National CIHR Research Training Program in Hepatitis C
Subvention nationale de formation des IRSC sur l'hépatite C

4th Canadian Symposium on Hepatitis C Virus

4ème Symposium canadien sur le virus de l'hépatite C

February 27, 2015 – 27 février 2015

The Fairmont Banff Springs Hotel,
Banff, AB

Alberta & New Brunswick Ballrooms
and Riverview Lounge

Program and Abstracts
Programme et résumés

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Welcome Message

Dear colleagues,

We would like to welcome you to the 4th Canadian Symposium on Hepatitis C Virus (HCV). Over the past 11 years, Canadian researchers have contributed to major discoveries in the field of Hepatitis C. Such discoveries include: the first proof of concept antiviral against HCV, the development of the first humanized mouse model for HCV infection, identification of novel biomarkers for HCV disease progression and treatment outcome and research assessing access to treatment in marginalized populations, in particular people with HIV, people who inject drugs, and First Nations people.

Despite this internationally recognized success, it is evident that interactions between Canadian scientists, clinicians and the affected community need to be strengthened in order to effectively respond to current and future challenges in the management of the disease. We believe that the Canadian HCV conference provides an ideal forum to exchange research findings, promote collaboration, and create synergy among Canadian researchers, practitioners and people living with HCV. We hope that this fourth annual symposium will build on the success of the first 3 meetings and continue to foster knowledge translation for researchers, healthcare practitioners and community-based groups working in the field of HCV. With the recently approved new antiviral drugs, it is indeed an exciting moment for anyone doing research, treating individuals and also for many who remain infected. Let us share our enthusiasm with all of you!

The National Canadian Research Training Program in Hepatitis C (NCRTP-HepC) has significantly contributed to advancing research training and knowledge translation in the field of hepatitis C. It has been very successful in its goal to improve research capacity by developing a network of collaborative investigators whose work encompasses the social, behavioural, clinical and basic sciences, crucial to develop, study and implement effective prevention and care programs to eradicate HCV-related diseases in Canada and worldwide. We are pleased that we are able to continue to contribute to the organization of this important meeting.

We would like to welcome you to this meeting and to the beautiful city of Banff. We look forward to finding out about your exciting research and to discussing together how we can shape the future of Hepatitis C research in Canada.

The organizing committee

Message d'accueil

Chers collègues,

Nous vous souhaitons la bienvenue au 4ème Symposium canadien sur l'hépatite C. Au cours des 11 dernières années, des chercheurs de partout au Canada ont contribué à certaines des découvertes majeures dans le domaine de l'hépatite C. Ces découvertes incluent la première démonstration clinique de l'efficacité de molécules antivirales ciblées contre le virus de l'hépatite C (VHC), le développement d'un modèle de souris humanisée de l'infection par le VHC, l'identification de nouveaux biomarqueurs de la progression de la maladie et les déterminants de l'accès au traitement antiviral chez les populations marginalisées, notamment les individus infectés par le VIH, les utilisateurs de drogues illicites (UDI) et les citoyens faisant partie des Premières nations. Malgré ces succès reconnus internationalement, il est apparu évident que les interactions entre les scientifiques et les cliniciens canadiens avaient besoin d'être renforcées afin de répondre efficacement aux défis présents et futurs que soulèvent l'infection par le VHC. Lorsque nous avons mis sur pied la première conférence canadienne sur le VHC, nous étions convaincus qu'un tel forum était devenu nécessaire de façon à disséminer les résultats de la recherche, promouvoir les collaborations, offrir un forum d'échange et créer une synergie entre les chercheurs canadiens de tous les horizons. Devant le succès remporté par nos 3 premiers symposiums, et à la l'aube de l'arrivée sur le marché de nouvelles molécules antivirales qui ont toutes les chances de révolutionner le traitement et le pronostic de cette infection, la quatrième édition de ce symposium s'annonce des plus excitante. Nous espérons que vous partagerez notre enthousiasme en ce sens.

Le Programme de subvention nationale de formation des Instituts de recherche en santé du Canada sur l'hépatite C (NCRTP-HepC) a contribué de façon significative à la formation de nouveaux étudiants et à la diffusion des connaissances dans le domaine de l'hépatite C. En particulier, ce programme a permis d'augmenter le nombre d'étudiants et l'étendue des travaux de recherche en développant un réseau de chercheurs dont les thèmes englobent les sciences sociales et du comportement ainsi que la recherche clinique et fondamentale. Les objectifs du programme sont, entre autres, de développer, étudier et mettre en œuvre des moyens efficaces de prévention et des programmes de soins dans le but d'éradiquer à long terme l'infection par le VHC au Canada et dans le reste du monde. Il était donc de mise que le NCRTP-Hep C prenne l'initiative de l'organisation de cette conférence.

Nous tenons à vous souhaiter la bienvenue à Banff. Espérons que nous pourrons découvrir les problématiques importantes que soulèvent encore l'infection par le VHC, l'étendue des travaux de recherche qui sont actuellement effectués sur le VHC au Canada et en profiter afin de discuter ensemble des moyens à prendre afin de façonner l'avenir de la recherche sur l'hépatite C au Canada.

Le comité organisateur

Program – Programme

Theme "Strategies to Manage HCV Infection in Canada: Moving Towards a National Action Plan"

07h15 - 08h00	Registration, Breakfast, Exhibition and Poster Area Opens
08h00 - 08h15	Welcome and Introductions **Dr. Naglaa Shoukry, Université de Montréal, Montréal, Canada** **Dr. Julie Bruneau**, **Université de Montréal, Montréal, Canada**

Clinical Sciences
Chairs: Dr. Curtis Cooper and Dr. Jordan Feld

08:15 - 08h45	Opening Keynote: Treatment for Chronic Hepatitis C Virus Infection: Challenges and Opportunities in the Era of Highly Effective Antiviral Therapy **Dr. Mark Sulkowski, Johns Hopkins University, Baltimore, USA**
08h45 - 09h05	HCV Care Clarity and Chaos in Canada **Dr. Curtis Cooper, University of Ottawa, Ottawa, Canada**
	Oral Presentations
09h05 - 09h15	Mucosal-Associated Invariant T (Mait) Cell Depletion and Exhaustion in HCV Infection and HIV/HCV Coinfection **Dr. Sonya MacParland, University of Toronto, Toronto, Canada**
09h15 - 09h25	Varying Efficacy of Sofosbuvir Treatment Regimens in Real Life Settings **Dr. Emmanuelle Huchet, Clinique l'Actuel, Montréal, Canada**

Biomedical Sciences
Chairs: Dr. Rodney Russell and Dr. Luis Schang

09h25 - 09h55	Viral and Host Factors of Hepatitis C Virus RNA Replication **Dr. Volker Lohmann, University of Heidelberg, Heidelberg, Germany**
09h55 - 10h10	Coffee Break
10h10 - 10h30	Resistance to HCV NS5A and NS5B Inhibitors: Significance and Mechanisms **Dr. Matthias Götte, University of Alberta, Edmonton, Canada**
	Oral Presentations
10h30 - 10h40	Small Interfering RNAs that Target the miR-122-Binding Region Inhibit HCV Replication by Multiple Mechanisms **Dr. Joyce Wilson, University of Saskatchewan, Saskatoon, Canada**
10h40 - 10h50	Biochemical Characterization of Potent Inhibitors of Hepatitis C Virus Polymerase Generated by -D-2'-C-Methyl-4-N-Hydroxycytidine Nucleoside Prodrug **Dr. Maryam Ehteshami, Emory University, Atlanta, USA**

Behavioural Sciences
Chairs: Dr. Julie Bruneau and Dr. Gerry Mugford

10h50 - 11h20	Confronting the Contradictions between Law Enforcement and Public Health: an Anthropological Perspective on the Hepatitis C Risk Environment in the US Inner-City with Notes from Canada **Dr. Philippe Bourgois, University of Pennsylvania, Philadelphia, USA**
11h20 - 11h40	Politics of HCV Infection in Aboriginal Peoples **Dr. Julia Rempel, University of Manitoba, Winnipeg, Canada**

Oral Presentations

11h40 - 11h50 LiveRLife: A Liver Health Promotion Campaign Among People Who Inject Drugs in the Drug and Alcohol Setting
Dr. Jason Grebely, University of New South Wales, Sydney, Australia

11h50 - 12h00 Resources for Engagement of Hepatitis C Infected Population in Care
Dr. Naveed Janjua, BC Centre for Disease Control & University of British Columbia, Vancouver, Canada

12h00 - 13h15 Lunch

13h15 - 13h30 CIHR Funding for HCV Research in Canada
Dr. Marc Ouellette, CIHR, Québec, Canada

Epidemiology and Public Health
Chairs: Dr. Jason Grebely and Dr. Mel Krajden

13h30 - 14h00 Scotland's Action Plan on Hepatitis C
Dr. Sharon Hutchinson, Glasgow Caledonian University, Glasgow, Scotland, UK

14h00 - 14h20 Burden of HCV in Canada and Management Strategies
Dr. Rob Myers, University of Calgary, Calgary, Canada

Oral Presentations

14h20 - 14h30 HCV, HIV and Risk: Characteristics of People Who Inject Prescription Opioids in Montreal
Dr. Svetlana Puzhko, McGill University, Montreal, Canada

14h30 - 14h40 Molecular Phylogenetics as a Tool for Monitoring Population Level Hepatitis C Virus Transmission Dynamics
Dr. Andrea D. Olmstead, University of British Columbia, Vancouver, Canada

14H40 - 14h55 Coffee Break

Strategies to Manage HCV Infection: Moving Toward a National Action Plan
Chairs and Discussion Leaders: Dr. Joyce Wilson and Dr. Marc Bilodeau

14h55 - 15h10 Responding to Hepatitis C in Canada: Status and Deliberations of the National HCV Task group
Dr. Mel Krajden, University of British Columbia, Vancouver, Canada

15h10-15h20 Beyond the medication - Resources Needed for Successful Treatment
Magdalena Kuczynski, Toronto Western Hospital, Toronto, Canada

15h20-15h30 The role for Patient Advocacy in building a Canadian HCV action plan
Daryl Luster, Steering /Executive Committees - Action Hepatitis Canada, President, Pacific HepC Network, Vancouver, Canada

15h30 - 16h00 Debate: 'Be it resolved that new HCV treatments should only be used on the sickest patients (F2 and above)'
Pro: Dr. Curtis Cooper, University of Ottawa, Ottawa, Canada
Con: Dr. Jordan Feld, University Health Network, Toronto, Canada

16h00 - 16h30 Panel and Audience Discussion
Issues Arising from the Debate and National HCV Priorities

16h30 - 16h45 Closing Remarks
Dr. Joyce Wilson, University of Saskatchewan, Saskatoon, Canada

16h45 - 18h30 Cocktail and Poster Session

Committees – Comités

Organizing Committee - Comité organisateur

Marc Bilodeau, Université de Montréal, Co-Chair
Frank Bialystok, University of Toronto
Julie Bruneau, Université de Montréal
Curtis Cooper, University of Ottawa
Maryam Ehteshami, Emory University
Jordan Feld, University Health Network
Jason Grebely, University of New South Wales
Mel Krajden, University of British Columbia
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Naglaa Shoukry, Université de Montréal
Hugo Soudeyns, Université de Montréal
Mark Tyndall, University of British Columbia
Nicholas van Buuren, Stanford University
Joyce Wilson, University of Saskatchewan, Chair

Session Chairs - Modérateurs de sessions

Clinical Sciences
Curtis Cooper, University of Ottawa and Jordan Feld, University Health Network

Biomedical Sciences
Rodney Russell, Memorial University and Luis Schang, University of Alberta

Behavioral Sciences
Julie Bruneau, Université de Montréal and Gerry Mugford, Memorial University

Epidemiology & Public Health
Jason Grebely, University of New South Wales and Mel Krajden, University of British Columbia

Abstract Reviewers - Réviseurs des résumés

Biomedical Sciences
Maryam Ehteshami, Emory University
Michael Houghton, University of Alberta
Sonya McParland, University of Toronto
Rodney Russell, Memorial University
Mohammed Sarhan, University of Alberta
Luis Schang, University of Alberta
Ragunath Singaravelu, University of Ottawa
Patricia Thibault, University of Saskatchewan
Nick van Buuren, Stanford University

Clinical Sciences
Jordan Feld, University Health Network
Rob Myers, University of Calgary
Lorne Tyrrell, University of Alberta

Epidemiology and Public Health
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Jason Grebely, University of New South Wales
Andrea Olmstead, University of British Columbia
Svetlana Puzhko, McGill University
Sahar Saeed, McGill University

Behavioural Sciences
Jordan Feld, University Health Network
Gerry Mugford, Memorial University
Sanjeev Sockalingam, University Health Network

Speakers Biographies and Abstracts – Biographies des conférenciers et résumés

Clinical Sciences

Dr. Mark S. Sulkowski, Johns Hopkins University, Baltimore, USA

Biography

Mark S. Sulkowski, MD, is a Professor of Medicine and serves as the Medical Director for the Viral Hepatitis Center at Johns Hopkins School of Medicine in Baltimore, Maryland. Dr. Sulkowski earned his medical degree at Temple University School of Medicine in Philadelphia, Pennsylvania followed by a residency in Medicine at Duke University Medical Center in Durham, North Carolina. He completed a fellowship in Infectious Diseases at John Hopkins School of Medicine concurrently with a post-doctoral position in Biostatistics and Epidemiology Advance Courses at Johns Hopkins University School of Hygiene and Public Health.

Dr. Sulkowski's clinical and research interests focus on hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) co-infection. He is currently the Principal Investigator for numerous clinical research trials involving novel HCV therapies in patients infected with HCV alone and in patients with HCV/HIV co-infection. Dr. Sulkowski is widely published, with over 100 scientific articles in peer-reviewed journals such as Gastroenterology, New England Journal of Medicine, Hepatology, and Lancet Infectious Diseases. In addition to his research, Dr. Sulkowski mentors fellows and residents, and lectures ad hoc on viral hepatitis and HIV at Johns Hopkins School of Medicine. He has authored over 20 review articles and book chapters, and serves as a peer reviewer for journals including the New England Journal of Medicine, Nature, AIDS, and Lancet. Dr. Sulkowski also serves on the editorial board for Hepatitis: Index and Reviews and Hepatology. He is a member of the Infectious Disease Societies of America, International AIDS society, American Association for Study of Liver Diseases, and European Association for the Study of the Liver, as well as an elected member of The American Society for Clinical Investigation. As an expert in his field Dr. Sulkowski has been an invited lecturer to over 60 national and international workshops, symposia, and conferences.

Abstract

Treatment for Chronic Hepatitis C Virus Infection: Challenges and Opportunities in the Era of Highly Effective Antiviral Therapy

In 1986, Hoofnagle and coworkers (NIH) reported the initial use of recombinant interferon alfa to treat patients with non-A, non-B hepatitis, demonstrating improvement in serum ALT levels in most patients. Over the ensuing decade, hepatitis C virus was identified cause of non-A, non-B hepatitis and molecular techniques were applied to test HCV RNA response during interferon therapy. With these advances, it quickly became apparent that the majority of patients treated with interferon alfa did not achieve HCV eradication known as sustained virologic response (SVR). Furthermore, the safety and tolerability of prolonged courses of interferon alfa prohibited treatment of many HCV-infected patients, limiting the effectiveness of this therapy. In 1995, several studies demonstrated that the addition of the guanosine nucleoside analogue ribavirin to interferon alfa led to higher SVR rates despite the lack of HCV RNA reduction with ribavirin monotherapy.

In 2003, the first report of a potent direct acting antiviral (DAA), an inhibitor of the HCV NS3/4A protease known as BILN 2061, was published, and, in 2011, nearly twenty-five years after the initial use of interferon to treat non-A, non-B hepatitis, two HCV NS3/4A protease inhibitors, telaprevir and boceprevir, entered clinical practice as part of "triple therapy" with interferon/ribavirin. While toxicity limited their effectiveness, the era of DAAs for the treatment of HCV was launched, and, in 2012, proof that HCV could be eradicated without interferon by the combination of two DAAs, a HCV NS3/4A protease inhibitor and HCV NS5A inhibitors, was published.

In 2015, multiple, interferon-free, combinations of DAAs which target several HCV nonstructural proteins, including NS3/4A protease, NS5B polymerase and NS5A, have been approved by regulatory authorities and, in some regions, been established as the "standard of care." Remarkably, 12 weeks of treatment with these DAA regimens can deliver high rates of HCV cure (> 95%) with minimal adverse effects across a diverse range of patients including those with HIV coinfection and/or decompensated liver disease. With the advent of highly effective DAAs, many of the historical barriers to HCV therapeutics linked to the necessity of interferon alfa have been overcome. In their place, new challenges have emerged including the treatment of persons with HCV genotype 3 infection and those with advanced liver disease for whom SVR rates are lower than observed in other patient groups as well as the specter of HCV drug resistance in persons who fail to achieve eradication of their HCV infection following treatment. However, the greatest challenge will be to rapidly translate these remarkable advances in HCV therapeutics to the large global community of persons chronically infected with hepatitis C to prevent the development of life-threatening complications of HCV disease.

Dr. Curtis Cooper, University of Ottawa, Ottawa, Canada

Biography

Dr. Curtis Cooper trained at the University of Saskatchewan (MD 1994). He received certification in Internal Medicine in 1997 and in Infectious Diseases in 1999 while at the University of Manitoba. He completed an HIV Research Fellowship and Masters of Epidemiology in 2002 at the University of Ottawa. He is currently an Associate Professor with the University of Ottawa, Scientist with the Ottawa Hospital Research Institute, Infectious Diseases Consultant with the Ottawa Hospital Division of Infectious Diseases and Director of The Ottawa Hospital Viral Hepatitis Program. He holds an Applied HIV Research Chair with the Ontario HIV Treatment Network. As a clinical researcher, his research activities encompass viral hepatitis, HIV, and vaccine development. His work is focused on the development of new therapeutic agents and the delivery of treatment that maximizes safety, adherence and safety. Is an active researcher with several cohort studies (CANOC, OHTN Cohort Study). He is co-chair of the CIHR Canadian HIV Trials Network Co-Infection Core research group, member of the Canadian Association of HIV Researchers executive and mentor with the National CIHR Research Training Program-Hep C.

Abstract

HCV Care, Clarity and Chaos in Canada

Well over 300,000 Canadians live with HCV. Only half are aware. Identification of infection is a pressing need that requires a national screening program, funding, and leadership. There is a growing burden of HCV-specific liver and extrahepatic complications which are applying an increasingly high financial cost to the Canadian health care system and will so for a least the next two decades. Canadian researchers have made significant contributions to advancing HCV knowledge and treatment. However, there remain many core research and Canadian-specific implementation questions that are unresolved. A continued commitment from the research community and funding agencies is required to address these issues. There is high calibre clinical expertise throughout Canada. However, patient access to care is haphazard. It is critical to expand this expertise and link it to all HCV-infected individuals. Continued development and implementation of novel HCV health care delivery models will facilitate access to care for remotely located and other marginalized HCV populations. HCV Direct Antiviral Therapies (DAA) are expensive and underfunded. Health care providers, industry and funders must do their part to maximize access to and the impact of HCV care and treatment. Action is required immediately to secure HCV DAA access for those with advanced HCV liver disease to avert needless morbidity and mortality.

Biomedical Sciences

Dr. Volker Lohmann, University of Heidelberg, Heidelberg, Germany

Biography

Volker Lohmann studied Biology at the University of Mainz and did his PhD on the biochemical characterization of the hepatitis C virus polymerase in the group of Ralf Bartenschlager. During his Postdoc in the same laboratory he established the HCV replicon model, which was the first efficient cell culture system for viral RNA replication, which set the ground for the development of direct acting antivirals, reverse genetics on viral genomes and the characterization of virus-host interactions. Since 2002 he is independent group leader in the Department of Infectious Diseases, section Molecular Virology at Heidelberg University.

Volker Lohmann's current research focuses on hepatitis C virus replication. This includes the understanding of viral and host cell factors contributing to viral RNA synthesis, innate and adaptive immune responses to viral infection and determinants driving viral pathogenesis. He is editorial board member of the "Journal of Virology" and "Virology".

Abstract

Viral and Host Factors of HCV Replication

Hepatitis C virus infections cause severe liver disease and are a major global health burden. Accessibility of in vitro models and cell culture systems allowed a detailed understanding of the viral replication cycle in molecular detail. HCV enters the cell by a well defined set of receptors and entry factors, the positive strand RNA genome is released into the cytoplasm and drives translation of a polyprotein, which is processed by cellular and viral proteases. The viral nonstructural proteins then induce membrane alterations mainly consisting of double-membrane vesicles, which harbor the sites of viral RNA replication. Viral translation, the formation of the viral replication sites and viral RNA synthesis involve a concerted action of viral nonstructural proteins and a number of host factors, e.g. microRNA-122 and the lipid kinase phosphatidylinositol 4-phosphate kinase (PI4KIIIα). Mir-122 is a liver specific micro-RNA binding to two well characterized sites in the 5' nontranslated region of the viral genome and supporting viral translation and RNA synthesis by yet poorly defined mechanisms. PI4KIIIα is a lipid kinase interacting with viral nonstructural proteins NS5A and NS5B. This interaction induces lipid kinase activity, resulting in an intracellular accumulation of PI4P. PI4KIIIα thereby contributes to the morphogenesis of viral replication sites and viral RNA synthesis. The presentation will focus on recent work on the mechanism of miR-122 in stimulating HCV translation and on our understanding how PI4KIIIα is involved in the generation of viral replication factories and viral RNA synthesis.

Dr. Matthias Götte, University of Alberta, Edmonton, Canada

Biography

In 1997, Dr. Götte obtained his Ph.D. degree at the Max-Planck-Institute for Biochemistry in Martinsried, Germany. He started his independent research in 2001 following a three-year period of postdoctoral training at the Lady Davis Institute for Medical Research at the Jewish General Hospital in Montreal. Dr. Götte later joined the Department of Microbiology & Immunology at McGill University, Montreal, and was promoted to Full Professor in 2011. In 2013, he received the Chercheur National award of the Fonds de la Recherche en Santé du Québec. Research in his laboratory is focused on the study of viral replication and its inhibition. His interests cover a broad range of important human pathogens, including the human immunodeficiency virus (HIV), the hepatitis C virus (HCV), and human herpesviruses. Results from his laboratory have contributed to the development of novel classes of viral polymerase inhibitors with antiviral activity against drug resistant variants. Dr. Götte has published approximately 100 peer-reviewed papers, reviews and book chapters in the field of virology and antivirals. He serves on the Editorial Board of Antimicrobial Agents and Chemotherapy and The Journal of Biological Chemistry. His research program is funded through grants from the Canadian Institutes of Health Research and contracts from industry. Since July 2014, he holds a position as Professor and Chair of the Department of Medical Microbiology & Immunology at the University of Alberta in Edmonton, Alberta, Canada.

Abstract

Resistance to HCV NS5A and NS5B Inhibitors: Significance and Mechanisms

The hepatitis C virus (HCV) can develop resistance to each of the available classes of direct-acting antivirals (DAAs). A complex interplay between genetic barriers, viral fitness, and drug-specific properties determine the outcome of the selection process, and, in turn, the clinical significance of mutational patterns. The error-prone nature of the HCV RNA-dependent RNA polymerase NS5B is the source for genetic variability and diversity. Mutant variants that are constantly generated during active viral replication are eventually selected under drug pressure and outgrow the population. The emergence of mutant variants commonly correlates with decreases in drug susceptibility. Hence, a logical goal in the development of effective drugs is to minimize the likelihood of resistance emergence in vivo. Conversely, the selection of resistance conferring mutations in vitro provides an important tool in drug discovery and development efforts. Success in selection experiments can validate the target and may also shed light on the binding site of the drug and mechanism of action. This presentation will focus on resistance patterns associated with drugs that target NS5B and NS5A, respectively. The clinical significance will be discussed in the context of molecular mechanisms and structural data.

Behavioural Sciences

Dr. Philippe Bourgois, University of Pennsylvania, Philadelphia, USA

Biography

Philippe Bourgois is a medical anthropologist conducting participant-observation fieldwork in the US inner city since 1985 on substance abuse, HIV-risk, poverty and violence. Supported by NIH research grants since 1988, he has been the Principle Investigator on a continuous R01 grant since 1996 examining the HIV risk environment of indigent drug injectors. He has published over 150 articles in public health, the humanities and the social sciences with a focus on social inequality, urban segregation, labor migration, ethnic conflict, homelessness, substance abuse, interdisciplinary methods, HIV and structural public health interventions. He is best-known for his best-seller academic books: In Search of Respect: Selling Crack El Barrio and Righteous Dopefiend. He has developed multiple protocols for interdisciplinary theoretical and methodological dialogue between epidemiology, clinical research and the humanities and social sciences and is currently the Co-I coordinating qualitative methods on three R01s including two RCTs and one meta-epidemiological analysis. He just received a Guggenheim Award for his work on US inner city poverty and substance abuse <http://www.philippebourgois.net>.

Abstract

Confronting the Contradictions between Law Enforcement and Public Health: an Anthropological Perspective on the Hepatitis C Risk Environment in the US Inner-City with Notes from Canada

Drawing on 25 years of participant-observation ethnographic data collection in the U.S. inner city among street-level crack and heroin users/sellers this paper develops the concept of the injection drug user risk environment to explore the possibility of upstream structural interventions that could lower hepatitis C prevalence among injection drug users. I provide two examples of public health prevention and health delivery services crucial to street-based drug abusers that are systematically undermined by punitive, zero-tolerance law enforcement priorities. 1) Syringe exchange and 2) Treatment on demand. Inverting the balance of forces between public health and law-enforcement/carceral priorities could transform one of the most crucial policy/services determinants of the risk environment. Contradictorily, the option of mandatory drug treatment in lieu of prison time and the outstanding adherence rates of AIDS patients in carceral settings, offer examples of the potential for the cooperative delivery of cost-effective prevention and harm reduction treatment services. The challenge for public health is to transform the criminal justice field from one of punishment to treatment and prevention guided by risk reduction priorities. New breakthroughs in HCV treatment open tremendous possibilities for vulnerable IDUs under legal/carceral supervision unless Big Pharma neoliberal logics render the expensive HCV treatment protocol inaccessible to the "undeserving poor".

Dr. Julia Rempel, University of Manitoba, Winnipeg, Canada

Biography

Dr. Julia Rempel's laboratory investigates the role of inflammation and innate immunity in liver associated diseases. They have worked with a First Nation community and the Assembly of Manitoba Chiefs, to determine whether genetic and cellular immune characteristics could contribute to an enhanced clearance of hepatitis C virus (HCV) infection observed in Canadian Aboriginal populations.
This work has made salient discoveries regarding the role of the immune system in Aboriginal health. The Rempel laboratory's findings have changed our understanding of the genetic causes of disease in Aboriginal peoples. Specifically, these findings indicate that Aboriginal populations have a stronger pro-inflammatory immune response compared to Caucasians supportive of more effective clearance of HCV, but also the onset of chronic inflammatory disease such as type 2 diabetes. Moreover, Dr. Rempel effectively sets these findings on genetic polymorphisms and immunity in the context of the historical and social events that shape Aboriginal health. Knowledge translation has also been a key component of her research. As an example of these activities, Dr. Rempel has produced research videos to make research assessable to all peoples.
Oji-Cree:https://onedrive.live.com/redir?resid=CE648B9696727432!120&authkey=!ACzTrW2LYG-Gx2s&ithint=video%2cmp4
English: https://onedrive.live.com/redir?resid=CE648B9696727432!121&authkey=!AJxzZtW3pM90&ithint=video%

Abstract

Politics of HCV Infection in Aboriginal Peoples

Canadian studies indicate that Aboriginal ethnicity is associated with enhanced clearance of hepatitis C (HCV) infection. My laboratory's genetic analyses indicate that this may be due to a heightened immune activity that is shared with indigenous people throughout the Americas, but is distinct from other peoples globally (evaluated by other investigators). One element that could have influenced these genetic profiles is the introduction of "old world" viruses which resulted in the eradication of large portions of indigenous peoples throughout the Americas. The survivors of these viral epidemics would have borne the heightened pro-inflammatory genetic profiles that are evident in their descendants today. In addition to the immune protection against HCV infection, genetic profiles supportive of pro-inflammatory immunity would also promote inflammatory diseases including metabolic disorders.
Despite the potential for more effective clearance, the incidence of HCV infection in Aboriginal communities is significantly higher than the general population. Studies have linked HCV infection in Aboriginals with the historical trauma of residential schools. Moreover, HCV infection in Aboriginal peoples is observed with a higher incidence of HIV co-infection, as well as mortality than non-Aboriginal counterparts. Overtime the face of the HCV infected Aboriginal, particularly with HIV co-infection, has also changed from middle-aged men to younger women; data that reflects the targeted trafficking of Aboriginal women. Aboriginals are also over represented in federal prisons, where a quarter to half of individuals are HCV infected. Although new treatments are available, stemming the burden of HCV infection in Aboriginal peoples also requires directly addressing the needs of our most vulnerable populations.

Dr. Marc Ouellette, CIHR, Québec, Canada

Biography

Dr. Ouellette is the Scientific Director of the Institute of Infectious Disease and Immunity of CIHR since January 1st, 2010. In this capacity, he has led the conception and implementation of several research initiatives in the areas of organ transplantation, inflammation, microbiome, vaccines and antibiotic resistance. Dr. Ouellette also oversees the development and outcomes of the HIV/AIDS and Hepatitis C Research Initiatives.

Dr. Ouellette obtained his Bachelor of Science (Honours) in Biochemistry at Ottawa University and received his PhD at Laval University on antibiotic resistance in bacteria. His postdoctoral studies were done under the mentorship of Pr. Piet Borst of the Netherlands Cancer Institute in Amsterdam, where he further developed his expertise in antimicrobial resistance studying protozoan parasites.

In 1990 he joined the Centre de Recherche en Infectiologie, of Laval University as an Assistant professor and is now full professor. Dr. Ouellette's research is focused on antimicrobial resistance where he has made seminal discoveries on resistance mechanism in protozoan parasites. More recently he has implemented proteomic and DNA microarray strategies to study antimicrobial resistance in the parasite Leishmania and the bacteria Streptococcus pneumoniae.

Dr. Ouellette has received numerous awards for his work including a New Investigator Award in Molecular Parasitology from the Burroughs Wellcome Fund, a MRC Scientist Award, a Scholar Award in Molecular Parasitology of the Burroughs Wellcome Fund, and a Tier 1 Canada Research Chair in Antimicrobial Resistance. He is a Fellow of the Royal Society of Canada and of the Canadian Academy of Health Sciences. He has served on numerous panels of national and international granting agencies and is a strong supporter of scientific exchanges with developing countries.

Abstract

CIHR Funding for HCV Research in Canada

Since its creation in 2000, the Canadian Institutes of Health Research (CIHR) and its Institute of Infection and Immunity have made supporting hepatitis C research a priority. As part of an ongoing partnership with the Public Health Agency of Canada (PHAC), CIHR has launched a number of strategic funding opportunities as a part of the PHAC-CIHR Joint Hepatitis C Research Initiative. The priority areas of this initiative have evolved alongside the knowledge base, from its initial goal of supporting broad-based hepatitis C research to reduce disease burden, to supporting a hepatitis C-focused training program, and to furthering epidemiological, clinical, biomedical and socio-behavioural hepatitis C research. A renewed agreement signed by CIHR and PHAC in 2014 emphasizes research in two overarching themes: biomedical and clinical research; and research with direct public health relevance. The current National Hepatitis C Collaborative Network funding opportunity, seeks to address both priorities by creating a cohesive research program that links researchers, knowledge users and decision makers from across Canada.

CIHR is constantly developing new programs to address the changing Canadian health research landscape. Signature Initiatives focusing on topics such Health Equity for Aboriginal Peoples, Community-based Primary Health Care, and Patient-Oriented Research have the potential to make an impact in the field of hepatitis C. These are in addition to the range of CIHR programs designed to support investigator-initiated research projects for investigators throughout the many stages of their careers. This presentation will outline the scope of research support tools currently available, and how CIHR is planning for the future.

Epidemiology and Public Health

Dr. Sharon Hutchinson, Glasgow Caledonian University, Glasgow, Scotland, UK

Biography

Sharon Hutchinson is a Professor of Epidemiology and Population Health at Glasgow Caledonian University. She leads a broad translational research programme on the epidemiology of hepatitis C virus (HCV) and other blood borne viruses, authoring over 130 publications. Her research has included a range of studies (involving population-based surveys, surveillance initiatives, novel record linkage studies, and statistical/economic models) designed to inform on the prevention, diagnosis, and treatment of HCV infection. Her research included the development of models to estimate the impact of antiviral therapy on the future burden of HCV related liver disease in Scotland, which provided the key evidence to guide a public health response and culminated in Scottish Government investing significantly (around £100 million since 2008) in their now globally recognised Action Plan on Hepatitis C. She was actively involved in all phases of this Action Plan – including the development, coordination and evaluation – and Chaired the National Network on Information Generating Initiatives. Beyond the UK, she has reviewed the evidence for and advised on the development of European guidance on the prevention of infectious diseases among people who inject drugs, and WHO guidance for the screening, care and treatment of HCV.

Abstract

Scotland's Action Plan on Hepatitis C

Six years has elapsed since the Scottish Government launched its Hepatitis C Action Plan – a Plan to improve services to prevent transmission of infection, particularly among people who inject drugs (PWID), identify those infected and ensure that diagnosed people needing therapy get it. Underpinned by industrial scale funding (around £100 million, additional to the general national health service funding, will have been invested in services by 2015), initiatives ranged from the introduction of testing in specialist drug services through finger-prick blood sampling by non-clinical staff, to the setting of government targets to ensure rapid scale-up of antiviral therapy. The Plan was informed by monitoring systems, indicating the extent of the problem not just in terms of numbers infected, diagnosed and treated but also the more penetrative data on end-stage liver disease and death, and also through compelling modelling work demonstrating the potential beneficial impact of scaling-up therapy on serious outcome trajectories. Achievements include around 50% increase in the proportion of the infected population diagnosed (38% to 55%); a sustained two-and-a-half fold increase in the annual number of people initiated onto therapy (450 to 1100) with more pronounced increases among PWID (300 to 900) and prisoners (20 to 140); and a reduction in the overall number of people living with chronic infection (39,000 to 37,000). The Action Plan has demonstrated that a Government-backed, co-ordinated and invested approach can transform services and rapidly improve the lives of thousands.

Dr. Robert P. Myers, University of Calgary, Calgary, Canada

Biography

Dr. Rob Myers is Associate Professor of Medicine and Director of the Viral Hepatitis Clinic at the University of Calgary. He received his Internal Medicine training at the University of Western Ontario, Gastroenterology fellowship at the University of Calgary, and Hepatology research fellowship at the Université de Paris VI in Paris, France. In 2008, Dr. Myers completed his MSc in Epidemiology at the University of Calgary.

His research interests include the noninvasive assessment of liver fibrosis, epidemiologic and health outcomes research in various liver diseases, and the investigation of novel therapies for autoimmune and viral liver diseases. He has been funded by the Canadian Institutes for Health Research (CIHR), the Alberta Heritage Foundation for Medical Research, and the Canadian Liver Foundation. Dr. Myers is actively involved in investigator-initiated and industry-sponsored clinical trials. Dr. Myers has published over 140 peer-reviewed scientific manuscripts, is an Associate Editor of the American Journal of Gastroenterology, and serves on the Editorial Boards of Hepatology, Annals of Hepatology, and the Canadian Journal of Gastroenterology and Hepatology. Dr. Myers is the past Chair of the Education Committee of the Canadian Association for the Study of the Liver (CASL) and a member of the National Education Advisory Committee of the CLF. In 2012, Dr. Myers was awarded a Queen Elizabeth II Diamond Jubilee Medal for his contributions to research and patient care in liver disease.

Abstract

Burden of HCV in Canada and Management Strategies

Chronic hepatitis C virus (HCV) infection is a significant medical and economic burden in Canada. In the Canadian Health Measures Survey (CHMS), the Public Health Agency of Canada reported an estimated anti-HCV prevalence of 0.5% or approximately 138,600 anti-HCV positive individuals in Canada. However, these figures are likely underestimates due to exclusion of high-risk populations including incarcerated individuals, Aboriginals, and people who inject drugs (PWID). Indeed, a recent modeling study suggests that approximately 252,000 Canadians were chronically infected in 2013. It has been estimated that approximately 60% of HCV cases in Canada are among current or former PWID, 20% are among infected immigrants, and 11% have received contaminated blood products. Of the nearly 8,000 incident cases in Canada in 2007, approximately 80% likely occurred via sharing of injecting equipment, and most of the remainder among immigrants from endemic countries. Approximately 30% of HCV-infected individuals remain undiagnosed. Complications of HCV are increasing due to aging of the infected population and progression of liver fibrosis. Modeling data suggest that over the next two decades, cases of decompensated cirrhosis, hepatocellular carcinoma, and liver-related mortality will increase drastically despite the use of highly-effective antiviral regimens. In this presentation, data regarding the burden of HCV in Canada and strategies for the management of HCV-infected cases will be reviewed.

Oral Abstracts – résumés oraux

Clinical Sciences

Oral presentation at 09h05

MUCOSAL-ASSOCIATED INVARIANT T (MAIT) CELL DEPLETION AND EXHAUSTION IN HCV INFECTION AND HIV/HCV COINFECTION

Sonya MacParland[1] Ali Fawaz[1] Warmond Chan[2] Colin Kovacs[3] Jordan Feld[4] David Wong[2, 4] Mario Ostrowski[1]
1. University of Toronto, Toronto, ON, Canada; 2. Toronto General Hospital, Toronto, Canada; 3. Maple Leaf Clinic, Toronto, Canada; 4. Toronto Western Hospital, Toronto, Canada

Mucosal-associated invariant T (MAIT) cells are innate-like T cells found in human blood, mucosal tissues and liver. MAIT cells respond to bacterial metabolites and can produce pro- and anti-fibrogenic cytokines such as TNFα, IL-17, IFNγ and IL-22. Due to their accumulation in the liver and potential to secrete pro- and anti-fibrogenic cytokines, these cells are of interest when examining why liver disease progresses more quickly in HIV/HCV coinfection. Previously, it was shown that in HIV monoinfected patients, there is a marked decline in circulating MAIT cells, associated with time since diagnosis of HIV. As well, with the decline of functional MAIT cells, a subset of impaired MAIT cells appeared. The purpose of this study was to assess potential impairment of MAIT cells in HCV monoinfection andHCV/HIV co-infection, to identify a possible pathway by which liver disease progresses more rapidly in co-infected individuals.

Methods: Peripheral blood mononuclear cells from 13 uninfected, 14 chronically HIV infected, 7 chronically HCV infected, and 13 HIV/HCV co-infected individuals were isolated from whole blood samples. Intracellular cytokine staining assay: Cells were stimulated with killed E. Coli in the presence of Brefeldin A/Monensin and stained with fluorescent antibodies targeting CD3 (T-cell marker), Vα7.2 and CD161 (MAIT cell markers), PD-1 and Tim-3 (exhaustion markers), and IFN-γ (anti-fibrogenic cytokine) and TNF-α and IL-17 (pro-fibrogenic cytokines). Flow cytometry was used to determine the MAIT cell phenotype within each patient subset.

Results: We found that there is a significant reduction in the circulating MAIT cell population between healthy and both HCV and HIV/HCV coinfected individuals ($P<0.05$). MAIT cells from the blood of coinfected individuals also expressed significantly more exhaustion markers including PD-1 and Tim-3 when compared to MAIT cells from healthy individuals ($P<0.05$). PD-1 and TIM-3 were also upregulated in the MAIT cells of HCV moninfected individuals, compared to healthy individuals but the effect was less profound.

Conclusion: In summary, we found that MAIT cell frequency and functionality appears to be altered in patients who are HCV infected and more so in those who are HIV/HCV coinfected. The impairment of these innate cells may represent a pathway by which HCV disease progresses more rapidly in HCV infected persons who are HIV coinfected.

Oral presentation at 09h15

VARYING EFFICACY OF SOFOSBUVIR TREATMENT REGIMENS IN REAL LIFE SETTINGS

Emmanuelle Huchet; Chrissi Galanakis; Benoit Trottier; Marc Poliquin; Stephane Lavoie; Daniele Longpre; Sylvie Vezina; Caroline Bedard; Rejean Thomas; Nima Machouf
Clinique médicale l'Actuel, Montreal, QC, Canada

Background: The efficacy and safety of Sofosbuvir (SOF) in HCV treatment regimens has been demonstrated in several clinical trials. Nevertheless, few studies have examined its effectiveness in clinical settings. The aim of this study therefore was to evaluate the impact of SOF on treatment outcomes in real life settings.

Methods: We prospectively assessed all genotype 1 (a,b) HCV infected patients attending our clinic who were receiving SOF-based treatments with or without pegylated interferon (PEGINF). According with our mDOT (modified Directly Observed Therapy) model of care, during their treatment period patients were followed each week at the clinic and had a consultation with a nurse, physician and pharmacist; and they had lab tests. Patients enrolled in RCTs were excluded. The primary outcome was sustained virologic response (SVR) at 12 weeks post-treatment by intent-to-treat analysis. Comparisons among treatment groups were assessed by chi-square.

Results: 56 patients were included in this study. Patients were mainly male (71%), treatment naïve (57%), their mean age was 54 y (IQR 49-58) at treatment initiation (baseline), 86% were infected by HCV genotype 1a and 14% by genotype 1b. More than half of patients (64%) were cirrhotic at baseline and 29% were co-infected with HIV. Patients were receiving 3 types of SOF-based regimens: Simeprevir (SIM) + SOF (61%); followed by SOF+PEGINF+Riba (30%); and SOF+Riba (9%). The majority of treatment regimens were 12 and 24 weeks long (91% and 7% respectively) with only 1 patient undergoing a 48 week treatment. 17 patients completed their treatment period and 12 weeks post treatment FU. Among the 17 patients having completed data, 16 out of 17 (94%) completed treatment and had undetectable viral loads at the end of treatment, and one died in the fourth week of treatment from decompensated liver cirrhosis. However, 12/17 (71% ITT analysis) achieved SVR 12-weeks post-treatment. The rate of SVR varied by regimen, treatment status at baseline and by presence of cirrhosis. Patients taking SIM+SOF had a SVR rate of 89%, while those on SOF+Riba had 25% SVR and those on SOF+Riba+PEGINF had 75% SVR (p=0.07). SVR was 100% (3/3) for relapsers or partial responders; 78% (7/9) for naïve patients and 40% (2/5) for nulls responders (p=0.11). Patients with cirrhosis had a SVR in 8/13 (62%) and those without cirrhosis in 4/4 (100%) of cases (p=0.21). Patients on SIM+SOF were more likely to achieve SVR even after controlling for cirrhosis (p=0.08). No patient had been lost to FU during the study period.

Conclusion: In real life settings, SOF offers a variable rate of SVR. The combination SIM+SOF was the most effective HCV treatment, including for patients with cirrhosis. An mDOT model of care, and close FU, could assure a good adherence to medication.

Biomedical Sciences

Oral presentation at 10h30

SMALL INTERFERING RNAS THAT TARGET THE MIR-122-BINDING REGION INHIBIT HCV REPLICATION BY MULTIPLE MECHANISMS

Adam Huys[2, 1] Michael Schinold[2] Patricia A. Thibault[2, 1] Joyce A. Wilson[2, 1]
1. Vaccine and Infectious Disease Organization, University of Saskatchewan, Saskatoon, SK, Canada; 2. Department of Microbiology and Immunology, University of Saskatchewan, Saskatoon, SK, Canada

Background: Robust HCV replication requires annealing of the liver-specific miRNA, miR-122, to two binding sites in the 5' UTR of the HCV genome. miR-122 anneals based on sequence similarity and in association with Ago2, a protein component of the RNA induced silencing complex (RISC). Because the sequence of the miR-122 binding site region is conserved and accessible by miRNA/Ago2 complexes we hypothesized that it would be an ideal target for siRNA based anti-HCV therapy.

Method and Results: We designed three siRNAs that target the miR-122 binding site region and found that they inhibited replication of HCV by up to 93% in transient HCV replication assays. A limitation to the use of siRNA therapeutics to treat viral infections is the development of resistance through incorporation of point mutations in the target sequence by error prone viral polymerases. Through serial siRNA treatments and selections we have identified multiple HCV RNA replicons having point mutations in the miR-122 binding sites, suggesting the miR-122 binding sites may not be as intolerant to sequence changes as generally believed. The replicative fitness, miR-122 dependence, and siRNA resistance profiles of the isolated mutants will be presented. By focusing on one replication competent HCV mutant, HCV S1:p3, our data shows that a single mismatch near the middle of the siRNA target sequences in the 5' UTR of HCV does not abolish siRNA inhibition. The point mutation reduces HCV RNA replicative fitness by about 10 fold, and imparted partial resistance to one of the siRNAs, but surprisingly, enhanced inhibition by two of the siRNAs. We hypothesize that these siRNAs may inhibit the mutant HCV genome by overlapping and blocking miR-122 binding to site 2. If this were the case, then their inhibition would be dependent on miR-122. However, when we tested inhibition of HCV S1:p3 by these siRNAs in an HCV replication system devoid of miR-122 they were still capable of impeding HCV replication, indicating that they can also inhibit HCV independent of both sequence identity and miR-122 binding. We speculate that they may impede other pro-viral events involving the 5' UTR. Finally, in the absence of endogenous miR-122 the siRNA that most efficiently inhibited the wild-type virus was able to augment replication of S1:p3, albeit much less efficiently than wild type miR-122. Thus, this siRNA appears to function as a miR-122 mimic, and thus we also speculate that it can likely also compete with wild-type miR-122.

Conclusions: These data suggest that the siRNAs that target the miR-122 binding sites are a possible therapeutic strategy. In addition, it appears that siRNA/Ago complexes impede the HCV life cycle by multiple mechanisms, including classical siRNA target cleavage, competition for miR-122 binding, and possibly modulation of pro-viral 5' UTR RNA structures or binding proteins present in the absence of miR-122.

Oral presentation at 10h40

BIOCHEMICAL CHARACTERIZATION OF POTENT INHIBITORS OF HEPATITIS C VIRUS POLYMERASE GENERATED BY B-D-2'-C-METHYL-4-N-HYDROXYCYTIDINE NUCLEOSIDE PRODRUG

Maryam Ehteshami[1] Sijia Tao[1] Sheida Amiralaei[1] Hao Li[1] Xiao Lu[1] Tugba Ozturk[1] Franck Amblard[1] Tamara McBrayer[2] Tony Whitaker[2] Steve J. Coats[2] Raymond F. Schinazi[1]
1. Emory University, Atlanta, GA, USA; 2. RFS PHARMA, LLC, Tucker, GA, USA

Introduction: Nucleoside analog inhibitors (NI) are an important class of anti-HCV agents that are pan-genotypic and have a high genetic barrier to drug resistance. Sofosbuvir, the only NI approved by the FDA to date, has demonstrated an excellent safety profile and treatment with sofosbuvir-containing regimens results in unprecedented cure rates for chronic HCV infections. Herein, we describe the discovery of a β-D-2'-C-methyl-4-N-hydroxycytidine prodrug, that, upon intracellular metabolism, can deliver multiple nucleoside 5'-triphosphate inhibitors (NI-TP). Upon cellular entry, this prodrug was metabolized to generate three distinct NI-TPs: 2'-C-methyl-CTP, 2'-C-methyl-UTP and 2'-C-methyl-4-N-OH-CTP. The two former NI-TPs are well characterized for their anti-HCV activity, whereas 2'-C-methyl-4-N-OH-CTP has not been studied.

Purpose: The aim of this study is to characterize the biochemical properties of 2'-C-methyl-4-N-OH-CTP as a novel inhibitor of HCV polymerase. We also aim to shed light on the mechanism of action of this prodrug in vivo through characterization of intracellular metabolism of β-D-2'-C-methyl-4-N-hydroxycytidine prodrug and cell-free enzymatic properties of the generated NI-TPs.

Methods: Prodrug was incubated in Huh-7 cells and LC-MS/MS was used to measure intracellular levels of metabolites. Inhibition of RNA synthesis by each NI-TP was separately evaluated using purified recombinant HCV polymerase and commercially available host DNA and RNA polymerases. In vitro enzymatic assays were employed to measure dissociation constants and rates of incorporation for each inhibitor.

Results: We observed that 2'-C-methyl-4-N-OH-CTP behaves as both a cytidine and uridine analog (C > U). We also established that 2'-C-methyl-4-N-OH-CTP effectively inhibited RNA polymerization when pre-incubated with purified NS5B enzyme, but was outcompeted when co-incubated with natural CTP and UTP substrates. Kinetic parameters, as well as intracellular NI-TP levels were taken into consideration in order to shed light on its intracellular mechanism and antiviral activity. Importantly, NI-TP metabolites of β-D-2'-C-methyl-4-N-hydroxycytidine prodrug were not substrates for host DNA polymerases and were poor substrates for host mitochondrial RNA polymerase even at 100 µM.

Conclusions: β-D-2'-C-Methyl-4-N-hydroxycytidine prodrug takes advantage of naturally occurring intracellular metabolism pathways to generate three bioactive NI-TP. These findings could have important implications for the development of a new class of NIs that mediate the intracellular delivery of multiple active nucleoside 5'-triphosphate analogs with distinct incorporation profiles.

Behavioral Sciences

Oral presentation at 11h40

LIVERLIFE: A LIVER HEALTH PROMOTION CAMPAIGN AMONG PEOPLE WHO INJECT DRUGS IN THE DRUG AND ALCOHOL SETTING

Jason Grebely[1] Michelle Micallef[1] Alison Marshall[1] Amanda Erratt[1] Remaliah King[1] Joanne Telenta[2] Sandra Jones[2] Nicky Bath[3] Carla Treloar[4] Dianne How-Chow[5] Jude Byrne[6] Paul Harvey[7] Adrian Dunlop[8] Gregory J. Dore[1]
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Background: Liver disease burden among people who inject drugs (PWID) continues to rise. Strategies are needed to enhance assessment and treatment.

Purpose: This study aimed to assess acceptability of non-invasive liver disease assessment (Fibroscan®) and liver fibrosis stage distribution among people with a history of injecting drug use participating in a liver health promotion campaign.

Methods: LiveRLife is a liver health promotion campaign designed to enhance liver disease assessment, developed through partnerships between researchers and community/peer groups. The three project phases includes: 1) campaign message/resource development; 2) campaign message/resource testing; and 3) campaign implementation. Between May and October 2014, participants with a history of injecting drug use were recruited from one community health centre, two opioid substitution treatment clinics and the Sydney Medically Supervised Injecting Centre in New South Wales, Australia, into a prospective observational cohort. Campaign implementation included resource material promotion (posters, videos, and booklets), surveys (including liver knowledge), Fibroscan®-based assessment, HCV RNA testing (dried-blood-spot), and nurse/specialist assessment. Acceptability of non-invasive liver disease assessment (Fibroscan®) and liver fibrosis stage distribution were assessed.

Results: Among 252 enrolled participants, the mean age was 43 (standard deviation, 10), 68% were male, 21% were of Aboriginal ethnicity, 27% had completed high school or higher education, 7% had full-time or part-time employment, 67% had ever been in prison and 33% had been in prison in the last 12 months. Seventy-five percent of people self-reported being infected with HCV. Overall, 75% (n=189) had injected drugs in the past six months and 71% (n=179) were currently receiving opioid substitution treatment. Prior to assessment, 88% of people were definitely willing to receive a Fibroscan®. Prior to assessment, 65% indicated that they would prefer Fibroscan® as a method for testing for liver disease, as compared to taking a sample of blood from a vein (16%) or a liver biopsy (19%). The overall median liver stiffness measurement was 6.1 kPa (range, 2.7-75.0). The mean number of valid measurements was 11.1, with a mean success of 91.3%. The proportion of people with mild fibrosis (F1, ≥2.5 – ≤7.4 kPa) was 68%, moderate fibrosis (F2, ≥7.5 – ≤9.4 kPa) was 13%, severe fibrosis was 10% (F3; ≥9.5 – ≤12.4 kPa), and cirrhosis (F4, ≥12.5 kPa) was 9%. The proportion with severe fibrosis/cirrhosis (≥9.5 kPa) was higher among those ≥40 years of age (24% vs. 12% among those <40 years, P=0.027). The proportion with severe fibrosis/cirrhosis (≥9.5 kPa) was also higher among males (22% vs. 11% among females, P=0.106), but this was not statistically significant.

Conclusion: Through effective partnerships, the LiveRLife campaign has been successfully developed for the drug and alcohol setting. Overall, one-fifth of participants had severe fibrosis/cirrhosis. Age ≥40 years was significantly associated with increased liver stiffness measurements, suggesting that this is an important group to monitor for advanced liver disease in this setting. Further research will inform whether this campaign will improve liver disease knowledge, assessment and treatment.

Oral presentation at 11h50

RESOURCES FOR ENGAGEMENT OF HEPATITIS C INFECTED POPULATION IN CARE

Terri Buller-Taylor[2] Liza McGuinness[2] Naveed Janjua[1] Gail Butt[3] Mel Krajden[4]
1. School of Population and Public Health, University of British Columbia, Vancouver, BC, Canada; 2. University of British Columbia, Vancouver, BC, Canada; 3. School of Nursing, University of British Columbia, Vancouver, BC, Canada; 4. Laboratory Medicine and Pathology, University of British Columbia, Vancouver, BC, Canada

Background: Benefits of > 95% curative and well tolerated emerging Hepatitis C therapies can only be realized if patient engagement and retention in care is dramatically increased through effective patient and provider education.

Purpose: To describe the process of establishing a national collaborative network and the development of accessible, linguistically and culturally appropriate educational resources for patients and providers that facilitate engagement and retention in hepatitis C care.

Methods: A collaborative, iterative process was employed with those affected and providers in the exploration of the issues, identification of the education needs, and the development of evidence-based and clinically relevant resources [1]. Network participation included membership in the project advisory committee and cultural and linguistic working groups, focus group participants for material testing, and dissemination planning groups.

Results: Eleven resources to increase knowledge and provide support along the entire cascade of care were developed for various population groups (English, French, Aboriginal, South Asian and those born between 1945-1965) in multiple formats: print, audio and video. Resources were distributed across Canada through multiple channels including workshops, conferences, community based partners and a website (http://hepatitiseducation.ca). Evaluations indicate the resources have broad uptake and address key patient and provider knowledge gaps that contribute to non-attendance.

Conclusions: Evidence-based resources educate providers and patients and support patients as they navigate through various stages of care. These resources will be crucial for engagement with HCV care in the future as treatment programs are scaled up.

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Epidemiology and Public Health

Oral presentation at 14h20

HCV, HIV AND RISK: CHARACTERISTICS OF PEOPLE WHO INJECT PRESCRIPTION OPIOIDS IN MONTREAL

Svetlana Puzhko[1, 4] Élise Roy[2] Didier Jutras-Aswad[4, 3] Andrea A. Artenie[4, 3, 1] Julie Bruneau[4, 3, 1]
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Introduction: As hepatitis C virus (HCV) is nowadays mainly transmitted through injection drug use, ongoing surveillance of drug injection trends is of crucial importance. Recently, prescription opioids (PO) use has dramatically increased in Canada and worldwide. In Montreal, the proportion of PO injectors tripled between 2005 and 2011 (1). The emergence of prescription opioid injection is of great concern for public health as PO injection was recently shown to be an independent predictor of HCV transmission (1) (2). Characterization of PO injectors is therefore important for improvement of HCV and HIV prevention and treatment interventions.

Purpose: The purpose of the study was to examine socio-demographic and behavioral characteristics associated with PO injection in Montreal.
Methods: Baseline characteristics of 1243 injection drug users participating in a prospective cohort study, the St Luc/HEPCO Cohort of Montreal, were examined. Eligibility criteria included being 18 years old or over, drug injection in the previous 6 months, and residing in Montreal area. Data was collected in 2004 - 2011 by validated interviewer-administered questionnaire and blood was obtained for HCV and HIV antibody testing. Bivariate comparisons were assessed by Chi-square statistics and Student's t-tests. Logistic regression modeling was applied to identify correlates of PO injection.

Results: Of the 1243 participants (83.8% males; mean age: 38.3 years), 380 (30.6%) reported PO injection in the past month. In multivariate regression analysis, the following socio-demographic characteristics were independently associated with PO injection: age (adjusted odds ratio (aOR) by 5-year increment: 0.78; 95% confidence interval (95%CI): 0.71,0.85); being Caucasian (aOR:1.93; 95%CI:1.16,3.19), and being non-francophone (aOR:1.87; 95%CI:1.28,2.72). Reporting co-use of the following drugs was positively associated with injecting PO: using injection heroin (aOR:2.98; 95%CI:2.17,4.11), non-injection stimulants (aOR:1.95; 95%CI:1.26,3.01), and smoking crack/cocaine (aOR:1.44; 95%CI:1.07,1.93). Other factors positively associated with PO injection were: living in unstable housing conditions (aOR:1.89; 95%CI:1.40,2.56), injecting in public place (aOR:1.58; 95%CI:1.10,2.26) or with other people (aOR:1.70; 95%CI:1.15,2.52), and HCV seropositivity (aOR:1.56; 95%CI:1.13,2.16). Conversely, HIV seropositive status was negatively associated with PO injection (aOR:0.50; 95%CI:0.28,0.88).

Conclusions: Our results suggest that PO injectors are younger than non-PO injectors. They are more likely to be HCV antibody positive and less likely to have HIV seropositive status. Moreover, PO injectors are more likely to report social and behavioural characteristics known to increase risks for HCV infection. A better understanding of PO injectors' characteristics and their living contexts will help develop HIV and HCV targeted prevention and treatment strategies in the future.

Funding sources: Funding sources: this project is funded by CIHR and FRQS-AIDS Infectious Disease network. Svetlana Puzhko receives FRQS Master's training award and the National CIHR Research Training Program in Hepatitis C fellowship.

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Oral presentation at 14h30

MOLECULAR PHYLOGENETICS AS A TOOL FOR MONITORING POPULATION LEVEL HEPATITIS C VIRUS TRANSMISSION DYNAMICS

Andrea D. Olmstead[3, 1] Vincent Montoya[3, 1] Jeffrey B. Joy[2] Iris Luo[3] Art F. Poon[2] Brendan Jacka[4] François Lamoury[4] Tanya Applegate[4] Julio Montaner[2] Yury Khudyakov[5] Jason Grebely[4] Darrel Cook[1] P R. Harrigan[2] Mel Krajden[1, 3]

1. BC Centre for Disease Control, Vancouver, BC, Canada; 2. BC Centre for Excellence in HIV/AIDS, Vancouver, BC, Canada; 3. University of British Columbia, Vancouver, BC, Canada; 4. The Kirby Institute, UNSW Australia, Sydney, NSW, Australia; 5. Centers for Disease Control and Prevention, Atlanta, GA, USA

Background: Improved surveillance methods are required to understand and monitor the impact of prevention and treatment interventions on hepatitis C virus (HCV) transmission.

Purpose: To develop a sequenced-based molecular epidemiology approach for identifying recent population level transmission clusters.

Methods: Sanger sequencing and maximum-likelihood phylogenetics (HCV NS5B, Core-HVR1 and HVR1 regions) were applied to individuals diagnosed with HCV in British Columbia, Canada in 2011, which included individuals with two or three sequential specimens collected less than one year apart. Patristic distances between sequential samples from the same individual were used to set cutoffs to identify recent transmission clusters at a population level. Logistic regression was used to identify factors associated with clustering. To further validate and characterize transmission events, deep amplicon sequencing was performed and the HCV intra-host diversity was measured in a subset of individuals.

Results: From 618 individuals, 647 sequences were obtained. Within the NS5B, Core-HVR1 and HVR1 phylogenies, depending on the cutoff used, a total of 63 (10%) to 92 (15%) unique individuals were identified within clusters that represent transmission events predicted to have occurred approximately a year or less before the date of sample collection. Compared to those not in clusters, individuals within clusters were more likely to be <40 years old (vs. ≥40 years; Adjusted Odds Ratio (AOR) 1.95, 95% CI 1.18 – 3.24), infected with HCV genotype 1a (vs. other genotypes; AOR 4.86, 95% CI 1.44 – 30.35), and to be seroconverters with an estimated infection duration of <1 year (vs. first time HCV positive; AOR 3.41, 95% CI 1.56 – 7.34) or seroconverters with an estimated infection duration of >1 year (AOR 2.53, 95% CI 1.47 – 4.40). Deep sequencing data provided additional support for 3 putative transmission pairs. The intra-host diversity along with estimated dates of infection were used to further characterize these transmissions.

Conclusion: Systematic application of HCV sequencing and molecular phylogenetics can be used to identify epidemiologically relevant population level transmission clusters. This information can be used to monitor the effectiveness of transmission reduction interventions and to target public health resources to populations at risk of onward transmission.

Posters - Affiches

Clinical Sciences

Poster presentation: CS001

SIMEPREVIR AND SOFOSBUVIR WITH MODIFIED DOSES OF RIBAVIRIN (RBV) THERAPY ON TELAPREVIR EXPERIENCED CO INFECTED (WITH HIV) CIRRHOTICS WITH CHRONIC HEPATITIS C (CHC). A RANDOMIZED OPEN LABEL CLINICAL PILOT STUDY: STOP C

P Patrick Basu[1] Niraj James Shah[2] Mark M. Aloysius[3] Robert S Brown Jr[1]
1. Columbia University School of Physicians and Surgeons, New York, NY, USA; 2. James J. Peters VA Medical Center, Icahn School of Medicine at Mount Sinai, New York, NY, USA; 3. King's County Hospital Medical Center, New York, NY, USA

Objectives: Cirrhotics with CHC still remains a challenge. Co-infected cirrhotics (HIV+CHC) are at a greater risk for rapid decompensation affecting QOL and have a higher transplant risk burden. Interferon based therapy entails a longer duration with an increased susceptibility of infections and marrow suppression warranting use of growth factors and even discontinuation of therapy/treatment failure. Telaprevir; a protease inhibitor (PI) based therapy have proved efficacious in co-infected patients. Newer generation PI coupled with polymerase inhibitors and adjusted doses of RBV have shown favorable outcomes. This clinical study evaluates the efficacy of Simeprevir, Sofosbuvir with RBV for 24 weeks in prior Telaprevir experienced co-infected cirrhotics.

Methods: Fifty (n=50) co-infected (HIV+CHC, non AIDS) cirrhotics with mean MELD 16, HIV RNA undetectable, mean CD 4 count 439, Hb 10.7, HCV RNA 1.7 million copies, mean platelet count 104, albumin 2.9 and WBC 4600. 18 genotype 1a and 32 genotype 1b. 16 null responders, 12 relapsers while 12 discontinued treatment.
Group A: Simeprevir 150 mg + Sofosbuvir 400 mg + RBV for 24 weeks
Group B: Simeprevir 150 mg + Sofosbuvir 400 mg + RBV 1000 mg for 16 weeks

Results: see table

Conclusion: The combination of Interferon free oral regimen in special population with prior experienced PI demonstrated no difference of SVR in 16th week over 24th weeks. This regimen was well tolerated and has a better safety profile than conventional trials.

	Group A, n=22, 24 weeks, Simeprevir 150 +Sofosbuvir 400 mg + RBV	Group B, n=28, 16 weeks, Simeprevir 150 mg + Sofosbuvir 400 mg + RBV 1000 mg
Undetectable 48 hours	2	4
Undetectable 1 week	3	7
Undetectable 2 weeks	16	19
Undetectable 8 weeks	17	22
Undetectable 12 weeks	17	23
Undetectable 16 weeks	17	23
Undetectable 24 weeks	18	

Poster presentation: CS002

IL-22 ENHANCES TGF-BETA PRO-FIBROTIC FUNCTION IN HEPATIC STELLATE CELLS IN A P38/MAPK DEPENDENT MANNER

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Background: Activation of hepatic stellate cells (HSCs) is a key event in liver fibrosis, characterized by enhanced extracellular matrix (ECM) production and altered degradation. The immune system modulates activation of HSCs through production of cytokine. IL-22 is an enigmatic cytokine, from the IL-10 family, with both pro- and anti-inflammatory properties. IL-22 deficient mice have high hepatic inflammation during acute injury compare to their wild type littermates. IL-22 is also elevated in the sera of patients with liver cirrhosis and carcinoma. However, in a mouse model of hepatitis B, IL-22 indirectly induces fibrosis by recruiting pro-fibrotic Th17.

Hypothesis: We hypothesized that IL-22 may modulate activation and induction of the fibrogenic process of HSCs.

Methods: The human HSC line LX2 and primary human HSCs were stimulated with increase doses of IL-22 and compared to TGF-β- and PBS- treated cells as positive and negative controls, respectively.

Results: IL-22 did not induce activation of HSCs. However, IL-22 enhanced the response of HSCs to suboptimal doses of TGF-β as observed by strong induction of alpha-smooth muscle actin (α-SMA), collagen type I (COL1A1) and tissue inhibitor of matrix metalloproteinase (TIMP-I). IL-22 stimulation did not enhance cell surface expression of TGF-β-RII. However, pretreatment of HSCs with IL-22 led to increase phosphorylation of SMAD2/3 in response to suboptimal doses TGF-β. This effect was dependent on the activation of the p38/MAPK pathway. IL-22 also downmodulated the expression of IL-22BP which may further increase the HSC response to IL-22.

Conclusion: Our results suggest a novel pro-fibrotic function for IL-22 through enhancement TGF-β signaling in a p38/MAPK dependent manner.

Poster presentation: CS003

TREATMENT FOR HEPATITIS C VIRUS INFECTION AMONG PEOPLE WHO INJECT DRUGS IN THE OPIOID SUBSTITUTION SETTING: THE ETHOS STUDY

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Background: Assessment and treatment for hepatitis C virus (HCV) among people who inject drugs (PWID) is low and strategies are needed to enhance access to care.

Purpose: This study aims to evaluate the effectiveness of HCV treatment among PWID.

Methods: Enhancing Treatment for Hepatitis C in Opioid Substitution Settings (ETHOS) is a prospective observational cohort, evaluating a model for the provision of HCV assessment and treatment among people with a history of injecting drug use and chronic HCV. Recruitment occurred through six opioid substitution treatment (OST) clinics, two community health centres and one Aboriginal community controlled health organisation in NSW, Australia. Participants initiating pegylated interferon/ribavirin (PEG-IFN/RBV) treatment between February 2009 and December 2012 (genotype 1, G1) or June 2013 (genotypes 2 and 3, G2/3) were included, to allow for adequate post-treatment follow-up. Statistical analyses were performed using Chi-squared or Fisher's exact tests, as appropriate.

Results: Among 415 participants, 27% (n=111) commenced treatment. Among those treated between 2009 and 2013 (n=104, mean age 43 years, 77% male), 36% (n=37) had injected drugs in the past six months and 62% (n=64) were currently receiving OST. In an intent-to-treat analysis, the sustained virological response (SVR) was 65% overall (68 of 104), 69% in G1 (20 of 29) and 64% in G2/3 (48 of 75). There was no difference in SVR between those never (74%, 23 of 31) and currently (66%, 42 of 64, P=0.484) receiving OST. SVR was similar among those who had injecting drugs in the past six months (70%, 26/37) compared to those who had not (64%, 43/67, P=0.665).

Conclusion: Response to treatment in this population was high and active injecting drug use did not compromise treatment response. This data suggests that targeted initiatives to enhance HCV treatment in OST or community health clinics can be successful.

Poster presentation: CS004

BENEFIT OF DAA-BASED HCV THERAPY IN MONO-INFECTED VS. HIV CO-INFECTED INNER CITY POPULATIONS

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Background: Historically, it had been demonstrated that interferon (PR)-based regimens were less effective in the setting of HIV co-infection. More recent data have shown that this is no longer the case with the availability of direct acting antiviral agents (DAAs). However, this has not been fully evaluated in vulnerable inner city populations, which account for 50% of the prevalent and 75% of the incident HCV infection in Canada.

Purpose: To determine treatment outcomes in mono-infected and HIV/HCV co-infected cohorts treated with DAAs in an inner city population in Vancouver.

Method: A retrospective observational study was conducted for individuals treated with DAAs (June 2009 – July 2014) and/or PR-based regimens (January 2002 – July 2014) at an inner city clinic in Vancouver. Data regarding HCV diagnosis and treatment, HIV co-infection status, and life-style co-morbid conditions were collected through chart review, with patient informed consent. The two-sample z-test was performed to compare the efficacy of DAA and PR-based approaches in mono-infected and HIV/HCV co-infected sub-populations, the treatment endpoint being a sustained virologic response (SVR or cure, undetectable serum HCV RNA 12 weeks after the end of treatment).

Results: Among a total of 372 treatment courses, 128 courses were completed with DAAs in 116 patients (97 male), median baseline age 53 years, 87% genotype 1, 34% treatment experienced, 13% compensated cirrhotic. Treatment regimens included 42% PI, 11% NS5A, 13% NUC, 13% non-NUC, and 22% mutli-class combination. Nineteen cases (15%) were HIV co-infected, with a median CD4 cell count of 482/mm^3, 84% HIV virologically suppressed and 89% on ARVs. Other baseline co-morbid conditions included: 76% PWID, 58% ethanol abuse, and 18% methadone maintenance therapy (MMT).

Treatment outcomes included SVR (overall 65%; 71% in non-genotype 1 infection), relapse (13%), null/partial response (9%), premature discontinuation due to toxicity (6%). Factors associated with achieving SVR included: treatment naïve to DAAs and PR (p=0.001), pegINF alpha-2a/2b free regimens (p=0.004), baseline HCV RNA ≤1,000,000 IU/mL (p=0.04), and rapid virologic response (RVR) (p<0.001). The overall SVR rate attained with DAAs was significantly higher than the 55% observed in 244 PR treatment courses (p=0.03). There was no statistically significant difference in SVR rates between mono- and co-infected individuals in either PR or DAA-based regimens. The majority (63%) cases of premature discontinuation due to toxicity were seen in the setting of PI-based regimens. In 22 patients on MMT, the cure rate was 41% (10/22 on pegINF alpha as a part of the regimens), and there was no requirement for methadone dose adjustment due to drug-drug interaction in any case.

Conclusions: We continue to observe high SVR rates in inner city populations treated for HCV within our program, with equivalent response rates demonstrated in mono- and HIV co-infected cohorts. The advent of DAA-based therapies has led to an increase in SVR rates in all target populations, and has not resulted in destabilization of MMT in any case. Going forward, the availability of safer and simpler therapies will allow us to reach a greater number of "core transmitters" more effectively.

Poster presentation: CS005

FAVOURABLE IFNL3 GENOTYPES AND LIVER FIBROSIS IN HIV/HEPATITIS C (HCV) CO-INFECTED INDIVIDUALS FROM THE CANADIAN CO-INFECTION COHORT

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Background: Liver fibrosis progression is faster in HIV/HCV co-infected individuals due to an elevated inflammatory profile. Interferon Lamda-3 (IFNλ-3), encoded by the human *IFNL3* gene (formerly *IL28B*), has both antiviral and pro-inflammatory properties, though reports of its association with liver fibrosis are inconsistent. Homozygous recessive SNPs (rs12979860CC, rs8099917TT) in this gene are linked to spontaneous HCV clearance and better treatment response, potentially via non-synonymous functional variant rs8103142,which leads to a lysine-arginine substitution at position 70(K70R).

Aim: Examining the relationship between specific IFNL3 genotypes and significant liver fibrosis as measured by the AST-to-platelet ratio index (APRI) ≥1.5 in HIV/HCV co-infected Canadians

Methods: From the prospective Canadian Co-infection Cohort (n=1176), HCV RNA-positive participants free of fibrosis, end-stage liver disease and chronic Hepatitis B at baseline (n=612) were included. Cases (n=126) developed an APRI≥1.5 over follow-up. Data were analyzed using Cox proportional hazards, adjusting for sex, ethnicity, alcohol use, age and baseline APRI. Multiple imputation was used to account for missing data.

Results: Overall 74% were male with median HCV duration=18 years.126 participants developed fibrosis over 1346 person-years of risk (9.40/100 person-years, 95% CI=7.90, 11.20/100 p-y). Univariate analyses suggested that each SNP may be linked to a higher risk of fibrosis. In multivariate analyses, rs8099917 had the strongest effect.

	rs12979860 CC	**rs8099917 TT**	**rs8103142 TT**
Univariate	1 (0.69, 1.45)	1.35 (0.93, 1.95)	1.15 (0.80, 1.66)
Multivariate	1.06 (0.72, 1.55)	1.46 (1, 2.14)	1.19 (0.82, 1.71)
Female	1.25 (0.82, 1.89)	1.27 (0.85, 1.90)	1.27 (0.84, 1.93)
Alcohol use	1.27 (0.87, 1.84)	1.24 (0.85, 1.81)	1.26 (0.87, 1.84)
Baseline APRI	3.29 (2.10, 5.16)	3.38 (2.15, 5.32)	3.28 (2.09, 5.13)
Age	0.99 (0.97, 1.01)	0.99 (0.97, 1.02)	0.99 (0.97, 1.01)
Aboriginal	1.10 (0.65, 1.87)	1.09 (0.64, 1.84)	1.09 (0.64, 1.84)

Conclusions: Our results suggest that among the IFNL3 SNPs analyzed, rs8099917 is linked to a higher rate of liver fibrosis among HIV/HCV co-infected Canadians. Larger studies are needed to confirm this finding.

Describe the implications of the above research on Hepatitis C and other basic/social/medical disciplines (max. 100 words):

Our study results could help identify important risk factors for significant liver fibrosis in HIV/HCV co-infected individuals, a population which has very critical clinical needs.

Poster presentation: CS006

TREATMENT OUTCOMES WITH TELAPREVIR-BASED THERAPY FOR HIV/HCV-COINFECTED PATIENTS ARE COMPARABLE TO HCV-MONOINFECTED PATIENTS: A CANADIAN EXPERIENCE

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Background: Hepatitis C virus (HCV) infection is an important cause of end-stage liver disease. Triple therapy including peginterferon (Peg-IFN), ribavirin (RBV) and telaprevir (TVR) has improved sustained virologic response (SVR) rates compared to Peg-IFN/RBV dual therapy, albeit with added toxicity.

Purpose: Our objective was to compare clinical outcomes of HCV-monoinfected and HIV/HCV-coinfected patients with HCV genotype 1 treated with TVR-based triple therapy at a regional referral center in Alberta, Canada.

Methods: All patients with compensated liver disease due to HCV genotype 1 treated with Peg-IFN/RBV/TVR from June 2011 to December 2013 were included. Demographic, clinical, and laboratory data was retrospectively collected including age, sex, HCV genotype, fibrosis stage, IL28B genotype, prior antiviral therapy, and where applicable, HIV viral load, CD4+ T cell count, and antiretroviral regimen. Outcomes included end of treatment virologic response (EOT), SVR at 24 weeks (SVR24), and safety. Multivariate logistic regression was used to examine the independent impact of HIV infection on SVR24 after adjustment for potential confounders.

Results: In total, 90 HCV-monoinfected and 12 HIV/HCV-coinfected patients initiated Peg-IFN/RBV/TVR therapy. The median age was 56 years (IQR 51-59), 73% was male and 43% had compensated cirrhosis. 72% were GT1a (available in n=47), 72% had IL28B non-CC genotype (n=58), and 9% were prior null responders (n=55). All coinfected patients had undetectable HIV RNA on antiretroviral therapy. Although HIV/HCV-coinfected patients were more likely to be prior null responders (25% vs. 5%; P=0.06), other baseline characteristics did not differ from HCV monoinfected patients. Compared with HIV-negative patients, HIV/HCV-coinfected patients had similar rates of EOT (73% [64/88] vs. 67% [8/12]; P=0.74) and SVR24 (65% [58/89] vs. 60% [6/10]; P=0.74). After adjustment for age and sex, HIV-infection was not associated with SVR24 (odds ratio [OR] 1.17; 95% CI 0.28-4.91); only advanced fibrosis (F3-F4) was a significant negative predictor of treatment response (OR 0.24; 95% CI 0.10-0.64). Treatment discontinuation due to adverse events occurred in 15% (13/89) of HCV-monoinfected patients vs. 8% (1/12) of HIV-coinfected subjects (P=1.00). Hepatic decompensation occurred in five patients (4/5 HIV negative) and two patients died (both HIV negative).

Conclusion: In our cohort of patients with compensated HCV genotype 1 infection treated with TVR-based triple therapy, HIV/HCV-coinfected patients had comparable treatment responses and tolerability to HCV monoinfected patients. The SVR24 rate observed in our cohort is similar to historical controls.

Poster presentation: CS007

INTERFERON INELIGIBLE NAIVE CHRONIC HEPATITIS C GENOTYPE I SUBJECTS TREATED WITH SIMEPREVIR AND SOFOSBUVIR IN SPECIAL POPULATION (PSYCHIATRIC). AN OPEN LABEL PROSPECTIVE CLINICAL PILOT STUDY; INSPIRE C STUDY

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Objectives: Chronic hepatitis C (CHC) is no longer a challenging clinical state with newer DAA's achieving SVR within a shorter duration of therapy. Pegylated Interferon Alfa 2a with Ribavirin was the main stay of therapy. Interferon is contraindicated in psychiatric population (Schizophrenic. major depression, bipolar and schizoaffective disorder). These population have a majority of co morbidities, substance abuse and advanced fibrosis along with a poor QOL score.

Exclusion criteria: Renal failure with CrCl<30, sickle cell, Thalassemic syndromes, Hemolytic syndrome, co infections (HBV, HIV) or CHF NYHA Stage IV

Methods: sixty CHC subjects [n=60, schizophrenia 20/60 (33.3%), major depression 15/60 (25%), bipolar disorder 20/60 (33.3%) and prior suicidal attempts with depression 5/60 (8.3%) with psychiatric disorder were recruited.

See table
GROUP A; (n=20); Simeprevir 150 mg + Sofosbuvir 400 mg + Ribavirin1000 mg daily, 12 weeks
GROUP B; (n=20); Placebo + Sofosbuvir 400 mg + Ribavirin1000 mg daily, 16 weeks
GROUP C; (n=20); Simeprevir 150 mg + Sofosbuvir 400 mg + Vitamin D 5000 mg daily, 16 weeks

Laboratory analysis:

HCV RNA viral load, CBC with ANC: Day 0 and 2 day, 1,4,8 and 12th week

TFT, haptoglobin, coombs test, renal function, liver function test: 14 th 30 th 40 th 60 th 90 th day]

Q89k polymorphism in 90 days

Fibroscan and serum fibrosis markers: Base line and one year post therapy

Results:

	Group A	Group B	Group C
Undetectable 48 hours	15	13	15
Undetectable 1 week	23	21	25
Undetectable 2 weeks	28	24	25
Undetectable 4 weeks	29	25	27
Undetectable 8 weeks	29	26	27
Undetectable 12 weeks	29	26	27
Retention	29/30, 96.7%	29/30, 96.7%	30/30, 100%

Conclusion: Oral combination therapy for Interferon ineligible group shows similar SVR rates with better tolerability and safety profile. This special population should be treated with this regiment to prevent cirrhosis and HCC.

Poster presentation: CS008

CLINICAL PROFILE AND TREATMENT OFFER IN HCV-INFECTED PATIENTS WITH END-STAGE-LIVER DISEASE

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Background: Antiviral treatment for chronic Hepatitis C Virus (HCV) infection has been shown to be moderately effective in RCTs but, in real life setting, only a minority of HCV-infected patients are treated and even less reach sustained virological response (SVR).

Purpose: Our goal is to evaluate, in a group of chronic HCV-infected patients with end-stage liver disease (ESLD), the proportion who received antiviral therapy before the appearance of ESLD. We hypothesized that the majority of patients would have been offered antiviral therapy, but either refused it or did note achieve SVR.

Method: HCV-infected patients with ESLD followed in our institution were screened for the study. ESLD was defined by the development of hepatocellular carcinoma, hepatic encephalopathy, esophageal variceal bleeding, spontaneous bacterial peritonitis, ascites or a MELD score of 14 or higher. We included 143 patients who filled a survey on sociodemographic data, risk factors, diagnosis and treatment of HCV infection. Medical charts were reviewed to complete data acquisition.

Results: Among 143 participants, 116 (81%) were diagnosed with HCV infection before ESLD developed. Overall, 77% of participants (n=111) received a treatment offer and 71% of patients (n=101) underwent antiviral therapy. Among the 101 treated participants, 67 (47% of the study population) were treated before the occurrence of ESLD and 32 only after ESLD had developed. 42 patients (29%) never received treatment. Among these, 33 (23% of study population) were never offered therapy and 9 declined treatment offer. Only 48 patients (34% of all patients) completed the full course of treatment. The most common causes for treatment discontinuation were side effects and treatment failure. Among the treated participants, 60 underwent 1 treatment trial and 40 underwent 2 trials or more for an average of 1.55 treatments per treated patient and a total of 155 treatment trials. Only 25 patients (17%) achieved SVR. When groups of treated vs non-treated individuals were compared, non-treated patients tended to have lower education levels, were more likely to live alone, to drink alcohol and to be active users of illegal drugs ($p<0,05$).

Conclusions: Among a group of HCV-infected patients with ESLD, we found that most patients (81%) were diagnosed before developing ESLD and a majority (71%) received antiviral therapy. However, a minority of patients (47%) received treatment before hepatic complications occurred, even less (34%) completed a full course antiviral therapy and the overall SVR rate was very low (17%). These results suggest that earlier, more effective therapy and fewer obstacles to treatment initiation are required in order to avoid the life-threatening complications of HCV infection.

Biomedical Sciences

Poster presentation: BS001

COMPARING GENETIC VARIATION WITHIN THE THREE DOMAINS OF THE HEPATITIS C VIRUS NS3 HELICASE BY DEEP SEQUENCING

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Background: Hepatitis C virus (HCV) subgenomic replicons are extremely useful for the study of viral replication. Replication enhancing mutations (REMs) [1], also referred to as cell culture adaptive mutations, have been identified that result in efficient RNA amplification in the highly permissive Huh-7 hepatoma cell line. Within the HCV replicon REMs cluster to a central region of NS5a, as well as to a cluster near the amino terminus of the NS3 helicase (NS3h), and at two distinct positions in NS4b [2]. In the context of the NS3h gene, the majority of REMs map to domain 1, with few REMs in domains 2 and 3. Domains 1 and 2 are highly structurally related, containing the ATP binding site and making the majority of the contacts with the RNA substrate, while domain 3 is primarily structural.

Purpose: To use a deep sequencing approach to investigate genetic variation in the helicase region of NS3 in the replicon system. We compared genetic variation in domain 1, which contains the majority of REMs reported in the literature, to that in domain 3 where few REMs have been reported.

Methods: Deep sequencing of amplicons covering the different domains of NS3h was performed to detect minor variants arising during replication. Briefly, in vitro transcribed replicon RNA was electroporated into Huh7 cells and replicon RNA was extracted following passaging. Replicon RNA was reverse transcribed to cDNA, and PCR amplicons were then amplified using barcoded PCR primers with 454 adaptor sequences. Amplicons were then subjected to pyrosequencing on a GS Junior sequencer (Roche). The raw reads were processed and minor variants calculated using Roche amplicon variant software.

Results: Consistently over five different sequencing runs, which included three independent RNA transfections and multiple time points, we found that the error rate calculated from the variants sequenced in a region of domain 1 in NS3h was approximately 2-fold higher than that for a region in domain 3. This result suggests a higher tolerance for variation in domain 1 as compared to domain 3, coinciding with a greater number of REMs reported in domain 1. In agreement with previous reports from our group, the majority of the variants consisted of transitions due to the high bias by the NS5b polymerase to form G/U or U/G mismatches [3], with only a small number of transversions. At the level of the individual variants, we noted five mutations in domain 1 and one mutation in domain 3 that were detected repeatedly over multiple sequencing runs covering as many as three independent RNA transfections.

Conclusions: We found that genetic variation was better tolerated in domain 1 as compared to the more structural domain 3. In addition, certain nucleotide positions were identified as hot spots for specific mutations. Further work will be required to follow up on individual mutations to establish their effect on RNA replication.

References:

1) Pietschmann et al. PLoS Pathog (2009), 5, e1000475.
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Poster presentation: BS002

INVESTIGATING THE ROLE OF MICRORNA-122-ASSOCIATED COMPLEXES IN HEPATITIS C VIRUS INFECTION

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Background: Approximately 200 million individuals worldwide are infected by hepatitis C virus (HCV), including more than 300 000 Canadians. HCV-infected individuals typically develop a persistent infection that leads to chronic hepatitis, cirrhosis and liver cancer. MicroRNA-122 (miR-122) is a highly abundant liver-specific microRNA shown to interact at two "tandem" microRNA-binding sites in the 5' UTR of the HCV genome. This unusual interaction promotes HCV RNA accumulation in both HCV-infected cells, and the livers of infected patients. Mutation, truncation, or exchange of the 3' terminal ribonucleotides of miR-122 for deoxynucleotides reduces HCV RNA accumulation. However, these nucleotides are not required for canonical miRNA activities (i.e. target cleavage and translational inhibition). This suggests that sequences in the 3' tail of miR-122 may mediate important interactions with viral or cellular factors involved in HCV RNA accumulation.

Aims: We hypothesize that miR-122 forms a distinct complex with host and/or viral proteins that together mediate HCV RNA accumulation in infected cells. Hence, we aim at identifying and characterizing host and viral factors associated with non-canonical miR-122 complexes in HCV-infected cells to identify novel antiviral targets that can be targeted with small molecules.

Methods: Alkyne-tagged miR-122 molecules are transfected into HCV RNA-harboring Huh-7 or Hep3B cells. Following miR-122 biotinylation by a click reaction, miR-122 ribonucleoprotein complexes from naïve and HCV-infected cells are isolated by streptavidin affinity purification. MiR-122-associated proteins are then analyzed by SDS-PAGE, liquid chromatography tandem mass spectrometry (LC-MS/MS) and multidimensional protein identification technology (MudPIT). Comparison of miR-122 complexes from naïve and cells infected with HCV RNA with mutations in either site 1 or site 2 of the miR-122 binding sites will allow the identification of proteins acting specifically at site 1 or site 2 of the HCV genome.

Results: Here, we demonstrate that alkyne-tagged miR-122 molecules are functional in mediating HCV RNA accumulation in Huh-7 cells. We show that the click reaction is stable under physiological conditions and permits efficient labeling and affinity purification of miR-122 molecules in cell lysates. Western blot of affinity purified miR-122 complexes show enrichment in the RNA-induced silencing complex (RISC) protein Argonaute 2.

Conclusions: We expect that the results will provide insight into a novel microRNA 'capping complex' as well as a non-canonical 'microRNA enhancing complex'. We anticipate that we will identify novel host-virus interactions important for viral replication that will provide new targets for therapeutic intervention.

Poster presentation: BS003

CHARACTERIZATION OF THE FUNCTIONALITY AND FLEXIBILITY OF THE VIRUS-SPECIFIC CD8 T CELLS ASSOCIATED WITH PROTECTION DURING HCV REINFECTION

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We have previously demonstrated that protection from viral persistence upon HCV re-exposure and reinfection correlates with expansion of virus-specific T cells. Protection was also associated with increased breadth of the immune response and shifting epitope dominance, suggesting the generation of de novo T-cell responses. In contrast, viral persistence was associated with limited expansion of T cells and reinfection with variant viral strains. Analysis of the virus-specific T cell receptor (TCR) repertoire demonstrated that effector HCV-specific CD8 T-cell clonotypes associated with protection upon reinfection are recruited from the memory population, with focusing of the repertoire following repeated infections. We hypothesized that the focusing of the T cell repertoire upon reinfection and resolution is associated with selection of T cell clonotypes with the highest functional avidity (i.e. a biological measure that describes how well a T-cell responds in vitro to a given concentration of its cognate antigen) and flexibility (i.e. the capacity to effectively detect different variants of the same epitope). We FACS sorted HCV-specific CD8 T cells using MHC class I tetramers to examine the diversity of the T cells repertoire. Limited dilution cloning was performed to generate individual clones corresponding to the most dominant clonotypes detected by high resolution bulk sequencing. The individual clones are currently being tested for their functional avidity and flexibility in detecting different variants of the targeted epitopes. The results obtained will establish a correlation between protective immunity and selection of T cell clonotypes with the highest functional avidity upon repeated re-exposure to the virus and will have implications for the development of vaccination regimens to boost generation and selection of such clonotypes.

Poster presentation: BS005

EVIDENCE OF GENERALIZED CD8+ T CELL IMPAIRMENT IN HCV INFECTION IS PRONOUNCED IN THE LIVER AND EXACERBATED IN HIV CO-INFECTION

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Background: Clearance of hepatitis C virus (HCV) infection requires cytotoxic CD8+ T cell (CTL) activity, yet HCV-specific CD8+ T cell function is impaired in chronic HCV infection. Similarly, CTL function is impaired in HIV infection, in which co-infection HCV is the most prevalent (25%) infectious co-morbidity leaving affected individuals with dual CTL impairment and faster liver disease progression. We and others recently observed generalized, antigen non-specific, CD8+ T-cell impairment in chronic HCV infection, implying a weakened immune system. The contribution of liver-infiltrating CD8+ T cells to the immunopathogenesis of this hepatotropic infection, and the mechanisms mediating this widespread CTL impairment, have not been elucidated.

Purpose: We investigated antigen non-specific CD8+ T-cell activity in blood and in the liver in HCV mono- and HIV-HCV co-infection to determine the effects of chronic infection on CTL activity and its correlation with liver disease.

Methods: Liver biopsies and blood samples from chronic, HCV mono-infected, treatment naïve individuals and HIV-HCV co-infected individuals on effective antiretroviral therapy. Blood samples from healthy individuals (controls) were also collected. The phenotype of isolated blood- or intrahepatic-CD8+ T cells and their activity (phosphorylated STAT5 signaling, anti-apoptotic Bcl-2 expression and proliferation) in response to the T-cell cytokine interleukin-7 (IL-7) were measured by flow cytometry.

Results: In HCV mono-infection, there were fewer naive CD8+ T cells in the blood compared to controls, with reduced STAT5 activation and Bcl-2 expression in response to IL-7, despite unchanged expression of the IL-7 receptor alpha (CD127). This impairment was particularly pronounced in central memory and naïve cells. In addition, low Bcl-2 expression correlated with higher fibrosis scores. Intrahepatic CD8+ T cells were comprised of more central and effector memory cells than blood, as determined by intra-individual analyses. These liver cells also expressed basal STAT5 activation yet this was not increased by cytokine stimulation. In contrast, their counterparts in blood lacked such basal activation yet responded to cytokine stimulation. Lastly, basal Bcl-2 expression was lower in IH-CD8+ T cells compared to blood-CD8+ T cells.

In HIV-HCV co-infection, CD8+ T-cell impairment appeared to be similar to that observed in HCV mono-infection. However, analysis of CD8+ T-cell subsets revealed a significantly more pronounced impairment compared to mono-infection, particularly among naïve and central memory cells. Also, the proliferation of stimulated CD8+ T-cells was significantly impaired in co-infection, unlike in mono-infection which was no different than healthy controls.

Conclusions: These phenotypic attributes suggest that CD8+ T cells are impaired in chronic HCV infection, irrespective of antigen specificity and in a novel manner to previous CTL impairment in HIV infection which we previously correlated with reduced CD127 expression. In the liver, this dysfunction is pronounced, and data suggests a correlation with liver fibrosis. In HIV-HCV co-infection, observed CD8+ T-cell activity is further reduced. Elucidating the mechanisms mediating the impairment of circulating and CD8+ T cells trafficking through the liver may improve our understanding of the effects of HCV infection on the immune system, identify targets to improve both HCV and non-HCV immunity among those with chronic HCV infection and prevent liver damage.

Poster presentation: BS006

HEPATITIS C VIRUS INFECTION REDUCES THE CHEMOSENSITIVITY OF HEPATOCELLULAR CARCINOMA CELLS

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Background: Hepatitis C virus (HCV) infection is a major risk factor for hepatocellular carcinoma (HCC). HCV is involved in the genesis of HCC but certainly also in its progression. Similarly, a strong association between liver fibrosis and the development of HCC is well established but poorly understood. We have previously demonstrated that type 1 collagen (COL1) could block apoptosis of normal hepatocytes through the ERK1 pathway. **Purpose:** The goal of this work was to evaluate the impact of HCV infection on the development of HCC and on its sensitivity against anti-cancer agents and to study the influence of COL1 on these effects.

Methods: We used 3 HCC cell lines: Huh7 (devoid of HCV proteins), 9-13 (a Huh7 cell line stably transfected with a genotype-1 replicon expressing HCV non-structural proteins) and JFH-1 (a Huh7 cell line infected with the JFH-1 HCV strain). Cell death was induced by exposure of the cell lines to cisplatinum (CP) [25 µg/mL] for 24hr, in the presence or not of COL1 [13.9 µg/cm2]. Cell viability (MTT assay), apoptotic bodies (apoptotic rate (AR)) and cell proliferation were measured.

Results: JFH-1-infected cells were more resistant to CP-induced apoptosis than the parental Huh7 cell line (AR: 21.5±1.8% in Huh7 vs 15.2±0.3% in JFH-1; $p<0.05$). This effect was not observed in 9-13 cells (15.8±1.8%; $p=0.1$). In presence of COL1, Huh7 and 9-13 cells were protected against CP-induced apoptosis (AR; Huh7: 9.8±2.2% on COL1 vs 21.5±1.9% on plastic; $p<0.01$, 9-13: 9.5±1.2% on COL1 vs 15.8±1.8% on plastic; $p<0.05$). Addition of COL1 did not increase the resistance of JFH-1-infected cells to CP (14.0±1.1% on COL1 vs 15.2±0.3% on plastic; $p=0.34$). Results were similar when measuring cell viability. Interestingly, the protective effect of COL1 was abolished when cells were co-treated with CP and the MEK inhibitor U0126 [20µM] (AR; Huh7: 16.3±2.1%, 9-13: 15.9±2.7%), a mechanism that was not observed in JFH-1-infected cells (AR; 13.1±1.6% vs 15.2±0.3%; $p=0.3$). There was no statistical difference in cell doubling time between HCC cell lines plated on COL1 or on plastic (Huh7: 53±6h vs 56±4h, 9-13: 63±4h vs 64±10h), nor between JFH-1 cells and the parental Huh7 cell line (Huh7: 53±6h; JFH-1: 64±3h).

Conclusions and perspectives: HCV infection reduces the chemosensitivity of HCC cells. This protective effect could be dependent on the presence of structural proteins and is not mediated by ERK pathway. The cellular pathways that are involved remain to be clarified. COL1 protects HCC cell lines against apoptosis by an ERK1/2-mediated mechanism without any effect on cell proliferation but this effect is lost in the presence of HCV infection.

Poster presentation: BS007

HCV REPLICATION REQUIRES THE RECRUITMENT OF THE AUTOPHAGY ELONGATION COMPLEX (ATG5-12/16) IN A LC3 INDEPENDENT MANNER

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Hepatitis C virus (HCV) infection is known to induce autophagosome accumulation as observed by the typical punctate cytoplasmic distribution of LC3-II in infected cells. Recently, we showed that viral RNA-dependent RNA polymerase (NS5B) interacts with ATG5, a major component of autophagy initiation. In this study, we evaluate the involvement of the autophagy elongation complex (ATG5-12/16) in HCV replication. We demonstrate that the elongation complex is recruited at the site of viral replication and acts as a proviral factor. Indeed, ATG5-12 as well as ATG16L1 colocalizes with the viral replicase and dsRNA in infected cells. Using in situ proximity ligation assay, we confirms that ATG5 can interact with two replicase component, namely NS5B and NS3, but not with the viral capsid (core). Furthermore, we show the capability of NS4B to induce autophagy. While NS4B-induced autophagy was escorted by colocalization of NS4B with LC3-II in NS4B-transfected cells, no colocalization has been observed during infection. Interestingly, LC3 is not recruited along with the elongation complex to the site of viral replication as no colocalization of LC3-II with viral proteins was observed. Finally, using dominant negative forms of ATG proteins and siRNA approach, we demonstrate that ATG5-12 conjugate is important for viral replication but not LC3-II formation. Together, these findings indicate that HCV uses the autophagy elongation complex as a proviral factor for its own replication while it impedes the formation of a genuine autophagosome at the site of viral replication.

Poster presentation: BS008

IDENTIFICATION OF CIRCULATING HUMAN MICRORNAS AS POTENTIAL BIOMARKERS OF HEPATITIS C VIRUS-ASSOCIATED LIVER FIBROSIS

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Background: Globally liver fibrosis is a major cause of liver related morbidity and mortality. To replace expensive and invasive liver biopsy and/or Fibroscan® testing for fibrosis staging, additional serum biomarkers are desperately needed. MicroRNAs (miRNAs) are small non-coding RNAs involved in the gene expression regulation and are highly abundant inside the cells and in blood. Changes in miRNAs levels have been associated with many human pathologies, including liver cancer.

Purpose: This study is designed to investigate a subset of circulating human miRNAs to serve as biomarkers for liver fibrosis in patients with chronic hepatitis C virus (CHC) infection.

Method: Expression of circulating miRNAs was measured in 100 CHC patients that received interferon-based HCV treatment. Liver fibrosis was scored by biopsy and/or FibroScan® prior to treatment. Plasma samples were collected before, during, and post-treatment. Levels of HCV RNA and the APRI score of liver inflammation were measured at each time point of sample collection. Circulating miRNAs levels were measured using qRT-PCR assays, which were normalized using synthetic miRNA (cel-miR-39). Healthy controls were used to establish baseline expression levels of circulating miRNAs from 15 people.

Results: MiR-122, miR-24 and miR-223 are the most abundant miRNAs in the liver and regulate genes essential for lipid and cholesterol metabolism. Based on preliminary data, these miRNAs are likely to be promising biomarkers for staging liver fibrosis because they show a significant expression upregulation in patients with CHC compared to HCV RNA negative controls. Average fold changes (±SEM) in CHC patients (n=8) compared to HCV RNA negative controls (n=2) of miR-122: 24.25±11.59 to 0.79±0.20, miR-24: 6.19±1.59 to 0.85±0.1, and miR-223: 6.53±2.12 to 0.74±0.25, respectively. Also, these miRNAs showed little variation within individuals whose blood was drawn 2-8 days apart and levels were not affected by multiple freeze-and-thaw cycles. Average changes (±SEM) in normalized Ct values within individuals (n=3) of miR-122: 1.69±0.78, miR-24: 0.87±0.5, and miR-223: 1.39±1.03.

Conclusions: By assessing miRNAs profiles in healthy control subjects and patients chronically infected with HCV, we identified a differentially expressed subset of three miRNAs that are associated with CHC. Our data support the potential use of quantifying the levels of serum miRNAs to assess HCV-associated liver fibrosis in CHC patients.

Poster presentation: BS009

MASS-SPECTROMETRY ASSISTED PROTEIN FOOTPRINTING PROVIDES KEY INSIGHTS INTO THE HEPATITIS C VIRUS NS5A-RNA COMPLEX

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Background: HCV NS5A is essentially required for viral replication, and therefore serves as a prime target in current drug development efforts. The RNA-binding protein is subdivided into three domains. Domain I has been crystallized in two structurally distinct, homodimeric conformations. However, the functional relevance of the dimer conformations for RNA binding remains elusive.

Purpose: Previously, we conclusively isolated 2 different species of NS5A in solution. We also provided strong evidence that only one of these conformations is competent for RNA binding. In this study, we aimed to further characterize the conformations of NS5A-DI and when it is in complex with RNA.

Methods: We employed a mass-spectrometry assisted protein footprinting technique to map the interaction surface of NS5A-DI at single amino acid resolution. Briefly, NS5A-DI was incubated in the absence and presence of a ligand. The two separate solutions were then treated with small molecules that modify arginines or lysines. The bound ligand protects these residues from the modifying agent and the protected residues can be identified through MS-assisted analysis [1].

Results: In the absence of a ligand, the 2 isolated species of NS5A-DI generated different protection patterns. In the presence of RNA, the protection pattern is consistent with a model that locates the RNA to the positively charged cleft of conformation I (PDB 1ZH1) [2]. Mutational analysis of these residues confirmed their involvement in RNA binding.

Conclusions: Our data further substantiates previous conclusions that at least two distinct species of NS5A-DI can be isolated in solution. In addition, we also identified a Lysine residue that is not part of the putative RNA binding cleft, although it shows a significant contribution to RNA binding. This suggests that the binding of RNA to NS5A-DI might involve alternative protein conformations or higher order structures.

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Poster presentation: BS010

HCV INFECTION INDUCES APOPTOSIS, BYSTANDER APOPTOSIS AND PYROPTOSIS

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Background: Apoptosis and pyroptosis are two distinct forms of programmed cell death (PCD) that are induced by different mechanisms and result in different pathological outcomes. Caspase-3 plays a central role in the induction of apoptosis, its activation results in a silent cell death that does not stimulate inflammation. Pyroptosis, on the other hand, is a caspse-1-dependent pro-inflammatory form of PCD that result in cell lysis. Studying the ability of HCV to induce these forms of cell death will contribute to our understanding of the mechanisms by which pathogenic events occur in the infected liver.

Purpose: To study the ability of HCV infection to induce apoptosis and/or pyroptosis in infected and/or neighboring hepatocytes, and to decipher the possible mechanisms responsible for their induction.

Method: Virus stocks were generated by transfecting Huh-7.5 cells with the adapted JFH1T HCV RNA followed by two rounds of virus passage. The viability of uninfected and infected cells was determined by MTT assay. Cell proliferation was assessed by CFSE assay. DNA fragmentation was tested by DNA laddering assay and propidium iodide (PI) DNA staining followed by detecting the presence of hypodiploid cells. Apoptosis was detected by measuring the percentage of cleaved-PARP-positive cells and was confirmed by testing the effect of treating the cells with caspase-3 inhibitor on the number of hypodiploid cells. Western blotting was used to determine the level of cleaved caspase-8, Grp78 and Chop proteins to decipher the pathway responsible for apoptosis induction. Infected or uninfected Huh-7.5 cells were stained with anti-HCV-core and PI to assess apoptosis in infected versus neighboring uninfected cells. This was confirmed by infecting a co-culture of Huh-7.5 cells and CD81-negative S29 cells followed by detecting the presence of cleaved-PARP-positive cells within the S29 population. The induction of pyroptosis was tested by staining the cells with a caspase-1 FAM-FLICA probe, and by testing the effect of treating the cells with caspase-1 inhibitor on the number of hypodiploid cells.

Results: Infection reduced the viability of infected cells and decreased the rate of cellular proliferation. PI staining and DNA laddering assays showed that HCV induces DNA fragmentation, a characteristic of both apoptosis and pyroptosis. HCV infection increased the number of cleaved-PARP-positive cells, a test used to specifically detect apoptosis. The induction of apoptosis was confirmed using a caspase-3 inhibitor, which inhibition resulted in a significant decrease in hypodiploid cells. Interestingly, hypodiploid cells were detected in both HCV core-positive and -negative populations, and cleaved-PARP-positive cells were found in both Huh-7.5 and S29 cell populations. These two results provide evidence that apoptosis is not limited to infected cells, but also occurs in neighboring uninfected cells. Infection caused an increase in the number of active-caspase-1-positive cells and inhibiting caspase-1 resulted in a significant reduction in the number of hypodiploid cells showing strong evidence for the induction of pyroptosis.

Conclusion: HCV infection causes a reduction in cellular proliferation rate. We were able to show that HCV infection induced multiple forms of PCD, including apoptosis in both infected and neighboring uninfected cells (bystander apoptosis), as well as pyroptosis.

Poster presentation: BS011

EFFECT OF MODIFIED TEMPLATES ON RNA SYNTHESIS BY THE HCV NS5B: IMPLICATIONS FOR THE MECHANISM OF RESISTANCE TO NUCLEOSIDE ANALOGUES

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Background: HCV NS5B is an RNA-dependent RNA polymerase responsible for viral RNA replication. Structurally, it resembles a human right hand with fingers, palm and thumb subdomains. The recently reported structure of HCV NS5B in complex with an RNA primer-template points to interactions between the enzyme and the 2'-OH group of specific RNA residues (1). However, the functional relevance of these interactions remained elusive.

Purpose: Based on the structural data, we hypothesize that the lack of 2'-OH groups of a DNA template can affect RNA synthesis and sensitivity to nucleotide analogue inhibitors. To test this hypothesis, we studied the effects of strategically introduced DNA residues in the template strand on nucleotide/nucleotide analogue incorporation and RNA elongation.

Methods: HCV NS5B was expressed and purified to near homogeneity. RNA synthesis was assessed in biochemical assays using defined, model RNA templates with specific chemical modifications.

Results: We found that NS5B is able to synthesize RNA on both RNA and DNA templates; however, we observed strong pausing sites associated with the following template modifications: dT, dU, and 2'F-U. 2'F-U adopts the RNA-like northern conformation of the sugar, despite the lack of the 2'OH group. These findings suggest that chemical modifications, and not the sugar pucker, cause enzymatic pausing. The chemical nature of the template can likewise affect the impact of resistance conferring mutations in NS5B. S282T decreases sensitivity to the inhibitor 2'C-Me-CTP when tested against the natural RNA template. When tested against DNA, the level of inhibition of RNA synthesis was similar with wild type NS5B and the S282T mutant.

Conclusions: The structure of the binary complex shows a hydrogen bond between the 2'OH group of the templated RNA residue and the backbone of G283. The lack of a 2'OH group at this position may therefore increase the flexibility of S282T at the priming site, which in turn helps to accommodate the modified nucleotide. Our results suggest that the 2'-OH group of the template nucleotide plays an important role in establishing the S282T-associated resistant phenotype.

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Poster presentation: BS012

EXAMINING THE ALTERATIONS IN THE HOST ENZYMATIC ACTIVITY DURING HEPATITIS C VIRUS REPLICATION USING ACTIVITY-BASED PROTEIN PROFILING

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Background: Host-pathogen interactions are indispensable for the replication of hepatitis C virus (HCV), and while numerous studies have demonstrated that HCV modulates the abundance of various host proteins, the systematic study of the virus's effect on the enzymatic activity has been relatively unexplored. For this reason, we applied activity-based protein profiling (ABPP) to study the changes in the activity of host enzymes during HCV replication. ABPP is a functional proteomics technique that employs active site-directed probe (ABP) to report on the activity of enzymes within complex proteomes, such as living cells. Herein, we employed broad-spectrum non-directed ABPs based on β-lactam scaffold for global profiling of the alterations in the activity of cellular enzymes during HCV replication. β-lactams are generally known as powerful antibiotics against bacterial infections, however, because the β-lactam moiety is a potent electrophile, it can react with the active residues of enzymes from different classes. Therefore β-lactam derived ABPs allows for functional examination of diverse enzyme families.

Aims: To identify the essential host enzymes that are differentially active during HCV infection. These enzymes can potentially be recognized as disease-associated biomarkers, with diagnostic and therapeutic significance.

Methods: Comparative ABPP was performed by employing novel β-lactam derived ABPs in situ on naïve Huh7 cells and Huh7 cells harboring HCV full-genomic replicon (HCV-FGR). In situ labeling allows for the identification of β-lactam targets in their native location, such as intracellular membranes and organelles. Subsequent to labeling, cells were lysed and proteome was extracted. The labeled proteins underwent streptavidin-enrichment, followed by on-bead trypsin digestion and analysis by nano LC-MS/MS.

Results: We were able to identify a variety of mechanistically distinct enzymes that demonstrate differential activity during HCV infection. Some of these enzymes have been previously reported to play important roles in HCV replication cycle, such as protein phosphatase 1B that is involved in interferon signalling, acetyl-CoA carboxylase, the rate-limiting enzyme in lipogenesis as well as cyclin-dependent kinase 2, which is the key enzyme in cell-cycle regulation. Importantly, we identified several other host enzymes that their activity dramatically change during HCV infection, but their role in the viral life cycle is yet unclear. Moreover, we developed a quantitative ABPP method for relative quantification of the cellular enzymes activity during HCV infection.

Conclusion: In conclusion, we successfully applied novel β-lactam derived ABPs for functional screening of enzyme activity in intact cells. We were able to identify a variety of mechanistically distinct target enzymes that show differential activity during HCV replication.These results will accelerate the discovery of protein activities associated with the pathogenic states of HCV infection, and therefore facilitate the discovery of new biomarkers as well as potential targets for therapeutic interventions.

Poster presentation: BS013

GSK3B INHIBITORS PREVENT HCV RELEASE THROUGH PERTURBATION OF VLDL ASSEMBLY

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Background: Hepatitis C virus (HCV) direct antiviral treatment has progressed in the past few years with better response rates. Although there has been considerable work in the field, there are still many unanswered questions and many untreated patients. Besides the detrimental liver cirrhosis and hepatocellular carcinoma (HCC) occurring late during the disease, HCV infection can be associated with liver steatosis and insulin resistance. This increases the disease morbidity and mortality and has been linked to disturbances in glycogen synthase kinase 3 (GSK3β) signaling [1]. In addition, the HCV nonstructural protein 5A (NS5A) activates the AKT pathway resulting in inhibition of GSK3β and the accumulation of B-catenin, which in turn inhibits cell apoptosis, thereby contributing to the development of HCC [2]. Researches also showed that HCV hijacks the very low density lipoprotein (VLDL) secretory pathway for its release from hepatic cells [3].

Hypotheses: Based on the possible role of the VLDL secretory pathway in HCV release, and preliminary data from work done in Dr. Houghton's laboratory, we hypothesized that down-regulation of GSK3β inhibits HCV release and could perturb the VLDL secretory pathway with subsequent trapping of the virus within the cells.

Methods: The expressions of WNT/ β-catenin pathway that includes GSK3β molecules in Huh7.5 cells were determined using quantitative RT-PCR. Proteins isolated from Huh7.5 cells were examined for total and phosphorylated GSK3β using Western blot and ELISA. Cells treated with GSK3β inhibitors were examined for the level of HCV replication and virion production in comparison to mock treated cells.

Results: Although, inhibition of GSK3β did not affect HCV replication in neither JFH-infected Huh7.5 cells nor HCV replicon cells, a significant reduction (P= 0.0003) in HCV virus genome copy number released into cell culture supernatants was observed in JFH-infected Huh7.5 cells. Moreover, GSK3β inhibitors treated-Huh7.5 cell culture supernatant showed intact intracellular virions infectivity with TCID50 significantly higher than that of the extracellular virions (P=0.0001). On the other hand, no differences in TCID50 between Intra and extracellular verions in cells treated with DMSO. Likewise, data from gene microarray analysis indicated that GSK3β inhibition is associated with downregulation of genes involved in the VLDL assembly. FPLC of the lipid profile in treated-Huh7.5 culture supernatant revealed a dose dependent reduction in the VLDL particles size and numbers in contrast to DMSO-treated cells.

Conclusion: GSK3β inhibitors affect HCV release and not assembly. This effect is predicted to be through interfering with VLDL assembly and secretion and further studies are required to explore factors involved in VLDL assembly pathway. Our work uncovers new aspects of virus-host interactions and the role of GSK3β and VLDL in HCV infectivity and pathogenesis.

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Poster presentation: BS014

25-HYDROXYCHOLESTEROL STIMULATED ANTIVIRAL MICRORNAS REGULATE HEPATIC LIPID METABOLISM

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Background: Recent work has demonstrated that interferon stimulated macrophages secrete an antiviral oxysterol, 25-hydroxycholesterol (25HC). While 25 HC has been implicated as an antiviral effector, its mechanism of action remains unclear. We have previously shown that 25HC represses hepatitis C virus (HCV) replication through regulation of coding genes associated with virus-associated host pathways.

Purpose: We sought to examine 25HC's regulation of microRNAs in order to further characterize its antiviral properties and regulation of hepatic lipid homeostasis.

Methods: Microarray profiling and TaqMan-based qPCR were used to identify the miRNA signatures associated with 25HC's antiviral effect and HCV infection. Predicted targets of miRNA candidates of interest were functionally validated using 3'UTR luciferase assays, and qPCR, and Western blot analyses. miRNA mimics and inhibitors were used to investigate these miRNAs' influence on hepatic lipid metabolism (using coherent anti-Stokes Raman (CARS) spectroscopy, triglyceride and cholesterol assays) and HCV infection.

Results: We demonstrate that 25HC activates the expression of microRNAs, miR-130b and miR-185, in HCV infected hepatoma cells. We show that miR-185 and miR-130b overexpression potently inhibits HCV replication. Conversely, miR-130b and miR-185 inhibition increases viral replication. miR-185 and miR-130b directly repress the expression of several host factors with regulatory roles in lipid metabolism, including SREBP, a master transcriptional regulator of cholesterol biosynthesis, SCD, a key enzyme in the synthesis of unsaturated fatty acids, and LDLR, a crucial receptor for cholesterol uptake. CARS microscopy demonstrates that inhibition of miR-185 or miR-130b activity results in lipid accumulation – highlighting HCV induced downregulation of these miRNAs' expression as a novel mechanism of HCV-induced steatosis. Furthermore, several of the 25HC induced antiviral miRNAs' direct targets correspond to host factors with crucial roles in the HCV life cycle, consistent with these miRNAs' antiviral activity. Interestingly, HCV infection downregulates the expression of miR-130b and miR-185 to potentially circumvent 25HC's antiviral effects.

Conclusions: With increasing evidence that cholesterol and unsaturated fatty acids play a crucial role in the entry and replication of several classes of virus, our work suggests that 25HC's activation of miRNAs regulating lipid metabolism could play a critical role in the broad antiviral response.

Poster presentation: BS015

DEVELOPMENT OF A NOVEL HEPATITIS C VIRUS (HCV) CLONING SYSTEM TO ISOLATE AND PROPAGATE INFECTIOUS HCV GENOMES FROM PATIENT BLOOD SAMPLE

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Background: HCV is responsible for 170 million infections worldwide and is a major cause of cirrhosis and hepatocellularcarcinoma. However, 30% of those infected with HCV never develop chronic disease and resolve during the acute stage of infection. This virus clearance is associated with the early development of board neutralising antibodies (Nab) response (Osburn et al., 2014).

Experiments with anti-HCV Nab have been extremely difficult to perform due to the inability to clone and/or propagate the autologous patient virus (or an E1/E2 chimeric virus) and study antibodies from the serum of the same patient. This is due to the high mutation rate of HCV (10-3 substitions/nt), approximately 1-10 mutations occurs within the 9.6 kilobase genome of HCV during every replication cycle. Only a few efficiently replicating particles (possibly 1 in 1000) may be present in the intrapatient HCV population or quasispecies.

To tackle this, nearly full length HCV genomes were generated from patient-derived blood samples and recombined with plasmid containing 5' and 3' UTR to create full length HCV genomes. Individual HCV clones will then be screened for its ability to propagate and replicate in a new reporter cell line that permits wild type infection.

Purpose: To develop of a novel hepatitis C virus (HCV) cloning system to allow rapid cloning and screening of infectious viral genomes from patients blood sample.

Method: To develop a novel HCV cloning system, nearly full length HCV cDNA genomes (5' and 3' UTR missing) were reverse transcribed from patient blood serum. cDNA genomes were then recombined with a selection plasmid containing the 5' and 3' UTR sequence to generate plasmids containing full length HCV genome. To select for an infectious HCV clone, a novel reporter cell line based on Huh7-J20 will be created and used to screen and propagate infectious HCV clones (Iro et al., 2009). These patient-derived infectious clones can then be sequenced for comparison and study.

Results:

- Nearly full length HCV1a genome was reverse transcribed and amplified from patient blood sample.
- Lentiviral vector constructs were created to generate a wild type HCV replication permissive reporter cell line.

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Poster presentation: BS016

DIFFERENTIAL DIVERSITY AND DYNAMICS OF HCV VARIANT SPECTRA ARE ASSOCIATED WITH MATERNAL HIV-1 CO-INFECTION AND MOTHER-TO-CHILD HCV TRANSMISSION

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Background: Mother-to-child transmission (MTCT) is responsible for most cases of pediatric HCV infection and its incidence is increased when the mother is co-infected with HIV-1. HCV is genetically diverse, and this diversity is especially high in hypervariable regions (HVRs) of the E2 envelope protein, which are subjected to host immune responses. In earlier studies [1, 2], we found that during pregnancy, the regions of E2 presenting the highest rate of positive selection were solvent-exposed, suggesting the potential involvement of neutralizing antibody responses or antibody-dependent cytotoxic activity (ADCC). Pregnancy is associated with a transient modulation of humoral and cell-mediated immunity. Thus, we hypothesize that modulation of humoral immune response influences the course of HCV infection during gestation.

Purpose: To investigate the potential association between HCV E2-based quasispecies diversity and maternal humoral immune responses, and to determine whether co-infection with HIV-I has an impact on maternal immunity, HCV quasispecies evolution, and the risk of MTCT.

Methods: Serial serum samples from women infected with HCV (n=17) or co-infected with HCV and HIV-1 (n=20) were collected during the first, second and third trimesters of pregnancy and in the *postpartum* period. Viral RNA was extracted, reverse transcribed, amplified using HCV genotype-specific barcoded primers and high-fidelity polymerase, and subjected to ultra-deep pyrosequencing (Roche 454 Titanium FLX+). Output files were subjected to a k-mer based error correction algorithm (KEC) (3) prior to calculation of variant frequencies based on HVR1 amino-acid sequences.

Results: Variant spectra obtained from each time point were then used to compute the Morisita-Horn similarity index, Shannon entropy, and Hamming distances. Association between these parameters and patient clinical and socio-demographic information were also tested to detect potential differences between mono-infected and co-infected subjects. Results based on the Morisita-Horn index showed that variant turnover was faster in mono-infected subjects than in the co-infected group. In addition, HCV viral load in mono-infected women decreased following childbirth while it increased in the co-infected group ($p = 0.0175$). Most interestingly, low values of Shannon entropy were associated with a 20-fold increased risk of MTCT of HCV in the mono-infected group ($p=0.006$).

Conclusions: Differential dynamics of HCV quasispecies diversity and viral load between the third trimester of gestation and the *postpartum* period suggests that maternal immunity could be driving HCV evolution during pregnancy and that maternal co-infection with HIV-1 impairs these mechanisms. Low entropy values could reflect the presence of neutralization escape variants or high fitness variants that are more likely to be transmitted to the child. These results could lead to improved care for pregnant, HCV-infected women and their children, for whom preventative and therapeutic options are presently limited.

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Poster presentation: BS017

CHARACTERIZATION OF PROTEASE INHIBITOR RESISTANT MUTATIONS AND ANALYSIS OF NOVEL INHIBITORS IN AN INFECTIOUS CELL CULTURE SYSTEM

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Background: In recent years, novel and effective direct-acting antivirals (DAAs) have become available and optimized in the clinic, but drug resistant variants can still emerge in treated patients (1). The primary goals of this project are to analyze fitness of resistant viruses, develop an EC50 assay for various DAAs in the context of a fully infectious virus, establish a protocol for selection of drug resistance, and assess the impact of genetic barrier on the emergence of resistant variants.

Purpose: The primary purpose of this project is to implement, improve or develop assays to characterize resistance and mechanisms of drug action. By identifying and analyzing resistance we can better understand the virus and improve therapies and/or therapeutic combinations.

Materials and Methods: To validate our system, four common PI drug resistant variants: L36M, T54A, R155K and I170A were generated in the JFH1T backbone through site-directed mutagenesis and viral fitness was assessed based on the levels of virus produced upon transfection of Huh-7.5 cells. Mutant viruses were cultured over 15 - 45 days and were sequenced to assess the impact of genetic barrier on reversion. Resistance of the mutants to Telaprevir was assessed by QRT-PCR. Appearance of mutants in culture was assessed through regional PCR amplification and sequencing.

Results: The L36M, T54A and R155K mutations resulted in titres of approximately 1 log lower than wild type JFH1T, I170A showed an approximately 3-4 log decrease. T54A and R155K showed ½ log and log increases of EC50 values, respectively, relative to wild type JFH1T. After passaging R155K for 45 days the lysine mutation was maintained in cell culture. After passaging T54A for 30 days the alanine mutation was maintained in cell culture. R155K also remained dominant when co-cultured with wild-type at a 10 to 1 ratio for three days. Several novel compounds, representing multiple drug classes were tested in our EC50 assay and the effective concentrations determined reflected the expected results based on chemical class.

Conclusions: Our results validate the efficacy and accuracy of our system. We intend to use this system to assess these characteristics in new and promising compounds. Considering the fully infectious nature of our system we believe it can accurately recapitulate the effects of new DAAs targeting multiple stages of the viral life cycle.

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Poster presentation: BS018

UNDERSTANDING BARRIER'S TO RESISTANCE: MOLECULAR MECHANISMS CONTROLLING SELECTION OF HCV VARIANTS

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Background: After 25 years of hepatitis C virus (HCV) research, we finally have interferon-free drug regimens that cure patients and have low toxicity! We are interested in learning from this grueling process in order to help lower the cost and time required to develop DAAs for other viruses. Specifically, we will focus on how to predict DAA targets that have a high barrier to drug resistance. Emergence of drug resistant variants follows the basic evolutionary principles and requires two things: Diversity and Selection. Our research aims to characterize mechanisms of selection and how these contribute to the development of drug resistance.

During the time in which a drug resistant virus is in the same cell as its drug susceptible parent, drug resistant virus can be subject to dominant suppression by drug-bound viral proteins within the same cell. Therefore, by choosing drug targets that are highly oligomeric, we can decrease the selection of drug resistant viruses. The Kirkegaard laboratory has established this paradigm, called the "Dominant Drug Target", using poliovirus as a model and identified the poliovirus capsid as our first dominant drug target. My goal is to screen a variety of HCV encoded viral proteins and corresponding DAAs to determine which proteins constitute dominant drug targets.

Methods: To mimic the situation in which a drug-resistant variant first arises, co-infection with wild-type and mutant viruses must be observed and quantified. To this end, we constructed a codon-altered JFH1 in which we introduced 247 non-coding mutations over a 918 nucleotide sequence located across NS2 and the N-terminus of NS3. This codon altered strain demonstrated wild-type growth kinetics in Huh7.5.1 cells. We then designed RNA in situ hybridization probes that differentiate between the codon-altered and wild-type JFH1 viruses to analyze growth of each strain independently and quantitatively using flow cytometry.

Results: Mutations conferring drug resistance to BILN-2061 (NS3), SR2486 (NS5A), and Daclatasvir (NS5A) have been cloned into the wild type JFH1 background. Huh7.5.1 cells have been co-infected with drug susceptible and drug resistant JFH1 strains in the absence and presence of drugs to quantify dominance relationships of these various strains of HCV. Our results indicate that the NS3/4A protease is a non-dominant drug target as we readily select drug resistant variants from our coinfected cells. This supports the observed low barrier to resistance of the protease inhibitors. However, our data suggest that NS5A is a dominant drug target. We observe repressed growth of NS5A variants in coinfected cells using our FlowRNA selection assay. Although the first-generation NS5A inhibitors have a barrier to resistance that could be labeled as moderate, the second-generation NS5A inhibitors have a high barrier to resistance. Our data indicates that dominance could play a role in decreasing the selection of resistant variants while targeting NS5A.

Poster presentation: BS019

A RECOMBINANT HCV ENVELOPE GLYCOPROTEIN E1E2 VACCINE ELICITS ANTIBODIES TARGETING MULTIPLE REGIONS ON E1E2 ASSOCIATED WITH BROAD CROSS-NEUTRALIZATION

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Background: Although effective Hepatitis C Virus (HCV) antivirals are on the horizon, a global prophylactic vaccine for HCV remains elusive. The diversity of the virus is a major concern for vaccine development; there are 7 major genotypes of HCV found globally. Therefore, a successful vaccine will need to protect against HCV infection of all genotypes. Despite the diversity, many monoclonal antibodies (mAbs) with broadly cross-neutralizing activity have been described suggesting the presence of conserved epitopes that can be targeted to prevent infection. Similarly, a vaccine comprising recombinant envelope glycoproteins (rE1E2) derived from the genotype 1a HCV-1 strain has been shown to be capable of eliciting cross-neutralizing antibodies in guinea pigs, chimpanzees, and healthy human volunteers.

Purpose/Method: In order to investigate the basis for this cross-neutralization, epitope mapping of anti-E1E2 antibodies present within antisera from goats and humans immunized with HCV-1 rE1E2 was conducted through peptide mapping and competition studies with a panel of cross-neutralizing mAbs targeting various epitopes within E1E2.

Results: The immunized goat antisera was shown to compete with the binding of all mAbs tested (AP33, HC33.4, HC84.26, 1:7, AR3B, AR4A, AR5A, IGH526, A4). Antisera showed the best competition against HC84.26/AR3B and the weakest competition against AR4A. Furthermore, antisera from five immunized human vaccinees were shown to compete with five pre-selected mAbs (AP33, AR3B, AR4A, AR5A, IGH526).

Conclusions: These data show that immunization with HCV-1 rE1E2 elicits antibodies targeting multiple cross-neutralizing epitopes. Our results further support the use of such a vaccine antigen to induce cross-genotype neutralization.

Future Directions: Characterization of the mechanisms of neutralization by vaccine antisera and at what entry step neutralization is taking place is currently being investigated and new data will be discussed.

Poster presentation: BS020

INTERACTION BETWEEN HCV CORE AND PTEN PLAYS A ROLE IN REGULATING HCV REPLICATION

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Background: Hepatitis C virus (HCV) infection causes serious global public health problems. The World Health Organization has established that there are more than 170 million chronic HCV patients worldwide. Hepatocellular carcinoma (HCC) is the most deadly clinical consequence of HCV infection. Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) can suppress PI3K-AKT pathway, one of the most critical cancer-promoting pathways PTEN is frequently mutated or deleted in tumors including HCC. However, the role of PTEN in HCV replication and pathogenesis is not well characterized. PTEN protein contains an N-terminal PIP2 (phosphatidylinositol-4,5-bisphosphate)-binding motif, a phosphatase domain, a C2 domain, a C-terminal tail containing two PEST (proline, glutamic acid, serine, threonine) sequences and a PDZ (PSD-95/DLG/ZO-1)-binding interaction motif at the end. Two naturally occurring mutations on the phosphatase domain disrupt PTEN's phosphatase activity: C124S mutation, which abrogates both lipid and protein phosphatase activity, and G129E mutation, which abrogates lipid phosphatase only.

Purpose: To determine the effect of PTEN on HCV infection and the underlying molecular mechanisms.

Methods: We characterized HCV infection after PTEN overexpression or knocking down. We also determined whether PTEN interacts with HCV viral proteins as a mechanism for its effect on HCV infection.

Results: PTEN negatively regulated HCV genotype 1a and 2a viral entry through PI3K-AKT pathway by using HCV pseudo-particles. In HCV-2a J6/JFH-1 genomic replicon cells, knocking down PTEN significantly enhanced HCV NS5A protein expression and viral replication; consistently, PTEN overexpression significantly inhibited HCV replication. We further showed that the phosphatase domain of PTEN was involved in HCV replication inhibition. Interestingly, PTEN with the lipid phosphatase defective mutation (G129E) could no longer inhibit HCV replication. In co-immunoprecipitation and pull-down assays, we showed that HCV core protein interacted with PTEN. HCV core aa. R50 and PTEN aa. 1-185 were required for the interaction. PTEN overexpression could no longer inhibit HCV genomic replication carrying core R50A mutation.

Conclusion: PTEN regulates HCV viral entry, protein expression and replication. The lipid phosphatase activity of PTEN is required for inhibiting HCV replication. HCV core interacts with PTEN, which may contribute to PTEN's effect on HCV replication. Our study may help justify further development of PTEN as a new drug target for HCV therapy.
Funding: NCRTP-HepC, CIHR, SHRF

Behavioural Sciences

Poster presentation: BES11

HEPATITIS C IS THERE HOPE?

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Background: There are people who have lived with Hepatitis C with behavioural issues that arose from abuse, poor coping skills and lack of knowledge. But is that how we help people, based on behaviour?

Purpose: To help people we need to understand what it is like. Compassion is needed, understanding that living with hepatitis has lead some people on in a path that has had a great impact on their lives. Hepatitis as a teacher has no boundaries or feelings. It impacts everyone involved including children. Hepatitis affects people holistically; emotionally, spiritually, mentally and physically.

Method: Oral/ story telling

Results: The only way to understand what it is like to live with a virus is to hear it first hand. Living with an illness impacts families whole, not only the individual. It has a financial impact as well, for instance can you imagine what it's like for the bred winner of the family and the head of the household to live with an illness that at the time there was no cure and only one result death, with four children who rely solely on her?

Conclusions: Having lived with an illness from many different perspectives, that include never hearing of the word hepatitis to watching loved ones die from the illness and burying friends. What would you do? Hear what it's like to disclose to your children. The only way to ever learn how to battle an issue is hear first hand experience of what it is like to live with it first. Until then do we ever really know? Do we have the empathy and compassion that comes from sincerity? The story promises to be gripping, with real live issues that people still face today. Hear what life was like from a young person's perspective to have a loved on live with hepatitis. Here is an epic journey of before hepatitis, their lives during and the outcome. The journal of a mother and a daughter with hepatitis. Is there hope?

Epidemiology and Public Health

Poster presentation: EPH001

HEPATITIS C INFORMATION NEEDS OF PATIENTS IN CARE IN CANADA

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Background: CATIE is Canada's source for up-to-date, unbiased information about HIV and hepatitis C. We connect people living with HIV or hepatitis C, at-risk communities, healthcare providers and community organizations with the knowledge, resources and expertise to reduce transmission and improve quality of life. CATIE is committed to informing the work we do with the needs of the communities we serve.

Purpose: CATIE developed a national needs assessment for patients engaged in hepatitis C care to better understand the information and educational needs of people living with hepatitis C. This information can be used to develop programs and resources in order to help people living with hepatitis C better manage their health.

Objectives: This national needs assessment will inform people working in hepatitis C of the information needs of people living with hepatitis C and how they would like to receive that information.

Methods: Twenty medical clinics across Canada serving people living with hepatitis C have been approached to request their help in recruiting people with hepatitis C to complete an online needs assessment. The needs assessment is available in both English and French through Fluid Survey. To date, eight clinics have agreed and 105 participants have been recruited. The aim is to recruit 500 participants by the spring of 2015.

Results: An interim analysis will be conducted to present information on demographics (age, gender, family background/place or origin) health and care (year of diagnosis, discussion of hepatitis C treatment options with physician; current hepatitis C treatment experience, involvement in decision making around hepatitis C care), knowledge needs (rating of current knowledge of hepatitis C, rating of need for hepatitis C information, importance of certain topic areas for hepatitis C information), and preferred formats to receive information on hepatitis C.

Conclusions: The information from this needs assessment will be shared broadly through various knowledge translation exchange mechanisms. This will include presentations at research conferences, providing results to participating clinics and making the results available on CATIE's website. This information will be used to inform CATIE's work in hepatitis C.

Poster presentation: EPH002

RECENT TRANSMISSION OF HEPATITIS C VIRUS IN AUSTRALIA IS ASSOCIATED WITH GENOTYPE 1A INFECTION AND HIV CO-INFECTION

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Background: Strategies to reduce hepatitis C virus (HCV) transmission are needed. Research has identified factors associated with HCV acquisition among people who inject drugs (PWID) and HIV positive men who have sex with men (MSM), but little is known about HCV transmission dynamics in these settings.

Purpose: The aim of this study was to investigate transmission dynamics and identify factors associated with phylogenetic clustering among people with acute HCV infection in Australia.

Methods: Data were derived from three cohorts- the Australian Trial in Acute Hepatitis C (ATAHC), the Hepatitis C Incidence and Transmission Study in prison (HITS-p) and the Hepatitis C Incidence and Transmission Study in the community (HITS-c). Participants from these cohorts with acute HCV infection with available plasma samples at the time of acute HCV detection were selected for inclusion. Viral RNA was extracted and the Core–E2 region of HCV was sequenced using Sanger sequencing. Phylogenetic trees were inferred using maximum likelihood analysis with 1000 bootstrap replicates and clusters were identified using ClusterPicker (90% bootstrap threshold, 5% genetic distance threshold). Factors associated with clustering were identified by comparing the characteristics of participants in either a pair or cluster with those not in a pair or cluster using Fisher's exact test or $\chi 2$ as appropriate. Logistic regression analyses were used to identify factors associated with being in a pair/cluster (compared to not being in a pair/cluster). All analyses were performed using STATA software.

Results: Among 234 participants with sequences (ATAHC, n=123; HITS-p, n=91; and HITS-c, n =20)), the HCV genotype prevalence was: G1a: 40% (n=94), G1b: 4% (n=10), G2a: 2% (n=4), G2b: 5% (n=11), G3a: 47% (n=110), G6a: 1% (n=2) and G6k 1% (n=3). Among participants with HCV G1a/G3a, 24% were in a pair/cluster (G1a-32%, 29/88, mean maximum genetic distance =0.034; G3a-18%, 19/108, mean maximum genetic distance=0.028). Of all G1a/G3a infected participants, 50% (14/28) of HCV/HIV co-infected participants were in a pair/cluster as compared to 20% (34/168) with HCV alone. In those with G1a/3a, factors independently associated with phylogenetic clustering included HIV co-infection [adjusted odds ratio (AOR) 3.21; 95%CI 1.35, 7.62], and HCV G1a infection (AOR 2.13, 95%CI 0.22, 0.97). Some clusters displayed distinct characteristics, demonstrating transmission within specific sub-populations (for example, within HIV positive MSM). However some clusters also demonstrated transmission between male and females in prison, injecting drug users in prison and in the community and MSM/PWID who were HIV positive and HIV negative.

Conclusions: In these three cohorts of acute HCV infection in Australia, the majority of participants were infected with HCV genotype 1a and genotype 3a infection, with 24% of these participants demonstrating phylogenetic clustering. HIV co-infection and G1a infection were independently associated with phylogenetic clustering. Strategies to enhance the delivery of prevention and/or treatment strategies to those with HIV should be explored, given an increased likelihood of HCV transmission. HCV transmission in acute infection demonstrates complex patterns of risk behaviours with overlapping modes of acquisition.

Poster presentation: EPH003

IDENTIFYING HCV TREATMENT BARRIERS AMONGST HIGH RISK POPULATION OF VANCOUVER DOWNTOWN EASTSIDE

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Background: Currently there is little information on what is preventing high risk vulnerable populations from engaging in HCV diagnosis and treatment. The aim of this study is to survey this population using a targeted questionnaire and to identify barriers to HCV care. This was administered during Portable Pop-up Clinics (PPCs) at specific locations frequented by people who inject drugs (PWID) where participants have the opportunity to access point-of-care testing.

Methods: Participants were recruited at PPCs held at two different community-based centers in Vancouver's Downtown East Side. During these PPCs OraQuick HCV Rapid Antibody point of care testing was offered. Participants who were tested were then offered to complete a questionnaire while they waited for test results.

Results: During January 2014 - May 2014, 171 individuals completed the questionnaire (38 female, 56% Caucasian, 40% First Nation; mean age: 46). Key demographic characteristics included: being single (81%), living alone (45%), not working (74%) and having finished high school (83%). Amongst all participants, 124 reported prior HCV testing (73%), 40 identified sharing needles or other injection equipment (23%), 74 injected drugs (43%), 95 were previously incarcerated (56%), 91 were aware of a cure for HCV (53%) and 138 stated they would consider treatment if they had HCV (81%). Among HCV positive individuals 44 of 51 participants reported prior HCV testing (86%), 32 were aware of their positive status (63%), 41 were previously incarcerated (80%) and 39 would consider treatment (76%). With regards to HCV treatment, 40% of participants believed that they did not need treatment, 20% did not know where to go for treatment, 30% expressed concern regarding side effects of treatment, 19% did not believe they can financially afford it, and 12% reported not wanting to consult a physician to receive treatment.

Conclusion: Despite awareness of their HCV infection, and their desire to get treated PWID do not routinely seek medical care. Barriers such as inaccessible medical care, unfamiliarity with available resources, and concerns regarding treatment side effects have been identified. Organized and targeted community events such as PPCs increase likelihood of reaching out to marginalized and high risk inner city populations to address these barriers in a systematic way.

Poster presentation: EPH004

METHAMPHETAMINE INJECTING IS ASSOCIATED WITH PHYLOGENETIC CLUSTERING OF HEPATITIS C VIRUS INFECTION AMONG STREET-INVOLVED YOUTH IN VANCOUVER, CANADA

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Background: Among prospective cohorts of people who inject drugs (PWID), phylogenetic clustering of HCV infection has been observed. The majority of studies have included older PWID, representing distant transmission events. This study aimed to investigate phylogenetic clustering of HCV infection among a cohort of street-involved youth.

Methods: Data were derived from a prospective cohort of street-involved youth aged 14-26 recruited from 2005-2012 in Vancouver, Canada (At Risk Youth Study, ARYS). Among participants who were HCV positive at the time of enrolment or had HCV seroconversion during follow-up, HCV RNA testing and sequencing (Core-E2/NS5B regions) were performed. Phylogenetic trees were inferred using maximum likelihood methods and clusters were identified using ClusterPicker (Core-E2, 90% bootstrap threshold, 0.05 genetic distance threshold).

Results: Among 945 individuals enrolled in ARYS, 16% (n=149) were HCV antibody positive at baseline (n=86) or demonstrated HCV seroconversion during follow-up (n=63). Of these, 100% (n=149) were recent injectors. Among HCV antibody positive participants with available samples (n=131), 75% (n=98) had detectable HCV RNA in the sample to be sequenced and 66% (n=65) had available Core-E2 sequences. The mean age of participants was 23 years, 32% (n=18) were female, 58% (n=33) reported recent (last six months) methamphetamine injection, and 3% (n=2) were HIV+. HCV genotype prevalence was: G1a: 42% (n=27), G3a: 51% (n=33), G2b 5% (n=3) and G1a 3% (n=2). Among participants with available Core-E2 (n=65), 14% (n=9) were in a cluster (one cluster of 3) or pair (three pairs), with all nine participants reporting recent methamphetamine injection. Recent methamphetamine injection was associated with membership in a cluster or pair (P=0.009).

Conclusion: In this study of street-involved youth with HCV infection and recent injecting, 14% demonstrated phylogenetic clustering. Phylogenetic clustering was associated with recent methamphetamine injection, suggesting that methamphetamine drug injection may play an important role in networks of HCV transmission.

Poster presentation: EPH005

HEPATITIS C VIRUS NETWORK DYNAMICS AMONG PEOPLE WHO INJECT DRUGS IN VANCOUVER, CANADA

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Background: There is excitement about treatment as prevention as a strategy for HCV control among people who inject drugs (PWID). But, little is known about characteristics that increase HCV transmission risk among PWID.

Purpose: This study evaluated whether new HCV infections among a cohort of young drug users are seeded from one or more transmission events from a cohort of long-term adult HCV-infected PWID.

Method: Data were derived from two prospective cohorts in Vancouver, Canada, including VIDUS (injected drugs in past month, 1996-2012) and ARYS (14-26 years, used illicit drugs other than or in addition to marijuana in the past month, 2005-2012). Participants who were HCV antibody (Ab+) at enrolment or had HCV Ab seroconversion during follow-up were tested for HCV RNA and sequenced (Core-E2 without HVR1). Phylogenetic trees were inferred using maximum likelihood. Phylogenetic segregation of the VIDUS and ARYS cohorts was assessed using Association Index (AI). Network analyses were performed using the 0.5th percentile of patristic distances of the ML tree in Cytoscape using NetworkAnalyser.

Results: Among 708 participants (VIDUS, n=657; ARYS, n=51), the majority were infected with HCV genotype 1a (48%, n=334) or G3a (34%, n=241). Among VIDUS participants (n=657), the mean age was 36 years and 25% (n=164) were HIV+. Among ARYS participants (n=51), the mean age was 23 years and 3% (n=2) were HIV+. Greater clustering was observed in VIDUS (31%) compared to ARYS (10%). A moderate degree of segregation between VIDUS and ARYS was observed with AI value of 0.763 (value >1 indicates no more segregation than would occur by chance). HCV infections from ARYS were seeded from multiple transmission events from VIDUS participants as compared to local HCV expansion among ARYS participants. Network analysis (0.5 percentile patristic distance threshold) identified 407 participants (nodes) with 1106 connections (edges), with 202, 21, 4, 16, and 164 nodes for genotypes 1a, 1b, 2a, 2b and 3a, respectively. The average number of neighbours was 4.6 and 7.3 for G1a and G3a, respectively. Meanwhile, participants with G1b, G2a and G2b had 2.2, 1.0 and 1.5 average neighbours each.

Conclusion: These data suggest that new HCV infections among a cohort of young drug users were seeded from several transmission events from a cohort of long-term HCV-infected injectors in Vancouver, Canada. Network analysis identified a high degree of linkage between participants with the most prevalent genotypes. Strategies to enhance the delivery of prevention/treatment strategies to high transmission foci could be explored, given an increased likelihood of HCV transmission in these sub-populations.

Poster presentation: EPH006

INFLUENCE OF GENDER ON HEPATITIS C VIRUS INFECTION MANAGEMENT AND TREATMENT OUTCOMES

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Background: Difficulties treating chronic Hepatitis C virus (HCV) are well documented [1][2][3]. It has been suggested that gender plays a role in disease progression [4]. However, the influence of gender on treatment outcomes is still relatively unknown.

Objective: The influence of gender on infection management and treatment outcomes in HCV infected individuals was evaluated.

Methods: We assessed patients with chronic HCV attending The Ottawa Hospital Viral Hepatitis Clinical between 1996 and 2013. Analysis focused on: (1) HCV risk factors and baseline characteristics, (2) pre-treatment work-up, and (3) treatment outcomes success. HCV risk factors and baseline characteristics included (injection drug use, cocaine use, blood products, tattoos, history of sexual activity, weight, ALT and AST levels. Pre –treatment workup was determined by FibroScan and Liver biopsy rates. Treatment outcomes success was determined by the SVR rates and side effect rates.

Results: We assessed 1878 individuals (32% female). Females were more likely to have received blood products (29.3% vs 21.6%, P<0.0001) and less likely to have engaged in IDU (48.4% vs 62.3%, P<0.0001). Alcohol abuse was lower in females (43% vs 69%, P<0.0001). Key baseline characteristics including HCV RNA (3.65x106 vs 7.38x106, P=0.06), ALT (75.8 vs 103.3, P<0.0001), and AST (60.4 vs 72.4, P<0.001) levels were lower in females. Liver biopsy rates were lower (44.6% vs 53.4%, P<0.001) and FibroScan rates were higher (14.4% vs 11.2%, p=0.054) in females. Mean fibrosis scores in women tended to be lower by biopsy (METAVIR: 1.8 vs 2.0, P=0.03) and FibroScan (1.8 vs 2.0, P=0.36). Overall, treatment rates (37.3% vs 45.1%, P=0.002) were lower in females. However, women were more likely to initiate interferon sparing regiments (4.2% vs 1.1%). Similar proportions of men and women completed treatment (61.1% vs 59.9%).Women cited higher side effects rates (22.7% vs 16.6%, P=0.057) while males had higher viral non-response rates at week 24 (6.9% vs 2.5%, P=0.02). SVR rates were higher in women (64.1%vs 55.0%, P=0.032). Compared to men in a multivariable logistic regression model adjusted for age, genotype, race, and HIV co-infection, the odds ratio for women achieving SVR was 1.27 (95% CI: 0.84-1.92).

Conclusions: HCV characteristics, management and treatment outcomes differ by gender. This should be considered when attempting to optimize HCV care and treatment outcomes.

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Poster presentation: EPH007

SUSTAINABILITY OF A HCV CURE IN A POPULATION WITH A HIGH RISK OF REINFECTION: MONTREAL'S EXPERIENCE

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Introduction: In Montreal the incidence rate of HCV infection is estimated at 26/100 py among the IDU population. Though treatment uptake among HCV infected patients remains low, the latter is likely to change given the recent development of promising INF-free treatments. Costs may be a barrier to treatment availability in many countries; HCV reinfection after successful treatment could be additional burden. HCV reinfection has been reported both in UDI and MSM patients but the extent to which reinfection occurs is unknown.

Purpose: The aim of this study was to evaluate the incidence of reinfection in a clinical cohort of HCV treated patients.

Methods: HEPVIRAC's (Hepatite Viral –l'Actuel's Cohort) patients with sustained virological response (SVR) to HCV treatment were included in this study. Censoring date was either the date of HCV reinfection or the date of the last negative HCV RNA test. Reinfection was defined as detectable HCV RNA with or without ALT elevation. Date of reinfection was estimated as the midpoint between the last negative and first positive HCV RNA tests. The rate of reinfection was calculated using the number of person-years of observation after the end of HCV treatment. Time from SVR to reinfection was estimated using Kaplan-Meier analyses.

Results: 338 patients cured from HCV were included. Men represent 77% and women 23% of the sample; mean age was 46 years; major risk factor for HCV infection was IDU for 275 (82%) patients, sexual transmission for 30 (9%) and the remaining patients were infected from tattoos (n=9), transfusion/contaminated liquid (n=11), sharing of non IDU material (n=4) or were from endemic regions (n=5). Patients were followed up (FU) for a median of 2.7 years after the end of treatment (IQR, 1.7-4.8), for a total of 1175 person-years of FU. 316 (94%) patients remained persistently negative, while 22 (6%) became reinfected during FU period with an overall reinfection rate of 1.7/100py [95% CI 1.07-2.58]. Median time from cure to reinfection was estimated at 14.7 years (95%CI 13.6-15.7). Cumulative incidence of seroconversion within 2 years of SVR was 4% (9/210) and 11% (10/88) within 5 years of SVR. Following adjustment for past or present drug use, the incidence rate of HCV reinfection was 0.43/100py [95% CI 0.02-0.11] for non drug users; 1.90/100py [95% CI 1.13-3.14] for past IDU and 3.60/100py [95% CI 1.44-7.39] for present IDU.

Conclusion: HEPVIRAC shows a relative low risk of HCV reinfection after successful treatment. Although the rate of HCV reinfection is higher in IDU than non IDU, it remains much lower than the overall incidence rate of the first HCV infection in drug users in Montreal.

Poster presentation: EPH008

ABOUT ACTION HEPATITIS CANADA

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Background: Action Hepatitis Canada (AHC), a national coalition of organizations responding to HBV and HCV, engages government, policy makers, and civil society across Canada to promote hepatitis prevention, improve access to care and treatment, increase knowledge and innovation, create public health awareness, build health-professional capacity, and support community-based groups and initiatives.

Purpose: To inform Canadian hepatitis communities about the activities of AHC

Method: In 2002, Canadian organizations involved in addressing viral hepatitis came together to form a national hepatitis network, calling on governments to develop hepatitis strategies, and introducing the document, "Responding to the Epidemic: Recommendations for a Canadian Hepatitis C Strategy". In 2010, organizations came together once again to form the "Canadian Coalition of Organizations Responding to Hepatitis B and C", which in 2013 became AHC.

Results: Organizational members of AHC come from across the country. Some are general members and others sit on the Steering and Executive Committees - meeting regularly. Patricia Bacon is the current Chair of AHC. Communications are on-going between AHC and the general membership. AHC also has websites in French and English. All AHC member organizations have one vote during elections. CATIE is currently the Secretariat for AHC. Many AHC members provide in-kind support, and all members involved with AHC activities do so voluntarily.

Conclusions: Beginning in 2010, AHC called on the federal, provincial and territorial governments to develop strategies to address viral hepatitis. In 2011, AHC produced, distributed and promoted the first "Report Card" capturing the achievements and gaps in research, prevention, testing, treatment, care and support programs and services for people living with and at risk for viral hepatitis. This report card was updated in 2012. Throughout 2013 and much of 2014, while continuing to call on governments to develop hepatitis strategies, and within a hopeful yet ever-changing climate of treatment advances, AHC has worked on strengthening its own capacity to act on behalf of those impacted by viral hepatitis.

AHC has engaged in media communications on issues such as the recent WHO hepatitis resolution - encouraging countries around the world to develop viral hepatitis strategies; and responding to patient treatment access concerns in light of the extremely high cost of new HCV drugs. The Steering Committee has also worked with the AHC Secretariat to develop a series of funding "asks" and approaches resulting in dedicated funds flowing to AHC for the first time to support meetings, media communications and community training and capacity-building efforts. Along with funds from the Canadian Hemophilia Society, AHC hired a researcher/writer to update the AHC "report card". The updated information gathered will be contained in a new, user-friendly, searchable database available for public viewing on AHC's websites. The first "Viral Hepatitis in Canada: Progress Report" will be launched in 2015 using the data collected. Community training and capacity-building initiatives will take place to strengthen AHC's capacity to advocate on behalf of those living with and affected by viral hepatitis.

Poster presentation: EPH009

THE REVOLUTION IN HEPATITIS C TREATMENT: USING ECONOMETRIC METHODS TO EVALUATE THE IMPACT OF THE NEW DIRECT ACTING ANTIVIRAL'S IN HIV/HEPATITIS C CO-INFECTED INDIVIDUALS

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Background: In Canada, approximately 20% of people living with HIV are also co-infected with Hepatitis C[1]. Despite controlled HIV viremia with the widespread use of combined antiviral therapy (cART), evidence suggests that co-infected individuals progress faster to endstage liver disease (ESLD) compared to mono-infected HCV patients.[2,3] Furthermore liver disease is now one of the leading causes of death among HIV-HCV infected individuals. Not since the introduction of cART in 1996 has there been a revolutionary therapy that could save so many lives. Until now, treating hepatitis C with standard of care, has yielded marginal success rates after patients endured debilitating side effects, lasting up to 48 weeks[4]. Direct Antiviral Agents (DAAs) are a new class of potent hepatitis C therapies that have the potential of eradicating Hepatitis C with minimal adverse events[5]. Sofosburvir and Simpeprevir are the latest DAA's that have been included on drug formularies in select provinces. In light of these new therapies one solution to reduce long-term clinical and health service utilization outcomes may be to treat all co-infected patients for their HCV infections. However these medications are extremely costly[6]. Clinical trials conducted thus far do suggest cost-effectiveness, however these trials have been conducted on a homogenous population that does not reflective the reality of the endemic. Moreover, a significant population that is often excluded from clinical trials are co-infected individuals mainly due to their many competing co-morbidities.

Methods: The Canadian HIV-HCV Co-infection Cohort (CCC), a multicenter national prospective study, is in a unique position to investigate the causal health and economic impacts of DAAs in a "real-world" setting. Our clinical outcomes of interests are the progression to advance liver disease, using a surrogate marker such as APRI or fibroscan results if available and death. Our health economic outcomes will include health service utilization such as ER, hospitalizations, specialist, GPs, and walk in clinic visits. Given the drugs were approved first in Quebec and our ability to use other provinces as control units; we will employ the difference-in-differences (DD) a quasi-experimental method to measure the impacts of DAA use. The DD method has been used in other contexts to evaluate the impacts of health policies. Other designs such as interrupted time series, individual fixed-effects, and marginal structural models may also be appropriate. We will undertake further investigation to clarify whether the clinical and policy contexts and our data can support the assumptions necessary for these methods to estimate unbiased causal effects.

Conclusions: We are in an era of new challenges, cures for Hepatitis C are available but due to financial constraints important decisions will have to be made as to who receives therapy. We hope the results from this research provide insight into improving clinical care not only in a cost-efficient manner but also to avoid health inequalities, in this vulnerable population.

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