly among governments and funders to prioritize and fund hepatitis B research and elimination programs. The group felt that a strong, vocal global advocacy movement is needed, as it would help us attract the attention of key policy and decision makers.

To improve overall awareness, the group discussed the idea of creating more messaging focused on the link between hepatitis B/D and liver cancer, as a strategy that would make all stakeholders take more notice. The group agreed that improving awareness among highly impacted countries was key to getting more people tested and diagnosed, but also thought that we need to improve access to affordable and accessible diagnostics, including point of care testing for both hepatitis B and hepatitis D. In addition to improving awareness and testing access, it was felt that we need to broadly normalize hepatitis B testing and remove the stigma and taboo associated with hepatitis B (Tu et al., 2020). The group also considered whether automatic reflex testing for hepatitis D of all people diagnosed with hepatitis B would help improve diagnosis of people living with co-infection.

The discussion also focused on the need to improve the affordability and accessibility of hepatitis B/D treatment. They questioned the role of governments and large global funds in helping to address treatment costs and remove cost-associated burden, especially for people in low- and middle-income countries (Howell et al., 2023; Polaris Observatory, 2023). To improve accessibility, the group discussed the role of primary care in managing and treating hepatitis B, and whether we could implement strategies to support primary care providers globally in improving their interest and capacity.

Addressing vaccine hesitancy and improving uptake of vaccine was thought to be a particularly important strategy towards hepatitis B/D elimination, and group consensus was that the vaccine is currently under-used – particularly in eliminating perinatal transmission. Overall, perinatal transmission was a key component of prevention (Khetsuriani et al., 2022), one that is both achievable and currently neglected.

Hepatitis Delta

Systematic screening & epidemiology

One of the main issues raised was the absence of systematic screening worldwide within the HBsAg carriers (Abdul Majeed et al., 2023). This reflects the relative difficulty to rely

on solid epidemiological data that would help to estimate the impact of HDV on public health, which was the second message from the participants. Major efforts in this direction are needed soon to fill this gap and get a clear evaluation of the importance of hepatitis D worldwide.

Therapeutic strategies

Collectively, we were all convinced that despite recent improvements in the management of patients (European Association for the Study of the Liver. Electronic address and European Association for the Study of the, 2023) and the use of bulevirtide in Europe (Dietz-Fricke et al., 2023), novel therapeutic strategies are urgently needed. In this context, innovative strategies, and combination therapies, including the development of specific siRNA and, although highly challenging, specific direct acting antivirals targeting HDAg (reviewed in (Asselah and Rizzetto, 2023)). It is important to keep in mind that HDV is highly difficult to target directly, given the nature of the replication cycle mostly relying on cellular polymerases and factors (Turon-Lagot et al., 2020). Alternative therapeutic strategies based on host-targeting agents require a deep understanding of the molecular interaction between the virus and cellular factors, which should be another research priority.

Pathogenesis

As a neglected disease, there is also an urgent need to better understand the nature of the disease, including the interaction between the virus and the immune response (e.g., the potential role of T-cell exhaustion (Oberhardt et al., 2022)) and the interaction between HDV and HBV genotypes. In this context, there is a lack of robust model for the study of HDV-induced pathogenesis. Innovative immunocompetent models are then required, that may include HBV/HDV-susceptible macaques that are currently under development. Taken together, multidirectional efforts are needed to better understand the disease and proposed new therapeutic strategies for the management of chronic hepatitis D, the most severe form of chronic viral hepatitis.

Support structures and research tools for Emerging Scientific and Medical Researchers

To assess primary needs and develop related supportive strategies, one section of the workshop focused on structures and tools for EMCRs. Participants were asked what major career barriers they have experienced, what upcoming challenges they foresaw, what types of resources, meetings, events, or support would be most helpful.

Attendees' primary concerns included:

Availability of training in core techniques and technologies.

Establishing a means to transfer key technological and procedural "know how" to emerging researchers was strongly desired. Attendees were encouraged to reach out to established members of the field to seek training and career advice as most members of the HBV community are very willing to share their expertise.

Limited career advancement options.

This was a frustration shared by many attendees. Unfortunately, there is no simple solution given the limited number of research positions and restricted funding world-wide, and the problem is exacerbated by the fundamentally different research structures around the world. Attendees were encouraged to be flexible, patient, and persistent as they sought to advance to the next level of their careers.

Expanded networking opportunities.

Networking with peers and senior members of the HBV community was recognized as essential to expanding career opportunities for junior researchers. Attendees were encouraged to initiate conversations with senior researchers, attend social functions at scientific meetings, and to reach out beyond their comfort zone when in networking situations.

Information on job opportunities.

Frustration with the difficulty in identifying career advancement opportunities was expressed by attendees. The highly variable nature of the job markets in academia and industry makes this difficult to resolve. Attendees were urged to consult as many sources of information as possible, and especially to seek guidance from their mentors.

Neglecting the importance of HBV.

HBV research is substantially underfunded relative to the virus' medical impact, and this was extremely frustrating to the attendees. Attendees were encouraged to work with lobbying and public health organizations such as the Hepatitis B Foundation to raise awareness of the need for resources to combat HBV among funders and decision makers in their countries. Advocacy to raise HBV awareness among the general population in their countries will also help make the need clear to the relevant decision makers.

Understanding of women's and family issues.

The unique demands and pressures on women scientists were discussed, and the conversation broadened into family matters that impact men also. This is very difficult to address as it is impacted by traditional expectations on women and the high expense of providing childcare support for young families. Attendees were encouraged to utilize all available support programs in their intuitions, to seek such services when negotiating a new position, and to preferentially take positions that provide such support.

Career transition funds.

The paucity of funds to help junior investigators transition to full independence was frustrating to attendees. Addressing this requires expanding funding opportunities for emerging investigators, which in turn requires advocacy for HBV research (such as that promoted by the Hepatitis B Foundation) among policy leaders and decision makers in their countries. Attendees were encouraged to become active in such advocacy efforts in their respective countries.

Participant feedback and lessons learned

All participants were also surveyed after the workshop to measure their experiences and to enable feedback on this networking approach. Across all panels, our approach stimulated strong engagement and free discussion amongst the participants. A post-event evaluation survey indicated that of respondents (n=17), 80% found the workshop to be very/extremely useful (4/5 on a 5-point scale) as a networking opportunity, and 73% found the workshop to be very/extremely useful for professional/career development. Additionally, 54% felt the workshop was very/extremely useful, and 40% felt it was somewhat useful, for identifying and discussing important questions in hepatitis B, hepatitis D and HCC research.

In terms of workshop format, 86% found the workshop to be very/extremely engaging, and 72% felt the format was very/extremely conducive for generating important conversation. When asked about interest in specific topics for a 2024 workshop, the most requested topics were research mentorship (79%), networking with other early career scientists (72%), clinical research (65%), career advancement (57%), career transition opportunities (50%) and prioritization of hepatitis B/D research (50%). Additional suggestions from respondents included allowing more time for in-depth discussion and networking among participants, as well as more focus on career development, career mentorship, and professional opportunities for students and post-docs.

The workshop organizers are committed to diversity and inclusion. A takeaway from this inaugural workshop is that organizers need to focus on recruiting more emerging scientists from Africa, both for the organizing committee and for workshop participation. With the 2023 meeting in Kobe, there was significant representation from emerging researchers in Asia and Europe, primarily due to travel constraints. Future effort will focus on ensuring significant representation of emerging African researchers.

In conclusion, the inaugural International Emerging Researchers Workshop was an effective approach for engaging and activating the EMCR community, with respect to both enabling networking and accumulating the knowledge of those directly working in the lab. We suggest that this approach can be widely applied to all scientific fields to support their future leaders and strengthen bonds within research communities.

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Table 1 –

Discussion topics

Topic	Led by
Experimental Models	Jochen Wettengel
Viral entry and cccDNA biology	Yuchen Xia
Expression, replication, and egress	Barbara Testoni
Viral genotypes, variants, and evolution	Margaret Littlejohn
Viral pathogenesis	Thomas Tu
Immunology	Nina Le Bert
Antivirals and immunotherapies	Gregor Ebert
Epidemiology and Hepatitis B Elimination	Chari Cohen
Hepatitis Delta	Eloi Verrier
Support structures and research tools for EMCRs	John Tavis

Table 2 –

Discussion questions and prompts

For scientific topics	**For research tools**
What do you think are the most significant scientific questions for this topic?	What are the major career barriers that you have experienced?
What do you think are the greatest challenges for this topic?	What challenges can you see coming up?
Imagine the next big Nature/Science/Cell paper in this field: what is it about?	At this point in your career, what resources or support would be helpful for you?
What aspects of this topic intersects with your main research topic? Are there synergies that you can see?	What types of events or meetings could we integrate into the annual HBV Meeting would be most helpful to you?