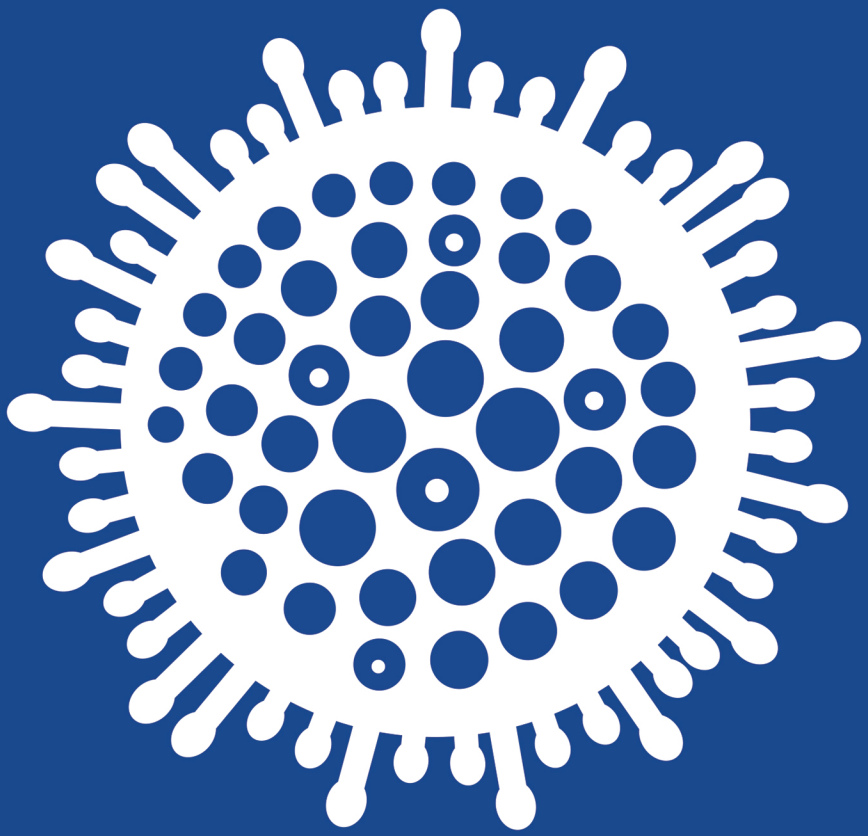
11th International Conference on

HHV-6 & 7

PROGRAM BOOK



June 23-26, 2019

Québec City, Canada

Thank you to our Conference Sponsors

HHV-6 FOUNDATION

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WISENT BIOPRODUCTS

WELCOME FROM THE CONFERENCE CO-CHAIRS

Dear Colleagues,

It is with great pleasure that we welcome you to the 11th International Conference on Human Herpesviruses 6 and *7* in Québec City. Together with the members of the International Organizing Committee, we aimed to put together an exciting and informative program that addresses all aspects of HHV-6 & HHV-*7* biology as well as new developments such as the possibility that HHV-6A & HHV-*7* plays a role in Alzheimer's disease progression.

We are honored to have Joel Dudley, PhD and Dr. Robert Moir as keynote and featured speakers. Their findings regarding Alzheimer's, published in Neuron last year, are provocative and potentially quite important.

We are fortunate to have outstanding scientists, clinicians and trainees from all over the world at the meeting to share their knowledge and novel findings. We hope that new collaborations will emerge and that existing ones will be strengthened.

We deeply thank Kristin Loomis, Jill Chase, and Jason Stanley of the HHV-6 Foundation for their administrative efforts to make this meeting possible as well as our colleagues on the International Organizing Committee who helped us build the program. We would also like to thank the sponsors of the conference including the HHV-6 Foundation, Universite Laval, Clinigen, Biken, Chimerix, Bio-Rad, Biocell, Wisent, ABI and CHU de Québec Research Center for their generous support.

Louis Flamand,
PhD, MBA Co-Chair

Danielle Zerr,
MD, MPH Co-Chair

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11TH INTERNATIONAL CONFERENCE ON HHV-6 & 7

CO-CHAIRS

Louis Flamand
PhD, MBA

Professor, Department of Microbiology-Immunology, Faculty of Medicine
Laval University & Senior Researcher
Division of Infectious and Immune Diseases
CHU de Quebec-Université Laval Research Center, Quebec City, CANADA

Dr. Flamand is an expert on HHV-6 and inherited chromosomally integrated HHV-6. He has published over 4 dozen publications related to HHV-6 and served as editor of the book *"Human Herpesviruses HHV-6A, HHV-6B & HHV-7: Diagnosis and Clinical Management"* 3rd edition.

Among his important contributions to HHV-6 are a large-scale study of iciHHV-6 in Canadians, establishing iciHHV-6 as a predisposing condition for the development of angina.

He currently serves as Chair of the department of microbiology-infectious-disease-immunology at the Faculty of medicine, Université Laval and senior researcher in the division of infectious and immune diseases at the CHU de Quebec research center.

Before joining Laval university, Dr Flamand obtained his PhD at the University of Montreal and post-doctoral training at the National Institutes of Health and at the Institute of Human Virology (Maryland, USA). He received his MBA in pharmaceutical management from Université Laval.

Danielle Zerr
MD, MPH

Professor & Division Chief University of Washington & Seattle Children's Hospital Pediatric Infectious Diseases & Affiliate Investigator, Fred Hutchinson Cancer Research Center Seattle, Washington, USA

Dr. Zerr is an expert on HHV-6 infection in both healthy children and transplant patients. She has published over three dozen papers on HHV-6 and is the author of *HHV-6 in Transplantation Summary for UPTODATE*, the widely used reference guide for physicians. Among Dr. Zerr's recent contributions to the literature are a meta-analysis on the impact of HHV-6B reactivation on acute GVHD, and several studies that define the relationship between HHV-6B reactivation and delirium and neurocognitive decline after hematopoietic cell transplantation.

Her 2005 study at Fred Hutchinson Cancer Research Center was one of the first to show that HHV-6 is associated with delayed engraftment, increased platelet transfusion requirements, CNS dysfunction, all-cause mortality and grade 3-4 GVHD in hematopoietic cell transplant recipients.

She currently serves as Division Chief in Pediatric Infectious Diseases at the University of Washington and Seattle Children's, where she holds the endowed chair for Pediatric Infectious Disease Research.

Dr. Zerr earned her medical degree at Temple University and then further training in Pediatrics, Infectious Diseases, and Epidemiology at the University of Washington.

2019 CONFERENCE – AGENDA AT A GLANCE

Sunday June 23rd	Monday June 24th	Tuesday June 25th	Wednesday June 26th
			SAB / IOC Breakfast 7:30-8:30 AM
	Introduction 8:30 AM	**Inherited ciHHV-6** 8:30-10:30 AM	**Transplantation** 8:30-10:30 AM
	Keynote – Alzheimer's 9:00-9:45 AM		
	Alzheimer's 9:45-10:45 AM		
		Break	**Break**
	Break		
	Alzheimer's 11:05-12:30 PM	**Inherited ciHHV-6** 10:45-12:00 PM	**Transplantation** 10:45-11:45 AM
		Malignancy 12:00-12:30 PM	
	Lunch 12:30-1:30 PM *Bio-Rad Workshop* *By Reservation Only*	**Lunch** 12:30-1:30 PM *IHC / ISH Workshop* *By Reservation Only*	**Treatment & Diagnostics** 11:45-1:00 PM
	Animal Models 1:30-2:35 PM	**Genes & Proteins** 1:30-2:15 PM	**Lunch** 1:00-2:00 PM
			DRESS / DIHS 2:00-2:45 PM
	HHV-6 & CNS Disease 2:35-3:35 PM	**Innate & Acquired Immunity** 2:15-3:30 PM	**Closing** – 2:45 PM
	Break	**Break**	**Workshop** *Annotation of the HHV-6 Genome* 3:00-5:00 PM
	HHV-6 & CNS Disease 3:55-5:55 PM	**Host-Cell Interaction** 3:45-5:00 PM	
Welcome Reception 5:30-7:30 PM *Hotel Le Concorde*		**High-Titer Virus Wksp** 5:00-6:00 PM	
	Posters and Reception 6:00-7:30 PM *Hotel Le Concorde*	**Reception** 6:00-7:00 PM *Le Parlementaire*	
		Banquet Dinner 7:00-8:30 PM *Le Parlementaire*	

INTERNATIONAL ORGANIZING COMMITTEE

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Yasuko Mori, M.D., Ph.D. Kobe University Graduate School of Medicine JAPAN	**Masao Ogata, M.D., Ph.D.** Oita University Faculty of Medicine JAPAN
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Tetsushi Yoshikawa, M.D., Ph.D. Fujita Health University School of Medicine JAPAN	**Danielle Zerr, M.D.** University of Washington & Seattle Children's Hospital USA

11TH INTERNATIONAL CONFERENCE ON HHV-6 & 7

GENERAL INFORMATION

CONFERENCE VENUE

Hôtel Le Concorde Québec – 2nd Floor Conference Room
1225 Cours du Général-de Montcalm
Québec City, Canada

hotelleconcordequebec.com
+1 (418) - 647 - 2222
+1 (800) - 463 – 5256

REGISTRATION

Registration packet pickup and walk-in registration is available at the following times at our registration tables at the Hôtel Le Concorde Ballroom Foyer, 2nd Floor:

Sunday, June 23 …… (*at Welcome Reception*) …… 5:30 pm – 7:30 pm
Monday, June 24 ……..……..7:00 am – 4:00 pm
Tuesday, June 25 …….. 7:00 am – 4:00 pm
Wednesday, June …… 7:30 am – 12:00 pm

GENERAL SESSIONS

All general sessions will be held in the Ballroom, which is on the second floor of Hôtel Le Concorde.

SPEAKER CHECK-IN

The presentations should be uploaded to the conference staff by 4:00 pm the day before your presentation. All speakers should utilize the computer provided by the conference to make their presentations; please let us know if there are extenuating circumstances. The computer used for the presentations will be equipped with the latest Windows operating system. PowerPoint (.pptx or .ppt) is the preferred presentation format. Speakers should use the link below for all uploads. As a backup, please bring your presentation on a USB drive that can be uploaded at the registration desk.

Presentation Upload Link: spaces.hightail.com/uplink/HHV6Foundation
Questions? Please Email: Jason_Stanley@hhv-6foundation.org

11TH INTERNATIONAL CONFERENCE ON HHV-6 & 7

GENERAL INFORMATION

POSTER SETUP, DISMANTLE AND SESSIONS

Posters can be handed in at the registration desk on Sunday June 23, 5:30 pm - 7:30 pm or Monday morning. Presenters may mount the posters themselves or turn them over to the conference staff. Posters should be mounted by noon on Monday. The posters will be removed Tuesday between lunch and afternoon break. You may collect your poster from the check-in desk any time after the afternoon break on Tuesday.

Poster Schedule	**General Viewing**	**Poster Reception**
Monday, June 24	12:00 pm – 6:00 pm	6:00 pm – 7:30 pm

All posters should be attended by the presenting author during the poster session.

INTERNET ACCESS

The Hotel Conference Center provides free Wi-Fi on site.

CONFERENCE MEALS AND RECEPTIONS

The following events and meals are included in your conference registration:

Welcome Reception

Sunday, June 23 ..... 5:30 pm - 7:30 pm
The Sunday Welcome Reception will be held in the Ballroom foyer on the second floor of the Hôtel Le Concorde. Food and beverages will be served.

Breakfast & Breaks

Complimentary morning breakfast pastries, fruit, coffee and tea will be provided adjacent the ballroom 7:00-8:30am Monday-Wednesday. Full breakfast is available daily at JAJA Restaurant on the ground floor of the hotel. (Meals at JAJA are not covered by conference registration).

Coffee breaks are offered every morning and afternoon during the conference.

Lunch

Lunch will be served adjacent to the meeting room daily, on Monday, Tuesday and Wednesday.

Poster Reception

Hôtel Le Concorde Ballroom Foyer, 2nd Floor
Monday, June 24 ..... 6:00 pm - 7:30 pm

11TH INTERNATIONAL CONFERENCE ON HHV-6 & 7

GENERAL INFORMATION

Tuesday Banquet Dinner (included with registration)
Location: Quebéc Parliament Building, 1045 rue des Parlementaires
The Parliament Building, home to the National Assembly and meeting place of Québec's 125 elected representatives, is a short walk from the Hôtel Le Concorde. Conference guests may take advantage of optional private tours of this national historical monument. Dinner will be served in the elegant beaux arts dining room.

Schedule:

Meet in the hotel lobby to walk down together in two groups …….. 5:30 pm & 5:45 pm
Cocktail Reception & Optional Tours …….. 6:00 pm - 7:00 pm
Dinner at Le Parlementaire Restaurant …… 7:00 pm - 8:30 pm

NOTE: *A current Photo ID is required at the security entrance.*

BONUS WORKSHOPS

Monday: BioRad ddPCR ……..…. during lunch, 2nd floor
Tuesday: Immunohistochemistry & ISH ……...…….. during lunch, 2nd floor
Tuesday: Growing High Titer Virus ……..……... 5:00 pm, main conference room
Wednesday: Annotation of the HHV-6/7 genome …….. 3:00 pm, main conference room

INSURANCE/LIABILITY

The conference organizers cannot accept liability for injuries or losses arising from accidents or other situations during or as a result of the conference.

CITY ATTRACTIONS

Old Québec is a charming and walkable UNESCO World Heritage Site. Public transportation is readily available, as are extensive bike paths and even horse-drawn carriages. Please consult the hotel front desk for a city map and tourism suggestions.

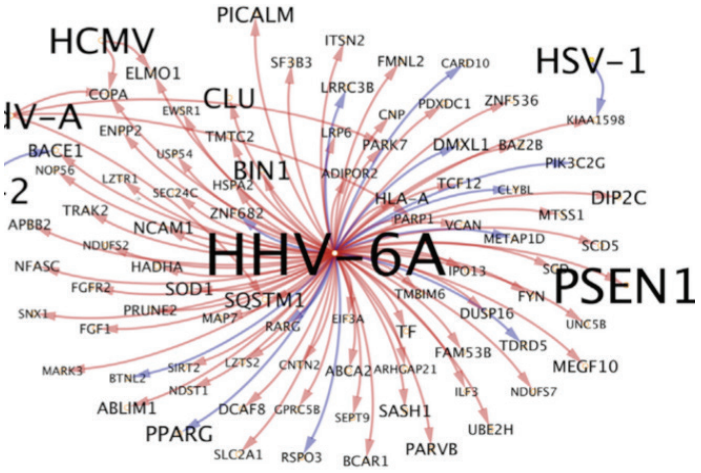
11TH INTERNATIONAL CONFERENCE ON HHV-6 & 7

GENERAL INFORMATION

Québec Parliament Building, location of the Tuesday reception and banquet

11TH INTERNATIONAL CONFERENCE ON HHV-6 & 7

KEYNOTE SPEAKER



Dr. Joel Dudley

Dr. Dudley is Associate Professor of Genetics and Genomic Sciences and founding Director of the Institute for Next Generation Healthcare and Director of Hasso Plattner Institute of Digital Health at the Icahn School of Medicine at Mount Sinai. In March 2018 Dr. Dudley was named Executive Vice President for Precision Health for Mount Sinai Health System. In 2017 he was awarded an Endowed Professorship by Mount Sinai in Biomedical Data Science. Prior to Mount Sinai, he held positions as Co-founder and Director of Informatics at NuMedii, Inc. and Consulting Professor of Systems Medicine in the Department of Pediatrics at Stanford University School of Medicine. His work is focused at the nexus of -omics, digital health, artificial intelligence (AI), scientific wellness, and healthcare delivery. His work has been featured in the Wall Street Journal, Scientific American, Neuron, MIT Technology Review, CNBC, Today Show and other popular media outlets. He was named in 2014 as one of the 100 most creative people in business by Fast Company magazine. He is co-author of the book *Exploring Personal Genomics* from Oxford University Press. Dr. Dudley received a BS in Microbiology from Arizona State University and an MS and PhD in Biomedical Informatics from Stanford University School of Medicine.

In the summer of 2018, Dr. Dudley and associates reported the results of a four-year, multi-cohort, multi-network "big data" analysis of what goes awry in the brains of Alzheimer's patients. To their surprise, they found that HHV-6A and HHV-7 DNA and RNA were not only more abundant in Alzheimer's brains vs. controls, HHV-6A appeared to directly interact or co-regulate known Alzheimer's genes.

11TH INTERNATIONAL CONFERENCE ON HHV-6 & 7

FEATURED SPEAKER

Dr. Robert Moir

Dr. Moir complete his Ph.D. at the University of Melbourne in Australia under the mentorship of Prof Colin Masters, one of the founders of the modern field of Alzheimer's disease (AD) research. Dr. Moir immigrated to the US in 1994 and joined the Genetics and Aging Research Unit (GARU) shortly after the group's formation. Dr. Moir now heads his own research lab within GARU and has been a faculty member of Harvard Medical School and Massachusetts General Hospital since 1998. Dr. Moir's research focus is the interaction of biomolecules involved in AD pathology. His work identified the low-density lipoprotein receptor protein (LRP) as the mediator of an important early step in production and clearance of the key molecular species in AD pathology- the Aβ peptide. Today, LRP-mediated Aβ clearance pathways are major targets for therapeutic intervention. His work revealed the importance of metals in the pathological aggregation of Aβ, leading directly to the investigation of the copper and zinc chelator clioquinol in clinical trials for AD. Moir has also helped establish Aβ's role in the formation of eye lens cataracts in AD patients, which could lead to better diagnosis of the disease. Dr. Moir's studies on the autoimmune response to amyloid have also revealed abnormalities in protective Aβ-reactive autoantibodies in AD patients that lead to development of the drug aducanumab. Dr. Moir's most recent studies have identified the normal biological function of Aβ. Aβ is a natural antibiotic that acts as part of the innate immune system to trap and kill pathogens invading the brain. This novel discovery suggests AD may be an inappropriate response to a real or falsely perceived infection in the brain. This finding may also shed light on the pathological mechanisms associated with other major amyloid-associated diseases, including diabetes and arteriosclerosis. Dr. Moir's findings have appeared in multiple high-profile journals including *Cell, Science, Lancet,* and *Nature Medicine, Neuron, and Science Translational Medicine*. Based on Dr. Moir's work on the normal biological function of Aβ, he and his collaborator Rudolph Tanzi have advanced the Antimicrobial Protection hypothesis of Alzheimer's disease that is emerging as a major new disease model.

BONUS WORKSHOPS

Digital droplet PCR: BIO-RAD

Sean Taylor, PhD of Bio-Rad

Monday Lunch
Leduc-Fortin Room, 2nd Floor
By reservation only
Lunch provided

This seminar will compare and contrast the data generated from qPCR and Digital PCR technology from identical samples with varying levels of contaminants to highlight how and when to use these technologies for applied and basic research applications. Digital PCR is a new technology that overcomes the pain points associated with qPCR including: 1) Standard Curve Reproducibility; 2) Variability in Detection and Quantification of Low Abundant Targets; 3) Interplate Variability and 4) Artifactual Data consequent to Differentially Contaminated Samples. These issues are particularly problematic and persistent with detection and quantification of circulating DNA or RNA biomarkers from biofluids, exosomes, micro RNA and long non-coding RNA due to their low abundance. Furthermore, the residual contaminants in nucleic acid extracts from paraffin blocks and in reverse transcribed cDNA samples can partially inhibit the qPCR polymerase activity, giving misleading or false negative results. Particularly problematic for virology is the detection of residual disease post-treatment due to the combined effects of inter-assay variability and low target abundance.

Immunohistochemistry & ISH

David Hudnall, MD (Chair)
Konnie Knox, PhD
Janos Luka, PhD
Bhupesh Prusty, PhD

Tuesday Lunch
Leduc-Fortin Room, 2nd Floor
By reservation only
Lunch provided

Very few laboratories perform immunohistochemistry and in situ hybridization for HHV-6A/B/7 on their samples. We gathered a few with expertise in this area, to share some of their techniques.

BONUS WORKSHOPS

Growing High Titer Virus

Louis Flamand, PhD (Chair)
Christine Birdwell, PhD
Janos Luka, PhD
Bhupesh Prusty, PhD

Tuesday 5PM
Main Conference Hall

During this session, the step-by-step procedures used by Louis Flamand's laboratory to produce infectious HHV-6A and HHV-6B viral stocks will be presented. Techniques and recommendations on procedures to avoid with be explained, along with titration methods. Following the presentation, other scientists generating cell-free viral stocks will be invited to provide insights on their techniques.

Annotation of the HHV-6A/B/7 Genomes

Alex Greninger, PhD (Co-Chair)
Philip Pellet, PhD (Co-Chair)
Louis Flamand, PhD
Ursula Gompels, PhD
Ruth Jarrett, PhD
Ben Kaufer, PhD
Yasuko Mori, MD, PhD

Wednesday 3PM
Main Conference Hall

Major advances in high-throughput experimental methodology have revealed that herpesvirus gene expression is surprisingly complex. Although the HHV-6A/B genomes in current databases are fairly accurate, gene architecture has mostly been deduced from other herpesviruses such as CMV or HSV. Recent evidence suggests that the HHV-6A/B genes are more complex than originally imagined. Possible changes to the HHV-6A/B gene nomenclature and annotation be will be discussed.

SCHEDULE OF ORAL PRESENTATIONS

MONDAY, JUNE 24TH		*Session Chairs / Presenter*
Introduction *8:30-9:00*		
0 - 0 *8:30*	Introduction to Conference - Day 1	*Flamand & Zerr*
0 - 1 *8:45*	Introduction to Alzheimer's Workshop: Thorny tangles: Roseoloviruses and Alzheimer's disease	*Pellett*
Keynote - Alzheimer's Disease *9:00-9:45*		
1 - 1 *9:00*	Multiscale Analysis of Independent Alzheimer's Cohorts Finds Disruption of Molecular, Genetic, and Clinical Networks by Human Herpesvirus	*Dudley*
Alzheimer's Disease *9:45-10:45*		*Jacobson & Komaroff*
1 - 2 *9:45*	HHV-6A and the amyloid-ε peptide- partners in Alzheimer's disease pathology	*Moir*
1 - 3 *10:15*	The viral protein corona directs viral pathogenesis and amyloid nucleation	*Järver*
1 - 4 *10:30*	Dissemination of HHV-6A in the olfactory tract of patients with increasing severity of dementia	*Alberi Auber*
Break *10:30-11:05*		
Alzheimer's Disease *11:05-12:30*		*Jacobson & Komaroff*
1 - 5 *11:05*	HHV-6A infection of microglial cells induces the expression of Alzheimer’s disease risk factors	*Rizzo*
1 - 6 *11:20*	Human herpesvirus in Alzheimer’s disease: Cause or effect?	*Readhead*
1 - 7 *11:35*	Autophagy, Herpesviruses and Neurodegenerative Disease	*Dong*
1 - 8 *11:50*	Discussion - Alzheimer's Disease	
1 - 9 *12:20*	The NIA high-priority topic of interest in infectious etiology of Alzheimer's disease	*Mackiewicz*

Lunch & Bonus Workshop

12:30-1:30

12:30	Digital Droplet PCR (By Reservation)	*Taylor*

Animal Models

1:30-2:35

Ablashi & Horvat

2 - 1 *1:30*	HHV-6A infection in CD46 transgenic mice	*Leifer*
2 - 2 *1:45*	Using murine roseolovirus to study HHV-6/7 reactivation	*Zhou*
2 - 3 *2:00*	Murine roseolovirus induces autoimmune gastritis and development of autoantibodies	*Bigley*
2 - 4 *2:15*	Transmission of HHV-6/7 porcine homolog and reduced survival in xenotransplantation	*Denner*

HHV-6 & CNS Disease

2:35-3:05

Caserta & Ward

3 - 1 *2:35*	Introduction to HHV-6 in CNS Disease	*Jacobson*
3 - 2 *3:05*	Human herpesvirus-6A induces proinflammatory multiple sclerosis-associated retrovirus envelope protein following CD46 receptor engagement	*Horvat*
3 - 3 *3:20*	HHV-6 U24 in multiple sclerosis: mechanism triggering autoimmunity?	*Straus*

Break

3:35-3:55

HHV-6 & CNS Disease

3:55-5:55

Fogdell-Hahn & Goodman

3 - 4 *3:55*	Latency associated U94A disrupts neuronal and glial cell function	*Mayer-Proschel*
3 - 5 *4:10*	Serological response against HHV-6A is associated with increased risk for multiple sclerosis	*Gustafsson*
3 - 6 *4:25*	MicroRNAs of HHV-6 in serum and cerebrospinal fluid of multiple sclerosis patients: correlation with activity and progression of the disease	*Domínguez-Mozo*
3 - 7 *4:40*	Phospho-RNA-seq enables access to cell-free RNA profiling in biofluids for disease detection and monitoring	*Tewari*

3 - 8 *4:55*	Neurovirulence of HHV-6B in pediatric patients; from febrile seizure to mesial temporal lobe epilepsy.	*Yoshikawa*
3 - 9 *5:25*	Human herpesvirus 6 alters mitochondrial function and can synergize with underlying disease state to promote disease progression in human T cells	*Birdwell*
3 - 10 *5:40*	Molecular detection of ε-herpesviruses in brain autopsies at University Teaching Hospital, Lusaka, Zambia	*Tembo*

Introduction to Poster Session

Posters & Reception

6:00-7:30

TUESDAY, JUNE 25TH

Session Chairs / Presenter

Introduction
8:30

Flamand

Inherited ciHHV-6
8:30-10:30

Kaufer & Royle

4 - 1 *8:30*	Chromosomally integrated HHV-6: from the integration mechanism to epigenetic silencing of the integrated virus genome	*Kaufer*
4 - 2 *9:00*	Comparative analysis of anti-HHV-6 antibody and T cell immune responses in subjects with or without inherited chromosomally-integrated HHV-6	*Flamand*
4 - 3 *9:15*	Investigation of HHV-6 integration and excision: frequencies and consequences for the telomere	*Royle*
4 - 4 *9:30*	When herpesviruses meet telomeres: toward a better understanding of HHV-6A/B chromosomal integration	*Collin*
4 - 5 *9:45*	Inherited chromosomally integrated HHV-6 demonstrates tissue-specific RNA expression in vivo	*Peddu*
4 - 6 *10:00*	Recurrent endogenization of human herpesvirus 6 on chromosome 22q in East Asians	*Parrish*
4 - 7 *10:15*	Chromosomally integrated human herpesvirus 6: reactivation in peripheral blood lymphocytes	*Allnutt*

Break
10:30-10:45

Inherited ciHHV-6
10:45-12:00

Kaufer & Royle

4 - 8 *10:45*	Revealing the natural history of integrated HHV6 using genomics and phylogenetics	*Aswad*
4 - 9 *11:00*	Analysis of the chromatin structures of integrated human herpesvirus 6A	*Mariani*
4 - 10 *11:15*	Excision of the integrated HHV-6 genome using CRISPR/Cas9 technology	*Aimola*
4 - 11 *11:30*	High human herpesvirus load and genome diversity affect infant development as shown by next generation genome deep sequencing	*Gompels*

4 - 12 *11:45*	Phylogeny of inherited chromosomally integrated (ici) and community HHV-6A and -6B strains	*Bonnafous*

Malignancy
12:00-12:30 — *Hudnall & Jarrett*

5 - 1 *12:00*	Does HHV-6 promote cancer development?	*Cesarman*
5 - 2 *12:15*	Presence of HHV-6 in the lymphoma microenvironment	*Crane*

Lunch & Bonus Workshop
12:30-1:30

	Immunohistochemistry & ISH (By Reservation)	*Hudnall*

HHV-6 Genes & Proteins
1:30-2:15 — *Medveczky & Pellett*

6 - 1 *1:30*	Molecular interaction of HHV-6B gH/gL/gQ1/gQ2 complex with the cellular receptor and neutralizing antibodies	*Nishimura*
6 - 2 *1:45*	Transformation of human peripheral blood T and B-cells by coinfection with human herpesvirus 6 and Epstein-Barr virus; possible role in MS	*Luka*
6 - 3 *2:00*	Mapping the HHV-6B transcriptome	*Gravel*

Innate & Acquired Immunity
2:15-3:30 — *Bouissiotis & Stern*

7 - 1 *2:15*	Basic study for vaccine development of HHV-6	*Mori*
7 - 2 *2:45*	Characterizing the CD4 T cell response to HHV-6	*Stern*
7 - 3 *3:00*	HHV-6A infection of endometrial epithelial cells modifies immune profile and induces a defective trophoblast invasion	*Bortolotti*
7 - 4 *3:15*	Downregulation of the protein tyrosine phosphatase CD45 in roseolovirus-infected T cells	*Hudson*

Break
3:30-3:45

Host-Cell Interaction
3:45-5:00 — *Gompels & Höllsberg*

8 - 1 *3:45*	Significance of CD46 isoforms for HHV-6A and HHV-6B infection	*Höllsberg*

8 - 2 *4:00*	HHV-6A small, non-coding RNA sncRNA-U14 alters human miR -30 processing through direct interaction	*Prusty*
8 - 3 *4:15*	Humanization of murine neutralizing antibodies against gQ1 and gH of HHV-6B	*Tjan*
8 - 4 *4:30*	Analysis of the functional domain of HHV-6A glycoprotein Q2 required for tetrameric formation and binding to receptor	*Wakata*
8 - 5 *4:45*	U94 of human herpes virus 6 induces apoptosis on triple negative breast cancer cells by blocking DNA repair mechanisms	*Caccuri*

Bonus Workshop
5:00-6:00

	Growing High Titer Virus	*Flamand*

Reception
6:00-7:00

Banquet Dinner
7:00-8:30

WEDNESDAY, JUNE 26TH

Session Chairs / Presenter

Transplantation
8:30-10:30

Boeckh & Yoshikawa

9 - 0 *8:30*	Intro to the day: HHV-6B and transplantation – what we know and what we have yet to learn	*Zerr*
9 - 1 *8:45*	Host Immunology in Transplantation	*Boussiotis*
9 - 2 *9:15*	Thymic function and reconstitution protect adult dUCBT recipients from HHV-6 viremia and HHV-6 encephalitis	*Tijaro-Ovalle*
9 - 3 *9:30*	Natural killer cells, viral reactivation, and relapse	*Rashidi*
9 - 4 *9:45*	Impact of HHV-6 in recipients of ex vivo T-cell depleted hematopoietic cell transplant	*Papanicolaou*
9 - 5 *10:00*	Association of CD134-positive T cells and HHV-6 reactivation evaluated by digital polymerase chain reaction in recipients of allogeneic hematopoietic stem cell transplantation	*Mori*
9 - 6 *10:15*	Low incidence of HHV-6 reactivation in haploidentical hematopoietic stem cell transplantation using GVHD prophylaxis with corticosteroids: a possible correlation with interleukin-6 suppression	*Tamaki*

Break
10:30-10:45

Transplantation
10:45-11:45

Boeckh & Zerr

9 - 7 *10:45*	Human herpesvirus 6B and lower respiratory tract disease after allogeneic hematopoietic cell transplantation	*Hill*
9 - 8 *11:00*	The biological plausibility of human herpesvirus 6 reactivation after allo-HCT inducing idiopathic pneumonia syndrome and aggravating acute GVHD	*Moore*
9 - 9 *11:15*	Reduction of hippocampal volume due to HHV-6B infection in pediatric HSCT recipients	*Kawamura*
9 - 10 *11:30*	Active HHV-6 infection is associated with unexplained acute liver failure in previously healthy children	*Knox*

Treatment & Diagnostics
11:45-1:00

Gautheret-Dejean & Ogata

10 - 1 *11:45*	TBD HHV-6 Diagnostics and Therpay	*Boeckh*
10 - 2 *12:15*	Oral Brincidofovir decreased HHV-6B viremia after HCT: results from a post-hoc analysis of a placebo-controlled phase 3 study	*Hill*
10 - 3 *12:30*	Novel therapeutic strategies for HHV-6	*Andrei*
10 - 4 *12:45*	Development of the Japanese guideline for management of HHV-6 encephalitis after allogeneic hematopoietic stem cell transplantation	*Ogata*

Lunch
1:00-2:00

DRESS / DIHS
2:00-2:45

Descamps & Yoshikawa

11 - 1 *2:00*	The characteristics of patients with persistent HHV-6 infection after drug induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS)	*Asada*
11 - 2 *2:15*	Heterologous immunity and the role of HHV-6 in drug hypersensitivity	*Ostrov*

Closing
2:45

Flamand & Zerr

Bonus Workshop
3:00-5:00

Annotation of the HHV-6/7 Genome — *Greninger & Pellett*

ABSTRACTS

Alzheimer's Disease

1-0 Introduction to Alzheimer's Workshop: Thorny tangles: Roseoloviruses and Alzheimer's disease

Oral

Philip E. Pellett

Department of Biochemistry, Microbiology & Immunology, Wayne State University School of Medicine, Detroit, MI, USA

In 1988, Yamanishi et al. showed that the virus we now know as HHV-6B causes roseola (exanthem subitum). Key elements of the etiologic proof included culture of the virus during the acute phase and detection of serological changes that were temporally-linked to the disease. The story made biological sense and was quickly confirmed internationally. In subsequent years, HHV-6B and other roseoloviruses have been associated with other diseases. Proof of causality for these viruses has been complicated because most of the diseases occur in individuals who are seropositive from prior infections. Most of us harbor populations of roseolovirus-infected cells that dynamically vary in their frequencies and in their ratios between lytic and latently infected cells. The spectrum of neurologic diseases associated with roseoloviruses includes HHV-6B-associated posttransplant acute limbic encephalitis (PALE), which is now robustly supported by evidence from numerous clinical studies internationally. Multiple lines of evidence support an etiologic contribution of HHV-6A and HHV-6B to multiple sclerosis in a subset of patients. Readhead et al. (2018) examined brain tissues collected from patients with Alzheimer's disease (AD) by unbiased methods of DNA and RNA sequencing. AD was associated with the presence of HHV-6A and HHV-7 DNA and transcripts, and detection of the viruses was associated with shifts in host gene expression in directions that might contribute to the pathogenesis of the disease. In addition to independent confirmation and analysis of virus activity at the single-cell level, it will be important to define the magnitude of the associations, including defining the fraction of AD patients linked to roseolovirus presence and activity. These intriguing, and even exciting, observations will hopefully motivate mechanistic studies of the molecular, cellular, and immunobiological basis of the role of these viruses in AD pathogenesis, as well as the development of diagnostic tools and algorithms, and novel therapeutic approaches for this important disease.

1-1 Multiscale Analysis of Independent Alzheimer's Cohorts Finds Disruption of Molecular, Genetic, and Clinical Networks by Human Herpesvirus

Oral

Joel T. Dudley[1,2,3,4]

[1]*Departments of Genetics and Genomic Sciences,* [2]*Icahn Institute of Genomic Sciences and Multiscale Biology,* [3]*Institute of Genomic Scientis and Multiscale Biology, Icahn School of Medicine at Mount Sinai, New York,* [4]*ASU-Banner Neurodegenerative Disease Research Center, Arizona State University, Arizona*

Investigators have long suspected that pathogenic microbes might contribute to the onset and progression of Alzheimer's disease, although definitive evidence has not been presented. Whether such findings represent a causal contribution or reflect opportunistic passengers of neurodegeneration, is also difficult to resolve. We constructed multiscale networks of the late-onset AD-associated virome, integrating genomic, transcriptomic, proteomic, and histopathological data across four brain regions from human post-mortem tissue. We observed increased human herpesvirus 6A and human herpesvirus 7 from subjects with AD compared with controls. These results were replicated in two additional, independent and geographically dispersed cohorts. We observed regulatory relationships linking viral abundance and modulators of APP metabolism, including induction of APBB2, APPBP2, BIN1, BACE1, CLU, PICALM, and PSEN1 by HHV-6A. This study elucidates networks linking molecular, clinical, and neuropathological features with viral activity and is consistent with viral activity constituting a general feature of AD.

1-2 HHV-6A and the amyloid-β peptide- partners in Alzheimer's disease pathology

Oral

Robert D. Moir, Deepak Kumar Vijaya Kumar, William A Eimer, Nanda Kumar Navalpur Shanmuga, and E. Tanzi

Genetics and Aging Research Unit, Harvard Medical School, Cambridge, MA USA and Department of Neurology, Massachusetts General Hospital. Charlestown, MA USA

An overwhelming body of data confirms that deposition of amyloid-β peptide (Aβ) as insoluble β-amyloid plaques in the brain drives the cascade of pathologies that leads to dementia in Alzheimer's disease. AD was long viewed as a functionless byproduct of catabolism and the peptide's propensity for fibrilization and β-amyloid generation as intrinsically abnormal. However, findings from our laboratory and those of independent investigators have recently identified a normal physiological role for Aβ: the peptide is an antimicrobial peptide (AMP) and effector protein of innate immunity. Aβ fibrilization mediates the peptide's protective activity against microbes, entrapping and permanently entombing microbial pathogens in β-amyloid deposits. While providing immediate protection for the brain against infection, accumulation of microbial-seeded β-amyloid may also contribute to amyloidosis and accelerate AD pathology.
We recently reported herpesviridae viruses are among the pathogens Aβ shows activity against, including HHV-6A, HHV-6B, and HSV-1. Moreover, herpesviridae neuroinfection accelerates amyloidosis in AD cell culture and animal models. Consistent with a role for HHV-6A in β-amyloidosis, an independent study recently reported the virus is elevated in AD brain. Here we characterize interactions between HHV-6 and Aβ. Data suggest Aβ not only directly inhibits HHV-6 but also targets and kills neuronal cells infected with herpes viruses. Aβ-mediated agglutination of HHV-6A also leads to an intense burst in oxygen radical generation within the peptide/virus agglutinates. Hypochlorite ions generated by newly formed plaques destroy entrapped pathogens, but may also damage neuronal structures through direct oxidation of host tissue and the promotion of local neuroinflammation. Our findings have implications for neuroinflammation, viral infectivity, and AD pathology.

1-3 *Oral* The viral protein corona directs viral pathogenesis and amyloid nucleation

Kariem Ezzat [1,2], Maria Pernemalm[3], Peter Järver[1], Samir El-Andaloussi[2], and Anna-Lena Spetz[1]

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Viruses rely on the intracellular host machinery for replication, production of viral proteins and assembly. However, outside cells, as nanosized obligate intracellular pathogens, viruses share many biophysical properties with nanoparticles. Based on this biophysical equivalence, we hypothesized that viruses accumulate a host-derived protein corona layer in extracellular environments similar to nanoparticles. Here we show that respiratory syncytial virus (RSV) and herpes simplex virus 1 (HSV-1) accumulate rich and distinctive protein coronae in different biological fluids including human plasma, human bronchoalveolar lavage fluid, non-human primate plasma, and fetal bovine serum. Moreover, we show that corona pre-coating differentially affects viral infectivity and immune cell activation. Additionally, we demonstrate that viruses can bind amyloidogenic peptides in their corona and catalyze amyloid formation via surface-assisted heterogeneous nucleation. Importantly, we show that HSV-1 catalyzes the nucleation and accumulation of the amyloid-beta ($A\beta_{42}$) peptide, which is the major constituent of amyloid plaques in Alzheimer's disease, *in-vitro* and *in-vivo* in Alzheimer's disease animal models. Our results provide a proof-of-concept for the presence of an extensive and dynamic viral protein corona layer that is critical for viral-host interactions. Unlike the viral genome-coded surface proteins, the viral protein corona is an acquired structural layer that is dependent on the viral microenvironment resulting in different viral identities based on the target tissue and the target organism. Additionally, the demonstration of corona-driven heterogeneous nucleation of amyloids illustrates convergence between viral and amyloid pathologies suggesting a direct physical mechanistic link that warrants further investigation.

1-4 *Oral* Dissemination of HHV-6A in the olfactory tract of patients with increasing severity of dementia

Praveen Bathini[1] and Lavinia Alberi Auber[1,2]

[1]Department of Medicine, University of Fribourg, Fribourg, Switzerland
[2]Swiss Integrative Center of Human Health, Fribourg, Switzerland

Olfaction declines with aging and appears to be a prodromal sign of cognitive decline in progressive neurodegenerative diseases (ND). Furthermore, the olfactory circuit is devoid of blood brain barrier protection and represents a port of entry for neurotoxic species that can spread to the brain. Nevertheless, very little is known about the pathophysiological

changes underlying smell loss that may reflect early network dysfunction.
A cross-sectional histo-anatomical study was conducted on post-mortem olfactory nerves of patients with increasing severity of dementia from mild cognitive impairment (MCI) to moderate and severe Alzheimer's disease (AD) (n=9 per staging). The olfactory bulbs and tracts show a prominent and progressive tauopathy in contrast to a weaker amyloid pathology localized to the glomerular region. The diffused amyloid pathology at the synaptic contact between olfactory sensory neurons (OSN) and mitral cells suggests an accumulation of amyloid deposits intracellularly as a result of neuronal damage or viral infection. Human herpesviruses, HHV-6A and HHV-7, have been recently implicated in the development of dementia and HHV-6B has been more frequently associated with multiple sclerosis. In the human olfactory nerve, HHV-6A and HHV-6B early antigens (3E3) are visible in the neurons' somata in both demented and control subject, whereas HHV-6A late antigens (gp82) are more abundant in demented patient and their appearance change with the advancement of the disease from few positive neurons to scattered puncta disseminated along the olfactory tract, colocalizing with pTau. In the olfactory target region of the entorhinal cortex, HHV-6A strongly colocalizes with A42 in plaques. No trace of HHV-7 could be detected across the olfactory nerve. These preliminary results indicate that HHV-6A and HHV-6B may spread from the nose, through the olfactory circuit to other brain areas. Despite the generalized presence of HHV-6A and HHV-6B, reflecting the incidence of Roseolovirus infections in the world population, lytic infections, as demonstrated by the late antigen labeling, are more prominent in demented subjects.

1-5 HHV-6A infection of microglial cells induces the expression of Alzheimer's disease risk factors

Oral

Daria Bortolotti[1], Valentina Gentili[1], Elisabetta Caselli[1], Antonella Rotola[1], Dario Di Luca[1], Roberta Rizzo[1]

[1]University of Ferrara, Department of Medical Sciences, Section of Microbiology and Medical Genetics, Ferrara, Italy

Introduction: In Alzheimer's disease (AD) brains three pathological characteristics are observed: extracellular insoluble senile plaques formed by amyloid-β (Aβ) peptide, apoE and its corresponding cholesterol that co-localize with Aβ plaques (Lazar 2013), and intraneuronal neurofibrillary tangles (NFT) formed by tau protein (Kumar 2015). Recent findings suggest a possible implication of HHV-6A in AD (Eimer 2018; Readhead 2018) and we showed the ability of HHV-6A to induce the expression of apoE, a risk factor for AD onset (Rizzo 2019). The control of viral infections in the brain involves the activation of microglial cells, the macrophages of the brain that are constantly surveying the central nervous system and, in response to small variations, rapidly change their morphology, become activated, secrete pro- and anti-inflammatory molecules, and phagocytose foreign material that can alter the equilibrium of the brain (Ransohoff 2016). We evaluated the effect of HHV-6A infection on microglial cell expression of the common risk factors for AD development: apoE, Aβ and tau.
Materials and Methods: We infected microglial cells (HMC3, ATCC®CRL-3304) in monolayer and spheroid 3D model with HHV-6A (strain U1102) cell-free virus inocula with 100 genome equivalents per cell. We collected the cells 1, 3, 7 and 14 days post infection (d.p.i.) and analyzed them for viral DNA and RNA, apoE, Aβ (1-40, 1-42), tau and phospho-tau, (Threonine 181) by real time immunofluorescence and immunoenzymatic assay.
Results: We observed a productive infection by HHV-6A. The expression of apoE increased at 3 d.p.i. and Aβ 1-40 expression increased at 7 d.p.i., while no significant induction was observed for Aβ 1-42 expression. The secretion of Tau started at 7 d.p.i., with an increasing percentage of the phosphorylated form.
Conclusions: Microglial cells are permissive to HHV-6A infection, which induces the expression of the common risk factors for AD development: apoE, Aβ and tau. Interestingly, there is a temporal induction of these molecules: apoE is induced at 3 d.p.i. followed by Aβ 1-40 at 7 d.p.i. and by phospho-tau at 14 d.p.i.

1-6 Human herpesvirus in Alzheimer's disease: Cause or effect?

Oral

Ben Readhead,[1,2,3,4], Jean-Vianney Haure-Mirande,[5] Michelle E. Ehrlich,[1,2,5,7], Sam Gandy,[5,6,8,9], and Joel T. Dudley[1,2,3,4]

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Objective: Investigators have long suspected that pathogenic microbes might contribute to the onset and progression of Alzheimer's disease (AD) although definitive evidence has not been presented. We recently reported an increased abundance of specific human *Herpesviridae* across multiple brain regions from subjects with AD compared with controls. Whether such findings represent a causal contribution, or opportunistic passengers of neurodegeneration has also been difficult to resolve. Causal inference methodologies offer a statistical approach for dissecting potential causal impacts of viral species on important aspects of AD pathophysiology. The APOE ε4 variant is recognized as the strongest genetic risk factor for AD, whereas the ε2 variant confers a protective effect. APOE is also known to drive host susceptibility to diverse viral, bacterial, and parasitic infections. In this preliminary study, we evaluate whether the activity of viruses detected in human post-mortem brain tissue is consistent with viral mediation of risk or protective effects conferred by the APOE ε4, and ε2 alleles, on AD-associated neuropathology.

Methods: We integrated matched genomic, transcriptomic, and neuropathological data collected post-mortem from 521 subjects within the Religious Orders Study, and Memory and Aging Project studies. We identified host DNA loci that are significantly associated with viral abundance, as well as AD-associated neuropathology (risk vQTL). We applied these risk vQTL as instrumental variables to a Causal Inference Test, and several Mendelian Randomization approaches to evaluate a causal role for viral species on AD-associated traits, and to identify the host biomolecular networks that participate in these relationships.

Results: Within the ROS/MAP cohorts, we observed that the HSV-1 Latency Associated Transcript is positively associated with APOE ε4 allele dosage, and that HHV-6B U90 gene is negatively associated with APOE ε2 allele dosage. Causal inference testing is consistent with a model whereby the lack of HHV-6B U90 gene causally mediates some of the protective effects of APOE ε2 on amyloid plaque density (FDR < 0.04), and increased HSV-1 mediates some of the effect of APOE ε4 on neurofibrillary tangle burden (FDR < 0.1). Host genes regulated by these viral species include known AD-associated and risk loci genes, including SPI1, NEFL, TOMM40, and MAPT-AS1, and may offer novel avenues for understanding interactions between virus and host biology in the context of AD. A further analysis evaluating AD risk loci beyond APOE also corroborates this picture, as well as implicating additional *Herpesviridae* species including HSV-2, HHV-6A, and HHV-7 with diverse facets of AD neuropathology in a species-specific manner.

Conclusions: This preliminary study offers evidence that is consistent with several human *Herpesviridae* exerting a causal effect on heterogeneous aspects of cellular and tissue neuropathology observed in early stages of AD. These findings suggest specific experimental validations that will be necessary to confirm and illuminate these interactions, as well as highlights host networks and biomolecules that may offer novel therapeutic opportunities for modulating host immune responses in AD.

1-7 Autophagy, Herpesviruses and Neurodegenerative Disease

Oral

Xiaonan Dong, PhD

Center for Autophagy Research, Department of Internal Medicine, UT Southwestern Medical Center, Dallas, TX 75390, USA

Autophagy is a lysosomal degradation pathway that plays a fundamental role in cellular, tissue, and organismal homeostasis. Definitive etiological links have been revealed between defective autophagy and human diseases, especially neurodegenerative disorders. Three recent animal studies demonstrated that genetic disruption of autophagy inhibitors (BCL2 and Rubicon) increases autophagy, extends lifespan, and improves healthspan in mice, including in providing protection against Alzheimer's like disease. Moreover, many viruses, particularly herpesviruses, have evolved a variety of mechanisms to suppress autophagy which promote viral infection and pathogenesis. Here, I will discuss the interactions between herpesviruses and autophagy from the perspective of understanding the pathophysiology of neurodegenerative diseases and implications for the development of novel therapeutic strategies and agents.

1-9 *Oral* The NIA high-priority topic of interest in infectious etiology of Alzheimer's disease

Miroslaw (Mack) Mackiewicz, PhD

National Institute on Aging, National Institutes of Health
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Alzheimer's disease (AD) is a progressive, degenerative disorder of the brain and is the most common form of dementia in the elderly. AD is the sixth leading cause of death in the United States. Prominent behavioral manifestations of AD include memory impairments and decline in other cognitive domains. Currently, at least 5.6 million Americans age 65 and older suffer from AD, and it is projected that the number of patients with AD will grow to 11.6 million by 2040.
The concept of a microbial etiology of AD was first proposed in 1952, and further investigated and strengthened through studies in the early eighties on the herpes simplex virus type 1 (HSV-1) in human brains. Discovery of HSV-1 in brains of AD patients made the concept of microbial etiology of AD plausible but raised questions such as whether pathogens are causal factors in AD or just "opportunistic passengers" of neurodegeneration. Since the early studies on HSV-1 in the CNS, numerous reports have associated AD with diverse bacterial, fungal, and viral pathogens, most frequently implicating herpes viruses.
NOT-AG-19-012 specifies a high-priority topic of interest in "Infectious Etiology of Alzheimer's Disease." This topic aims to: (1) determine whether microbial pathogens represent a causal component of Alzheimer's disease (AD), (2) establish mechanisms by which microbial pathogens impact neurodegenerative processes in AD, and (3) inform aspects of future translational studies in AD, including discovery of candidate therapeutics aimed at regulating pathogen-associated networks and molecules in AD.
Dr. Mack Mackiewicz will discuss NOT-AG-19-012 funding opportunity announcement, NIH/NIA policy issues, and grant writing and review process.

1-10 *Poster* Generating a mix pool of iPSCs from hundreds of genetically different donors for genetic studies

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Performing genotype associations on cellular phenotypes for many different donors is a laborious process. It involves the reprogramming of donor adult tissue into induced pluripotent stem cells (iPSCs) and subsequently differentiating them into the tissue of interest. To make this process more efficient, we proposed a method to multiplex this process by reprogramming a mixed pool of cells from many different donors. We pooled a 120 different lymphoblastoid cell lines (LCLs) from the Personal Genome Project (PGP) and reprogrammed them into iPSCs as a pool by nucleofection of Yamanaka factors. To track donor identity, we employ our method that accurately predicts donor proportion of the pool by extracting the pool's genomic DNA, performing next-generation sequencing and running our algorithm with exploits single nucleotide polymorphisms (SNPs) of the donors to track donor identity. It is not straightforward to use SNPs because they are usually biallelic and are distributed sparsely throughout the genome. Current commonly used sequencing technologies have short read lengths, making it nearly impossible to ascribe any read to any particular donor. This algorithm employs an Expectation Maximization algorithm that uses an iterative process to discover the proportions that best fit the observed sequencing data given whole genome SNP profiles of every donor. Finally, we applied our method to test for genotype specific effects of inducing pluripotency from a mixed pool. Our method can also be broadly applied to test genotype specific effects for other cellular phenotypes and enables the multiplexed testing of diverse donor cells en masse.
Recent research suggests that HHV-6 is potentially an infectious agent that infects neurons and cause an increased risk for developing Alzheimer's disease (AD). To test this hypothesis, we plan to infect a mixed pool of neurons derived from iPSCs from AD patients (as well as control individuals) with viruses such as HSV-1, HHV-6A, and HHV-7. We then plan to determine if neurons from AD patients are more susceptible/protected against such infections or have a differential immune response against the viruses compared to control individuals. Our method for multiplex reprogramming and testing of a mixed pool of many different donor cells will enable such analyses.

Animal Models

2-1 HHV-6A infection in CD46 transgenic mice

Oral

Benjamin Charvet[*], Christa Heyward[#], Bhupesh K. Prusty[°], Géraldine Gourru-Lesimple[*], Claire Dumont[*], Tuan Phan[#], Cynthia Leifer[#], Branka Horvat[*]

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Several clinical studies have correlated human herpesvirus 6 (HHV-6) infection to the induction of neuroinflammation and development of a number of neurologic pathologies. However, a direct link of causality is still missing, mainly due to the lack of suitable small animal models for HHV-6 infection. Human CD46 (hCD46) is the identified receptor for HHV-6A. We have demonstrated that HHV-6A intracranial infection of hCD46 transgenic mice (hCD46tg) results in persistent brain infection, characterized by long-term detection of viral genome in murine brains (up to 15 months after infection) and infiltration of immune cells. As HHV-6 viral genomes have been found chromosomally integrated in human cells we have analysed whether it could take place in murine cells as well. Our preliminary results suggest that HHV-6A infection of hCD46tg mice results in chromosomal integration in brain cells. This infection was correlated with behavioral changes in the mice. HHV6-infected hCD46tg mice exhibited significantly decreased active time in the tail suspension test, and reduced movement in the open field test, but no change in a marble burying test. The tail suspension test is often used as a proxy for depressive phenotypes, the open field test is a proxy for fear response and depression, and the marble burying test measures obsessive compulsive symptoms. HHV-6A FISH in brains of infected mice showed a preferential localization to the Purkinje cells in the cerebellum and in Raphe Nuclei, which is part of the serotonergic pathway, are a target of antidepressant therapies like specific serotonin reuptake inhibitors and thus play a role in behavior. Consistent with these small animal model results, HHV-6A infection has been reported recently in human post mortem brains of individuals with bipolar depression and major depressive disorder. Furthermore, recent studies show that HHV-6A infection can accelerate neuroinflammation in non-human primates. Thus, our observations of behavioral changes and chromosomal integration in the mouse model of HHV-6A infection suggest that infection may be linked to behavior phenotypes. This model opens new perspectives in the study of viral and host factors involved in behavioral disorders.

2-2 Using murine roseolovirus to study HHV-6/7 reactivation

Oral

Xiaofeng Zhou

Division of Pulmonary and Critical Care Medicine, Dept. of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI, USA

Murine roseolovirus (MRV) has recently been determined to be a mouse homolog of HHV-6A/B and HHV-7 based on genomic DNA sequence and structure. It was later confirmed that MRV has an identical DNA sequence as the virus previously described as mouse thymic virus (MTV) or mouse T lymphotropic virus (MTLV) and designated as murid herpesvirus 3 by ICTV. MRV was first identified by Rowe and Capps in 1961, and the virus causes necrosis of the thymus as well as other lymphoid organs and acute immunosuppression when experimentally inoculated into newborn mice. MRV infection in newborn mice temporarily depletes $CD4^+$ and $CD4^+CD8^+$ T cells in the thymus and spleen. While the affected lymphoid tissues regenerate within one to two months after infection, the latently infected mice continue to shed virus for life. Spontaneous infections with MRV appears to be asymptomatic. The prevalence of MRV in wild house mice is about 78%. MRV can be transmitted by a long period of contact, presumably through saliva, and by nursing newborns. Given that MRV is genetically and biologically closely related to HHV-6/7, MRV infections in mice can be used as an important model for studying the reactivation of HHV-6/7. The clinical significance of HHV-6 infection is mostly evidenced in immunocompromised patients such as recipients of allogeneic hematopoietic stem cell transplantation (HSCT) or solid organ transplants. The reactivation of HHV-6 is a cause of encephalitis and is associated with myelosuppression, acute graft-versus-host disease (GVHD), cytomegalovirus reactivation and disease, non-infectious pulmonary complications, and solid organ allograft dysfunction. We have performed a minor histocompatibility antigen-mismatched bone marrow transplant (BMT) in latently infected mice. We found that MRV is reactivated and establishes lytic infection in the lungs, skin, and liver after BMT. The reactivation of MRV in BMT

recipients induces an idiopathic pneumonia syndrome-like lung injury and aggravates acute GVHD. We are currently investigating the immune responses against the reactivation of MRV in BMT recipients. We are also starting to look into the impact of MRV reactivation on the gut microbiome, which may contribute to the BMT recipients' immune responses. Further modeling and understanding MRV reactivation and its pathogenesis should provide new concepts in designing therapeutic and management strategies to cope with the outcomes of herpes viral reactivation in immunocompromised patients.

2-3 *Oral* Murine roseolovirus induces autoimmune gastritis and development of autoantibodies

Tarin M. Bigley[1,3], Jose B. Saenz[2], Liping Yang[1], Jason C. Mills[2], Wayne M. Yokoyama[1]

Washington University School of Medicine, Divisions of [1]Rheumatology and [2]Gastroenterology, Department of Medicine, and [3]Department of Pediatrics, Saint Louis, MO

The human roseoloviruses, HHV-6 and HHV-7, have been associated with autoimmune disease but demonstrating causality has been difficult due to the ubiquitous and chronic nature of roseolovirus infections. Murine roseolovirus (MRV) is a recently sequenced beta-herpesvirus that is a natural murine pathogen and is genetically highly related to HHV6 and HHV7 by sequence analysis. Based on phenotype and sequence, MRV is closely related to murine thymic virus (MTV, aka murine thymic lymphotropic virus). Neonatal infection with MTV was previously shown to result in the development of autoimmune gastritis. Herein we have demonstrated that neonatal MRV infection also induces autoimmune gastritis in adult mice and have performed a more extensive analysis than previously reported with MTV. Our studies show that autoimmune gastritis occurs in the absence of detectable virus in gastric mucosa, develops in multiple mouse strains, and is characterized by T cell, neutrophil and eosinophil infiltrate into the gastric mucosa. Neonatal MRV infection results in a transient reduction in thymic and peripheral $CD4^+Foxp3^+$ regulatory T cells (Treg). Ganciclovir (GCV), a potent inhibitor of replication of other beta-herpesviruses, inhibits MRV replication and MRV-mediated $CD4^+$ T cell and Treg depletion. Treatment with GCV early in life also reduces autoimmune gastritis, suggesting that MRV causes autoimmune disease by altering immunologic tolerance early in life. Interestingly, neonatal MRV infection results in the production of a wide array of autoantibodies in adult mice, beyond gastric autoantibodies, indicating that neonatal infection may have a broad effect on immunologic tolerance. These findings strongly suggest that a roseolovirus infection results in disruption of immunologic tolerance, leading to the development of autoimmune disease.

2-4 *Oral* Transmission of HHV-6/7 porcine homolog and reduced survival in xenotransplantation

Joachim Denner

Robert Koch Institute, Berlin, Germany

A roseolovirus found in pigs is closely related to the human herpesvirus 6 (HHV-6) and HHV-7. Unfortunately, it was named porcine cytomegalovirus (PCMV), a misleading name because it is not closely related to the human cytomegalovirus (HCMV, human herpesvirus 5). PCMV is an immunosuppressive virus. However, its immunosuppressive properties are not well studied. PCMV modulates the expression of immune-related genes, and PCMV infection in pigs is often associated with opportunistic bacterial infections. PCMV is widely distributed among farm pigs and wild boars. Most infections in pigs are subclinical. Only infections in pregnant sows and piglets are associated with the disease.

Xenotransplantation is under development in order to alleviate the shortage of human donor organs. Pigs are for several reasons, the best-suited donors. However, xenotransplantation may be associated with the transmission of zoonotic viruses. Indeed, PCMV was transmitted in numerous preclinical xenotransplantation trials to different non-human primates: pig kidney transplantations into baboons and cynomolgus monkeys and pig heart transplantations into baboons. In all cases, infection with PCMV was associated with a significant reduction of the survival time of the transplant. Transmission was even observed when PCMV was not detected in the blood of the donor pig. When we analyzed the transplanted PCMV-positive baboons, the virus was found in all organs by PCR and immunohistochemical methods using PCMV-specific antibodies. The mechanism of action of PCMV is still unclear. The effect on the coagulation system and endothelial cells, as well as immunosuppressive properties of the virus, are discussed. PCMV

was not found in Auckland Island pigs used as islet cell donors in the first clinical trials to treat diabetes in humans, and therefore, no virus was transmitted to the patients. We developed highly sensitive and specific PCR-based and immunological detection methods for PCMV screening of the donor pigs and the transplant recipients.

Although it was shown that PCMV might be transmitted via the placenta, we succeeded in eliminating PCMV from a pig herd by early weaning. When we screened butchers and blood donors for antibodies against PCMV, we found positive reactions in Western blots using recombinant PCMV proteins. However, we could show that cross-reacting antibodies against HHV-6 are the reason. It would be interesting to know whether antibodies against HHV-6 protect from PCMV infection.

HHV-6 & CNS Disease

3-1 Introduction to HHV-6 in CNS Disease

Oral

Steven Jacobson

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Controversy will invariably exist when attempting to make associations with a ubiquitous virus and human disease. Despite compelling human molecular and pathological studies that have associated HHV-6 with several CNS disorders (our lab has mainly focused on multiple sclerosis), it has been difficult to prove causation. This is due to several factors, including i) the ubiquity of HHV-6 in the general population; ii) observational studies often yield associative results that cannot demonstrate causation; and iii) there has been no well-established animal model of HHV-6. Interventional studies such as clinical trials are effective for demonstrating the involvement of an agent in a disorder, as the agent can be targeted, and specific outcomes monitored and correlated with measurable clinical changes. However, to date, there is no effective, specific anti-HHV-6 intervention to employ in clinical trials of patients with MS or other HHV-6 associated CNS disorders.

Using a small nonhuman primate, we demonstrate that prior intranasal (IN) inoculations (a common route of infection for HHV-6) with HHV-6A and HHV-6B viruses accelerate an experimental MS-like neuroinflammatory disease (experimental autoimmune encephalomyelitis (EAE)). Marmosets received intranasal HHV-6A, 6B or mock inoculations, and six months later were induced with EAE. Post EAE induction, brain MRI scans were performed every two weeks until predetermined clinical endpoints.

Animals receiving intranasal virus alone were asymptomatic, and patterns of viral DNA detection were observed that reflected the compartment-specific tropism observed in humans. HHV-6 infected animals prior to EAE induction demonstrated low levels of viral antigen the brain, suggesting that the olfactory pathway is sufficient for viral entry into the CNS. Following EAE induction, virus-inoculated marmosets exhibited reduced time to inflammatory perivascular brain lesions and significantly reduced overall survival compared to uninfected, EAE-induced controls. By intracellular cytokine staining, levels of the most highly proinflammatory CD8 T cell subset significantly correlated with post-EAE survival time in the virus-inoculated marmosets, suggesting that a (viral?) antigen-driven expansion may have occurred in the periphery during the EAE disease course. By histopathology, viral antigen in the brain of virus-inoculated EAE marmosets was markedly elevated and concentrated in lesions relative to the surrounding parenchyma. This observation in particular mirrors the finding of HHV-6 viral antigen localized to MS brain lesions, which has provided some of the strongest evidence linking this virus with MS. Collectively, we demonstrate that in a nonhuman primate model of MS, asymptomatic intranasal viral acquisition accelerates and exacerbates subsequent neuroinflammatory disease, likely through a combination of peripheral activation and CNS infection.

Our experience in how to associate a ubiquitous virus such as HHV-6 with MS has provided us with the tools and resources to begin preliminary investigations into the potential role of this virus in another chronic, progressive neurologic disease, Alzheimer's disease. Recent, novel and exciting findings have been reported in this field of investigation, and we have tasked to explore the possible association or HHV-6 in human brain material obtained from cohorts of patients with Alzheimer's disease and controls. As we have established a highly sensitive and specific digital droplet PCR assay for the detection of HHV-6, we applied these techniques to the detection of HHV-6A and HHV-6B sequences in AD human brains. Our ongoing results will be presented. In addition, we have applied a pathogen sequence bioinformatical tool to screen RNA-seq data from a large Alzheimer's disease and control human brain data base. These results will also be presented.

3-2 Human herpesvirus-6A induces proinflammatory multiple sclerosis-associated retrovirus envelope protein following CD46 receptor engagement

Oral

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Human herpesvirus 6 (HHV-6) infection has long been suspected to play a role in the pathogenesis of MS and

neuroinflammation. The aberrant expression of human endogenous retrovirus (HERV) elements of the HERV-W family has been associated with different diseases, including multiple sclerosis (MS). In particular, the expression of the envelope protein (Env) from the multiple sclerosis-associated retrovirus (MSRV), a member of HERV-W family and known for its potent proinflammatory activity, is repeatedly detected in the brain lesions and blood of MS patients. We show here that both HHV-6A and stimulation of its receptor, transmembrane glycoprotein CD46, induce the expression of MSRV-Env. The engagement of extracellular domains SCR3 and SCR4 of CD46-Cyt1 isoform was required for MSRV-Env transactivation, limiting thus the MSRV-Env induction to the CD46 ligands binding these domains, including C3b component of complement, specific monoclonal antibodies, and both infectious and UV-inactivated HHV -6A, but neither HHV-6B nor measles virus vaccine strain. Induction of MSRV-Env required CD46 Cyt-1 singling and was abolished by the inhibitors of protein kinase C. Finally, both membrane-expressed and secreted MSRV-Env triggered TLR4 signaling, displaying thus a proinflammatory potential, characteristic for this viral protein. These data expand the spectrum of HHV-6A effects in the modulation of the immune response and support the hypothesis that cross-talks between exogenous and endogenous viruses may contribute to inflammatory diseases and participate in neuroinflammation. Furthermore, they reveal a new function of CD46, known as an inhibitor of complement activation and receptor for several pathogens, in transactivation of HERV-Env genes, which may play an important role in the pathogenesis of inflammatory diseases.

3-3 HHV-6 U24 in multiple sclerosis: mechanism triggering autoimmunity?

Oral

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Introduction: We have reported that Killer Ig-like receptor (KIR)2DL2 inhibitory receptor expression on the surface of $CD56^{bright}$ natural killer (NK) cells impairs the response to human herpesvirus (HHV) infections in a subgroup of MS patients (Rizzo 2012; Ben Fredj 2014; Rizzo 2016). NK cells may affect anti-viral response directly by causing infected cell lysis or indirectly by stimulating B-cell responses or affecting T-cell activation. In pathological conditions, NK/B cell interactions could be modified by viral antigens that present homologies with self-antigens. It is known that HHV-6 U24(1-15) peptide has a potential role in mimicking myelin basic protein (MBP) in MS (Tejad-Simon 2003; Sang 2014; Sang 2017) with a potential effect on both cellular and humoral immune response.

Materials and Methods: We enrolled 40 MS patients with a stable RRMS, 40 healthy controls, and 40 patients with neuropsychiatric systemic lupus erythematosus (NLES). We analyzed HHV's (EBV, VZV, HHV-6, HSV-1, HSV-2) serology (IgM/IgG) and immune-phenotyped NK cells. We investigated the effect of HHV-6 pU24(1-15) peptide on humoral (IgG) and cellular immunity (Fluorospot assay).

Results: MS patients positive for KIR2DL2 expression on $CD56^{bright}$ NK cells demonstrated significantly increased IgG titers towards HHV-6 compared to controls and NLES ($p<0.01$), which also correlates with higher EDSS scores. In particular, MS patients presented with higher levels of IgG towards HHV-6 U24(1-15) peptide than controls ($p<0.01$), comparable to levels observed of the corresponding MBP (93-105) self-peptide (p=NS). Considering antibody production of the phosphorylated form of U24(1-15) peptide (pU24), we reported higher IgG levels, in particular in KIR2DL2+ MS patients, in both plasma and CSF ($p<0.01$; $p<0.001$, respectively). When we stimulated NK and T cells from MS patients with pU24(1-15) peptide, we observed a higher activation status in KIR2DL2+ MS patients in terms of IFN-gamma production by T cells, but not of Granzyme-B by NK cells, compared to controls.

Conclusions: These results suggest that the expression of KIR2DL2 receptor on $CD56^{bright}$ NK cells might affect not only NK cells functions but also the immune response towards U24 HHV-6 protein, in particular in its phosphorylated form.

3-4 Latency associated U94A disrupts neuronal and glial cell function

Oral

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Objective: Human herpesviruses have been proposed to play a role in neurodegeneration for decades, although their

contributions to disease onset and progression are not fully characterized. The CNS is a reservoir for latent HHV-6A and the viral gene U94A is the major latency-associated transcript, making U94A a primary candidate for studying the effect of latent HHV-6A on neural cell populations. We have focused on understanding the impact of U94A on human oligodendrocyte progenitor cells (hOPCs), which are essential for myelin repair in the context of multiple sclerosis (MS) and, based on recent data linking HHV-6A to AD, we extended our studies to include human neurons, which are a direct target of Alzheimer's disease (AD).

Method: We examined the effects of U94A in primary human neurons and hOPCs *in vitro* with a focus on cellular survival, motility, morphology, differentiation, and maturation. To determine the possible pathways that are affected by U94A we are also applying a combination of unbiased, genome-wide, proteomic and genomic approaches, combined with analysis of target genes that are known to be involved in the cellular processes that we have shown to be affected by U94A expression.

Results: While infection with whole virus impaired hOPC proliferation, expression of U94A alone impaired cell migration without affecting cell survival proliferation, or differentiation into oligodendrocytes. We showed dysregulation of genes associated with progenitor cell migration consistent with both the experimental pathology of U94A+ hOPCs and the incomplete remyelination characteristic of MS. In primary human neurons expressing U94A we found significant defects in neurite complexity and reduced expression of genes that play a critical role in neurite morphogenesis.

Conclusion: U94A expression as a model of HHV-6A latency impairs the cytoskeletal function of glial cells and neurons. While we think that the specific consequences of these dysfunctions are cell type and disease-specific, it is likely that U94A increases vulnerability to secondary insults and/or impairs repair processes. In hOPCs the migration defect impairs the cells' ability to contribute to repair function and is thus likely to contribute to demyelinating disease progression. Cytoskeletal dysfunction in neurons could render U94A+ neurons more vulnerable to secondary insults associated with AD and is likely to affect the progression and severity of AD.

3-5 *Oral* Serological response against HHV-6A is associated with increased risk for multiple sclerosis

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Background: Human herpesvirus (HHV)-6A or HHV-6B involvement in multiple sclerosis (MS) etiology has remained controversial. By using a novel serology method, we investigated this further in a large, national, case-controlled cohort and in persons before MS disease onset.

Methods: A multiplex serological assay measuring IgG reactivity against the immediate early protein 1 (IE1) from HHV-6A (IE1A) and HHV-6B (IE1B) was used in a MS cohort (8742 persons with MS and 7215 matched controls) and a pre-MS cohort (478 individuals and 476 matched controls). Adjusted linear and logistic regression models were conducted to investigate associations with MS. Genome-wide association analyses were performed and associations with HLA haplotypes were investigated.

Results: The IgG response against IE1A was positively associated with MS (OR = 1.55, $p = 9x10^{-22}$), and with increased risk of future MS (OR = 2.22, $p = 2x10^{-5}$). An interaction was observed between IE1A and Epstein-Barr virus (EBV) antibody responses on MS risk (attributable proportion = 0.24, $p = 6x10^{-6}$). In contrast, the IgG response against IE1B was negatively associated with MS in the established MS cohort (OR = 0.74, $p = 6x10^{-11}$). The genetic control of HHV-6

antibody response was located on the HLA region.
Conclusion: Increased serological response against HHV-6A, but not HHV-6B, IE1 proteins with an interaction with EBV and association to HLA complex genes supports a role for HHV-6A in MS etiology.

3-6 *Oral* MicroRNAs of HHV-6 in serum and cerebrospinal fluid of multiple sclerosis patients: correlation with activity and progression of the disease

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Objective. To analyze the expression levels of the Human Herpesvirus-6 (HHV-6) A/B miRNAs in the serum and cerebrospinal fluid (CSF) of multiple sclerosis (MS) patients and patients with other neurological disorders (OND).
Methods. This study, approved by the local Ethics Committee of the Hospital Clínico San Carlos (Madrid) and funded by the HHV-6 Foundation, included 43 untreated MS patients and 36 patients with OND, inflammatory or non-inflammatory, who were matched by age and gender. Among the MS patients, 24 had clinically isolated syndromes (CIS), which can develop into clinically definite MS, and 19 had relapsing-remitting MS (RRMS). Clinical data about the evolution of the disease was available for all MS patients. The data included radiological measurements, relapse rate, EDSS, and MSSS over time.
We assessed the expression levels, in paired samples of serum and CSF, of the known HHV-6 A/B-synthesized miRNAs: hhv6b-miR-Ro6-1-5p, 2-3p, 3-3p, 3-5p, 4-3p, and miR-U86. For this purpose, we used Taqman MicroRNA Assays (Applied Biosystems). Intrathecal HHV-6 A/B antibody production, and anti-HHV-6-A/B IgG and IgM levels in serum were also measured by ELISA kit (Vidia).
Results. We detected the following miRNAs:

	hhv6b-miR-Ro6-2-3p		hhv6b-miR-Ro6-3-3-p		hhv6b-miR-Ro6-3-5p		miR-U86	
	Serum	CSF	Serum	CSF	Serum	CSF	Serum	CSF
MS	97.7	81.4	4.7	2.3	95.3	51.2	55.8	11.6
OND	91.7	82.4	0	2.9	88.9	55.9	50	5.9

We did not find any differences in the prevalence and expression levels of the HHV-6 A/B miRNAs between MS and OND patients in serum or CSF.
The MS patients who were positive for hhv6b-miR-Ro6-3-5p in CSF had higher HHV-6 IgG levels in serum (p=0.021), and CSF (p=0.0005) compared to those who were negative. In addition, the time from the last relapse was significantly lower among the patients who were positive for hhv6b-miR-Ro6-3-5p in CSF than among those who were negative, although this difference did not reach a significant value (p=0.053).
In both groups, levels of certain miRNAs were correlated with each other in the serum (2-3p and 3-5p: r=0.814, p=1.93E-18; 2-3p and miR-U86: r=0.603, p=2.98E-5; 3-5p and U86: r=0.681, p=9.39E-7).
Conclusions. We describe, for the first time in the scientific literature, the expression levels of HHV-6 miRNAs in serum and CSF samples from MS and OND patients. HHV-6 IgG levels in serum and CSF were found to be higher in MS patients, but not in OND patients, when the hhv6b-miR-Ro6-3-5p was detected in CSF. Further studies are necessary for understanding the role of these miRNAs in MS.

3-7 *Oral* Phospho-RNA-seq enables access to cell-free RNA profiling in biofluids for disease detection and monitoring

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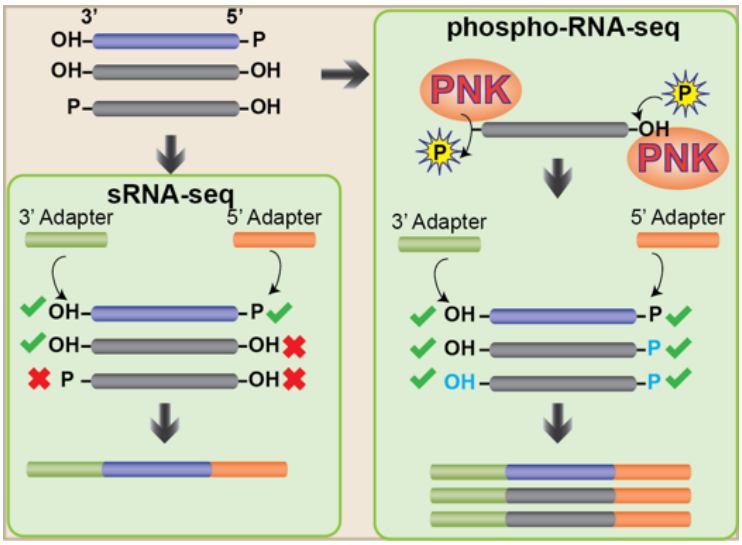
Measuring disease biomarkers in biofluids (e.g., blood, urine, cerebrospinal fluid, etc.) to detect and monitor pathophysiologic events in tissues has enormous appeal. While detecting specific DNA sequences in blood plasma has been successful in informing about the presence of cancers, viruses and a range of pathogens, this approach does not provide reveal gene expression information, which could reveal more detailed information about disease activity. Measuring cell-free RNA in plasma would provide such information and has the potential to be more sensitive, given that one DNA molecule in a cell gives rise often to thousands or more RNA molecule.

However, with the exception of microRNAs, broad sequencing of cell-free messenger RNA (mRNA) profiles in plasma has been difficult to achieve. We discovered that a major barrier to cell-free RNA sequencing from plasma is that the large majority of mRNAs are both fragmented and have modified phosphorylation states at their 5' and 3' ends that make them invisible to standard small RNA-seq methods. We present a modified RNA-seq methodology, called phospho-RNA-seq, which revealed thousands of mRNA in blood plasma. The key to this approach is (i) the incorporation of T4-polynucleotide kinase treatment to change the phosphorylation states at the RNA ends, and (ii) a stringent bioinformatics analysis pipeline to reduce false positive alignments.

Phospho-RNA-seq identified cohorts of gene transcripts in plasma, including ones expressed in a tissue-specific manner. As proof-of-concept validation of the approach for biomarker identification, we used phospho-RNA-seq to longitudinally profile plasma specimens collected from patients undergoing hematopoietic stem cell transplantation. We detected bone marrow-enriched and liver-enriched transcript sets in plasma, which tracked with bone marrow recovery and hepatic injury, respectively. By providing expanded access to the transcriptome in plasma and potentially other biofluids, phospho-RNA-seq enables the discovery of new cell-free RNA biomarker signatures for a wide variety of clinical applications. We propose that this could include minimally-invasive detection of viral activity in a range of diseases, including those of the central nervous system.



3-8 Neurovirulence of HHV-6B in pediatric patients; from febrile seizure to mesial temporal lobe epilepsy.

Oral

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Primary HHV-6B infection can cause exanthem subitum, which is a common febrile exanthematous illness in infants and young children. Clinical course of the disease is benign and self-limiting. However, central nervous system (CNS) complications are major a problem from a clinical standpoint. Most pediatric cases with HHV-6B encephalitis at the time of primary viral infection have been reported in East Asia, including Japan. HHV-6B is the second most common pathogen of childhood encephalitis/encephalopathy in Japan. Meanwhile, an association between HHV-6B and febrile status epilepticus has been studied worldwide. It has been suggested that febrile status epilepticus caused by HHV-6B infection may play an important role in the development of mesial temporal sclerosis resulting in mesial temporal lobe epilepsy (MTLE) in adults. This presentation will review various types of CNS complications in association with HHV -6B infection in children. It will also discuss the possible role of HHV-6B infection in the pathogenesis of MTLE, which is one of the refractory adult epilepsies.

3-9 Oral Human herpesvirus 6 alters mitochondrial function and can synergize with underlying disease state to promote disease progression in human T cells

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Objective: As a family, herpesviruses are known modulators of mitochondria/mitochondrial dysfunction has been implicated in the pathology and progression of neurological disease. In particular, Human Herpesvirus 6A and 6B (HHV-6A/B) have been linked to the progression of neurological diseases, such as Alzheimer's, and have been reported to trigger the onset of inherited mitochondrial disease. However, how HHV-6 infection modulates host mitochondria and the consequences to cell homeostasis and disease states remain largely unknown.

Methods: We hypothesize that betaherpesvirus infection can alter mitochondrial function and exacerbate a multitude of disease states. To determine how HHV-6 infection modulates mitochondrial function we utilized a human T cell model of infection. Mitochondrial function was assayed through multiple avenues after both HHV-6A and B infection. Knock down of the mitochondrial DNA (mtDNA) polymerase, polymerase gamma (POLG), in T cells was used to model an underlying mitochondrial dysfunction to mimic a disease state in conjunction with HHV-6 infection.

Results: We have discovered that HHV-6A and 6B increase mitochondrial DNA replication and transcription, while also imbalancing the expression of nuclear and mtDNA encoded oxidative phosphorylation components (OXPHOS). In particular, components encoded by mtDNA in complex IV were increased after HHV-6 infection. The alterations to the OXPHOS machinery resulted in a decrease in the mitochondrial membrane potential of infected T cells and an increase in glycolysis. Additionally, HHV-6 infection increased mitochondrial reactive oxygen species (mtROS) production. The increase in OXPHOS complex IV was, in part, related to stabilization of p53 and a subsequent increase in the complex IV assembly factor SCO2. Inhibition of p53 resulted in a decrease in infection and partially restored normal mitochondrial function. When underlying mitochondrial dysfunction was present, HHV-6 replication was decreased but infected T cells had augmented pro-inflammatory cytokine production. In addition, the metabolic shift in POLG deficient cells after infection was more severe.

Conclusion: Overall, our data suggest that human herpesvirus 6A and 6B dramatically alter mitochondrial function to benefit their life cycle, which can have detrimental consequences in an existing disease state when metabolic deficiencies are present.

3-10 Oral Molecular detection of β-herpesviruses in brain autopsies at University Teaching Hospital, Lusaka, Zambia

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Background: The β-herpesviruses (Human Cytomegalovirus, Human herpesviruses 6A, 6B and 7) are all neurotropic and neuro-pathogenic DNA viruses that cause central nervous system-related diseases and deaths. In low-income countries, such as Zambia, infections with these viruses are rarely diagnosed in patients with central nervous system-related symptoms because of limited diagnostic and treatment capacity. Current antibiotic regimens are not working for many patients, and so there is a great need for surveillance studies of possible viral causes of meningitis and encephalitis in this high mortality patient group.

Methods: Cross sectional study to determine the prevalence of β-herpesvirus DNA in autopsy brain tissues archived at the University Teaching Hospital in Lusaka, Zambia and correlations with histopathology and pre-mortem clinical data. Real Time PCR was used to screen 91 hippocampus specimens obtained at autopsy (35 from children, 56 from adults), from the year 2012-2014.

Results: β-herpesviruses were detected in 29.7% (27/91) of the hippocampus tissue specimens analysed. In children HHV-6B DNA was detected in 14.3% (5/35) of cases, and there was one case positive for HHV-7. CMV or HHV-6A were not detected in children. Among adult mortalities, HHV-6B DNA was detected in 21.4% (12/56), CMV DNA in 14.3% (8/56), HHV-7 DNA in 5.4% (3/56) and there was one case that was positive for HHV-6A. HHV-6B was strongly associated with a histopathologically confirmed diagnosis of bacterial meningitis (OR: 8.65 (95% CI, 1.077-69.075; p=0.042). In adults, cytomegalovirus was independently associated with anatomical central nervous system pathology.

Conclusion: β-herpesvirus DNA was readily detected in brain autopsies at UTH, and both CMV and HHV-6B appear to be more readily detectable in the hippocampus tissue of patients who died with central nervous system pathology. There is a need for more proactive surveillance of these ubiquitous viral pathogens in CSF, and pilot trials of anti-herpesvirus treatment to evaluate whether or not such therapy can prevent deaths in this neglected and high mortality patient group in the African setting.

3-11 Diagnosis of primary minor immunodeficiencies in patients undergoing encephalitis HHV-6/HHV-7 etiology

Poster

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HHV-6 and HHV-7 are opportunistic viruses that are reactivated in an immunocompromised host. In addition to AIDS and immunosuppressive drugs, primary immunodeficiencies, including minor, can be the cause of reactivation.
Objective: To evaluate the immune status of patients undergoing HHV-6/HHV-7 encephalitis.
Methods: This study included 34 adult patients who had HHV-6/HHV-7 encephalitis (limbic, multifocal demyelinating, and/or diencephalic lesions on MRI) without AIDS or immunosuppressants. PCR of the CSF confirmed the diagnosis. The immune status was assessed using flow cytofluorimetry and ELISA. Diagnosis of deficiencies of IgG subclasses (deletions of constant regions), mannose-binding lectin (polymorphisms of MBL2 gene), myeloperoxidase (MPO gene polymorphisms), natural killer and/or natural killer T-lymphocytes (MTHFR 677 C/T), IgA (deletions of constant regions), and IgE (AICDA polymorphisms) were performed using genetic tests.
Results: In 27 of 34 patients (79%), primary minor immunodeficiency was diagnosed: selective deficiencies of IgG subclasses (7 patients), mannose-binding lectin (8), myeloperoxidase (3), natural killer and/or natural killer T-lymphocytes (7), IgA (1), IgE (6), combined – in 5 cases.
Conclusions: Immunocompetent patients without AIDS or immunosuppressants undergoing HHV6/HHV-7 encephalitis suffer from primary minor immunodeficiencies, which can explain viral reactivation and possibly should be the object of immunotherapeutic interventions.

3-12 Children infected by HHV-6B with febrile seizures are more likely to develop febrile status epilepticus: a case control study in a referral hospital in Zambia.

Poster

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Background: Human herpesvirus 6 (HHV-6), the causative agent of roseola infantum has two species HHV-6A and HHV-6B. They have both been shown to be neurotropic and possibly involved in pathogenesis of febrile seizures. We describe a case control study comparing the frequency of HHV-6A and/or HHV-6B infections in children with febrile seizures and a control group of febrile children without seizures.
Methods: We recruited children aged 6-60 months admitted with a febrile illness with seizures (cases) or without seizures (controls) presenting within 48 hours of commencement of fever. 3mls of whole blood was centrifuged and plasma stored at -800C for pooled screening for HHV-6B and HHV-6A by Taqman Real Time PCR.
Result: 102 cases and 95 controls were recruited. The prevalence of HHV-6B DNA detection did not differ significantly between cases (5.8% (6/102)) and controls (10.5% (10/95)) but HHV-6B infection was associated with febrile status epilepticus (OR 15; 95% CI, [1.99-120]; p=0.009). HHV-6A was not detected.

Conclusion: HHV-6B infection was strongly associated with febrile status epilepticus suggesting HHV-6B infections could play an important role in the pathogenesis of FSE, and treatment of this underlying CNS infection could lead to better treatment outcomes for patients with FSE.

3-13 The first case of HHV-7 related epilepsy

Poster

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HHV-7 related neurological disorders are usually observed in patients with compromised immune systems. Reports of healthy individuals with HHV-7 infection and diverse neurological disorders are limited. Later onset and higher frequency of seizures are also more characteristic of pediatric HHV-7 than with HHV-6 infection. Here we present the first case of HHV-7 related epilepsy in an immunocompetent child. An 11-year old male patient presented with first and sudden onset tonic-clonic seizures. He had a history of acute febrile illness with drowsiness and headache without nausea and vomiting a few days previously. Cerebrospinal fluid (CSF) examination revealed 1 white blood cell (WBC) /mmc, glucose 48 mg/dl, and protein 10 mg/dl. All results, including immunological and virological tests by polymerase chain reaction (PCR) in blood and CSF were negative. Cranial magnetic resonance imaging (MRI) and electroencephalogram (EEG) results were normal. Dehydration therapy and steroids (dexamethasone 0.5-1mg/kg) were prescribed. The patient's clinical condition improved temporarily – there were no seizures for 3 weeks. Unfortunately, convulsions resumed. When monotherapy with a first-line antiepileptic drug (carbamazepine) failed, combination therapy with lamotrigine and valproate was established under plasma-valproate concentrations routine monitoring. Over six months the patient had polymorphic focal seizures with secondary generalization and tonic-clonic seizures 1-3 times per week. Focal seizures were often preceded by sensory (visual, hearing), psychic, autonomic, and motor auras. His physical examination revealed only slight tremor of the distal extremities. His WBC count and C-reactive protein (CRP) were normal. The EEG showed diffuse slowing with theta (θ) and delta (δ) waves, bilateral synchronous bursts of delta waves with bifrontal dominance (moderately expressed cerebral changes with interest of the deep median structures of the brain). Repeat MRI revealed cerebellopontine angle cyst (8x13mm). CSF testing showed 9 WBC/mmc (60% lymphocytes) an increased level of protein 40 mg/dl, glucose and lactate levels were normal. Parasitic, tick-borne, and anti-NMDA receptor encephalitis were excluded. Real-time qualitative polymerase chain reaction (PCR) DNA test of CSF was positive only for HHV-7 (10^2 copies/ml). HHV-7 DNA was also detected in urine (10^2 copies/ml) and saliva. IgM, IgG HHV-7 antibody levels were not determined. The patient received intravenous immunoglobulin (IVIG) 2g/kg and methylprednisolone (initial dosage 10 mg/kg), resulting in a disappearance of all types of seizures for 3 weeks. At present, 3 months later, he still has only focal seizures. Treatment with IVIG and methylprednisolone improved the patient's condition in absence of etiotropic therapy.

3-14 Clinical characteristics of complex febrile seizure caused by primary infection of human herpesvirus 6B

Poster

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Objective: Exanthem subitum (ES) is a common infectious disease in infants and young children which is caused by primary human herpesvirus 6B (HHV-6B) infection. Encephalitis or complex febrile seizures (CFS) are important complications in the disease. In this study we investigated clinical characteristics of CFS patients with primary HHV-6B infection.

Method: Patients younger than 5 years old who were admitted to our hospital for CFS between 2014 and 2017 were enrolled in this study. Serum was collected at the time of admission. HHV-6B DNA was detected by a real-time PCR and HHV-6B antibody was measured by an indirect immunofluorescence assay. Primary HHV-6B infection was confirmed by HHV-6B DNA detection and negative HHV-6B antibody in the serum. We compared the clinical characteristics between the patients with and without HHV-6B infection.

Result: Sixty-two patients (39 boys and 23 girls) were enrolled. Thirty patients with primary HHV-6B infection were

identified and 32 patients were non HHV-6B cases. Patients with primary HHV-6B infection (median age: 13 months, 7 -39 months) were significantly younger than those without HHV-6B infection (19 months, 9-59 months) (p=0.008). The frequency of patients who had seizures later than 24 hrs after fever onset was higher in patients with primary HHV -6B infection (15 cases, 50%) than those without the infection (2 cases, 6%) (p=0.001).
Conclusion: These results suggest different mechanism of CFS due to primary HHV-6B infection from other causes.

3-15 Presence of roseolavirus infection markers in adult patients with epilepsy
Poster

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Human herpesviruses (HHV)-6A, HHV-6B, and HHV-7 are considered to be involved in the pathogenesis of the frequent neurological disorder epilepsy. Prognosis of the disease depends on the pathogen, the diverse routes of CNS entry, viral tropism, and immune system state of the host. The aim of this study was to determine the role of roseoloviruses infection in pathogenesis of epilepsy, analysing 53 patients with epilepsy and 104 apparently healthy controls.
Methods: To determine the presence of virus-specific antibodies ELISA and IFA was used. Virus-specific genomic sequences were revealed by nPCR and restriction endonuclease, but viral load was determined by qPCR, virus gene expression by RT-PCR.
Results: Seroprevalence of HHV-6 and HHV-7 IgG class antibodies were similar between patients with epilepsy and control group individuals (81.1% vs 78.8% and 81.7% vs 83.0%, respectively). Roseolovirus genomic sequences in DNA samples from whole blood were found in 86.8% of patients with epilepsy versus 64.4% in control group individuals. HHV-6A was identified in two epileptic patients and HHV-6B in 13/15 epileptic patients and in all positive for HHV-6 control individuals' blood samples. Plasma viremia (marker of infection active phase) was revealed only in patients with epilepsy (9/46, 19.56%). Significantly higher viral load was detected not just in patients compared to the controls, but was also higher in patients with persistent viral infection in active phase than in those with latent phase. HHV-6 U89/90 immediate early gene expression in PBMC was found in 73.3% (11/15) of the epileptic patients with previously detected HHV-6 genomic sequence in blood DNA samples, but was not found in any of the HHV-6 positive samples from control individuals. HHV-7 U57 gene expression, was detected in 37.5% (15/40) of epileptic patients positive for HHV-7 genomic sequences and in (1/63) control samples. Changes in the levels of pro-inflammatory and anti-inflammatory cytokines were determined in patients with elevated viral load.
Conclusion: Results on frequent active HHV-6 and HHV-7 infections in epileptic patients' peripheral blood indicate possible involvement of these viruses in the disease development. However, more evidence is required, such as data on viral presence in tissue from specific regions of the brain.

3-16 HHV-6A infection of U373 cells reduces autophagy, activates UPR, and induces protein tau phosphorylation
Poster

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Objectve: HHV-6A and HHV-6B have been recently reported to dysregulate autophagy and induce ER stress during their replicative infection in HSB-2 and Molt-3, respectively. HHV-6A has been strongly linked to Alzheimer's disease in which autophagy dysregulation, ER stress, and UPR activation play a role. Indeed these cellular processes have been shown to contribute to amyloid plaque formation and protein tau hyper-phosphorylation, two hallmarks of Alzheimer's disease. In this study, we evaluated the impact of HHV-6A infection of U373, a glioblastoma cell line, on autophagy and ER stress and correlate these effects with the capacity of the virus to induce protein tau phosphorylation.
Methods: U373 cells were infected with HHV-6A derived from supernatant of HSB-2 cells concentrated 100x. Infection was evaluated by IFA while ER stress/UPR, autophagy, and protein Tau phosphorylation were assessed western blot analysis. The role of ER stress was evaluated by using 4-phenylbutyric acid, a chemical chaperone known to reduce ER stress.
Results: The results obtained in this study suggest that HHV-6A infection of U373 cells reduced the autophagic flux,

as indicated by the reduction of LC3II in the presence of Chloroquine. As a strong connection between autophagy and ER stress exists, we then assessed the impact of HHV-6A infection on UPR activation. Our results show that UPR was activated by viral infection and that both the pro-apoptotic UPR molecules BIP and CHOP were up-regulated. We then investigated whether these effects could correlate with tau protein phosphorylation and found the HHV-6A infected cells displayed and increase phosphorylation of this protein at several residues. The role of ER stress in increase of tau protein phosphorylation was then assessed using 4-PBA, a molecular chaperone that reduced ER stress and partially counteracted the effect induced by HHV-6A infection.

Conclusion: All together these results suggest that HHV-6A infection reduced autophagy, activated ER stress/UPR, and induced protein tau phosphorylation in U373 cells. Since glial cells strongly contribute to Alzheimer's disease by promoting the phosphorylation of tau protein, this study suggest that HHV-6A infection of these cells could activate this pathologic process by infecting glial cells. Indeed ER stress may lead to the activation of kinases such as GSK3 beta that may in turn phosphorylate tau protein in several residues. Moreover, the virus induced ER stress could also promote the release of soluble factors involved in the neuro-inflammation that characterizes Alzheimer's disease.

3-17 *Poster* Identifying cell type specific driver genes in autism from cerebral organoids

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There is great interest in using cerebral organoids for modeling disease processes in complex neuropsychiatric disorders such as autism spectrum disorders (ASD). In our work, we adapted a cerebral organoid framework to identify critical cell types and candidate driver genes in ASD-associated copy number loci (15q11-13 duplications and 16p11.2 deletions). However, two main limitations are hindering the applications of organoids for this purpose. First, huge inherent variability in organoids has been widely reported. Second, to identify cell types in the organoids we can apply high-throughput single-cell RNA sequencing technologies, but these are costly for a large number of patient-derived organoids and cover only 10-20% of all transcripts presently. As such, we develop a scalable and cheap transcriptome-based framework termed Orgo-Seq where we identified critical cell types and candidate driver genes in cerebral organoids differentiated from 25 individuals by deconvoluting bulk RNA sequence data.

We are working on using the deconvolution approach on bulk RNA sequence data developed from our ASD work to phenotype cerebral organoids infected with HSV-1, HHV-6, and HHV-7. This allows us to leverage on both bulk RNA sequence data and previously published single-cell RNA sequence data to identify cell types and cell type specific driver genes/networks that are perturbed in cerebral organoids post infection. Since the cerebral organoids are comprised of 10 major cell types identified from single-cell RNA sequencing (such as stem cells, neural progenitor cells, and various neuronal cell types), we can evaluate if there are one or more cell type(s) that are preferentially affected upon infection with HSV-1, HHV-6, and HHV-7, and identify cell type specific transcriptomic networks that are common or specific to these viruses.

Inherited ciHHV-6

4-1 *Oral* Chromosomally integrated HHV-6: from the integration mechanism to epigenetic silencing of the integrated virus genome

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Human herpesvirus 6A/B (HHV-6A/B) and a few other herpesviruses have been shown to integrate their genomes into host telomeres of host chromosomes. Virus integration is mediated by telomere sequences present at the ends of the virus genome. These viral telomere sequences are dispensable for virus replication, but ensure efficient virus integration and maintenance of the virus genome in latently infected cells. In case of HHV-6A/B, integration also occurs in germ cells, resulting in individuals that harbor the integrated virus genome in every single cell of their body and transmit it to their offspring. This condition has been termed inherited chromosomally integrated HHV-6 (iciHHV -6). About 1% of the human population has this condition, while the biological and medical consequences for these individuals remain poorly understood. This presentation will highlight the recent advances in the understanding of the integration mechanism and the epigenetic changes that contribute to the silencing of the integrated virus genome.

4-2 *Oral* Comparative analysis of anti-HHV-6 antibody and T cell immune responses in subjects with or without inherited chromosomally-integrated HHV-6

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Objectives: Individuals with inherited chromosomally-integrated human herpesvirus 6A/B (iciHHV-6A/B) carry a copy of the entire viral genome in every somatic cell of their body. Viral reactivation and/or spontaneous viral gene expression in iciHHV-6$^+$ subjects have been documented by several research groups arguing that iciHHV-6$^+$ individuals might be exposed to higher HHV-6A/B antigenic burden than non-iciHHV-6 subjects. Consequently, our working hypothesis is that iciHHV-6$^+$ subjects display a more robust anti-HHV-6 immune response due to increase antigenic burden than non-iciHHV-6$^+$ subjects.

Methods: The Montreal Heart Institute biobank was screened for the presence of iciHHV-6 using QPCR. All positive cases were confirmed by digital droplet PCR. iciHHV-6$^+$ and control subjects matched for age and sex were contacted for blood donation. Plasma and peripheral blood mononuclear cells were isolated and stored frozen until assayed. Plasmatic cytokine levels (IL-10, GM-CSF, IFNg, IL-12, IL-1b, IL-2, IL-5, IL-13, IL-17A, IL-4, IL-23, IL-6, IL-8, TNF-a) were measured using the Human High Sensitivity 14-plex discovery assay (Eve technology, Calgary, Alberta). Antibodies against HHV-6 (U11A, U11B, gL, IE1A, IE1B, U57, gp82/105, gM, and gO) and control antigens (EBV, CMV, FLU) were determined using the LIPS assay. T cell responses were determined by IFNg ELISPOT using HHV-6B U11, U14, U71, and U90 antigens.

Results: Out of 15484 subjects, 85 were identified as iciHHV-6$^+$. Of these, 46 were iciHHV-6A+ (54%). Cytokine analysis indicates that none of the 14 measured analytes differed significantly between controls and iciHHV-6$^+$ subjects. No differences in the antibody response of controls (n=20) and iciHHV-6$^+$ subjects against control antigens (FLU, CMV, and EBV) were observed. In contrast, iciHHV-6A$^+$ subjects had more robust antibody responses against U11A, U11B, and IE1A antigens relative to control subjects. iciHHV-6B$^+$ subjects also demonstrated a stronger antibody response against IE1A and IEB than controls. For T cell analysis, participants had to be recontacted. Out of 85 re-contacts, 55 iciHHV-6$^+$ subjects agreed to make a blood donation. Blood was obtained from a similar number of controls matched for age and sex (n=57). ELISPOT analysis indicates that T cell responses against control antigens (CMV, EBV, and FLU) were equivalent between iciHHV-6$^+$ and control subjects. While the T cell responses against U11, U14, and U71 antigens were equivalent between controls and iciHHV-6+ subjects, the latter displayed reduced IFNg$^+$ reactive T cells against U90 peptides.

Conclusion: Our results indicate that iciHHV-6$^+$ subjects had significantly higher antibody reactivity against the U11 and IE1 antigens relative to controls. In contrast, the T cell responses of iciHHV-6$^+$ subjects were either comparable or slightly lower to that of control subjects.

4-3 *Oral* Investigation of HHV-6 integration and excision: frequencies and consequences for the telomere

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Human herpesvirus 6, HHV-6, contains telomere-like repeat sequences within its genome and has the capacity to integrate into human telomeres. Approximately 1% of the population carry an inherited, chromosomally-integrated copy of HHV-6 in every cell (iciHHV-6), originating from germline integrations in a small number of ancestors. Previously we showed that the iciHHV-6 genome can be partially or completely released as circular molecules from its integrated state, leaving a novel telomere at the point of excision. We have now measured the frequencies of telomere formation at telomere-like repeat sequences within the iciHHV-6 genome, at DR_L-T2 (short array $(TTAGGG)_n$ repeats) and DR_R-T1 (long array of degenerate telomere-like repeats). Novel telomeres were detected at both locations at surprisingly high, albeit different frequencies in lymphoblastoid cell lines, circulating white blood cells and sperm from iciHHV-6 carriers. In cell lines, the majority of truncations at DR_L-T2 generate a very short telomere that is expected to trigger a DNA damage response, potentially leading to exit from the cell cycle. However, some of the newly formed telomeres at DR_L-T2 are longer than expected, and the proportion that is lengthened is approximately ten-fold higher in white blood cells compared to cell lines from iciHHV-6 carriers. This suggests that a subset of the partial iciHHV-6 excisions occur in hematopoietic progenitor cells *in vivo* where telomerase is expressed. In iciHHV-6B carriers, novel telomere formation at the internally located DR_R-T1 is consistent with the release of the iciHHV-6B genome as a circular molecule (with a single intact DR) and our preliminary analysis suggests a higher frequency in sperm than blood DNA. To address whether these processes of release could play a role in the biology of telomeres and HHV-6 following childhood infection we are measuring viral DNA load in saliva from healthy adults and using sensitive approaches to detect telomere formation at DR_L-T1, indicative of integration.

4-4 *Oral* When herpesviruses meet telomeres: toward a better understanding of HHV-6A/B chromosomal integration

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Objective: Human herpesviruses types 6A and 6B (HHV-6A/B) readily integrate their viral genome into host's telomeres. To date, actors involved in HHV-6A/B integration remain poorly characterized. The immediate-early protein 1 of HHV-6A/B (IE1A/B) is one of the first proteins expressed during infection and is found invariably associated with the promyelocytic leukemia protein (PML). PML, a protein involved in various cellular regulatory mechanisms such as DNA damage responses (DDR), oligomerizes and forms nuclear bodies (PML-NBs) to recruit interacting partners. Additionally, PML-NBs are a hub for SUMOylation, a post-translational modification by binding SUMO proteins to target proteins through their SUMO acceptor and/or SUMO-interacting motif (SIM) sites. IE1A/B contains a bona fide SUMO acceptor site and putative SIM sites. Accordingly, we hypothesized that PML influence IE1A/B's SUMOylation, facilitating its interaction with proteins involved in DDR present in PML-NBs and located at damaged telomeres.
Methods: Colocalization of IE1A/B with PML at telomeres was demonstrated in wild-type (WT) and PML deficient (PML- / -) U2OS cells by immunofluorescence conjugated with in situ hybridization. Detection of SUMOylated IE1A/B was carried out by co-transfecting HA-SUMO proteins, PMLI and IE1A or IE1B expression vectors in HEK293T, WT and PML- / - U2OS cells. Cells were co-immunoprecipitated for IE1 and SUMO-IE1A/B was detected with by western blot. IE1B SUMO/SIM mutants and PML lacking E3 SUMO ligase activity were generated by site-directed mutagenesis. IE1B and PML mutants were also characterized by immunofluorescence for their phenotypes. HHV-6A/B integration was determined as described by Gravel et al. (J Virol 2017).
Results: Twenty percent of IE1A/B foci were found to localize with PML at telomeres during infection. In PML- / -

cells, the number of IE1A/B at telomeres decreased by 66% and 48%, respectively ($p<0.0001$; $p<0.001$). Ectopic or endogenous expression of PML enhanced IE1A/B SUMOylation 3 to 6 folds and resulted in IE1A/B hyperSUMOylation. Moreover, in the presence of PML lacking E3 SUMO ligase activity, a two-fold reduction in IE1A/B SUMOylation was observed and IE1A/B hyperSUMOylation was lost. Mutation of the SUMO acceptor site and SIM sites abolished IE1A/B SUMOylation entirely leading to a diffuse nuclear distribution with IE1A/B no longer colocalizing with PML and telomeres. Lastly, HHV-6A/B integration in PML-/ - cells was severely affected.

Conclusion: Our results suggest that PML is a key player in bringing SUMOylated IE1A/B to telomeres, the site of integration. We are currently generating SUMO/SIM mutant viruses to study the importance of this modification in HHV-6A/B telomeric integration.

4-5 *Oral* Inherited chromosomally integrated HHV-6 demonstrates tissue-specific RNA expression in vivo

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Objective: HHV-6 integrates into every cell of the human body in approximately 1% of the world's population. It remains unclear whether inherited chromosomally integrated HHV-6 (iciHHV-6) reactivates and whether it demonstrates gene expression *in vivo*. Our goal was to determine tissue-specific gene expression of iciHHV-6.

Methods: 650 whole genome sequences corresponding to unique donors from the Genotype-Tissue Expression (GTEx) project were aligned to HHV-6A/B genomes to find iciHHV-6 positive individuals. Tissue-specific RNA-seq data for iciHHV-6 individuals were aligned against HHV-6A/B reference genomes to evaluate gene expression. A similar approach was used to screen whole exome sequence from the Mount Sinai Brain Bank (MSBB) data.

Results: From the DNA-Seq data available for 650 GTEx individuals we detected 6 consistent with iciHHV-6 – 4 iciHHV-6B and 2 iciHHV-6A. These samples had an average normalized depth of coverage of HHV-6 that was approximately half (0.45 +- 0.035) that of human housekeeping genes EDAR and beta-globin. Notably, iciHHV-6 status could be determined from exome sequencing data based on off-target reads mapping to HHV-6 at higher rates than non-iciHHV-6 individuals. From the six iciHHV6 individuals, RNA-seq data was available from 111 tissues.

Analysis of these transcriptomes showed variable tissue-specific activity with highest expression in the U90-U100 genes in both iciHHV-6A and iciHHV-6B. For iciHHV-6B, the highest expression was seen in the U90 IE-1 transactivator and U100 glycoprotein Q genes. HHV-6 expression was detected in the brain, testis, breast, adrenal gland, lungs, salivary gland, esophagus, skeletal muscle, colon, tibial nerve and artery, adipose tissue, heart, skin, and thyroid. Tissues with the highest levels of HHV-6 gene expression included the testis, esophagus, and brain tissue. Expression of iciHHV-6A genes were notably higher than those of iciHHV-6B in brain tissue.

Conclusions: In this analysis we detected an iciHHV-6 prevalence of 6/650 (0.92%). Both whole genome and whole exome sequences were found to be reliable metrics for detecting iciHHV-6. During our analysis of the RNA-seq data we found iciHHV-6A and 6B are variably expressed through the human body. iciHHV-6A was observed to be dominantly expressed in brain tissue, which was recapitulated in the MSBB dataset. This suggests that there is tissue-dependent activity, however in most actively expressed tissue there was consistently higher expression of the U90 and U100 genes relative to other genes. Future studies are required to confirm protein expression of these HHV-6 genes and replicate the work performed here, given the low number of samples examined.

4-6 *Oral* Recurrent endogenization of human herpesvirus 6 on chromosome 22q in East Asians

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Objective: While an ancient origin of some inherited chromosomally integrated HHV-6 (iciHHV-6) has recently been proposed, the population genetics of iciHHV-6 remain unclear, especially in non-European populations. Some of us recently proposed a "founder" iciHHV-6A in the Japanese population on the basis of microsatellite analysis.

Methods: We analyzed 8,498 whole-genome sequencing (WGS) datasets from Japanese subjects, aligning unmapped reads to HHV-6 reference genomes. We determined iciHHV-6 species based on viral sequence and analyzed chromosome haplotype using phylogenetic trees of phase-estimated microarray data or analysis of rare variants. We confirmed physical linkage of haplotypes to iciHHV-6 using FISH-mapped samples.
Results: The frequency of iciHHV-6 in this population was 0.41%, with a ratio of iciHHV-6A to iciHHV-6B around 1:2 (0.14%vs 0.27%). We confirmed a rare haplotype on the distal long arm of chromosome 22 that is physically linked with iciHHV-6A and is present in all subjects who harbored iciHHV-6A, a telomere-proximal rare variant in this haplotype co-segregates perfectly with iciHHV-6A. Based on recombinations observed within this rare founder HHV-6A-linked haplotype, it entered the Japanese population around 2,300 years ago, a time of known influx of continental East Asians into Japan called the Yayoi Period. Furthermore, we observed another haplotype on chromosome 22q bearing a founder iciHHV-6B allele that accounts for 39% of Japanese iciHHV-6B carriers.
Conclusions: We observed a striking enrichment for iciHHV-6 on chromosome 22q, with two independent integration events on this chromosome arm accounting for 71% of all Japanese iciHHV-6. This study is the first to demonstrate that the chromosome of iciHHV-6 integration can be assigned based on subtelomeric haplotype, underscoring the ancient, endogenous nature of most iciHHV-6 alleles and documenting enrichment on a specific chromosome that suggests viral targeting and/or natural selection.

4-7 *Oral* Chromosomally integrated human herpesvirus 6: reactivation in peripheral blood lymphocytes

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Human herpesvirus 6 (HHV-6) is a ubiquitous beta-herpesvirus present in over 90% of adults worldwide. HHV-6 is present in two distinct species, HHV-6A and HHV-6B. HHV-6B is the more common form, and is the cause of fever and roseola in children under 2, while the effects of HHV-6A are less well known. Both species of HHV-6 remain latent in the host after primary infection during childhood and, similar to other human herpesviruses, have been shown to be capable of reactivation. The most common, direct, clinical consequence of HHV-6 reactivation is in immunocompromised patients, such as patients who have received transplant organs, in whom HHV-6 reactivation can cause encephalitis or bone marrow suppression. A unique feature of HHV-6 among herpesviruses is that in approximately 1% of the human population HHV-6 DNA can integrate into chromosomal DNA. The clinical consequences of ciHHV-6 remain unclear and it is unknown how HHV-6 reactivates from its integrated chromosomal state. Individuals affected by ciHHV-6 can be readily identified using a novel droplet digital PCR (ddPCR) technique. A ddPCR assay designed to detect HHV-6 DNA, targeting the highly conserved *u57* region of the viral genome, was developed to assess the presence of the virus in patient samples. The presence of a single copy of HHV-6 DNA per cell in a patient's whole blood, PBMC, or brain tissue sample identifies him or her as having ciHHV-6. A variety of small molecules known to activate HHV-6, including Trichostatin A, Amoxicillin, and Hydrocortisone, were tested and it was found that phorbol 12-myristate 13-acetate (PMA), an activator of T cell proliferation, caused an increase in ciHHV-6B expression in an infected T cell line. Based on this observation ciHHV-6B peripheral blood mononuclear cells (PBMCs) were stimulated with PMA and measured for reactivation by ddPCR assays targeting HHV-6B DNA or RNA and RNAscope in situ hybridization (ISH). The viral load remained constant throughout stimulation, with HHV-6B DNA maintaining a concentration of 1 copy per cell. RNA expression showed a strong positive correlation with increasing PMA stimulation, the highest RNA expression at 1 ug/mL PMA. These ddPCR results were confirmed by the RNAscope assay, which demonstrated a qualitative increase in positive signal after PMA treatment. This study demonstrates the ability of ciHHV-6 lymphocytes to alter levels of HHV-6 expression without changing the amount of viral DNA present.

4-8 *Oral* Revealing the natural history of integrated HHV6 using genomics and phylogenetics

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Objective: HHV-6A and B are unique among human herpesviruses in that they integrate into the telomeres of latently infected and germ cells. Germline integration has resulted in about 1% of humans carrying the integrated virus, which is then inherited in mendelian fashion. To date, FISH is the only reliable method of identifying the chromosomal location

of the integrated virus. Here, we set to establish a simple sequence-based approach to identify the chromosome harboring the HHV-6 genome.
Methods: We developed a bioinformatic approach using HHV-6 genomics data in combination with evolutionary reconstruction using phylogenetics. By analyzing more than 250 HHV-6 and iciHHV-6 genomes, we are able to reconstruct the evolutionary history of different HHV-6 strains and their integrated relatives. Moreover, we performed a strategic panel of FISH analyses to determine the integration site of the iciHHV-6 lineages in order to test if this approach could be used as a diagnostic tool.
Results: Using these approaches, we were able to identify HHV-6 lineages with a distinct evolutionary history to a high degree of confidence, linking each to specific chromosomal integration events and specific genomic characteristics. We confirm that carriers of inherited chromosomally integrated HHV-6 (iciHHV6) do not all descend from a single ancestor and that there were at least a dozen independent integration events, but not all integrations are independent.
Conclusion: By combining NGS data and a phylogenetically-informed analytical framework our approach provides a relatively cheap and rapid diagnostic screen to identify the chromosomal location of integrated HHV-6 without the need for FISH in most cases. Moreover, our reliable and statistically robust phylogenetic results allow us to draw a more comprehensive picture of the history of different integrations in order to consider the evolutionary consequences of each integration separately.

4-9 Analysis of the chromatin structures of integrated human herpesvirus 6A

Oral

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Objective: Human herpesvirus-6A is a ubiquitous betaherpesvirus that infects most humans. HHV-6A establishes a life-long latent infection where the virus genome integrates into the telomeres of infected cells. Upon integration the virus genome is transcriptionally silenced. While epigenetic modifications, including posttranslational modification of histone proteins, are known to play a critical role in herpesvirus latency, little is known regarding the role of chromatin structures in HHV-6A gene regulation and latency. Here we test the hypothesis that the HHV-6A integrated genome forms specific higher-order chromatin structures within the host cell nucleus.
Methods: We employed circular chromosome conformation capture coupled with high-throughput sequencing (4C-seq) to map the genome-wide chromatin contact maps of integrated HHV-6A genomes in two relevant cell models. We have further generated genome-wide ChIP-seq data for regulatory histone modifications and CTCF insulator protein to allow us to assess the functional impact of HHV-6A contact with host cell chromatin structures.
Results: We developed a 4C-seq approach to identify specific chromatin interactions formed between HHV-6 genomic and human chromosomal loci. We applied this approach to inherited chromosomally integrated HHV-6A (iciHHVA) cells and to experimentally infected HHV-6A cells. Using independent replicates and reciprocal viewpoint primers we defined reproducible chromatin interactions between HHV-6A and regions affecting human gene expression. Interestingly, as intra-chromosomal interactions (cis) tend to occur more frequently in the context of higher-order chromatin structure, we found clusters of HHV-6A interactions near the telomeres of different human chromosomes in these cell models. These results suggest that the virus forms compact structures with the host telomeres. We plan to use this assay to explore the functional consequences of these virus-host chromatin interactions further.
Conclusions: We have developed an experimental and bioinformatic approach to facilitate the analysis of higher order chromatin structures formed by integrated HHV-6 genomes within infected cells.

4-10 Excision of the integrated HHV-6 genome using CRISPR/Cas9 technology

Oral

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Objective: Human herpesvirus 6A and 6B integrate their genomes into the telomeres of latently infected cells. In addition, integration can also occur in germ cells, resulting in individuals that harbor the integrated viral DNA in every cell. This condition is called inherited chromosomally integrated HHV-6 (iciHHV-6) and affects about 1% of the world's population. The integrated HHV-6A/B genome can be mobilized from the host telomeres, resulting in virus reactivation that is associated with various diseases. Until now, it remains impossible to cure cells from the integrated virus genome

and prevent reactivation.
Methods: Here we set to develop a system that allows removal of HHV-6 from the chromosomes of infected cells using the CRISPR/Cas9 system. First, we generated Cas9 expressing cells harboring the integrated HHV-6 genome. Subsequently, a string of ten different gRNAs targeting the terminal HHV-6 DR regions were transfected into the Cas9 expressing cells. The removal of the HHV-6 genome was analyzed by FACS, qPCR, and FISH.
Results: Our preliminary results demonstrate that the virus genome could be efficiently excised using the CRISPR/Cas9. Genome copies detected by qPCR were reduced and the majority of the cells lost the FISH signal of the integrated virus genome. In addition, most experimental cells were not able to reactivate, while almost all control cells reactivated and expressed GFP.
Conclusion: Our preliminary results revealed that our CRISPR/Cas9 allows efficient removal of the integrated virus genome. We are currently optimizing the excision efficiency as a complete removal is required to test this approach in a clinical setting.

4-11 *Oral* High human herpesvirus load and genome diversity affect infant development as shown by next generation genome deep sequencing

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Objectives: High throughput methods using next generation sequencing have been instrumental in advancing analyses of human herpesvirus genetic diversity. We have been analysing populations where the number of strains infecting individuals is increased due to immunosuppression facilitating opportunistic infections. Cohorts include those in sub-Saharan Africa where mothers are HIV positive and their children, even those who remain HIV negative, are influenced by increased maternal infections and viral load. This was associated with adverse childhood development and we aimed to analyse the contributions of genetic diversity.
Methods: We have developed new methods to analyse genome diversity in order to determine mixed infections with multiple strains. To do this we used Illumina based deep sequencing combined with SNP and motif analyses.
Results: We focused on betaherpesviruses, analysing first the highly diverse human cytomegalovirus followed by investigations on human herpesvirus 6A/B, for both endogenous integrated and exogenous virus genomes. This allowed evaluation of contributions for both viral load as well as burden of infection in determining disease outcomes. Methods and relative outcomes are reviewed.
Conclusions: Overall results indicate interventions are required which can address both aspects of viral load and genome diversity with mixed infections.

4-12 *Oral* Phylogeny of inherited chromosomally integrated (ici) and community HHV-6A and -6B strains

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Objectives: Over 90% of people around the world harbor HHV-6B strains. HHV-6A strains seem to be rarer as well as inherited chromosomally integrated (ici) forms whose frequency is below 2.9%. In this study, we compared the distribution and the genetic diversity of HHV-6 strains collected in French hospitals to other published data from different areas in order to define their relationship and propose evolutionary patterns.
Methods: HHV-6 DNA was extracted from various samples (whole blood, hair follicles, biopsies, etc.) of 228 infected patients. The species was determined by specific qPCR or sequencing analysis and the integrated status was established by detection of viral DNA in hair follicles/nails, FISH, and/or longitudinal analysis of viral load. For a sub-group of 111 patients (41 HHV-6A, 70 HHV-6B) and 10 laboratory strains the complete U38 and U39 genes (3039 and 2493 pb)

were amplified and sequenced. Twenty four HHV-6A and 141 HHV-6B published sequences from different countries in Europe, America, Asia, and Africa were included to build phylogenetic trees in maximum likelihood using Mega 7 software (Gompels 1995; Isegawa 1999; Dominguez 1999; Gravel 2013; Tweedy 2015 & 2016; Zhang 2016 & 2017; Telford 2018; Greninger 2018).
Results: The community strains (n = 109) were predominantly HHV-6B (94%) whereas HHV-6B accounted only for 58% of well defined iciHHV-6 (n = 104). The intra-species variability calculated on all studied sequences was 2.9 to 3.7% for both genes. Phylogenetic analysis showed that all but two iciHHV-6A strains formed a clade related to U1102 strain and separated from others HHV-6A strains. 14 different sequences were found among iciHHV-6A, some shared by several strains, whereas the other HHV-6A sequences were unique. For HHV-6B, the same sequence, except for a SNP, was shared by 93 iciHHV-6B and 11 community HHV-6B strains. They were very close to different sequences from several other iciHHV-6B and HHV-6B, including Z29 strain. This group is the sister-clade of a group containing most community HHV-6B (n = 75), including HST strain and 6 iciHHV-6B, including the 3 Asian ones, all with a much higher variability. For both species clades can be identified by nucleotidic signatures.
Conclusions: The low variability of most iciHHV-6A and iciHHV-6B could reflect, for each, an ancient integration event into germ cells followed by co-evolution with the human genome. The frequencies observed around the world today would depend on the population migrations and expansions, as well as the beneficial or harmful biological effects of iciHHV-6. As integrated genomes can reactivate they could be at the origin of community strains, some of which could have, in turn, integrated more recently into germ cells.

4-13 Preliminary data on the prevalence of inherited chromosomally integrated human herpesvirus 6 in Russia

Poster

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Human betaherpesvirus 6A and 6B (HHV-6A, HHV-6B) are ubiquitous in human populations worldwide. Growing evidence suggests that HHV-6A/B can integrate into subtelomeric/telomeric regions of host cell chromosomes and be inherited through the germline (inherited chromosomally integrated (ici) HHV-6A, 6B). The prevalence of iciHHV -6A/B positive individuals vary between 0.6% and 2% depending on the geographical region sampled (Flamand, 2018). Similar research has not been conducted in Russia previously.
Objective: The study of iciHHV-6A/B prevalence in healthy individuals in Russia.
Methods: The study involved 262 healthy individuals (75 male, 187 female with median age 36 years, (ranged 19–67 years) from Moscow and the Moscow region. In addition, 3 members of one family (2 male, 1 female: 21, 50, and 69 years old) were studied for confirmation of the genetic transmission of iciHHV-6B. HHV-6A/B DNA in samples of whole blood, hair follicles, and fingernail clippings were detected using quantitative real time PCR assay. The iciHHV -6B whole genome sequence was determined by next-generation sequencing. De novo assemblies of the genome were generated with SPAdes, contigs were aligned with reference HHV-6B (AB021506) genome. Phylogenetic tree was made using RAxML.
Results: HHV-6A/B DNA was detected in whole blood in 5.7% (15/262) cases. The levels of HHV-6A/B DNA (log_{10} copies/10^5 cells) were: <1.0 – 4.5% (12/262), 1.0–2.0 – 0.8% (2/262), >5.0 – 0.4% (1/262). IciHHV-6B was documented by analysis of hair follicles and nail clippings in the case of one female (41 years old) with high load viral DNA (5.25 log_{10} copies/10^5 cells) in whole blood. We examined relatives of the iciHHV-6B–positive female for interpretation of the genetic transmission. IciHHV-6B was documented by analysis of hair follicles and fingernail clippings over three generations: mother–daughter–grandson. Phylogenomics analysis of the Russian isolates showed that they belonged to HHV-6B group and formed a unique lineage within the European clade. The genome sequences from this study will be available in NCBI GenBank BioProject PRJNA524981.
Conclusions: There were no previous studies on the prevalence of iciHHV-6A/B in Russia. We have obtained preliminary data on iciHHV-6A / B, establishing the prevalence of iciHHV-6B at 0.4% (1/262, 95% CI 0–2.1). IciHHV-6B was detected and laboratory confirmed in three generations. IciHHV-6A was not found. Further research and larger studies of iciHHV-6A, -6B population in the Russian population are required to understand the genetic variety and geographical stratification of HHV-6A and -6B in the world.

4-14 Visualizing the effects of telomere-integrated HHV-6A on the host cell

Poster

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Human herpesvirus 6A (HHV-6A) and 6B (HHV-6B) integrate their genome into the host telomeres of latently infected cells. Both viruses can also integrate into germ cells, resulting in transmission of the virus genome from parent to child. This form of HHV-6 is termed inherited chromosomally integrated HHV-6 (iciHHV-6) and affects about 1% of the human population. Reactivation of acquired and inherited HHV-6A/B is associated with several diseases including encephalitis and graft rejection. The integrated HHV-6 genome has been detected in the shortest telomere of somatic cells and is associated with chromosomal instability. However, little is known about its impact on telomere regulation and homeostasis. Currently, no method exists to visualize the integrated virus genome in living cells. Therefore, we developed a visualization system to study the homeostasis of ciHHV-6$^+$ telomeres.

To study telomere homeostasis and interaction of the HHV-6 genome with the shelterin complex, we developed a system to visualize the virus genome in living ciHHV-6A cells. We visualized the HHV-6 genome using specific gRNAs and a nuclease-dead Cas9 (dCas9) containing tandem repeated epitope tags that are bound by scFv-GFP proteins. To increase the signal-to-noise ratio we developed and validated a polycistronic gRNA vector that delivers 10 gRNAs targeting Cas9 to the HHV-6A DR regions. In vitro derived ciHHV-6A cells and patient-derived cell lines were stably transduced with the imaging constructs and the polycistronic gRNA vector. We are currently using this system to investigate the movements of ciHHV-6 containing telomeres and its interaction with host factors. This study will elucidate the real-time behaviour of ciHHV-6 containing telomeres and its implications towards altered chromosomal stability.

4-15 rs73185306 SNP prevalence and inherited chromosomally-integrated human herpesvirus 6A/B

Poster

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Objective: The mechanisms leading to HHV-6A/B chromosomal integration are still poorly understood. In a recent populational study from China it was reported that the presence of the rs73185306 C/T SNP represented a predisposing factor for HHV-6A/B integration. The SNP is located in the MOV10L1-MCL1 genes. Interestingly, the MOV10L1 gene codes for a PIWI-interacting RNA helicase that can act as a repressor of retrotransposons during spermatogenesis. It was hypothesized that the presence of the rs73185306 SNP could alter the MOV10L1 repressor function and in turn favor HHV-6A/B integration. Our goal was to validate whether the rs73185306 SNP was more prevalent in iciHHV -6A/B$^+$ compared to control individuals using samples from the Montreal Heart Institute biobank.

Methods: To validate a possible association between the rs73185306 C/T SNP and iciHHV-6A/B the DNA samples from Montreal Heart Institute biobank were screened for the presence of iciHHV-6A/B. Fifty-five iciHHV-6A/B$^+$ subjects were identified. DNA from 57 age and sex-matched healthy individuals were used as controls. Using High Resolution Melt assay followed by sequencing, the presence of the rs73185306 C/T SNP was determined. Three genotypes were identified: WT homozygous (C/C), heterozygous (C/T), and homozygous (T/T).

Results: We observed that 80% of the iciHHV-6A/B$^+$ individuals were WT homozygous (C/C) while 20% carried the C/T SNP. In controls, a similar proportion of the rs73185306 C/T SNP (19%) was found, indicating that no significant statistical difference exists in rs73185306 C/T SNP prevalence between iciHHV-6$^+$A/B subjects and controls. At 20% prevalence the rs73185306 SNP (C/T) was more frequently detected than expected considering its 10% prevalence worldwide. We also confirmed that the presence of integrated HHV-6A/B in chromosome 17p was preferentially over represented in our biobank, with 29% of our iciHHV-6$^+$ subjects having ciHHV-6A/B in 17p. The rs73185306 C/T SNP was also distributed equally between subjects carrying integrated virus in 17p compared to those with integration in other chromosomes, indicating that the presence of this SNP is not predictive of integration in the 17p chromosome.

Conclusions: Altogether our study indicates that despite a higher prevalence for the rs73185306 C/T SNP in the Canadian population relative to the rest of the world the presence of this polymorphism does not correlate with increased presence of iciHHV-6+A/B, suggesting that rs73185306 C/T SNP does not influence chromosomal integration.

4-16 Identification of ancestral iciHHV-6B lineages

Poster

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Background: We previously screened almost twenty-four thousand individuals from the Generation Scotland: Family Health Study for inherited chromosomally integrated HHV-6 (iciHHV-6) and identified 647 positive individuals (2.7%). The majority were iciHHV-6B-positive (n=603). Genome-wide association analysis (GWAS) revealed associations between iciHHV-6B and single nucleotide polymorphisms (SNPs) at the telomeric ends of chromosomes 7p, 9q, 11p, 17p and 19q suggesting the presence of ancestral viral integrations in these locations. PCR and sequence analysis confirmed that 22% of iciHHV-6B-positive individuals had an ancestral integration in chromosome 17p. The present study aimed to develop simple bioinformatics and lab-based methods for identification of ancestral viral lineages.
Methods: We analysed host haplotypes adjacent to suspected HHV-6B integration sites in families carrying iciHHV -6B genomes to identify SNP combinations associated with ancestral viruses. In addition, we performed a k-mer analysis to identify oligonucleotides that are unique to these iciHHV-6 lineages. These viral sequences were then used to develop assays to identify the most common ancestral viruses in populations of European descent.
Results: We defined the host haplotypes associated with ancestral iciHHV-6B integrations in chromosomes 9q, 11p, 17p, and 19q. Around one-third of iciHHV-6B-positive individuals in the Generation Scotland study possessed a 9q haplotype associated with iciHHV-6B; we also identified a second 9q haplotype associated with iciHHV-6B. K-mer analysis identified oligonucleotides that are unique to the iciHHV-6B genomes integrated in chromosomes 9q, 11p, and 17p. Further analysis of available iciHHV-6 genomes identified ten groups of genomes suggesting that there are at least ten distinct ancestral HHV-6B lineages.
Conclusion: By analysing iciHHV-6B-associated host haplotypes and iciHHV-6B genomes, we have developed bioinformatics and lab-based approaches for the identification of ancestral lineages of iciHHV-6B.

4-17 Inherited chromosomally integrated HHV-6 genomes with unusual compositions result from duplication and deletion occurring following viral integration

Poster

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Background: We previously identified 647 individuals with inherited chromosomally integrated HHV-6 (iciHHV-6) in the Generation Scotland: Family Health Study. The majority of individuals carried an iciHHV-6 genome with a single unique region and two direct repeats (DR); however, 23% of iciHHV-6A genomes and 6% of iciHHV-6B genomes had more unusual compositions. The most common were genomes lacking all or most of the unique region (n=25), but genomes with extra unique regions and DRs were also identified. The present study aimed to determine the configuration of these aberrant genomes.
Methods: We developed a novel method of linkage analysis using droplet digital PCR and statistical modelling to resolve unusual HHV-6 genome configurations. Targeted enrichment and whole viral genome sequencing were also performed to characterize an iciHHV-6A deletion mutant with a single DR but also some residual unique region sequences. Viral integration sites and adjacent host haplotypes were analysed to determine whether aberrant genomes were derived from ancestral viruses.
Results: Linkage analysis identified concatemers of iciHHV-6A and B genomes, and iciHHV-6A genomes with duplication of DR sequences. All individuals with iciHHV-6B concatemers carried the host haplotype associated with the common ancestral integration in chromosome 9q, suggesting that viral genome duplication occurred following integration. None of the aberrant compositions was associated with two different integration events. We identified 21

genomes with just a single DR, two HHV-6A genomes with two linked DRs, and two HHV-6A genomes with a single DR and a small part of the unique region. These unusual deletion mutants were derived from the ancestral iciHHV-6A integration in chromosome 17p. Sequence analysis revealed that these genomes comprised a single DR and part of the U100 gene, but the remainder of the unique region was missing.

Conclusion: Unusual iciHHV-6 genome compositions largely result from duplication and deletion occurring following viral integration. We found no evidence to suggest that these aberrant viral compositions result from looping out and reintegration of viral genomes.

Malignancy

5-1 Does HHV-6 promote cancer development?

Oral

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There is substantial evidence that HHV-6 can be found in a number of tumors, including Hodgkin lymphoma, oral and gastrointestinal carcinomas, and gliomas. However, while HHV-6 DNA and antigens, including the possible oncoprotein DR-7, have been detected in tumor cells in certain types of cancer, they are also frequently present in the surrounding cells of the tumor microenvironment. The gammaherpesviruses, EBV (HHV-4) and KSHV (HHV-8) are present within tumor cells, where they express viral oncoproteins and have clear associations with specific cancers. While EBV infection is ubiquitous, KSHV is not, which has allowed epidemiologic demonstration of causality of KSHV in Kaposi sarcoma. In contrast, HHV-6 has not been consistently found solely inside the tumor cells, nor in the majority of tumor cells. In addition, given its highly prevalent infection, questions arise about whether it plays a causal role or is simply reactivating in the tumor microenvironment, with spurious spread to tumor cells.

Techniques like PCR are prone to contamination, and the sensitivity of the assay can pick up circulating viral DNA. In contrast, immunohistochemistry, while less sensitive, is prone to cross-reactivity with other proteins that can result in false-positive findings. These technical limitations have cast doubt on some studies describing associations of HHV-6 and cancer, and the relative dearth of studies using immunohistochemical techniques has resulted in limited data on specific localization of HHV-6 in tumors. Nevertheless, it is highly plausible that HHV-6 plays a contributory role in cancer development, although HHV-6A and HHV-6B likely contribute in different ways and to different types of cancer.

An example of a virus that does not directly induce transformation, yet is able to contribute to cancer, can be found in HIV. HIV is not found in tumor cells and is not an oncogenic virus, but is recognized by the WHO/IARC as a biologic carcinogen. Immunodeficiency is a major mechanism by which HIV increases the risk of cancer, but immune activation and effects of HIV proteins may also play roles. There has also been evidence of HIV affecting replication of tumor viruses even if the same cells are not co-infected. Similarly, HHV-6 present in the tumor microenvironment may modulate factors involved in oncogenesis, including immune mediators, in ways that promote tumor development or support tumor growth. In addition, there have been studies documenting the ability of HHV-6 to cooperate with other viruses, including HIV and HPV. New methodologies should lead to molecular mechanistic information to settle this interesting question.

5-2 Presence of HHV-6 in the lymphoma microenvironment

Oral

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Introduction: While Epstein-Barr virus (EBV) and human herpesvirus 8 (HHV-8) have shown a definite association with lymphoproliferative disease, a role for the T-lymphotrophic herpesvirus HHV-6 has been less clear. HHV-6 proteins have been variably detected in lymphoma, including Reed-Sternberg cells (HRS) in classic Hodgkin lymphoma (CHL) with more recent data suggesting they may be confined to tumor-associated lymphocytes. Particularly intriguing in this context is its potential to modulate the inflammatory microenvironment by altering T cell subsets, function and/or cytokine production. More robust tools are needed to investigate this question. We combine flow cytometric isolation of tumor and background lymphoid cells with RNA sequencing to report the first pattern of HHV-6B viral gene expression within T cells in the CHL microenvironment.

Methods: We utilized flow sorting to enabled rapid isolation of viable HRS cells from primary CHL tumors to generate full transcriptome RNA sequence data from primary cases of CHL including both HRS cells and associated lymphoid populations (Reichel et al, Blood 2015). Intratumoral B cells were used as control for transcriptional signatures. RNA from purified intratumoral T cells was also extracted and sequenced in one case. We examined the data for the presence of known and unknown infectious agents. Expression of HHV-6 at the protein level was validated by

immunohistochemistry for gp60/110 and p41 110 (Millipore Sigma, MAB8537 and Santa Cruz Biotechnologies, 9A5D12). A range of other CHL, large B cell, T-cell, and post-transplant lymphoproliferative processes also underwent HHV-6 antibody screening using tissue microarrays.

Results: EBV transcripts were found in HRS cells in one case, but no other known or unknown viruses were identified in the CHL tumor cells. However, we identified HHV-6B transcripts within CHL intratumoral T cells, with very high expression of the U51 and U24A transcripts. Immunohistochemistry for HHV-6 in an expanded panel of lymphoid malignancies revealed that both the fraction of lymphocytes within individual lymphomas and the frequency of HHV-6 involvement between different lymphoma subtypes varied significantly. T cell lymphomas had the highest frequency of HHV-6 detection followed by post-transplant lymphoproliferative disorders, while diffuse large B-cell and follicular lymphomas only rarely contained such positive cells. HHV-6 expression was not identified within the tumor cells themselves.

Conclusions: This is the first report to our knowledge confirming HHV-6 expression using RNA sequencing and demonstrating the expression pattern within an intratumor T cell population. The most highly expressed genes U24 and U51 have the potential to affect production of cytokines and alter the tumor microenvironment. The identification of specific patterns of viral gene expression may enable a more mechanistic approach to investigating the potential role of HHV-6 in lymphomagenesis and progression.

HHV-6 Genes & Proteins

6-1 Oral Molecular interaction of HHV-6B gH/gL/gQ1/gQ2 complex with the cellular receptor and neutralizing antibodies

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Objective: Human herpesvirus 6B (HHV-6B) equips a tetrameric glycoprotein complex gH/gL/gQ1/gQ2 as viral ligand and it recognizes the host receptor CD134 specifically expressed on activated T-cells. The engagement between gH/gL/gQ1/gQ2 and CD134 is critical to initiate the entry process, and thus antibodies for them are able to inhibit the HHV-6B infection by interfering with this interaction. In previous studies anti-gQ1 monoclonal antibody (Mab) KH-1 and anti-gH Mab OHV-3 were produced and exhibited neutralizing activities against HHV-6B. However, the receptor recognition mechanism and mode of action of the neutralizing Mabs are still a mystery due to the lack of knowledge about the structure and molecular function of gH/gL/gQ1/gQ2. The objective of this study is to analyze the affinities and modes of interaction between gH/gL/gQ1/gQ2 and its receptor for these neutralizing antibodies from a molecular aspect and thereby clarify the interplay between these key molecules.
Methods: The recombinant HHV-6B gH/gL/gQ1/gQ2 ectodomain was expressed by a mammalian cell-based system and was purified to homogeneity. Ectodomain of the human CD134 was prepared by the similar procedure. For the preparation of Fab domains of Mabs KH-1 and OHV-3, their Fc parts were cleaved by papain digestion and trapped. The affinities were evaluated by surface plasmon resonance (SPR) analysis with a Biacore T200 instrument. Competition between Mabs and the receptor was analyzed by an enzyme linked immunosorbent assay (ELISA). The shape of gH/gL/gQ1/gQ2 complex alone or with the Mabs was analyzed by negative stain electron microscopy (EM).
Results: The affinity of CD134 with gH/gL/gQ1/gQ2 was sufficiently high as an entry receptor and comparable to those of the neutralizing Mabs. The anti-gQ1 Mab KH-1 competed with the interaction between CD134 and gH/gL/gQ1/gQ2, indicating the Mab could directly inhibit its interaction. On the other hand, the anti-gH Mab OHV-3 showed no effect, implying different inhibition mechanisms. The negative stain EM analysis visualized a curved and elongated shape of the gH/gL/gQ1/gQ2, similar to the pentameric glycoprotein complex of human cytomegalovirus. The Fabs of the Mabs were located on different sites, indicating multiple positions of neutralizing epitopes.
Conclusions: Analyses with the purified gH/gL/gQ1/gQ2 revealed the shape and molecular basis for the interaction with the receptor and the Mabs and it will be useful for the future evaluation of viral and host factors which interact with the gH/gL/gQ1/gQ2.

6-2 Oral Transformation of human peripheral blood T and B-cells by coinfection with human herpesvirus 6 and Epstein-Barr virus; possible role in MS

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Objective: In vitro studies indicate that EBV positive B-cell lines can be infected with HHV-6A and in some cases HHV-6A may undergo replication in these cell lines.
B-cell and T-cell lymphomas with dual HHV-6 and EBV positivity have also been described. EBV and HHV-6 have both been implicated in multiple sclerosis. The objective of this study was to identify whether cell lines of hematological or neurotropical origin could be established based on coinfection with EBV and HHV-6A. Establishment of doubly-infected cells may reveal an important role for HHV-6 and EBV coinfection in both lymphoproliferative and neurotropic disease. The established cell lines could be used in future studies to study the role of HHV-6 in the etiopathogenesis of lymphoid neoplasias and autoimmune diseases such as multiple sclerosis.
Methods: HHV-6A strain S was propagated in Molt-3 cells. EBV containing supernatant was used for coinfections either from B95-8 cells or P3HR-1 cells
Results: We demonstrated that HHV-6A infection induces CD21 on T-cells and allows entry of EBV and establishment of doubly-infected cells. Human B and T-cell lines were established from normal donors by

coinfection with HHV-6 and EBV. T-cell lines were grown in tissue culture for over ten years without losing the EBV genome and expressed EBNA-1, EBNA-2, and EBNA-3 while LMP and EBER expression varied. They retained the HHV-6 genome for several weeks, however ~80% of the established T-cell lines lost the HHV-6 genome months in tissue culture. The B-cell lines established by coinfection produced human antibodies with increasing antibody production after conversion to plasmablasts. Coinfection of U251 or U87 glial cell lines led to dual positivity but the EBV genome was lost after a few weeks in culture. In long-term culture they expressed HHV-6 associated proteins but no viral replication was observed. Coinfection of human primary astrocytes led to short term proliferation of the astrocytes and establishment of dual infection but the cell lines underwent morphological changes and lost proliferation after four months in culture. They expressed several HHV-6 associated proteins. Some of these viral proteins were also detected in biological fluids of MS patients by an antigenemia assay.
Conclusions: These studies suggest that HHV-6 could be a helper factor for EBV infection of T-cells *in vivo*. Furthermore, both EBV and HHV-6 may play a role in the development of some T-cell malignancies and in the development of immunodysfunction in humans.

6-3 Mapping the HHV-6B transcriptome

Oral

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Objective: With the "omics" revolution in recent years the study of RNA transcripts produced during infection and under specific conditions becomes relatively easy. In the quest for finding new transcripts expressed differentially in the course of an HHV-6B infection we made use of large-scale RNA sequencing to analyze and compare the HHV-6B transcriptome during infection of human Molt-3 T-cells.
Methods: Molt-3 T-cells were infected with HHV-6B Z29. Infected cells were harvested at 6, 9, 12, 24, 48, and 72 hours post-infection. Infected cells were also harvested at 6 hours post-infection in the presence of cycloheximide, a protein synthesis inhibitor, and 72 hours post-infection in the presence of phosphonoacetic acid, a viral DNA polymerase inhibitor. Total RNA was subsequently isolated from all these time points and used for library generation. Libraries were sequenced on the Ion Torrent S5 system.
Results: A total of 50-55 million reads per sample were obtained with more than 95% of bases over a quality score of 30 (Q30; 99.9% = base call accuracy). We first looked for HHV-6B specific reads matching new HHV-6B transcripts. We observed differential expression of new HHV-6B transcripts in all samples analyzed. The presence of many of these new HHV-6B transcripts were confirmed by RT-PCR. Many of these new transcripts represent new splicing variants of previously reported transcripts. A detailed analysis of these newly found transcripts will be presented.
Conclusions: We observed several HHV-6B transcripts differentially expressed in the course of an infection. This is the first time that these transcripts have been identified during an HHV-6B infection. The presence of these new transcripts was also confirmed by RT-PCR and Sanger sequencing, demonstrating the relative abundance of these transcripts. Whether these new transcripts lead to new proteins is presently under investigation. Overall, our work demonstrates the diversity and the complexity of the herpesvirus transcriptome.

7-1 Basic study for vaccine development of HHV-6

Oral

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Human CD134/OX40 has been found to be a specific receptor for HHV-6B, and it binds to the HHV-6B gH/gL/gQ1/gQ2 complex which expresses on viral envelope, and its components, gQ1 and gQ2 are unique to HHV-6. The interaction of the viral glycoprotein complex and CD134 is essential for virus entry into the target cells, and antibodies for the complex inhibit virus infection. Therefore we had an idea that the viral complex might be a target for HHV-6B vaccine strategy. Immunization of the glycoprotein complex into mice showed that it could induce specific antibodies that could protect HHV-6B infection and also induce cellular immunity against the complex. In addition, we identified gQ1 epitope that could induce cellular immunity in mice, indicating that the viral complex containing gQ1 and gQ2 could be a target for HHV-6 vaccine strategy.

7-2 Characterizing the CD4 T cell response to HHV-6

Oral

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Objective: To determine the functional capacity, frequency, specificity, and phenotypic characteristics of T cells that control HHV-6 in chronically infected individuals.
Methods: T cell epitopes were defined using comprehensive genome-wide screening of overlapping peptide series and by elution of MHC-bound peptides from HHV-6B-infected cells. In most cases blood samples were obtained from healthy adult donors, with some responses also analyzed in samples from healthy infants (<2 yrs) or from solid organ transplant recipients pre and post-transplantation. T cell responses were characterized using peripheral blood mononuclear cell samples or short-term T cell lines expanded in vitro. Phenotypic and functional assays included IFN-γ ELISpot and intracellular cytokine staining for IFN-γ, TNF-α, IL-2, IL-10, granzyme B, and perforin, with CD107a mobilization and in vitro killing assays used to assess cytolytic capacity. DRB1*03:01 tetramers were used to sort the antigen-specific population for RNA analysis and TCR sequencing using the Illumina platform.
Results: Phenotypic and functional analysis by flow cytometry indicated that the T cell populations responding to HHV-6 in healthy donors were mainly CD3+CD4+ from the effector memory (CD45RA-/CCR7-) subset. Responding cells produced IFN-γ, TNF-α, and low levels of IL-2, alone or in combination, highlighting the presence of polyfunctional T cells in the overall response. Many of the responding cells mobilized CD107a, stored granzyme B, and mediated specific killing of peptide-pulsed target cells. In most individuals T cell populations that respond to HHV-6 target both viral structural components and infected cell proteins, with particular specificities present at low levels (<0.01% of total T cells). Ongoing experiments are using single-cell and bulk RNASeq transcriptional analysis to better characterize T-cell subsets involved in the HHV-6-specific response and using TCR repertoire deep-sequencing to evaluate clonotype diversity and persistence.
Conclusions: The T cell response to HHV-6B targets a wide variety of antigens including both structural components of the virion as well as viral proteins found only in infected cells and is characterized by polyfunctional CD4 effector T cells with cytolytic capacity.

7-3 HHV-6A infection of endometrial epithelial cells modifies immune profile and induces a defective trophoblast invasion

Oral

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Objective: We first reported HHV-6A DNA presence in 43% of endometrial cells from women with idiopathic

infertility, whereas no fertile control women harbored the virus (Marci 2016). Growing evidence confirms this implication (Caselli 2017; Coulam 2018). We investigated the effect of HHV-6A infection on the immunological status of the endometrium.

Materials and Methods: Endometrial biopsies, uterine flushing and whole blood samples were collected during the implantation (mid secretory phase) windows in 67 idiopathic infertile women. The presence of HHV-6A infection was evaluated by DNA and mRNA analysis (U22, U42, U94 genes) on endometrial cellular subsets (Marci 2016). We analyzed the endometrial immunological status: i) the endometrial immune profile analyzing the ratio of IL–15/Fn–14 mRNA as a biomarker of endometrial (e)natural killer activation/maturation and the IL–18/TWEAK mRNA ratio as a biomarker of both angiogenesis and the Th1/Th2 balance (Ledee 2016); ii) endometrial receptivity to trophoblasts in an endometrial 3D in vitro model; iii) eNK cells and regulatory T cells immune-phenotype in endometrial biopsies and peripheral blood by flow cytometry.

Results: We confirmed the presence of HHV-6A infection in 40% of idiopathic infertile women. We observed a low immune activation profile in HHV-6A negative infertile women, characterized by a low IL-15/Fn-14 mRNA and IL -18/TWEAK mRNA ratio, reflecting a low eNK maturation and local angiogenesis. On the contrary, HHV-6A positive infertile women presented a high IL-15/Fn-14 and IL-18/TWEK mRNA expression, representative of eNK cell cytotoxic activation. As a proof of concept, we observed a lower percentage of $CD56^{bright}CD16$- eNK cells in women positive for HHV-6A infection ($p<0.01$) and a slight increase in cytotoxic $CD56^{dim}CD16$- eNK cells. We observed no differences in the percentage of peripheral blood T, B and NK cells. On the contrary, when we evaluated the immune-phenotypes, we observed a decrease in $CD4+CD25+CD127^{dim}$/- regulatory T cells in women positive for HHV-6A infection ($p<0.01$). We assessed the effect of HHV-6A infection on endometrial receptivity by co-culturing endometrial epithelial cells with spheroids generated from the EVT-derived cell line JEG3. Within 3 days, spheroids on ESC culture surface from HHV -6A negative women expanded 2-fold while spheroids on ESC culture surface from HHV-6A positive women failed to expand.

Conclusions: The identification of an effect of HHV-6A infection on endometrial immune status opens new perspectives in idiopathic infertile women care management. In addition, it would be possible to select antiviral therapies as novel, non-hormonal therapeutic approaches to those idiopathic infertile women characterized by the presence of endometrial HHV-6A infection.

7-4 *Oral* Downregulation of the protein tyrosine phosphatase CD45 in roseolovirus-infected T cells

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Like all herpesviruses, the *Roseoloviruses* establish lifelong infection within their host, requiring these viruses to evade host anti-viral responses. One common host-evasion strategy is the downregulation of host-encoded, surface-expressed glycoproteins. Herpesviruses have been shown to downregulate adhesion molecules, class I MHC molecules, and NK-activating ligands, presumably in an effort to escape host anti-viral responses. The Roseoloviruses have also been shown to downregulate NK-activating ligands, class I MHC molecules, and the TCR/CD3 complex. To more globally identify glycoproteins that are differentially expressed on the surface of HHV-6A infected cells we performed cell surface capture of glycoproteins present on the surface of T cells infected with HHV-6A and compared these to proteins present on the surface of uninfected T cells. We found that the protein tyrosine phosphatase CD45 is downregulated in T cells infected with HHV-6A. We also demonstrated that CD45 is similarly downregulated in cells infected with HHV -7. CD45 is essential for signaling through the T cell receptor and as such, is necessary for developing a fully functional immune response. Interestingly, the closely related betaherpesviruses MCMV and HCMV have also separately evolved unique mechanisms to target CD45, suggesting that CD45 is an important viral target (Thiel *et al.* 2016, Gabaev *et al.* 2011). To identify the HHV-6A ORF responsible for downregulating CD45, we deleted a block of four roseolovirus-unique open reading frames from the HHV-6A genome (ORFs U21-U24). T cells infected with this deletion mutant virus displayed CD45 expression similar to that of uninfected T cells, suggesting the downregulation of CD45 in HHV -6A-infected T cells is mediated by the virus, and more specifically, by one of these deleted gene products.

7-5 Poster MHC-class II tetramers identify CD4 T cells responding to HHV-6B antigens in expanded T cell lines and ex vivo in PBMCs from an HLA-matched population

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Objective: The goal of this work was to develop a set of MHC-II tetramers able to identify and isolate specific CD4 T cells responding to HHV-6B antigens. The set of tetramers is being used to study and better understand the nature of the CD4 T cells responding to HHV-6B in HLA-matched donors.
Methods: PE and APC-labeled DRB1*03:01 tetramers were obtained using five previously identified DRB1*03:01 naturally processed and presented epitopes derived from the HHV-6B proteins U85, U48, U11, U56, and U63. The five tetramers were used to analyze short-term T cell lines expanded in vitro or ex vivo PBMCs, both from HLA-matched donors, to identify the double-positive population. In addition, DRB3*02:02 (U39 epitope) and DRB3*01:01 tetramers (U48 and U56 epitopes) were also prepared and testing is ongoing.
Results: The pool of DRB1*03:01 tetramers was able to recognize HHV-6B-specific T cells in ex vivo PBMC samples from each of 8 HLA-matched donors tested. Low frequencies of double-positive cells were observed (0.03 ± 0.02% of total cells vs. 0.002 ± 0.002% of total cells for control CLIP-peptide containing tetramers). For detailed characterization tetramer staining experiments were performed using T cell populations expanded in vitro. Optimal staining conditions determined were a final concentration of tetramer of 2 ug/mL in the reaction mix and incubation at room temperature for 2 hours. Each individual tetramer showed staining in 2-3 out of 5 donors tested. Each donor showed different patterns of staining, in terms of frequency of cells recognized and individual tetramer(s) recognized. When tetramers were used as a pool to analyze the expanded T cells staining was observed in 9 out of 9 donors, with an average of 11.8 ± 6.2% of total cells that were double-positive for the tetramer pool and background staining of 0.05 ± 0.02% of total cells for CLIP-tetramer.
Conclusions: The DRB1*03:01 tetramers described in this work efficiently recognized T cells in an HLA-matched population. We are using the pool of tetramers to isolate and characterize antigen-specific CD4 T cells using in vitro expanded cell populations. Future work is aimed at isolating and studying ex vivo antigen-specific T cells.

7-6 Poster Donor-derived memory CD4 T cells and HHV-6 reactivation after allogeneic hematopoietic cell transplantation

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Objective: To determine if there is a correlation between HHV-6B specific CD4+ T cells in donor peripheral blood mononuclear cells (PBMCs) and the risk for HHV6 reactivation after allogeneic hematopoietic cell transplantation (aHCT).
Methods: We identified patients who received HLA-matched, non-T-cell-depleted, myeloablative aHCT, who underwent once or twice-weekly quantitative plasma PCR testing for HHV-6 and CMV in the first 100 days post-HCT, and for whom we had cryopreserved stem-cell donor PBMCs. PBMCs were stimulated with mock or HHV-6B-infected lysate and tested by flow cytometry for IL-2, IFN-γ, TNF-α, and CD154. The frequency of HHV-6B-specific T cells was calculated as the percentage of specifically activated CD3+CD4+ cells and used to estimate the number of donor-derived HHV-6B-specific T cells infused in the stem cell product. We compared the estimated number of transplanted CD4 T cells and the estimated number of HHV-6B-specific (expressing ≥3 activation markers) donor T cells in two groups based on either peak HHV-6 ≤ median or none detected or peak HHV-6 > median. We performed the same analyses based on CMV detection.
Results: The cohort consisted of 28 patients in group 1 (five patients with HHV-6 detected, median viral load 100 copies/mL; 23 patients with no detection) and five in group 2 (median viral load 550 copies/mL). Patients in group 1

received a greater estimated number of HHV-6B-specific T cells (median, 5.1×10^4 cells/kg) compared to group 2 (median 5.0×10^3 cells/kg; p=0.047) as well as a greater estimated number of total CD4 T cells (1.35×10^8 compared to 1.33×10^7 cells/kg respectively; p=0.047). The number of total or HHV-6B-specific donor T cells had no appreciable effect on subsequent CMV reactivation.

Conclusion: A higher number of total donor CD4 T cells or non-manipulated HHV-6B-specific donor memory T cells infused at transplant may reduce the risk for HHV-6 reactivation after aHCT. Prophylactic co-infusion of enriched or HHV-6B-specific T cells with aHCT products could confer clinical benefit in high risk patients, particularly those receiving infusions that are T-cell depleted or cord-blood origin.

Host-Cell Interaction

8-1 Significance of CD46 isoforms for HHV-6A and HHV-6B infection

Oral

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CD46 has been shown to be the receptor for HHV-6A, whereas HHV-6B has recently been suggested to use CD134. CD46 may, however, express various isoforms that potentially could influence infection. CD46 gene editing using recombinant Cas9 with the CRISPR system allows later reconstitution of CD46 isoforms. This talk will address how separate isoforms of CD46 may guide infection by HHV-6A and HHV-6B in distinctive ways.

8-2 HHV-6A small, non-coding RNA sncRNA-U14 alters human miR-30 processing through direct interaction

Oral

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Small, non-coding RNAs (sncRNAs), including miRNAs, encoded by pathogenic organisms have been shown to play key roles during disease/infection progression. Extensive work during the last two decades has greatly contributed to our understanding of the mechanisms by which sncRNAs regulate mRNA degradation and translation. Moreover, recent evidence supports the role of ncRNAs in RNA-mediated RNA regulation, including an interesting example of long ncRNAs modulating pri-miRNA processing. However, it has yet to be described if pathogenic sncRNAs target the human miRNA processing machinery through RNA-RNA interaction. We here show a novel example of inhibition of human miR-30 processing by HHV-6A sncRNA-U14. Our results provide a biochemical basis for viral sncRNA-human miRNA interaction that results in altered mitochondria architecture through p53-Drp1 axis. Pathogenic regulation of human miRNAome through RNA-RNA interaction is a novel example that goes against the common scientific paradigm and shows the intricate nature of host-pathogen interaction. We thus provide the first ever molecular evidence behind HHV-6A reactivation mediated neuronal damage upon viral reactivation that does not require any viral protein. Our results might explain the high rate of association suggested for HHV-6A and several neurodegenerative disorders, including Alzheimer's disease, multiple sclerosis and bipolar disease.

8-3 Humanization of murine neutralizing antibodies against gQ1 and gH of HHV-6B

Oral

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Objective: Human herpesvirus 6B (HHV-6B) infection is a common concern of child health since it causes exanthema subitum and, in severe cases, encephalopathy/encephalitis accompanied by neurological sequelae. The reactivation of HHV-6B is a serious problem for the recipients of hematopoietic stem cells transplantation, frequently causing lethal encephalitis. Drug-induced hypersensitivity syndrome (DIHS) or drug rash with eosinophilia and systemic symptoms (DRESS) are also related to the HHV-6B reactivation. Despite the clinical importance there is little established prophylaxis for HHV-6B infectious diseases. Because HHV-6B initiates infection via an essential interaction between viral ligand gH/gL/gQ1/gQ2 and cellular receptor CD134, neutralizing antibodies which inhibit the interaction have potential for clinical use. In previous studies, we have isolated several murine monoclonal antibodies (Mabs) which neutralize HHV-6B. In this study, we focused on two Mabs, KH-1 and OHV-3, which recognize the gQ1 and gH respectively and humanized them as candidates of therapeutic antibodies.
Methods: The sequences of heavy and light chains of the Mab KH-1 and OHV-3 were determined. The variable region of each fragment was subcloned into the respective mammalian expression vector which contains the sequences of human IgG1 constant regions. The expression vectors of chimeric heavy and light chains were co-transfected into 293T cells, and the expressed antibodies were purified by Protein A resin. Murine antibodies were purified from supernatants of hybridoma cells by Protein A resin and used for the positive controls. The antigen recognition was analyzed by indirect immunofluorescence assay (IFA). Neutralizing activity was assessed by evaluating the inhibition efficacy for HHV-6B infection to MT4 cells.

Results: Chimeric Mabs KH-1 and OHV-3, which have human IgG1 constant domains, were successfully produced without changing their specificity to the antigens. Pre-treatment of HHV-6B with these chimeric Mabs resulted in inhibition of the infection in a dose dependent manner. Quantitative analysis of the inhibition efficacy revealed that the chimeric Mabs and original mouse Mabs have no significant difference in their neutralizing activities. The analysis also revealed a difference in the neutralizing activity between Mab KH-1 and OHV-3.
Conclusions: In this study we succeeded in creating humanized neutralizing antibodies for HHV-6B based on murine antibodies, which could lead to a breakthrough for development of HHV-6B prophylaxis and treatment. Furthermore, these results show the availability of the murine-human chimeric Mabs without losing their neutralizing activities. The recombinant system also provides a basis for further humanization of the chimeric Mabs by replacing frameworks of mouse variable domains.

8-4 *Oral* Analysis of the functional domain of HHV-6A glycoprotein Q2 required for tetrameric formation and binding to receptor

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Objective: HHV-6A and HHV-6B have specific envelope glycoprotein complex gH/gL/gQ1/gQ2 on the viral envelope and this complex-receptor interaction is essential for viral entry. Although gQ1 appears to directly bind to the receptor, formation of HHV-6A gH/gL/gQ1/gQ2 complex or HHV-6B gQ1/gQ2 complex is important for receptor binding. Furthermore, we previously reported that HHV-6A complex whose gQ2 was replaced with that of HHV-6B (AgH/AgL/AgQ1/BgQ2) bound to CD46, but less efficient than the original HHV-6A complex. Interestingly, although a complex of HHV-6B with AgQ2 (BgH/BgL/BgQ1/AgQ2) lost the ability to bind to CD134, it bound to CD46 much weakly. It suggests that gQ2 plays a role for complex-receptor binding. In this study, we focused on HHV-6A gQ2 and analyzed its functional domain in more detail.
Methods: We constructed two types of C-terminal truncated HHV-6A gQ2 mutants and six types of chimeric gQ2 mutants in which amino acid residues of HHV-6A were replaced with the homologous regions of HHV-6B. The gH, gL, gQ1 and each gQ2 mutant were co-transfected into 293T cells, and immunoprecipitation was performed using antibody against the gH/gL complex, and then gH, gL, gQ1, gQ2 or CD46 was blotted with each antibody.
Results: We found that AgQ2 with C-terminal truncation beginning at residue 162 (AgQ2Δ163-214) could not form tetrameric complex with gH, gL, and gQ1 because AgQ2Δ163-214 could not interact with AgQ1. Furthermore, the tetramer containing chimeric gQ2 in which residues 130-185 of AgQ2 were replaced with those of BgQ2 did not bind to CD46. Conversely, the tetramer with chimeric BgQ2 which has a replacement with AgQ2 at residues 130-185 could bind to CD46.
Conclusions: These results suggested that the residues 130-185 of AgQ2 contains the functional domain, and it is important for tetrameric complex to recognize CD46.

8-5 *Oral* U94 of human herpes virus 6 induces apoptosis on triple negative breast cancer cells by blocking DNA repair mechanisms

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Objective: The expression of U94, the latency gene of human herpesvirus 6, in the absence of any other viral transcripts, strongly inhibits in vitro angiogenesis and migration of both vascular and lymphatic human endothelial cells (ECs) and *ex vivo* vasculogenesis. More recently, we demonstrated that U94 induces a partial mesenchymal-to-epithelial transition and impairs cell migration, invasion, and proliferation in different human cancer cell lines through inhibition of the S phase of the cell cycle, and down-modulation of Src, and downstream signaling pathways. Moreover, the viral protein exerts a long-term control of tumor growth, invasiveness, and metastasis in vivo. U94 exerts its activity upon its arrival to the nucleus. Based on these evidences, in order to gain deep insight on the mechanisms of action of the viral protein, we characterized its molecular targets.
Method: For all the experiments, MDA-MB 231 cells were transduced for twenty-four hours with amplicon vectors

expressing or not U94 at MOI 1. The Affymetrix array on DNA repair genes was conducted, and its analysis results were confirmed using real-time PCR. Chemotherapeutic drugs cytotoxic rate after U94 transduction was evaluated by counting cells using the COULTER COUNTER® Analyzer. Apoptosis was assessed by western blot analysis.
Results: The Affymetrix array analysis demonstrated that U94 is able to inhibit not only genes involved in cell cycle regulation but also in DNA repair processes such as BRCA1.
Based on this evidence, in order to understand whether U94 could act as a chemo-sensitizer in triple negative breast cancer cells, we exposed transduced cells to cisplatin and doxorubicin, DNA-damaging drugs, and taxol, a microtubule inhibitor. As expected, U94 transduction was able to reduce cell proliferation. Interestingly, U94 transduction of MDA-MB 231 cells enhanced cisplatin and doxorubicin cytotoxicity, whereas no differences were detected after taxol treatment.
Western blot analysis showed that U94 induces an apoptotic process in MDA-MB 231 cells through down-modulation of the anti-apoptotic protein Bcl-2 and activation of the pro-apoptotic proteins Bax and BAD. Moreover, U94 increased poly (ADP-ribose) polymerase cleavage, and the apoptosis effector Caspase-3 was also activated.
Conclusions: In the current study, we provide evidence that U94 exerts a pro-apoptotic effect against breast cancer cells following down-modulation of DNA repair gene expression. Collectively, our data suggest that U94 may interfere with the DNA damage response pathways, making cancer cells more sensitive to chemotherapy.

8-6 *Poster* Human herpesvirus 6A and 6B inhibit in vitro angiogenesis by induction of human leukocyte antigen G

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Objectives: We have previously reported that human herpesvirus 6 (HHV-6) infection of endothelial cells (ECs) induces the loss of angiogenic properties through the expression of HHV-6 U94, likely associated with the release of a soluble mediator. On the other hand, the soluble isoform of HLA-G is known to exhibit an anti-angiogenic function, important in implantation, neoplastic, and transplantation settings. This study was therefore aimed to analyze the expression of HLA-G in HHV-6 infected ECs to explore any potential role in virus-induced anti-angiogenic activity.
Methods: To evaluate HLA-G induction human umbilical vein endothelial cells (HUVECs) were infected with HHV -6A or 6B, nucleofected with plasmids expressing virus U94 gene, or treated with recombinant U94 protein. HLA-G induction was analyzed by RT-PCR, flow cytometry, and ELISA. Ability of virus genes to directly induce HLA-G expression was investigated by luciferase reporter assay. Induction of cell transcription factors was analyzed by RT-PCR microarray analysis. Angiogenesis ability was analyzed by capillary-like formation on basal membrane extract.
Results: Results showed that both HHV-6A and HHV-6B infection induce a potent up-modulation of HLA-G expression in infected ECs, including both membrane and soluble isoforms. Interestingly, HHV-6A and HHV-6B preferentials induced different isoforms of HLA-G. The virus-induced increase of HLA-G is likely due to the expression of the U94 virus gene, which reproduced the effect of whole virus. This U94 effect is mediated by the induction of the human transcription factor ATF3, which recognizes a consensus sequence on the HLA-G promoter, inducing its activation. Notably, the virus-induced inhibition of ECs angiogenetic ability was directly correlated with HLA-G expression and release, since the addition of an anti-HLA-G antibody restored the angiogenic properties of HHV-6-infected ECs.
Conclusions: Our data show for the first time that HHV-6A and 6B infections induce up-modulation and release of HLA-G in human endothelial cells. This remodulation, and in particular the release of the soluble HLA-G isoform from infected cells, is directly related to the inhibition of angiogenic properties observed in ECs upon HHV-6 infection. Furthermore, the results indicate that virus infection induces ATF3, which is able to interact directly with the HLA-G promoter, finally inducing the HLA-G production associated with virus infection. It will be interesting to further analyze the virus effects in different cell types, since these might be important in diverse clinical conditions involving not only the regulation of angiogenesis but also the development of immune response and inflammation.

8-7 Poster Human herpesvirus 6A induces dendritic cell death and HMGB1 release without virus replication

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Human herpesvirus 6A (HHV-6A) is a common virus involved in several clinical syndromes and has important immunomodulatory effects. Dendritic cells (DC) are key players in innate and adaptive immunity and are implicated in the pathogenesis of many human diseases, including infections. We have previously demonstrated suppressive effects of HHV-6A on key DC functions. In the current study, we show that a non-productive infection of HHV-6A in DC leads to titre dependent cell death and the release of high mobility group box 1 (HMGB1) protein and interleukin (IL)-6. These immune responses aimed to clear the infection may also imply risks for inflammatory pathologies associated with HHV-6 such as allergies and autoimmunity.

8-8 Poster Cytokine production by inactivated HHV-6B compared to the effect of infectious virus

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Roseoloviruses infect CD4 cells, and the infection has an effect on the immunity of the attacked cell. Human herpesviruses HHV-6A, HHV-6B and HHV-7 bind different cell surface receptors consequently inducing different messenger RNAs and cytokine/chemokine release. Of the three roseoloviruses, HHV-6B seems to elicit the most severe immunosuppression in both primary and reactivated infections. The cytokine production by roseoloviruses have already been partially described both *in vivo* and *in vitro*.

Beside infectious particles, herpes viruses produce a high quantity of non-infectious particles that might also affect the biology of immune cells. In our study, we used heat and UV-inactivated virus preparations to infect MOLT-3 cells *in vitro*. At different time intervals, aliquots of mock-infected, infected and inactivated-virus-infected cells as well as supernatants were collected during the whole infection cycle. Production of key anti-inflammatory and pro-inflammatory cytokines were quantified by measuring protein release with commercial ELISA kits and the synthesis of mRNA by commercial RT-PCR kits.

Through the whole infection cycle, cytokine production by inactivated viruses significantly differs from the mock-infected and infected (but not virus inactivated) cultures. HHV-6B infection drastically altered the release of several cytokines, especially during the first four days post-infection. UV inactivated virus can enter cells, thus gene expression is limited, but proteins of the infecting virus can cause immunomodulatory effects. In the early phase of virus absorption this can be seen on the pro- inflammatory IFN- γ level, and also on the gene expression of this cytokine. On the other hand, in the late phase of infection, anti-inflammatory TNF-β1 cytokine level increases parallel to the expression of the pro-inflammatory GM-CSF. Heat-inactivation damages the structure and enzymes of the viruses. In the early phase of heat-inactivated virus absorption the pro-inflammatory IL-12 level is decreased, and meanwhile pro-inflammatory IL-1β gene expression is increased to compensate for the loss by the heat-inactivation. In the late phase of treatment with heat-inactivated virus, anti-inflammatory IL-2 cytokine level decreases along with the expression of the anti-inflammatory IL-10 and IL-4.

Our results suggest that cytokines elicited by non-replicating particles might regulate the immune system. This data draws attention onto further studies in which cytokine and chemokine production, as well as regulatory mechanisms, can be investigated by single gene expression.

Transplantation

9-1 HHV-6B and transplantation – what we know and what we have yet to learn

Oral

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As a brief introduction to the transplant sessions, the epidemiology of HHV-6B reactivation and disease associations after transplantation will be reviewed and gaps in our knowledge will be highlighted.

9-2 Host Immunology in Transplantation

Oral

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Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a curative therapy for patients with leukemia resistant to chemotherapy and immunotherapy. Immune reconstitution after allo-HSCT has a critical role in the clinical outcome of transplantation, including control of leukemia by graft versus leukemia effect (GVL) and opportunistic infections. Although the first phase of T cell reconstitution after HSCT depends on the peripheral expansion of T cells transferred with the graft, permanent T cell reconstitution is mediated via a central mechanism, which depends on de novo production of naïve T lymphocytes by the recipient's thymus from donor-derived lymphoid-myeloid progenitors (LMP). Thymopoiesis can be assessed by quantification of Recent Thymic Emigrants (RTEs), T cell receptor excision circles (TRECs) levels, and T Cell Receptor (TCR) repertoire diversity. These assays are valuable tools for monitoring post-transplantation thymic recovery, but more importantly have shown the significant prognostic value of thymic reconstitution for clinical outcomes after HSCT, including opportunistic infections, disease relapse, and overall survival. Human Herpesvirus-6 (HHV-6) is an important opportunistic pathogen for patients undergoing allogeneic HSCT with the highest incidence among recipients of umbilical cord blood transplantation (UCBT). HHV-6 reactivation after allogeneic HSCT, in particular of the HHV-6B subtype, has been associated with numerous post-transplant complications including encephalitis, acute graft-versus-host-disease, delayed engraftment, fever, and rash, although a causative relationship and negative impact on HSCT outcomes has not been consistently demonstrated and remains controversial. As all opportunistic pathogens, reactivation of HHV-6 after HSCT is related to impaired reconstitution of immunity and use of therapeutic immunosuppression for the prevention or treatment of graft versus host disease (GVHD). Reactivation of HHV-6 has implications on the function of multiple immune cell types due to the nearly ubiquitous distribution of the primary cellular receptor, CD46. More importantly, the receptor of HHV-6B, CD134 (OX40), a member of the TNF receptor superfamily is expressed in activated T cells. CD134 is upregulated in alloreactive T cells during GVHD thereby indicating that development of GVHD may facilitate infection of immune cells by HHV-6 via a mechanism that positively regulates viral entry and is independent of thymic recovery and post-thymic immune reconstitution. In addition to T cells, key immune cells that can be targeted by HHV6 include monocytes, macrophages, and dendritic cells. HHV-6 infected cells produce various cytokines and chemokines, which can alter the inflammatory milieu and activate inflammatory cascades that may trigger GVHD, thereby compromising the clinical outcomes of HSCT in a manner independent of thymic reconstitution. We will discuss mechanisms of thymic recovery, new strategies to improve thymic entry and differentiation of LMPs and to accelerate recovery of thymic stromal microenvironment in order to improve reconstitution of the T cell repertoire, and will consider tentative implications of HHV-6 reactivation in these processes.

9-3 Thymic function and reconstitution protect adult dUCBT recipients from HHV-6 viremia and HHV-6 encephalitis

Oral

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Introduction: HHV-6 reactivation is a common complication of umbilical cord blood transplantation (UCBT), often manifested as limbic encephalitis. Rabbit anti-thymoglobulin (ATG) has been used in UCBT conditioning to achieve T cell depletion, reduce the risk of graft rejection and decrease the incidence of graft-versus-host disease (GVHD). However, ATG-induced immunosuppression has been associated with delayed immune reconstitution, increased infectious complications and higher non-relapse mortality. In a clinical trial of reduced intensity double-unit UCBT (dUCBT), we substituted low dose total body irradiation to determine whether dUCBT without ATG would improve protective immunity to HHV-6 and other viral infections.
Objective: Evaluate reconstitution of hematopoiesis and immunity in ATG-spared dUCBT recipients and their association with HHV-6 reactivation and clinical manifestations.
Methods: Twenty-eight adult patients with hematologic malignancies were treated with reduced intensity conditioning without ATG followed by dUCBT. We examined thymic regeneration by quantifying T cell receptor excision circles (TREC) and reconstitution of hematopoiesis and immunity by assessing neutrophils, platelets, T and B lymphocytes, monocytes and NK cells. Correlation analysis was performed between immunological endpoints and HHV -6 reactivation after dUCBT, as documented by HHV-6 viremia or HHV-6 end-organ disease. Correlation analysis between HHV-6 reactivation and clinical outcomes of dUCBT was also performed.
Results: HHV-6 reactivation, as determined by assessment of HHV-6 viremia, was observed in 24 of 28 (86%) patients during the first month after dUCBT. 6 of these 24 (25%) patients developed HHV-6-related encephalitis. There was a correlation between the development of encephalitis and HHV-6 viral load of ≥50.000 copies/ml ($p=0.007$). Pre-transplant TREC levels of ≥1.200 copies/ml negatively correlated with subsequent HHV-6 reactivation ($p=0.04$). On day 30, day 60 and day 100 post-transplant, higher TREC levels correlated with lack of HHV-6 viremia ($p<0.04$). A tendency of higher $CD4^+$ lymphocyte counts in the first 100 days after transplant was also observed in patients who did not develop HHV-6 reactivation. No association between GVHD and HHV-6 viremia or encephalitis was observed. Neutrophil and platelet engraftment did not correlate with HHV-6 reactivation or its absence.
Conclusions: High incidence of HHV-6 reactivation was observed in adult dUCBT recipients despite avoiding ATG. HHV-6 viral load correlated with HHV-6 encephalitis. Improved baseline and early post-transplant thymic function had a protective effect on HHV-6 reactivation.

9-4 Natural killer cells, viral reactivation, and relapse

Oral

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Natural killer (NK) cells, the first lymphocyte subset reconstituting after allogeneic hematopoietic cell transplantation (HCT), are a major component of innate immunity with important roles in defense against viruses and cancer. NK cells contribute to immune function by secreting cytokines such as TNFα and IFNγ and are also able to kill target cells by natural cytotoxicity and antibody-mediated cellular cytotoxicity (ADCC). Adoptive transfer of NK cells can yield clinical responses in highly refractory malignancies. A unique subset of terminally differentiated NK cells, termed adaptive, are $CD56^{dim}NKG2C^+CD57^+$ cells that exhibit properties of immune memory with potent ADCC. Adaptive NK cells have decreased expression of inhibitory checkpoints such as PD1 and NKG2A and are inherently resistant to cellular suppression by Tregs and MDSCs. CMV reactivation results in expansion of adaptive NK cells, which will persist long after resolution of infection. In addition, several studies have reported a protective effect of CMV reactivation after allo-HCT against relapse of the underlying hematologic malignancy. Our group and others have shown that adaptive NK cells may mediate this effect. It is not clear whether or how the CMV-NK-relapse paradigm can be extended to other viruses that commonly reactivate after HCT. EBV and HSV do not seem to leave an imprint on NK cells. Our group showed that absence of HHV-6 reactivation by day +28 of cord blood transplantation predicts a remarkably low rate of leukemia relapse. The mechanism of this association is unknown and may involve NK cells. For example, HHV-6 reactivation may prevent normal reconstitution of adaptive NK cells. Finally, long-term effects of alterations in NK cell repertoire after viral reactivations (e.g., chronic GVHD, secondary malignancies, recurrent late infections, and immunosenescence) need to be studied.

9-5 Impact of HHV-6 in recipients of ex vivo T-cell depleted hematopoietic cell transplant

Oral

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Introduction: HHV-6 viremia occurs in approximately 40% of T-cell-replete hematopoietic cell transplant (HCT) recipients. Ex vivo T-cell depletion by $CD34^+$ selection archives in 4-5 $\log_{10}$ T-cell depletion (TCD) of the allograft and is associated with increased risk for viral infections. We examined TCD HCT recipients monitored prospectively for HHV -6 at our institution. Our objectives were to 1) report the incidence of HHV-6 viremia, end-organ disease (EOD) and HHV-6 viral kinetics; 2) examine the association between HHV-6 viremia and acute GVHD and overall survival at 1-year post-HCT.

Methods: From January 2012 to December 2016, HHV-6 was routinely monitored by quantitative PCR in plasma (lower limit of quantification [LOQ] 188 copies/mL) weekly from D+14 through D+60 and at clinician's discretion through+100. HHV-6 viremia was defined as ≥1 HHV-6 VL >LOQ. Persistent HHV-6 viremia was defined as ≥2 consecutive VLs ≥500 copies/mL. had ≥3 HHV-6 plasma PCR tests by day (D)+100. $CD34^+$ selection was performed by the CliniMACS CD34 Reagent system (Miltenyi Biotec, Germany). All pts received myeloablative conditioning. The cumulative incidence function was used, and death, relapse, and second transplant were treated as competing risk factors.

Results: Of 312 pts, 183 (59%) were male, 210 (67%) had acute leukemia or MDS, 160 (51%) received a graft from a mismatched donor, 184 (59%) pts were CMV R+. HHV-6 viremia occurred in 172 (55%) pts and persistent HHV-6 viremia in 83 (27%) pts at a median of 33 days (IQR, 25-44) post-HCT. The median duration of viremia was 67 days (IQR, 56-75). Of 83 pts with persistent viremia, 23 (28%) pts had maximum VL ≥10,000 copies/mL. Seven pts (2% of the cohort and 8% of pts with persistent HHV-6 viremia) developed HHV-6 EOD by 1-year post-HCT: encephalitis in (1), pneumonitis (4,) and organizing pneumonia (2p). The maximum VL for pts with EOD was median 71,700 copies/mL (IQR, 8,211-123,500). Acute GVHD grades 2-4 was in 17% pts with and without persistent HHV-6 viremia (P=NS). Persistent HHV-6 viremia was associated with lower 1-year overall survival (57% vs. 71% without viremia) P=0.02.

Conclusion: 1) 83 (27%) of TCD HCT developed persistent HHV-6 viremia; 2) 8% pts with persistent viremia developed HHV-6 end-organ disease; 3) Rates of acute GVHD grades 2-4 were similar between patients with and without persistent HHV-6 viremia; 4) Persistent HHV-6 viremia was associated with lower overall survival at 1-year.

Figure 1. Cumulative incidence of HHV-6 viremia by D+100 after HCT.

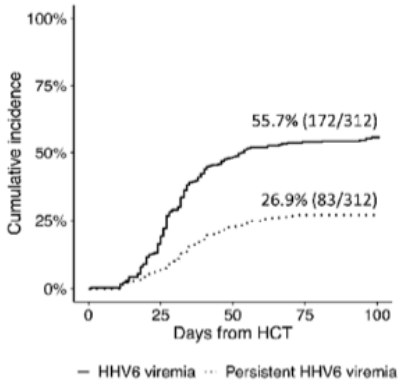
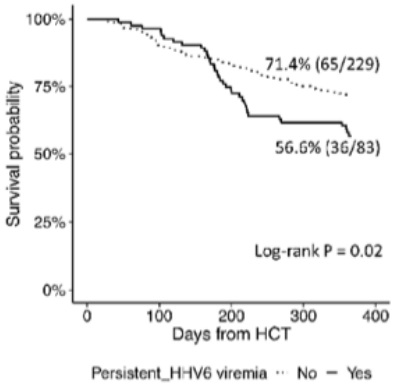


Figure 2. One-year overall survival by persistent HHV-6 viremia.



9-6 Oral Association of CD134-positive T cells and HHV-6 reactivation evaluated by digital polymerase chain reaction in recipients of allogeneic hematopoietic stem cell transplantation

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Introduction: Human herpesvirus 6 (HHV-6) can cause life-threatening central nervous system disorders such as encephalitis and myelitis after allogeneic hematopoietic stem cell transplantation (HSCT). Recently, it has been reported that CD134 could be a specific receptor of HHV-6B and that its expression levels in CD4-positive T cells after allogeneic HSCT could be related to the reactivation of HHV-6. In our prospective study, the relationship between HHV -6 reactivation and $CD134^+$ T cells were evaluated in the recipients of allogeneic HSCT.

Patients and methods: HHV-6 viral load in plasma was quantitatively measured weekly after allogeneic HSCT by digital polymerase chain reaction (dPCR) in 34 patients. The ratio of CD134 in $CD4^+$ T cells (CD134/CD4 ratio) was serially measured by flow cytometry before and after transplantation.

Results: HHV-6 reactivation was detected in 23 patients (68%) with dPCR. The CD134/CD4 ratio before conditioning was significantly higher in patients with HHV-6 reactivation than in those without (median [range], 3.8% [0.4–25.6] vs. 1.5% [0.4–3.7], $P < 0.01$), whereas the CD134/CD4 ratio was not associated with HHV-6 reactivation at any point after transplantation. In multivariate analysis, stem sources from HLA-mismatched donor and a higher CD134/CD4 ratio before conditioning were significantly associated with HHV-6 reactivation (odds ratio = 10.5, 95% confidence interval: 1.3–85.1; $P = 0.03$).

Conclusion: A higher CD134 expression rate in $CD4^+$ T cells before conditioning was associated with a higher risk of HHV-6 reactivation, suggesting that the rate, as well as stem source, may be a promising marker for predicting HHV-6 reactivation after allogeneic HSCT.

Univariate and multivariate analysis for factors associated with HHV-6 reactivation

Factors	Univariate *P* value	Multivariate		
		Odds ratio	95% CI	*P* value
Stem cell source				
HLA-mismatched donor (including CBT) versus HLA-matched donor	*< 0.01*	15.4	2.0–121.0	*0.04*
CD134/CD4 ratio before conditioning				
≥ 3.0% versus < 3.0%	*<0.05*	10.5	1.3–85.1	*0.03*

CI, confidence interval; HLA, human leukocyte antigen; CBT, cord blood transplantation; HSCT, hematopoietic stem cell transplantation.

9-7 Oral Low incidence of HHV-6 reactivation in haploidentical hematopoietic stem cell transplantation using GVHD prophylaxis with corticosteroids: a possible correlation with interleukin-6 suppression

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Human leukocyte antigen (HLA) mismatch and the administration of immunosuppressive agents are considered risks for HHV-6 reactivation after stem cell transplantation (SCT). Recent HLA-haploidentical SCT (haplo-SCT) studies using posttransplantation cyclophosphamide infusion reported a higher incidence of HHV-6 reactivation (35%–78%). We perform haplo-SCT using methylprednisolone (1–2 mg/kg) and low-dose anti-thymocyte globulin as graft-versus-host disease (GVHD) prophylaxis and in a reduced-intensity conditioning regimen, respectively. However, using weekly qPCR monitoring, plasma HHV-6 DNA was detected in only 3 of 42 haplo-SCT patients (7.1%). Their serum IL-6 levels were significantly reduced, whereas increases in the levels of pro-inflammatory cytokines such as IL-6 in the early stage of SCT are reported to be associated with HHV-6 reactivation. No HHV-6-associated encephalitis developed. In

conclusion, the administration of immunosuppressive agents potentially prevented HHV-6 reactivation and encephalitis after haplo-SCT by suppressing IL-6 production. Recently, tocilizumab, a humanized anti-IL-6 receptor antibody, has been reported to harness GVHD. Given the relationship between HHV-6 reactivation and subsequent acute GVHD, it would seem that tocilizumab might be helpful to limit both HHV-6 reactivation and GVHD.

9-8 *Oral* Human herpesvirus 6B and lower respiratory tract disease after allogeneic hematopoietic cell transplantation

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Human herpesvirus 6B (HHV-6B) DNA is frequently detected in bronchoalveolar lavage fluid (BALF) from immunocompromised subjects with lower respiratory tract disease (LRTD). Whether HHV-6B is a pulmonary pathogen is unclear. We tested BALF for HHV-6B DNA using PCR in allogeneic hematopoietic cell transplantation (HCT) recipients who underwent a BAL for evaluation of LRTD from 1992-2015. We used multivariable proportional hazards models to evaluate the association of HHV-6B$^+$ BALF with overall mortality, death from respiratory failure, and the effect of anti-HHV-6B antivirals on these outcomes. We used branched chain RNA in-situ hybridization (RISH) to detect HHV-6 messenger RNA (U41 and U57) in lung tissue. We detected HHV-6B$^+$ BALF from 147 of 553 (27%) individuals. Subjects with HHV-6B$^+$ BALF, with or without copathogens, had significantly increased risk of overall mortality (adjusted hazard ratio [aHR], 2.18; 95% CI, 1.41-3.39) and death from respiratory failure (aHR, 2.41; 95% CI, 1.54-3.78) compared to subjects with HHV-6B$^-$ BALF. Subjects with HHV-6B$^+$ BALF who received antivirals within 3 days pre-BAL had an approximately 1 $\log_{10}$ lower median HHV-6B BALF viral load, as well as a lower risk of overall mortality (aHR, 0.42; 95% CI, 0.16-1.10), compared to subjects with HHV-6B$^+$ BALF not receiving antivirals. We detected intraparenchymal HHV-6 gene expression by RISH in lung tissue in all 3 tested subjects with HHV-6B$^+$ BALF and sufficient tissue RNA preservation. These data provide evidence that HHV-6B detection in BALF is associated with higher mortality in allogeneic HCT recipients with LRTD. Definitive evidence of causation will require a randomized prevention or treatment trial.

9-9 *Oral* The biological plausibility of human herpesvirus 6 reactivation after allo-HCT inducing idiopathic pneumonia syndrome and aggravating acute GVHD

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Objectives: Idiopathic pneumonia syndrome (IPS) and acute graft-versus-host disease (aGVHD) are significant causes of morbidity and mortality post allogeneic hematopoietic cell transplant (allo-HCT). The reactivation of human herpesvirus 6 (HHV-6) is also common within the first 100 days after transplant. Our study was to determine the biological plausibility of HHV-6 occurrence as the first viral infection post-HCT to induce the development of IPS and aGVHD.
Methods: We performed a retrospective review of 738 allogeneic hematopoietic cell transplant patients enrolled from 2005 to 2011. We also established a novel bone marrow transplantation (BMT) animal model to demonstrate that reactivation of murine roseolovirus (MRV, a murine homolog of HHV-6) promotes IPS and aGVHD after transplant.
Results: First onset viral infections of HHV-6 within 100 days post-transplant increased the risk of developing IPS after adjusting for confounding factors (adjusted hazard ratio [aHR], 5.52; 95% CI, 1.61-18.96; P = 0.007). First onset viral infection with HHV-6 also increased risk of grade II to IV aGVHD (aHR, 1.83; 95% CI, 1.02-3.28; P = 0.04). In an allogeneic minor histocompatibility mismatched (miHA-mismatched) murine BMT model, MRV can be reactivated in

the lung and other organs following the BMT procedure. Reactivation of MRV induced an IPS-like phenotype in the lung and aggravated aGVHD. Interestingly, reactivation of MRV reduced the number of natural killer (NK) cells, which have been previously implicated in suppressing severe aGVHD in BMT mice.
Conclusions: First onset HHV-6 infection within 100 days post allo-HCT is associated with increased risk of IPS and aGVHD. Experimentally reactivating a mouse homolog of HHV-6 caused organ injury similar to phenotypes seen in human transplant recipients. Thus, it is biologically plausible that reactivation of HHV-6 post allo-HCT can predispose recipients to development of IPS and aGVHD. Our work suggests prophylactic or preemptive therapy controlling early HHV-6 reactivation post allo-HCT may reduce non-relapse mortality and morbidity caused by IPS and aGVHD.

9-10 *Oral* Reduction of hippocampal volume due to HHV-6B infection in pediatric HSCT recipients

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Objective: After HHV-6B primary infection the virus can establish lifelong latency in various sites, including the central nervous system, and can reactivate in immunocompromised patients, such as hematopoietic stem cell transplant (HSCT) patientS. Moreover, the virus is a leading cause of the post-transplant acute limbic encephalitis (PALE). Abnormal intensity of the hippocampal area by brain MRI and memory loss are characteristic findings of PALE. These data suggest that HHV-6B replication in the hippocampus may cause direct neurological impairment in PALE. PALE cases have been reported mainly in adult, but also some pediatric patients. Therefore, the aim of this study was to determine whether HHV-6B infection can impair the hippocampus in pediatric HSCT transplant recipients or not. We sought to evaluate hippocampal damage due to HHV-6B infection based on the volumetric analysis of the hippocampus before and after HSCT by using brain MRI.
Methods: Twenty HSCT recipients, who received brain-MRI before and after transplantation and were monitored weekly for HHV-6B infection, were enrolled in this study. Patients were identified as having HHV-6B infection when more than 1×10^4 copies/mL of viral DNA were detected from peripheral blood post-transplant. All subjects were measured on the 3T MRI scanner using a 12-channel head coil. Hippocampuses were automatically segmented by the Freesurfer 6.0 image analysis suite. Median volume ratio of the hippocampus before and after transplantation were compared between the recipients with and without HHV-6B infection.
Results: HHV-6B infection was demonstrated in 8 of the 20 recipients after HSCT. The median age of the patients with and without HHV-6B infection was 10.5 and 8.5 years old respectively. Two out of the 8 HHV-6B infected patients had encephalopathy at the time of viral infection. No encephalopathy was observed in 12 recipients without HHV-6B infection. In the right hippocampus the median volume ratio before and after transplantation was 0.93 in the HHV-6B infected recipients and 1.02 in the recipients without viral infection (P=0.0069). In the left hippocampus, the median volume ratio before and after transplantation in recipients with and without HHV-6B infection was 0.92 and 1.00 (P=0.0031).
Conclusions: In this retrospective analysis, significant reduction of both sides of hippocampal volumes is observed in recipients with HHV-6B infection. HHV-6B infection may cause hippocampal atrophy. Present studies suggest that HHV-6B infection can cause mild neurological symptoms and neurological sequela such as mild cognitive dysfunction in pediatric HSCT recipients. Therefore, long term neurological follow up is required for pediatric HSCT recipients with HHV-6B infection.

9-11 *Oral* Active HHV-6 infection is associated with unexplained acute liver failure in previously healthy children

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Objective: Several studies have suggested that human herpesvirus 6 (HHV-6) may be a cause of acute liver disease in children. In these cases HHV-6 PCR analysis of plasma or serum is frequently negative, and presence of viral genome in liver tissue does not correlate with hepatic disease markers such as elevated serum transaminases (Ozaki Y et al. 2017).

In order to further explore these observations we evaluated liver biopsies from eight children presenting with acute liver failure (ALF) for evidence of active HHV-6 Infection.

Methods: A retrospective review of diagnostic laboratory cases evaluated for active HHV-6 infection in children presenting with ALF was performed. Sections of formalin fixed paraffin embedded (FFPE) tissues from patients' native liver and accompanying surgical pathology reports were submitted to Coppe Laboratories from multiple healthcare institutions. Specimens were analyzed by immunohistochemical (IHC) staining using a monoclonal antibody specific for a late structural glycoprotein antigen shared by both HHV-6A and HHV-6B.

Results: Eight cases of ALF in children aged 13 months to 14 years were identified with all cases progressing to orthotopic liver transplantation. One case has been previous reported (Szewc AM et al. 2018). Another case involved what proved ultimately to be a child with chromosomal integrated HHV-6 (ciHHV-6) wherein high HHV-6 DNAemia during antiviral treatment provoked serious clinical confusion and concern. In all cases the explanted livers were positive for expression of an HHV-6 glycoprotein, with viral antigen primarily identified in the glandular epithelium of bile ducts and degenerating hepatocytes.

Conclusions: This report emphasizes important aspects of the diagnosis of clinical disease associated with HHV-6 infections. The use of a structural glycoprotein as the virologic infection marker assures that the infection is fully productive and associated with spread of the virus within the tissues. Analyses based upon PCR and viral serology indicate that the patient has a history of HHV-6 infection. However, these technologies have limited clinical predictive value when trying to confirm a specific association between viral detection and the pathological changes present in the tissue at the time of sample acquisition. Early detection and confirmation of active HHV-6 infection in livers of children with acute and rapidly progressing liver insufficiency could provide for early intervention with effective antiviral agents, preservation of the native liver and avert the need for liver transplantation.

9-12 *Poster* Quantitative serum PCR argues against long-term persistent HHV-6 viremia after cord blood HCT

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Objective: HHV-6 reactivation is common after cord blood hematopoietic cell transplantation (CBT). However, the ability of the new immune system to clear HHV-6 viremias is unclear. A previous study of 23 CBT recipients suggested that ~50% of patients had detectable HHV-6 by whole blood PCR over a year after transplant (Illiaquer et al. J Infect Dis 2014; 567-570). Our objective was to re-evaluate this finding in a larger cohort, including T-cell subsets and adaptive NK cells.

Methods: We reviewed the records of CBT recipients at the University of Minnesota (2011-2017) in whom HHV-6 had reactivation (whole blood quantitative PCR) by 1 year post-transplant. A subset (n = 59) had a research serum sample at 1 year post-transplant on an IRB-approved biorepository protocol. Samples were subjected to quantitative PCR for HHV-6A and 6B (Rotor-Gene, Qiagen) in a CLIA-certified laboratory. We quantified T-cell subsets and adaptive NK cells by a LSR II flow cytometer.

Results: Median (range) age was 44 (1-72) years and 40 patients were male. 24 cord units were single. The most common underlying diseases were acute leukemia (n = 29), lymphomas (n = 10), and non-malignant disorders (n = 9). 33 patients were CMV seropositive. ATG or alemtuzumab were used in 16 patients. Ex-vivo T cell depletion was not used. Conditioning was reduced-intensity in 31 patients. GVHD prophylaxis was cyclosporine-based in 41 patients and sirolimus-based in 18. Grade II-IV acute GVHD occurred in 25 patients and chronic GVHD by 1 year in 12. HHV-6A (14,556 copies/mL) and 6B (less than lower limit of quantification) were detectable each in one sample. The patient with detectable HHV-6A had previous whole blood levels >$2x10^6$ copies/mL, suggesting chromosomal integration. The patient with detectable HHV-6B had previous whole blood levels ranging 9.900-164.800 copies/mL. The frequencies (median [range]) of T-cell subsets were: [CD3+]CD4+ (62 [7-90]%), [CD4+]CD45RA+ (19 [0.5-91]%), [CD4+]CD45RA-CCR7+ (39 [0.6-74]%), [CD4+]CD45RA-CCR7- (32 [0.2-76]%), [CD3+]CD8+ (31 [7-91]%), CD8+CD45RA+ (56 [6 -100]%), [CD8+]CD45RA-CCR7+ (22 [0-76]%), and [CD8+]CD45RA-CCR7- (6 [0-25]%). Adaptive (NKG2C+CD57+) NK cells comprised a median (range) of 7 (0-68)% of NK cells.

Conclusions: Long-term persistent HHV-6 viremia was rare in our 59 patients. Using serum in our study permitted exclusive detection of cell-free HHV-6 DNA, which is a more accurate indication of HHV-6 reactivation. This may explain the discrepancy of our results with those by Illiaquer et al. using whole blood. We found no evidence for the inability of cord blood allografts to clear HHV-6 viremia.

9-13 Oral shedding of human herpesviruses in renal transplant recipients

Poster

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Objective: To describe the profile of human herpesviruses shedding in the oral cavity of renal transplant recipients.
Methods: This cross-sectional study investigated the prevalence of eight types of herpesviruses in saliva from immunosuppressed patients of the Renal Transplant (RT) Unit and healthy controls (non-transplanted and immunocompetent) in Brazil. 137 subjects (aged 20-61), divided into the RT group (RTG, n=86) and healthy group (HG, n=51), were evaluated. Mouthwash samples were collected via oral rinse and then submitted to screening for the presence of eight types of herpesvirus by using a PCR with Restriction Fragment Length Polymorphisms (RFLPs).
Results: We detected high prevalence of HHV-7 in both RTG (74.5%) and HG (61.6%). Higher frequency of EBV (p=0.016) and HSV-1 (p=0.009) was found in RTG than HG. Higher frequency of co-infections was found in saliva from RT (51.0%) than HG (31.4%) (p=0.029), specially involving HHV-7 and EBV, HSV-1, VZV, and/or HHV6B. The frequencies of VZV and HHV-6 were low in both groups. We found HHV-6B in RT (5.9%) and in HG (1.2%). Additionally, we found HHV-6A only in HG (2.3%). HHV-2, CMV, and HHV-8 were undetectable in saliva.
Conclusion: Renal transplant recipients excreted herpesviruses more often than controls, especially HSV-1 and EBV and they present with co-infections more frequently than the control group. HHV-7 seems to be constitutive in saliva of renal transplant patients and the control group.

9-14 Evolution of HHV6- and CMV-specific T cell functional responses and immune cell populations after kidney transplant

Poster

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Background: HHV-6 reactivation can occur after solid organ or hematopoietic stem cell transplantation, and has been associated with many complications, including all-cause mortality and graft rejection. Inadequate understanding of the interplay between viral replication, host immune response, and clinical immunosuppression has limited progress toward better management strategies and outcomes.
Objectives: To understand the immunopathogenesis of HHV-6 infection, this study aims to: 1) Track the longitudinal kinetics of cellular reconstitution after induction immunosuppression with alemtuzumab (anti-CD52) or anti-thymocyte globulin (anti-CD3), HHV-6 specific CD4 and CD8 T cell responses, and HHV-6 DNAemia in the PBMC of kidney transplant recipients, and 2) Compare these parameters for HHV-6 and HCMV, and for patients receiving each type of induction approach.
Methods: Patient population - Blood samples were collected prospectively from kidney transplant recipients before (within 3 months) and after (monthly for 6 months and at 12 months) transplant and healthy adults as controls . Virus-specific CD4 or CD8 T cell responses - CD4 or CD8 T cell functional responses (proliferation, cytokine production and cytolytic capacity) are measured by multi-parameter flow cytometry after short-term stimulation with HHV-6 or HCMV whole virus or controls. Lymphocyte (T and B cells), NK, dendritic cells and monocytes populations were measured at concurrent time points to follow immune reconstitution. Viral DNA detection - HHV-6 and HCMV DNA were measured in plasma by real-time PCR.
Results:
1. For fifteen patients with full sample sets, studies have been completed for pre- and 2, 6, and 12 months post-transplant timepoints.
2. HHV-6 or HCMV-specific T cell responses showed distinct qualitative features and kinetics of evolution over time.
3. The following patterns have been identified: a) IFNg responses were not common, but detected more frequently than IL2 or TNFa. b) HHV6- but not HCMV-specific CD4 T cell responses primarily involved mobilization of CD107a/b, suggesting a cytolytic population. c) CD8 but not CD4 T cells expressed granzyme B against either virus. d) T cells infrequently expressed PD1 (primarily CD4 not CD8 T cells), perforin, IL2, or TNFa.

Conclusions: In healthy immunocompetent adults and transplant recipients, HHV-6-specific T cells are minimally detectable in PBMC ex vivo, but can be expanded *in vitro* for detailed characterization. CD4 and CD8 T cell responses evolve differentially over the first year, between related chronic viruses, and among individuals after kidney transplant. The role of induction immunosuppression regimen and/or viral reactivation on these patterns will be identified when data analysis is completed and blinding to these factors is broken.

10-1 Development path for novel therapeutics

Oral

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HHV-6 has been associated with a number of clinical conditions and syndromes, but no antiviral agent or immunotherapy has been specifically licensed to treat of prevent HHV-6 infection of disease. This presentation will summarize the current state of HHV-6 clinical association research with emphasis on the immunocompromised host, and discuss possible development paths to novel therapeutics and preventative interventions using transplant patients as a key target populations.

10-2 Oral Brincidofovir decreased HHV-6B viremia after HCT: results from a post-hoc analysis of a placebo-controlled phase 3 study

Oral

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Objectives: Human herpesvirus 6B (HHV-6B) is detected in plasma in ~40% of allogeneic hematopoietic cell transplant (HCT) recipients and ~75% of cord blood recipients. HHV-6 viremia, particularly at levels >10^4 copies/mL, is associated with increased risk for HHV-6 encephalitis. Ganciclovir and foscarnet have antiviral activity against HHV -6B, but studies of pre-emptive or prophylactic treatment have not been successful in preventing HHV-6B encephalitis. Prophylaxis for HHV-6B has never been studied in a randomized trial. Brincidofovir (BCV, CMX001) has potent in vitro activity against HHV-6B (EC50=0.007 μM), and unlike cidofovir, has good CNS penetration.

Methods: In vivo activity of BCV against HHV-6B has not been demonstrated. We tested plasma from participants in the SUPPRESS clinical trial to explore the potential of BCV to prevent HHV-6B viremia. SUPPRESS was a randomized, double-blind, placebo (PBO)-controlled trial of oral BCV for cytomegalovirus (CMV) prophylaxis after allo-HCT. 452 adult CMV-seropositive HCT recipients without CMV viremia at screening were randomized 2:1 to receive BCV or PBO twice-weekly until week 14 post-HCT. We selected subjects who were randomized within 2 weeks of allo-HCT, who did not have HHV-6 viremia at baseline, and who received at least 6 doses of BCV or PBO within the first 3 weeks of randomization. Weekly plasma samples from the first six weeks post-HCT were tested for HHV-6B with quantitative PCR (Viracor Eurofins, Lee's Summit, MO; species testing at University of Washington Clinical Virology Laboratory, Seattle, WA). We compared HHV-6B viremia between subjects who received BCV and those who received PBO using the Kaplan-Meier method, log rank test, and Cox proportional hazards model. Analyses were stratified by high and low risk of HHV-6B reactivation; high risk was defined as T-cell depletion, cord blood stem cell source, or HLA-mismatched HCT.

Results: 92 subjects in the BCV group and 61 in the PBO group met criteria for inclusion: 64% were male, 84% were white, and median age was 57 years (range: 18-76). 6% of subjects received cord blood grafts, 5% had haploidentical donors, and 8% had mismatched donors. 41% had ex vivo T-cell depletion or received serotherapy with antithymocyte globulin or alemtuzumab. Baseline characteristics were balanced between groups. 15% of BCV subjects vs. 31% of PBO subjects had detectable HHV-6B viremia within 6 weeks after HCT. The cumulative incidence of HHV-6B viremia was significantly lower in BCV subjects. When stratified by high and low risk of HHV-6B reactivation, the cumulative incidence of HHV-6B viremia was significantly lower in high risk but not low risk subjects. Two subjects (2%) in the BCV group had HHV-6B viremia >10^3 copies/mL compared to 7 (11%) in the PBO group. In the study overall, no subject in the BCV group and one in the PBO group developed HHV-6 encephalitis. 9% of subjects receiving BCV reported rash as an Adverse Event during the first 7 weeks post-HCT compared to 26% of subjects receiving PBO ($p = 0.006$).

Conclusions: BCV reduced the incidence of detectable HHV-6B viremia in HCT recipients enrolled in a randomized, placebo-controlled clinical trial. BCV prophylaxis could prevent the morbidity and mortality associated with HHV-6B in HCT recipients.

10-3 Novel therapeutic strategies for HHV-6

Oral

Graciela Andrei

Rega Institute for Medical Research

No antiviral drugs are approved for the treatment of HHV-6, which can be explained because the majority of the infections caused by HHV-6 are asymptomatic and transient and do not require antiviral therapy. In transplant recipients, therapeutic options are based on anti-cytomegalovirus drugs (ganciclovir, foscarnet, and cidofovir) with a common target, the viral DNA polymerase. These therapies are associated with dose-limiting toxicities, and resistance can be an issue in immunosuppressed individuals. Recent studies argued that the use of anti-herpetic medications might decrease the risk of central nervous system diseases, but the dose-limiting toxicities of ganciclovir, foscarnet, and cidofovir, the need for intravenous administration, and their poor blood-brain-barrier (BBB) penetration hamper their use for this indication.

Thus, novel drugs, preferably orally bioavailable, with good efficacy and desirably with broad-spectrum anti-herpesvirus activity, safe toxicology profile and acceptable BBB penetration are needed. In the last few years, new classes of anti-herpesvirus molecules targeting different steps of the viral lytic replicative cycle were identified, and some of them also proved active against HHV-6. Inhibitors of viral genome replication include those targeting not only the DNA polymerase, encompassing nucleoside analogs (cyclopropavir and other methylenecyclopropane analogs), nucleotide analogs (acyclic nucleoside phosphonates related to cidofovir) and prodrugs (brincidofovir), but also the helicase/primase complex, and protein-protein interactions]. Viral gene expression, virion assembly, and egress [inhibitors of DNA cleavage/packaging, protein kinase (maribavir and other benzimidazole analogs)] were also validated targets.

In addition to direct-acting antiviral agents, drugs that target cellular proteins essential for viral replication (inhibitors of cyclin-dependent kinases, proteasome, and cyclooxygenase 2) have also been described. Besides, several drugs with anti-herpesvirus activity have been repurposed for the treatment of drug-resistant herpesvirus infections, such as leflunomide (approved for the treatment of rheumatoid arthritis), artesunate (an antimalarial agent), and the mammalian target of rapamycin drugs sirolimus and everolimus.

Alternative approaches using compounds that modulate the chromatin state of viral genome may suppress infection and reactivation or boost antiviral immunity. In addition, strategies to target the latent infection are nowadays conceivable with the use of technologies such as CRISPR/Cas9 that may be adapted for antiviral treatment by specifically editing latent viral genomes that potentially cure the patient of latent infection.

10-4 Development of the Japanese guideline for management of HHV-6 encephalitis after allogeneic hematopoietic stem cell transplantation

Oral

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In Japan, HHV-6 encephalitis is now recognized as one of the main etiologies of central nervous system (CNS) dysfunction after allogeneic hematopoietic stem cell transplantation (HSCT). As previously presented (10th conference, and BMT 2017), we performed a nationwide retrospective study to obtain data that can guide clinical decisions. Analysis showed that development of encephalitis concentrated within 2–6 weeks after HSCT. Recipients of cord blood transplantation (CBT) or HLA-mismatched unrelated HSCT and presence of an immune reaction were risk factors of HHV-6 encephalitis. Brain MRI findings were negative in 28% of patients at the time of development. With both foscarnet and ganciclovir, full-dose antiviral therapy was associated with better response rate. Use of foscarnet was associated with lower incidence of death within 30 days of encephalitis.

Referring to results of this nationwide study and other reports (particularly, UpToDate written by Dr. Zerr), we (HHV-6 subcommittees, guideline committee of the JSHCT) developed a practical guideline. The guideline was completed in February 2018. The main description is given below.

Disease associations: At present, the established HSCT complications caused by HHV-6 reactivation include HHV-6

encephalitis only.
Diagnostic methods: Quantitative PCR testing is recommended to diagnose HHV-6 reactivation. Chromosomally-integrated HHV-6 should be excluded.
Diagnosis: Criteria to diagnose HHV-6 encephalitis include the presence of neurological symptoms, positive HHV-6 DNA in cerebrospinal fluid (CSF), and the absence of other identified etiology of CNS dysfunction. In patients who show CNS dysfunction, high levels of plasma HHV-6 DNA suggest HHV-6 encephalitis, but this is insufficient for definitive diagnosis.
Empiric therapy: For patients with CNS symptoms for whom PCR results cannot be obtained within several hours empiric foscarnet therapy should be considered if the patient has factors associated with HHV-6 encephalitis as follows: CBT, HLA-mismatched unrelated HSCT, onset between weeks 2 and 6 after HSCT, memory loss, dysesthesia, presence of immune reaction, steroid use, and hyponatremia.
Treatment: The first choice is foscarnet. In patients whose condition is unsuitable for foscarnet therapy, ganciclovir is recommended. Combination therapy can be used. Full dose therapy is recommended. Antiviral therapy should be for 3–4 weeks in patients whose CNS symptoms are improving and HHV-6 DNA in CSF converse negative. In other cases, extension of therapy duration is recommended.
We included foscarnet as an agent that should be covered by insurance. In November 2018, the Ministry of Health, Labour and Welfare of Japan approved foscarnet for HHV-6 encephalitis after allogeneic HSCT.

10-5 Measuring the viral load of HHV-6: comparison between qRT-PCR and ddPCR

Poster

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Objective: HHV-6 is responsible for opportunistic diseases in immunocompromised patients. Follow-up is performed by the measure of viral load (VL) in blood, usually by the use of quantitative polymerase chain reaction (PCR). According to the symptomatology, viral load may be measured in other biological matrices, such as cerebrospinal fluid (CSF) in case of encephalitis.
In about 1% of the population, HHV-6 DNA is integrated into the human genome and is present in every cell of the organism (iciHHV-6). In those subjects, VL is ≥ 6 log of viral genome per million cells, no matter the biological matrix used.
The objective of our study was to compare two methods of PCR, qPCR and droplet digital PCR (ddPCR) to measure HHV-6 VL for the diagnosis and follow-up of patients.
Methods: A total of 94 blood samples from 50 patients followed for an HHV-6 infection in the laboratory were analyzed. The majority of these patients were hospitalized in Hematology (80%). The number of samples per patient ranged from 1 to 9 (mean 1.9).
DNA extraction has been performed using QIAamp DNA Blood MiniKit (Qiagen). Viral load, expressed as the number of HHV-6 genomic DNA copies per million cells (cop/M cell), has been measured using qPCR in U65-66 genes (Gautheret-Dejean et al. J Virol Methods 2002) and U42 gene (developed by P. Bonnafous) and ddPCR in U42 gene (P. Bonnafous). The number of HHV-6 genomic copies per cell has been calculated by the quantification in parallel of the gene coding for albumin (Laurendeau et al. Clin Chem 1999).
Results: HHV-6 DNA was detected in 94, 97, and 93% of the samples for U65-66 qPCR, U42 qPCR, and U42 ddPCR respectively. Median VL were 12,950 cop/M cell [4-102x10^6] for U65-66 qPCR, 27,800 cop/M cell [43-208x10^6] for U42 qPCR, and 17,900 cop/M cell [5-26.6x10^6] for U42 ddPCR. Correlation was observed between the two qPCR methods ($p<0.0001$; $R^2=0.984$) and between U42 qPCR and U42 ddPCR ($p<0.0001$; $R^2=0.952$).
Conclusions: Results of HHV-6 VL measured by U65-66 or U42 qPCR and U42 ddPCR were correlated. However, VL using U42 qPCR was slightly higher than that obtained using the other methods. Otherwise, sensitivity of U42 ddPCR was inferior when compared to either qPCR method.

10-6 Structure-based design of small-molecule inhibitors to block virus latent infection

Poster

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Epstein-Barr virus (EBV) is a DNA tumor virus responsible for 1 to 2% of human cancers including subtypes of Burkitt's lymphoma, Hodgkin's lymphoma, gastric carcinoma, and nasopharyngeal carcinoma (NPC). Persistent latent infection drives EBV-associated tumorigenesis. Epstein-Barr nuclear antigen 1 (EBNA1) is the only viral protein consistently expressed in all EBV-associated tumors and is, therefore, an attractive target for therapeutic intervention. It is a multifunctional DNA binding protein critical for viral replication, genome maintenance, viral gene expression, and host cell survival. Using a fragment-based approach and x-ray crystallography, we identify a 2,3-disubstituted benzoic acid series that selectively inhibits the DNA binding activity of EBNA1. We characterize these inhibitors biochemically and in cell-based assays, including chromatin immunoprecipitation and DNA replication assays. In addition, we demonstrate the potency of EBNA1 inhibitors to suppress tumor growth in several EBV-dependent xenograft models, including patient-derived xenografts for NPC. These inhibitors selectively block EBV gene transcription and alter the cellular transforming growth factor-β (TGF-β) signaling pathway in NPC tumor xenografts. These EBNA1-specific inhibitors show favorable pharmacological properties and have the potential to be further developed for the treatment of EBV-associated malignancies. Importantly, this novel, fragment-based approach for targeting EBNA1 has potential applications for targeting other herpesvirus proteins involved in regulating viral latency.

10-7 Genotyping of human herpesvirus 6 by real time PCR and DNA sequencing

Poster

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Objective: Human herpesvirus 6 (HHV-6) isolates are classified into two variants, HHV-6A and HHV-6B, by distinct genetic, antigenic, and biological characteristics. The prevalence of HHV-6 variants in Russia is unclear due to the lack of genotyping commercial test-systems.
The goal of the study was to develop a new real-time PCR-based approach for genotyping of HHV-6.
Methods: The DNA was extracted using Multi-DNA-Extraction kit (Ref. 80-01, Astra Biotech GmbH) from the 200 plasma samples of young children with Exanthem subitum or hematopoietic stem cell transplantation (HSCT) admitted at St. Petersburg Filatov's Children Hospital and at St. Petersburg Raisa Gorbacheva Memorial Research Institute for Pediatric Oncology, Hematology and Transplantation. The HHV-6 DNA was detected by qualitative and quantitative real-time PCR using commercially available diagnostic kits (AmpliSens, Russia).
Genotyping of HHV-6 was performed by real-time PCR for the detection of the polymorphisms in genes U67 (in-house developed, Russian patent # 2627607, 2017), U38 (patent CN103820574B, 2014), and U41 (Yavarian et al., 2015). The 49 DNA samples were available for the Sanger sequencing of the U86 gene (fragment 547 bp, both strands) to confirm the results of genotyping. The whole genome sequences of HHV-6A NC_001664 and HHV-6B NC_000898 were used as reference sequences.
Results: Using the developed real-time PCR genotyping kit for the diversity of the U67 gene, we defined the HHV-6B variant in all 200 HHV-6-positive DNA samples. The 120 randomly selected HHV-6 DNA samples were further confirmed HHV-6B by real-time PCR assays of U38 and U41. The Sanger sequencing of the U86 gene (fragment 547 bp) verified HHV-6B genotype. This fragment shows <70% identity between variants A and B and it includes easily recognizable 15 bp deletion in HHV-6A (Isegawa et al., 1999).
Conclusions: New real-time PCR-based approach for HHV-6 genotyping allows distinguishing between variants HHV -6A and HHV-6B. It will be useful for diagnostics and assessing the genetic diversity and geographical distribution of these HHV-6 genotypes in Russia and elsewhere.

11-1 The characteristics of patients with persistent HHV-6 infection after drug induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS)

Oral

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Background: Drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS) is characterized by fever, cutaneous eruptions, hematological abnormalities, organ disorder, and reactivation of human herpesvirus 6 (HHV-6). In addition, autoimmune diseases such as type 1 diabetes and thyroiditis may develop long-term after resolution of DIHS. Reactivation of HHV-6 has been reported to be involved in clinical condition of DIHS. We found that quite a few patients harbor high levels (>1,000 copies / 1,000,000 PBMC) of HHV-6 DNA in PBMC over 4 months after onset of DIHS. However, it is unclear what effect persistent HHV-6 infection has on DIHS patients in chronic phase.

Methods: We analyzed clinical symptoms, blood test findings, reactivation of herpesvirus, expression of serum cytokines (IL-4, IL-5, IL-10, IFN-γ), and soluble IL-2 receptor for 8 DIHS patients with persistent HHV-6 infection and 10 patients with transient HHV-6 infection.

Results: Compared to the transient HHV-6 infection group, persistent HHV-6 infection group showed more severe acute phase cutaneous and mucosal eruptions, higher levels of HHV-6 DNA in acute phase, higher rate of cytomegalovirus reactivation, higher levels of IL-4 in acute phase and higher levels of soluble IL-2 receptor in late phase, higher rate of long-term complications (i.e., interstitial nephritis, arthritis, thyroiditis).

Conclusion: Persistent HHV-6 infection may have some influence on the pathological and immunological conditions of DIHS and may be involved in the clinical course and prognosis.

11-2 Heterologous immunity and the role of HHV-6 in drug hypersensitivity

Oral

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Adverse drug reactions are commonplace and can occur when a drug binds to its intended pharmacological target or off-targets. Immunologically mediated adverse drug reactions, such as drug hypersensitivity syndrome, drug reaction with eosinophilia and systemic symptoms (DRESS), and Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis (SJS/TEN), can be severe and result in a diverse set of clinical manifestations that include fever and rash as well as multiple organ failure (liver, kidney, lungs, and/or heart). A number of drug hypersensitivity reactions have been shown to occur in virally infected patients (e.g., abacavir hypersensitivity in HIV patients). A set of drugs that induce hypersensitivity responses have demonstrated strong HLA associations (e.g., abacavir hypersensitivity occurs exclusively in individuals that carry HLA-B*57:01). The mechanistic roles of the viruses in the initiation, or perpetuation, of T cell mediated adverse drug reactions are complex and have been proposed to involve stimulation of virus-specific T cells that cross-react with the drug presented to T cells in the context of HLA (the heterologous immunity model). The recent report of occupational trichloroethylene hypersensitivity syndrome associated with carrying HHV-6 and HLA-B*13:01 (Odds ratio 91.8) reveals mechanistic links between the virus and T cell mediated adverse drug reactions.

POSTER LIST

Alzheimer's Disease

HHV-6 & CNS Disease

Inherited ciHHV-6

POSTER LIST

Innate & Acquired Immunity

Host-Cell Interaction

Transplantation

Treatment & Diagnostics

The HHV-6 Foundation is pleased to sponsor and provide administrative support for the 11th International Conference on HHV-6 & 7.

The HHV-6 Foundation is a non-profit foundation that operates the conference, awards pilot grants and manages a repository of reagents to offer to the scientific community at nominal rates.

Kristin Loomis – Founder, President & Executive Director
Dharam Ablashi – Scientific Director
Jill Chase – VP & Repository Manager
Jason Stanley- Conference Administrator
Eva Eliassen – Research Associate & Newsletter Editor
Kent Van Donge – Intern

HHV-6 Foundation Repository

Go to our website for details at: hhv-6foundation.org/repository. Reagents include:

- HHV-6A & HHV-6B infected and uninfected cells
- HHV-6A, HHV-6B and HHV-6A/B specific monoclonal antibodies
- Spiked samples for laboratory reference
- Control slides

DHARAM ABLASHI RESEARCH FUND -- PILOT GRANT PROGRAM

Private donations for this research fund have been made in honor of Dharam Ablashi, co-discoverer of HHV-6 and scientific director of the HHV-6 Foundation. These funds have enabled pilot grants to the following individuals in 2017-2018:

2018 Pilot Grants

- **Mara Cirone, MD, PhD**, Sapienza University of Rome Department of Experimental Medicine, ROME, *Autophagy dysregulation, HHV-6 and Alzheimer's disease*
- **Niza Frenkel, PhD,** Tel Aviv University, Daniel Abraham Institute of Virology, ISRAEL, *Testing the effect of immunosuppressive drugs employed in transplantations on HHV-6 infection*
- **Alex Greninger, MD, PhD**, University of Washington, Laboratory Medicine, USA, *Characterization of the HHV-6A/B U15 as a vFLIP*

- **Ruth Jarrett, MBChB,** University of Glasgow, Centre for Virus Research, SCOTLAND, *Characterisation of iciHHV-6 integration sites using targeted enrichment coupled with sequencing on the Oxford Nanopore Technologies platform*
- **Stefan Nierkens, PhD,** University Medical Center Utrecht, NETHERLANDS, Department of Immunology/Haematology, *The effect of HHV6 viremia on thymopoiesis after hematopoietic transplantation*
- **Darby Oldenburg, PhD,** Gunderson Medical Foundation, USA, *Construction & Characterization of a Murine Roseolovirus BAC*
- **Swapneel Patel, MD, PhD**, Washington University School of Medicine, USA, *Immunomodulation by murine roseolovirus*
- **Tuan Phan,** medical student, Tulane University Medical School, USA *Retrospective Analysis of Human Herpesvirus 6 (HHV-6) Infections in Skin Tissue Biopsy Samples Obtained from Hematopoietic Stem Cell Transplant Recipients with Acute Graft- Versus-Host Disease and In Vitro Analysis of CD134 Upregulation by Toll-Like Receptor 9 (TLR9)/HHV-6B Binding Interaction*
- **Phillip West, PhD,** Assistant Professor, Texas A&M Health Science Center, Dept. of Microbial Pathogenesis & Immunology, USA, *Defining how HHV-6 Induces Mitochondrial Dysfunction and Metabolic Alterations in Human T-lymphoblast Lines*
- **Darren Wight, PhD,** Freie Universität Berlin, Institut für Virologie, GERMANY, *Role of shelterin in HHV-6 integration and its association with disease in iciHHV-6 individuals*

2017 Pilot Grants

- **Roberto Alvarez-Lafuente, MD,** Hospital Clinico San Carlos, SPAIN, *MicroRNAs of HHV-6 in serum and cerebrospinal fluid of multiple sclerosis patients: correlation with activity and progression of the disease*
- **Elisabetta Caselli, PhD,** University of Ferrara, ITALY, *HHV-6 induced alterations of microRNAs and transcriptional factors expression: focus on autoimmunity*
- **Mara Cirone, PhD,** La Sapienza University of Rome, ITALY, *Autophagy Interaction with HHV-6A Infection*
- **Bibhuti Das, MD,** University of Texas Southwest Medical Center, USA, *Cardiotropic Viral Infections in Children with Dilated Cardiomyopathy and Clinical Significance of Pre-transplant*
- **Elin Engdahl, PhD,** Karolinska Institute, SWEDEN, *Human herpesvirus 6B affects the MAPK pathway, possibly involved in vacuolization of infected cells*
- **Louis Flamand, PhD,** Laval University, CANADA, *Induced pluripotent stem cells to study the biological impacts of inherited chromosomally-integrated HHV-6*
- **Joshua A. Hill, MD**, Fred Hutchinson Cancer Research Center, USA, *Cancer Risk and Inflammatory Profile in Patients with Inherited Chromosomally Integrated Human Herpesvirus 6*
- **Ruth Jarrett, MD,** University of Glasgow, UNITED KINGDOM**,** *Characterisation of iciHHV-6 integration sites using targeted enrichment coupled with sequencing on the Oxford Nanopore Technologies platform*
- **David Koelle, MD,** University of Washington, USA, *Do donor T cell responses to HHV6B predict reactivation after hematopoietic stem cell transplantation?*
- **Mauro Malnati, MD, PhD,** San Raffaele Scientific Institute, ITALY, *HHV-6 A/B infection and Multiple sclerosis: triggering factor or epiphenomenon?*
- **Roberta Rizzo, PhD**, University of Ferrara, ITALY, *Assessment of the effects of HHV-6A infection on the endometrial (e)NK cells of infertile women*
- **Suzana Straus, PhD,** University of British Columbia, CANADA, *Investigating the potential link between HHV-6B and MS: interaction between U24 and WW domains*
- **Xiofeng Zhou, PhD,** University of Michigan, USA, *A Mouse Model of HHV-6 Reactivation Post Allogeneic HSCT*

OUTSTANDING TRAINEE ABSTRACT AWARDS

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11TH INTERNATIONAL CONFERENCE ON HHV-6 & 7

YOUNG INVESTIGATOR AWARDS

Caroline B. Hall Award for Excellence in Clinical Research

2017: Roberta Rizzo, PhD

University of Ferrara, Department of Medical Sciences, Ferrara, Italy

2015: Joshua Hill, MD

Department of Medicine, Division of Allergy and Infectious Diseases, Fred Hutchinson Cancer Research Center, University of Washington, Seattle, WA

2013: Yoshiki Kawamura, MD, PhD

Fujita Health University School of Medicine, Fujita, Japan

Koichi Yamanishi Award for Excellence in Basic Research

2017: Ben Kaufer, PhD

Institute of Virology, Freie Universität Berlin, Germany

2015: Bhupesh Prusty, PhD

Group Leader, Department of Microbiology, University of Würzburg, Germany

The 2019 recipients will be announced

Tuesday evening at the banquet

PAST RECIPIENTS OF THE HHV-6 FOUNDATION'S

Dharam Ablashi Lifetime Achievement Award

Dario Di Luca, 2017
University of Ferrara
ITALY

Philip E. Pellett, 2015
Wayne State University
USA

Caroline Breese Hall, 2013
University of Rochester Medical Center
USA

Yoshizo Asano, 2011
The Zambia Project, Hokkaido University
Fujita Health University (1979-2010)
JAPAN

Koichi Yamanishi, 2008
National Institute of Biomedical Innovation
JAPAN

Dharam Ablashi, 2006
HHV-6 Foundation
USA

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Tuesday evening at the banquet

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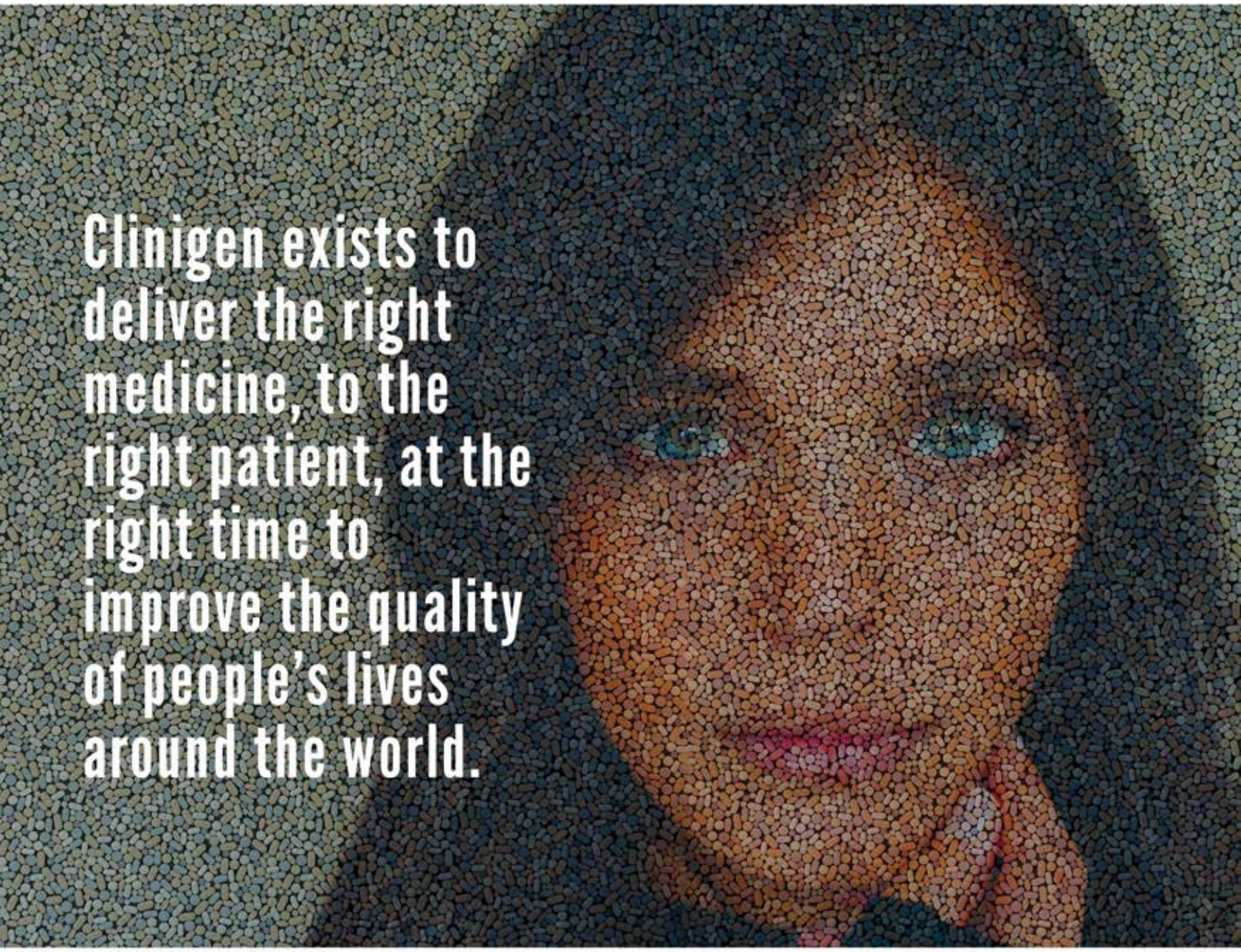

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