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Addressing the next challenges: A summary of the 22nd international symposium on hepatitis C virus and related viruses

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Summary

Following the discovery of the hepatitis C virus (HCV) more than 25 years ago the field has succeeded to develop methods that have changed the safety of blood products, understand the molecular virology, epidemiology and clinical disease of HCV, and identify specific targets for the development of direct-acting antivirals for HCV cure. Nevertheless, major clinical and scientific challenges remain: therapy is still only available to a fraction of infected patients worldwide and many patients remain undiagnosed and/or live in countries where therapy is unattainable. An urgently needed HCV vaccine to eradicate infection remains still elusive. Scientifically, major questions remain regarding the life cycle, pathogenesis and mechanisms of viral clearance and persistence. Addressing these challenges, this meeting report reviews key findings of the 22nd

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Conflict of interest

The authors declare no competing financial interests. TFB has served as advisor for Gilead, Biotest and Vironexx. Inserm, the University of Strasbourg and Genovac/Aldevron Freiburg have filed a patent application on monoclonal anti-claudin1 antibodies for the inhibition of hepatitis C virus infection (US Patent # 8,518,408; WO2010034812; PCT/EP 08 305 597 0).

Authors' contributions

All authors contributed by writing the manuscript and editing the final version.

International Symposium on Hepatitis C Virus and Related Viruses in Strasbourg, France from October 9 to 13, 2015.

Keywords

Virus; Liver disease; Therapy; Immune responses; Viral hepatitis; Meeting report; Hepatitis C virus; Vaccine; Treatment; Resistance; Molecular virology

Introduction

Around 500 researchers from around the world attended the 22nd International Symposium on Hepatitis C Virus and Related Viruses in Strasbourg, France from October 9 to 13, 2015. This meeting report summarizes key findings of the 76 oral presentations and 261 posters and review topics of keynote, primer and plenary lectures.

In their opening remarks the meeting organizers reviewed the progress and next challenges: within the last decade, tremendous progress has been made in the understanding of the hepatitis C virus (HCV) life cycle, viral pathogenesis, diagnosis and treatment. The recently developed direct-acting antivirals (DAAs) now enable high cure rates but DAAs are currently only available to a fraction of patients and there is still no vaccine to prevent HCV infection [1]. Furthermore, the HCV life cycle, pathogenesis of virus-induced liver disease and cancer as well as the mechanisms of viral evasion are only partially understood. Clearly, HCV is a highly relevant human viral infection that offers significant challenges to clinicians and scientists in the future.

Clinical challenges

In his primer, Jean-Michel Pawlotsky highlighted the recent progress in the management of chronic hepatitis C with new combination treatments based on DAAs and reviewed the emerging clinical challenges regarding treatment failure and resistance [2]. He emphasized the need to address these challenges by better understanding of treatment failure and providing novel treatment approaches for patients with resistance to the DAA combination therapies where treatment options are not defined. In her keynote, Brigitte Autran reviewed immunopathogenic mechanisms in HCV-HIV co-infection. Several presentations reported new insights gained from clinical cohorts. O'Brien *et al.* reported how precision medicine based on subgroup identification taking into account gender, viral load and rs12979860 genotype could identify patients likely to be cured with shorter treatment duration. Dahari *et al.* indicated that early viral kinetic analysis showed that only a minor fraction of patients might require full-length DAA treatment, suggesting that individualizing the duration of DAA therapy while on treatment enables cost saving. Foster *et al.* reported that 12 weeks interferon (IFN)-free therapy with sofosbuvir, a NS5A inhibitor and ribavirin enables SVR12 in most of the patients with decompensated cirrhosis. Noteworthy, daclatasvir was more efficient in genotype 3 infected patients than ledipasvir. Malespin *et al.* pointed out that for sofosbuvir-based combination therapy, detection of HCV RNA at the end of treatment should not be viewed as a treatment failure given that the vast majority of these patients achieved SVR12. Larouche *et al.* revealed that low quasispecies diversity is a key

risk factor for mother-to-child transmission of HCV. Moreover, Gambato *et al.* showed that distribution of HCV quasispecies is associated with cholestatic hepatitis C patients undergoing liver transplantation (LT), the major strain before LT remained the major strain post-LT. Addressing the challenges of DAA-based therapy, two studies focused on potential novel strategies to prevent infection of the graft during LT and/or cure chronic HCV infection using entry inhibitors. Using human liver-chimeric uPA-SCID mice, Mesalam *et al.* showed that HCV infection can be prevented and/or delayed by administration of a broadly neutralizing human monoclonal antibody (mAb) targeting HCV, while Tawar *et al.* showed efficient HCV clearance following monotherapy treatment of humanized mice using a humanized mAb targeting CLDN1 on the hepatocyte cell surface. Finally, two studies performed in cell culture models reported new data on viral resistance to DAAs. Serre *et al.* showed viral fitness of protease resistant HCV genotypes 1–6 *in vitro* and highlighted the role of substitutions at NS3 positions 155, 156 and 168, while Liu *et al.* indicated that anti-miR-122 therapy can act in an additive or synergistic manner in combination with DAAs and can prevent the emergence of DAA-resistant variants.

Molecular virology – entry

The first molecular virology session of this meeting was dedicated to HCV entry into cells [3]. Following a plenary lecture by Didier Trono on human-specific transcriptional networks and transposable elements, the entry session was introduced by Thomas Pietschmann, who highlighted recent important discoveries in this area. Jackson *et al.* presented a detailed mapping of the subdomains of the HCV E2 glycoprotein that are involved in protein folding and viral functions using a mAb panel and a library of 552 alanine mutations spanning the protein sequence, which will be useful for immunogen design. Using a novel computational method to characterize the functional coevolution of E1 and E2 and the experimental assessment of their coevolution clusters, Douam *et al.* discussed the HCV fusion mechanism during virus entry, highlighting HCV membrane fusion as a unique mechanism among the viral world, with both glycoproteins acting as interdependent members of a single protein complex that mediates virus fusion. The presentation by Jannick Prentoe extended previous findings that the hypervariable region 1 (HVR1) of E2 shields critical epitopes and is a major contributor to genotypic differences in neutralization sensitivity *in vivo*. Using affinity enrichment coupled to quantitative mass spectrometry (AE-qMS), Gerold *et al.* described serum-response factor binding protein 1 (SRFBP1) as a novel pan-genotypic HCV-specific host entry co-factor that is rapidly recruited by CD81 at the initial steps of infection, likely at the level of membrane fusion. Fukuhara *et al.* revealed through analysis of HuH7 cell lines deficient in scavenger receptor class B type-I (SR-BI) and low-density lipoprotein receptor (LDLR) genes by using CRISPR/Cas9 system that SR-BI, LDLR and very low-density lipoprotein receptor (VLDLR) redundantly participate in HCV entry. Finally, Griffin *et al.* uncovered novel potent inhibitors of HCV p7 protein and presented novel evidence that p7 ion channel activity may play a role during virus uncoating, analogous to the influenza M2 proton channel. Altogether, the findings presented in this session highlighted the relevance of host cell entry factors as targets for novel antivirals and highlighted novel functions or epitopes of HCV E1E2 proteins for vaccine design.

Viral assembly

HCV non-structural proteins are known to be involved in virion morphogenesis [4]. Zayas *et al.* focused on the role of NS5A in HCV assembly, and they identified a conserved cluster of basic amino acid residues at the N-terminus of NS5A DIII domain that is also critical for particle assembly. Furthermore, their data suggest that NS5A DIII contains two determinants regulating assembly at different, but closely linked steps: (i) recruitment of replication complexes to core protein; and (ii) RNA genome delivery to core protein to trigger encapsidation, which is tightly coupled to particle envelopment. Other presentations focused on cellular factors involved in HCV assembly. Lussignol *et al.* showed that, in HCV-infected cells, the nucleoporin Nup98, a component of the nuclear pore complex, re-localizes to cytosolic lipid droplets, where core protein accumulates. They also showed that Nup98 interacts with the viral genome and is incorporated in HCV viral particles. In another study, Roesch *et al.* showed that the host factor annexin A3 is recruited to lipid droplets in HCV-infected cells. Knockdown of annexin A3 had no effect on HCV RNA replication, but progeny virion production was severely impaired. Core envelopment was not compromised, but the specific infectivity of secreted virions was decreased in annexin A3-knockdown cells, suggesting that this host factor regulates the production of infectious HCV particles. Wozniak *et al.* hypothesized that HCV might alter trafficking machinery to promote its secretion. Their data indicate that Rab7-inter-acting lysosomal protein (RILP), a key regulator for late endosomal/lysosomal trafficking, is cleaved during HCV infection and that HCV-induced cleavage of RILP generates a novel adaptor protein that enhances outward, kinesin-mediated trafficking to increase HCV particle secretion. The HCV virion has the unique feature of being associated with lipoprotein components [5]. However, viruses produced in hepatoma cells do not fully recapitulate the composition of patient-derived virus. To produce *bona fide* viral particles, Calattini *et al.* infected liver humanized FRG mice with cell culture-derived HCV and characterized the viral subpopulations produced *in vivo*. By this approach, they demonstrated that the composition and biophysical properties of these viral subpopulations modulate their levels of infectivity and receptor usage.

HCV translation and replication

In his keynote lecture Ralf Bartenschlager reviewed the replication strategies of HCV and Dengue highlighting differences and similarities [6,7]. HCV is known to induce rearrangement of intracellular membranes to replicate its genome [6], and understanding these modifications is a hot topic in HCV research. Gastaminza *et al.* performed cryo soft X-ray tomography to investigate in whole, unstained cells, the ultrastructural alterations induced by HCV replication, and obtained native tridimensional maps of cellular modifications caused by persistent HCV RNA replication. HCV replicating cells show enlarged tubules with prominent pseudospherical extrusions. They have also observed a profound alteration of the mitochondrial morphology that correlated with the extent of ER modifications, and their data indicate a topological relationship between altered mitochondria and modified ER tubules. Romero-Brey *et al.* have dissected the determinants within NS5A responsible for double membrane vesicle (DMV) formation in HCV-infected cells. They found that the RNA binding domain 1 (D1) as well as the amino-terminal membrane anchor are indispensable for this process, whereas D2 and D3 are not involved.

Importantly, accelerating cleavage kinetics at the NS4B-5A site diminished DMV formation, indicating that regulated polyprotein cleavage is essential for efficient DMV biogenesis. Eyre *et al.* used mass spectrometry to identify several phosphorylation sites on NS5A in the context of HCV-infected cells. By site-directed mutagenesis, they demonstrated that Ser-235 phosphorylation is essential to HCV RNA replication and membrane-bound replication complex formation and may be regulated by PI4KIII α and CKI- α kinases. Fogeron *et al.* reported the expression, purification, characterization and membrane reconstitution of NS2. For this purpose, they developed a wheat germ cell-free expression system to overcome cellular toxicity of this protein. This system allowed them to successfully produce and purify milligram amounts of a detergent-solubilized full-length NS2 that should be suitable for solid-state NMR analyses in a native-like membrane lipid environment. McCormick *et al.* showed data suggesting the existence of two functionally distinguishable compartments in the membranous web: a NS3-5B derived compartment housing RNA-dependent RNA polymerase activity, and another compartment, accessible by either NS3-5A or NS3-5B, also orchestrating replication dependent events. Catanese *et al.* performed proteomic analyses of mature HCV particles isolated from cell culture. They identified 4 viral proteins and 46 cellular proteins associated with extracellular HCV virions, revealing complex virus-host interactions.

Viral replication – host factors

Takaji Wakita introduced the session by giving a detailed overview on host cell factors involved in HCV replication and membranous web formation. Addressing the role of microRNA-122 (miR-122) in HCV replication, Schult *et al.* using reporters and IRES mutant constructs demonstrated the importance of miR-122 interaction with the HCV IRES region in order to avoid misfolding of the IRES sequence leading to loss of translation/replication fitness. As a result of an unbiased miRNA screening, Li *et al.*, observed a dual role for miR-135a by a direct interaction with HCV IRES sequence and by interfering with antiviral host factors. In the same line, Kang and Luo described miR-122 and miR-194 as required for HCV replication in mouse cellular models. Progress in the understanding of the relationship between lipid metabolism and replication was addressed in several presentations: based on a genome-wide screen, Wong *et al.* observed that during HCV infection human Choline Kinase α (hCK α) is upregulated. In regards to their results, hCK α acts, independently of the CDP-choline pathway, as a modulator of membranous web formation and a partner of NS5A to ease its interaction with the RC and lipid droplets. Proper membranous web formation depends on numerous cell host factors: Harak *et al.* observed that non-adapted strains led to activation of PI4KA and accumulation of PI4P, as it might happen *in vivo*, to build a favourable membranous environment. Inversely, abrogation of cellular lipid kinase PI4KA activation is a consequence of adaptive mutation usage in NS5A and NS5B HCV proteins. Lingbao *et al.* identified the prolactin regulatory element binding protein as a new NS4B interactor, overexpressed during HCV infection, required for proper membranous web formation. In the same line Humphreys *et al.* demonstrated that autotaxin and the lysophosphatidic acid-dependent autocrine pathway, two actors of the lipid metabolism involved in hepatocellular carcinogenesis, favour HCV replication. In contrast, specific inhibition of HCV replication observed by Crouchet *et al.*, following lipid-free

apolipoprotein treatment of the cells was due to a marked cholesterol efflux orchestrated in an ABCG1-dependent manner.

Pathogenesis

Recent evidence indicated that the risk of HCC development persists even in patients who successfully cleared HCV infection [8,9]. It is therefore of utmost importance to better understand HCV-induced pathogenesis including the molecular mechanisms underlying HCC development. In his primer presentation, Francesco Negro summarized the complex interplay between HCV and its host leading to liver disease, including lipid and glucose metabolic alterations induced by the virus. Jessica Zucman-Rossi gave a comprehensive overview of the genomics of HCC in her keynote lecture, highlighting the impact of next-generation sequencing on our understanding of the mechanisms underlying malignant transformation and the identification of potential novel therapeutic targets [10]. Most groups focused on HCV-induced changes in host cell gene expression. Chida *et al.* reported their finding about the role of the transcription factor CREBH in HCV-induced expression of TGF- β 2 and fibrogenesis using a hepatoma/stellate cell co-culture model. Bandiera *et al.* performed a genome-wide study of the epigenetic changes in chronically HCV-infected hepatocyte-like cells and demonstrated epigenetic modifications at loci of genes with clinical relevance for HCV-induced HCC, including *EGF*. Interestingly, several groups reported virus-induced changes in host cell gene expression that may contribute to HCV-induced pathogenesis and at the same time have a proviral effect. Using miRNA profiling in persistently HCV-infected hepatocyte-like cells, liver tissue and computational analysis, Pernot *et al.* identified miR-146a as a putative miRNA involved in liver disease and a proviral host factor. Interestingly, Pène *et al.* reported that ethanol can increase the production of HCV lipo-viro-particles in primary human hepatocytes most likely by increasing VLDL secretion. Furthermore, Okamoto *et al.* showed in HCV core-transgenic mice that signal peptide-peptidase is a crucial host factor for HCV particle production and pathogenesis. Another major interest was the interplay between HCV and the hepatocyte metabolism. Using *in vitro* models and liver tissue, Lévy *et al.* showed that the HCV-induced modulation of metabolic genes, particularly glutaminolytic genes, correlated with fibrosis grades. Noteworthy, glutaminolysis appeared to be required for HCV replication. Likewise, Clément *et al.* reported that HCV may benefit from the insulin-resistant state induced by the virus given that insulin-mediated activation of AS160 increased infectious viral particle production. Furthermore, Lerat *et al.* demonstrated that HCV proteins induce insulin resistance by impairing insulin signaling in HCV transgenic mice. Using HCV transgenic mice and patient-derived liver tissue, Moreau *et al.* reported that a subset of HCV-expressing cells is sufficient to induce global alteration of hepatic metabolic zonation via paracrine communication between HCV-positive and -negative cells. It was also shown by Sharma *et al.* that HCV NS5A can regulate cell growth and apoptosis by stabilizing human GU-rich element-containing transcripts. On the other hand, Harouka *et al.* were interested in potential viral compartmentalization within livers with HCC and reported that HCV genetic diversity was higher in livers containing HCC than in non-HCC cirrhotic livers suggesting that the tumor acts as a viral reservoir. Finally, Zhuang *et al.* highlighted an interplay between HCV and the circadian clock by showing evidence for a circadian regulation of HCV entry and replication. Taken together, this session shed new light on the complex interactions between

the HCV life cycle and the host cell metabolism that likely contributes to liver disease pathogenesis.

Related viruses

The related virus session covered *Flaviviridae* of the genera *Flavivirus* and *Pestivirus* and as a novelty the *Orthohepevirus* hepatitis E virus (HEV). In his keynote Michael Diamond pointed out the critical role of IFN- λ for tightening the blood brain barrier to protect mice from Dengue virus (DENV) and West Nile-induced brain inflammation [11,12]. Tavenau *et al.* presented structural studies on DENV 2 NS5 by small angle X-ray scattering clarifying the relative orientations of methyl-transferase- and RdRp-domains. Scaturro *et al.* demonstrated that the secretion of NS1 from DENV infected cells is dispensable for RNA replication as well as virion morphogenesis. Moreover, he observed novel interactions between NS1 and the structural proteins as well as NS1 residues critical for virion morphogenesis. Hengli Tang introduced human stem cell-derived hepatocytes as a model system to study the biology of DENV infection. Einav *et al.* explained that the inhibition of host cell kinases AAK1 and GAK by anti-cancer drugs affects the replication of HCV, DENV and Ebola virus via targeting cellular sorting adapters. Mortality of DENV in mice could be significantly reduced by these drugs. Acosta *et al.* presented a high-content siRNA screen identifying peptidyl-arginine deiminase type IV as a host factor critical for DENV production. Inhibition of this enzyme affected all DENV serotypes, West Nile virus and HCV, qualifying such drugs as candidates for developing broad-spectrum antivirals. Brett Lindenbach disclosed an innovative approach using a bacterial toxin for a highly specific inhibition of autophagy by cleavage of LC3, which interfered with the replication of yellow fever virus but not HCV. This demonstrated the value of bacterial toxins as a novel tool-box for virologists. Rouillé *et al.* reported an optimized purification protocol for bovine viral diarrhea virus particles and offered first high-resolution EM images as well as insightful lipidomic analysis. Tautz *et al.* presented functional studies based on the crystal structure of a pestiviral single chain NS3/4A-protease demonstrating that the interaction of the C-terminal part of NS4A with the NS3 surface determines whether the NS3/4A complex is used as building block for viral replicases or for the formation of virion morphogenesis complexes. Heather Eccleston reported on the importance of the T cell response to resolve HEV infections in rhesus macaques. The crucial contribution of CD4⁺ T cells was demonstrated by antibody-mediated depletion, which resulted in the induction of viral persistence. Taken together, the session demonstrated the complementary character and impact of the related viruses for HCV research.

Adaptive immunity – cellular and humoral

An overview of the adaptive immunity session was given by Barbara Rehermann. She connected the presented data concerning all key players of the adaptive immune response in HCV infection including their regulation and emphasized the importance of the remaining challenge to develop an active HCV vaccine [13]. Regulation of the adaptive immunity was also the topic of the plenary lecture given by Mala Maini. She summarized current knowledge about the cross-talk of NK cells and myeloid-derived suppressor cells with T cells in HBV infection [14]. A focus of this year's presentations was the definition of molecular determinants of CD8⁺ T cell responses. Andrea Cox showed that PD-1 expression

on antigen-specific T cells is enhanced by TGF β - mediated Smad3. Wolski *et al.* presented data of a gene co-expression network approach that analyzed PBMC-derived HCV-specific CD8⁺ T cells from patients with chronic or resolved HCV infection at several time-points during the acute phase. Importantly, Wolski *et al.* identified modules of genes associated with leukocyte function in correlation with HCV resolution. In another transcriptomic analysis Rosenberg *et al.* defined innate and adaptive immune mechanisms that are active before, during and after acute HCV infection in people who inject drugs (PWID). In PWID, HLA-B*27 allele is protective against HCV GT3 infection due to cross-reactive CD8⁺ T cells that were likely primed in the context of a previous HCV GT1 infection as depicted by Jörg Timm. With respect to T cell functionality, Wieland *et al.* demonstrated that IFN-free therapy-mediated antigen withdrawal leads to a redistribution of HCV-specific CD8⁺ T cell subsets that correlates with an increase in proliferative capacity. Cellular and humoral immunity was linked by the talk of Tobias Boettler who described strong follicular T helper cell signatures on virus-specific CD4 T cells during acute HCV infection in association with the induction of virus-specific antibodies. Merat *et al.* analyzed the E1E2-reactive antibody repertoire of seroconverters from the Amsterdam Cohort Studies among drug users and observed that spontaneous resolvers harbor a broad antibody repertoire recognizing E1E2 from several HCV genotypes. Fong *et al.* reported that the specificities of human mAbs to HCV derived from a subject who spontaneously cleared three successive HCV infections of different genotypes and subtypes did not differ from chronic infection. Regarding epitopes of neutralizing antibodies, Thomas Krey described that the central immunoglobulin (Ig)-like β -sandwich domain in HCV glycoprotein E2 that contributes to the composite binding site for CD81 exhibits extensive structural dynamics.

Vaccines

Despite their effectiveness, DAAs are unaffordable for the majority of HCV-infected individuals; thus, the development of prophylactic HCV vaccines with high efficacy and low cost remains a high priority for the global control of HCV infection [15,16]. This Vaccine session was introduced by a presentation by Hiroshi Yokokawa who showed results of immunization of common marmosets with adjuvanted, purified HCVcc particles, which induced cross-neutralizing antibodies (nAbs) against strains of several genotypes. Wong *et al.* then described a vaccine comprising the two envelope glycoproteins of HCV (E1E2) or only E2 and immunization of goats. Either immunogen induced a strong strain-specific nAbs and E1E2 antisera had broad cross-neutralizing activity at pre-binding and/or early post-binding steps by inhibiting HCV interaction with heparan sulfate proteoglycan (HSPG), SR-BI, and/or CD81. The presentation by Dapeng *et al.* reported a soluble form of E2 protein (sE2) that was produced at high levels in stably transfected *Drosophila* S2 cells. sE2 induced antibodies in mice, rabbits and rhesus macaques that had neutralizing activity against HCV of all seven genotypes and that developed memory T cells specific for E2 in macaques. Finally, Matti Sällberg presented an immune competent mouse model that is based on the syngenic transplantation of H-2b-restricted Hep56 cells containing a self-replicating subgenomic HCV replicon RNA, which can be rapidly applied for the study of HCV-specific cellular immune responses. Altogether, this session highlighted promising HCV vaccine candidates that warrant further pre-clinical and clinical developments.

Innate immunity

The Innate Immunity session illustrated the complexity of the innate immune responses during HCV infection [17]. In a plenary lecture, Percy Knolle gave an overview of his contributions to the current knowledge of the T cell/innate cell cross-talk in the liver including intrahepatic myeloid-cell aggregates for T cell population expansion [18]. Addressing the relevance of NK cells in HCV infection, Felmlee *et al.* observed that peripheral NK cells are enriched, expressed more NKp30 and exhibited enhanced killing activity in HCV exposed yet uninfected subjects. With respect to the regulation of NK cells in HCV, Jacob Nattermann described that monocytes derived from HCV-infected patients who carry the IL28B T/T genotype secreted less IL-12 and IL-18 and thus exhibited a reduced ability to stimulate IFN γ production by NK cells. This finding provides a link between the IL28B genotype with NK-cell function. The type-I IFN response is a key player in the immune response to HCV and thus a target of viral interference with the immune system. Ratna Ray reported that HCV-associated upregulation of miR-373 leads to increased HCV RNA replication by targeting JAK1 and IRF9 and consequently to the interference with IFN signaling. Another immune evasion strategy described by Grünvogel *et al.* showed that HCV replication intermediates are released in exosomes. This secretion of dsRNA reduced activation of TLR3 responses in the exosome-releasing hepatocyte and thus maintained HCV persistence. Additionally, HCV dsRNA-containing exosomes led to cell contact-dependent activation of human PBMC. Hei *et al.* presented that the RIG-I like family member LGP2 is a positive regulator of HCV-induced IFN signaling. Moreover, Hansen *et al.* demonstrated that HCV-triggered autophagy is regulated by Golgi-located human immunity-related GTPase M (IRGM) via UNC-51-like kinase complex (ULK) activation.

Finally, Charles M. Rice presented in his key-note lecture an overview on unmet needs in HCV research and reviewed recent data from his laboratory on miRNA-HCV interactions and recently discovered related viruses in other mammals [19,20].

In summary, HCV2015 was a great success allowing virologists, immunologists, epidemiologists and hepatologists to share the latest findings addressing the next challenges in HCV and related viruses. The 23rd International Symposium on Hepatitis C Virus and Related Viruses will be held October 11 to 15, 2016, in Kyoto, Japan (<https://www.c-linkage.co.jp/hcv2016>).

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